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Development of a modified acetonitrile-based extraction procedure followed by ultra-high performance liquid chromatography–tandem mass spectrometry for the analysis of psychiatric drugs in sediments

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ABSTRACT

An analytical method based on a modified Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction procedure followed by ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) was developed for the analysis of psychiatric drugs in sediments. An optimized approach was applied in sample preparation by using basic acetonitrile as extraction solvent. Extraction was followed by a clean-up using dispersive solid phase extraction (dSPE) to remove matrix interfering compounds. The analytical method was validated in terms of sensitivity, linearity, recovery, intra- and inter-day precisions and method detection and quantification limits. Under optimized conditions, limits of detection ranged from 0.01 ng g^{-1} to 2.08 ng g^{-1}; and recoveries between 47 and 110% with relative standard deviation (RSD) below 5%. The developed methodology was applied to sediments of two Portuguese rivers (Douro and Lima rivers) and nine out of eleven psychiatric drugs were detected in sediments at concentrations up to 26.4 ng g^{-1} (dry weight). To the best of our knowledge, it was the first time that the human metabolites norfluoxetine and norsertraline were detected in river sediments at levels of few nanograms per gram.

1. Introduction

Mental disorders such as major depression, bipolar disorder, schizophrenia, and substance abuse, are among the most common classes of human diseases [1]. For instance, depression has been recognized as an important global burden disease and in 2010 it was pointed out as the second cause of disability worldwide [2]. The most frequent approach for the treatment of mental disorders requires the use of pharmaceuticals. For that reason psychiatric drugs world consumption has increased with time and this trend is expected in the future [3].

Due to the high consumption rate, psychiatric drugs are among the most frequently detected pharmaceuticals in the environment. They have been ubiquitously detected in wastewaters [4,5], surface waters [6,7], drinking water [8,9], and to a less extent in soils [10,11] and biota [12]. Furthermore, psychiatric drugs can persist in the environment, as is the case of the antiepileptic carbamazepine that remains unchanged in soil for 40 days [13], or the antidepressant fluoxetine and its human metabolite norfluoxetine that have also showed a tendency to resist to biodegradation [14].

Currently several analytical methodologies are described in the literature for the quantification of psychiatric drugs in the environment, however most of these methods are focused on their analysis in aqueous matrices [8,15–18]. Analytical methodologies for the determination of psychiatric drugs in sediments are still sparse [19,20]. Extraction methods such pressurized liquid extraction (PLE) [19–21], ultrasonic extraction [22,23], microwave assisted micellar extraction [24] have been described for the analysis of pharmaceuticals in sediments, usually combined with a purification step using solid phase extraction (SPE) [19,20,22].

In 2003, a new extraction and clean-up technique – QuEChERS (acronym name for Quick, Easy, Cheap, Effective, Rugged,

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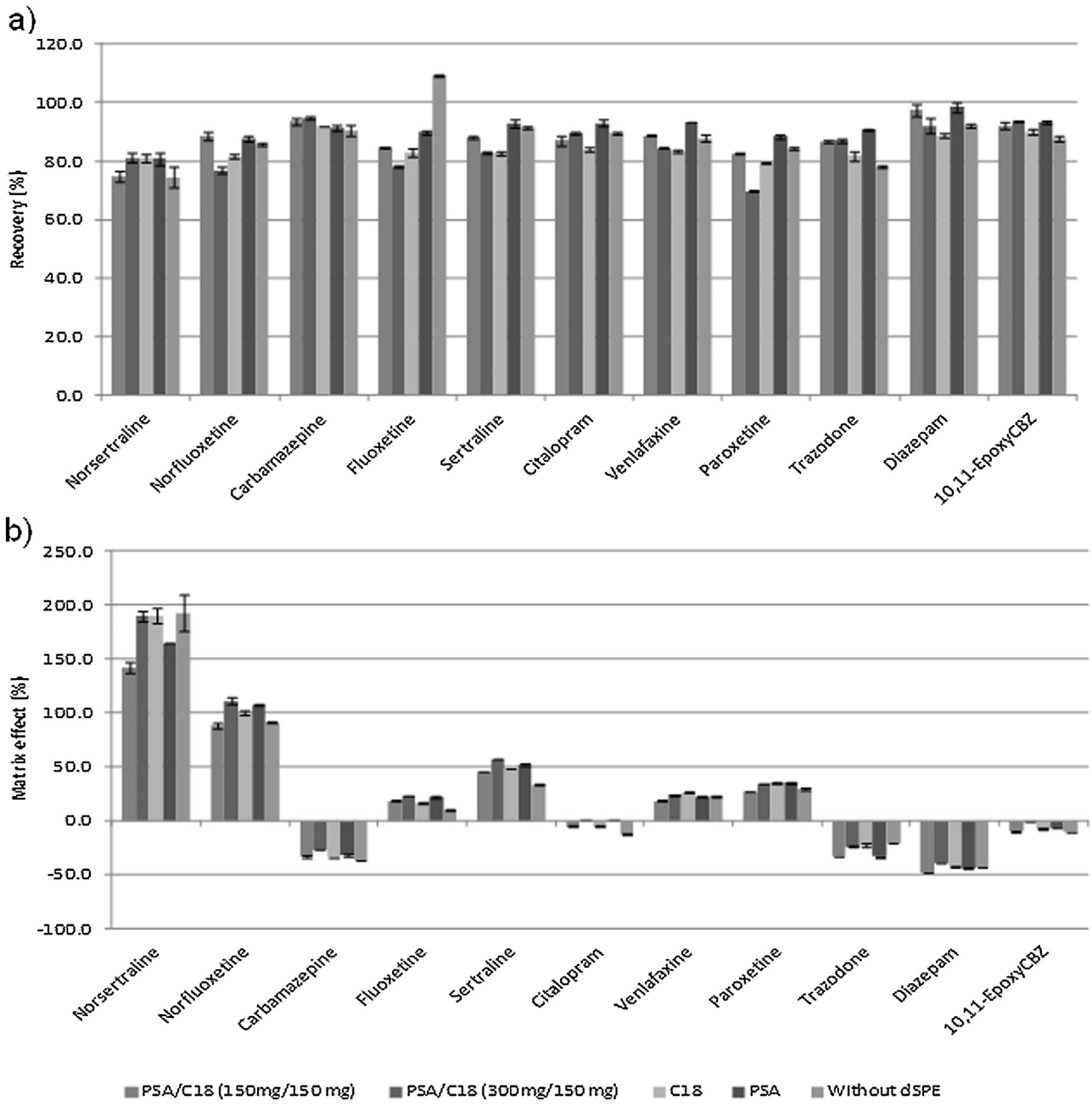


Fig. 1. (a) Recoveries and (b) matrix effects of the selected pharmaceuticals using different dSPE sorbents and without clean-up.

and Safe) – was developed by Anastassiades et al. [25] for the analysis of pesticides in fruits and vegetables. This procedure is based on an extraction with an organic solvent, usually acetonitrile, followed by a liquid–liquid partition induced by the addition of salts, and a dispersive solid phase extraction (dSPE) to clean-up the extract. The QuEChERS method is a simple and rapid extraction procedure that requires low solvent consumption, allowing a more environmentally-friendly approach in sample preparation [26]. These advantages have contributed to the increase of the QuEChERS analysis over the years. Recently, this procedure was also applied to the analysis of pharmaceuticals in different environmental samples such as sewage sludge, soil and sediment [27–31]. Nevertheless, only few psychiatric drugs have been extracted using the QuEChERS method as, for example, the antiepileptic carbamazepine [27,29,31,32].

Since pharmaceuticals are found in trace levels in the environment, powerful hyphenated techniques are necessary for their reliable identification and quantification. Usually liquid chromatography coupled to electrospray ionization (LC-ESI-MS) and low-energy collision dissociation tandem mass spectrometry (CID-MS/MS) has been the technique of choice for this purpose. The use of the CID-MS/MS in the monitoring reaction mode scans (MRM) permits the improvement of the selectivity, specificity and sensitivity of the quantification method.

The aim of this work was to develop a rapid, robust and sensitive modified QuEChERS extraction method for the simultaneous analysis of eight psychiatric drugs described in Table 1. The analysis pertained to different antidepressants, such as fluoxetine, sertraline, citalopram, venlafaxine, paroxetine and trazodone; the antiepileptic carbamazepine; the anxiolytic diazepam; and three human metabolites norsertraline, norfluoxetine and

Table 1
Physical-chemical properties of the selected pharmaceuticals.

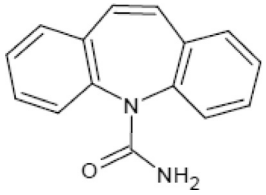
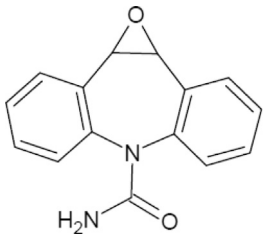
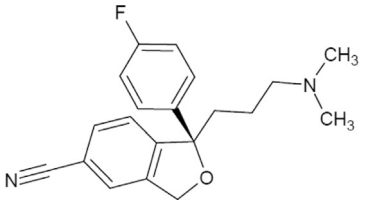
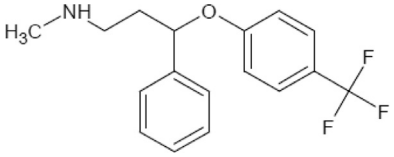
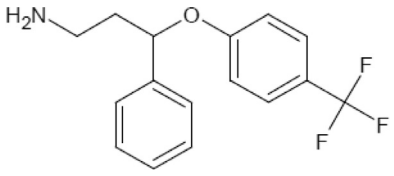
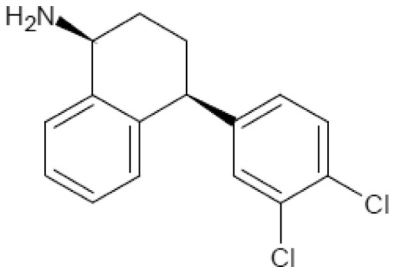
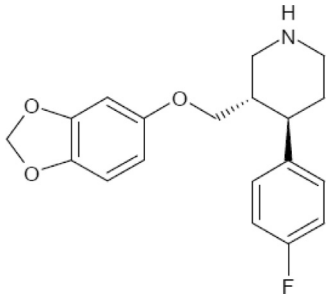
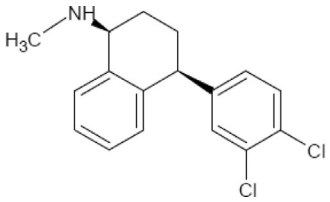
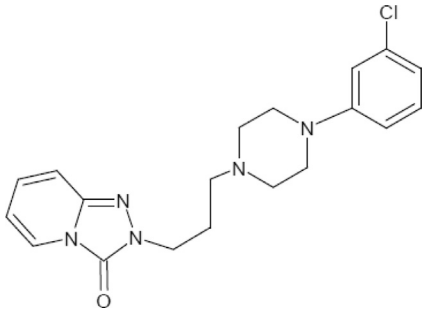
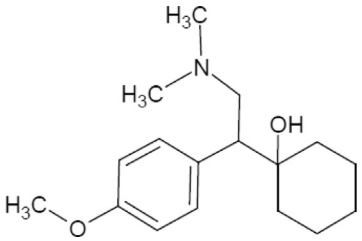
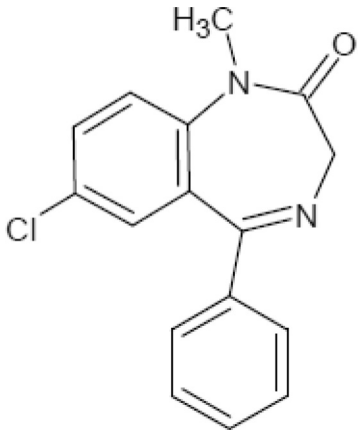
Pharmaceutical	Chemical structure	Formula	M_w	pKa[b]	Log P[b]	Log K_{OC}[c]	Log K_{OW}[c]
Anti-epileptics							
Carbamazepine		$C_{15}H_{12}N_2O$	236.09	15.96	2.77	3.59	2.45
10,11-Epoxycarbamazepine[a]		$C_{15}H_{12}N_2O_2$	252.09	15.96	1.97	2.59	0.95
Antidepressants							
Citalopram		$C_{20}H_{21}FN_2O$	324.16	9.78	3.76	4.40	3.74
Fluoxetine		$C_{17}H_{18}F_3NO$	309.13	9.80	4.17	5.32	4.05
Norfluoxetine[a]		$C_{16}H_{16}F_3NO$	295.12	9.77	3.74	5.18	4.18
Norsertraline[a]		$C_{16}H_{15}Cl_2N$	291.06	9.73	4.72	5.37	4.82
Paroxetine		$C_{19}H_{20}FNO_3$	329.14	9.77	3.15	2.64	2.57

Table 1 (*Continued*)

Pharmaceutical	Chemical structure	Formula	M_w	pKa[b]	Log P[b]	Log K_{OC}[c]	Log K_{OW}[c]
Sertraline		$C_{17}H_{17}Cl_2N$	305.07	9.85	5.15	5.53	5.29
Trazodone		$C_{19}H_{22}ClN_5O$	371.15	7.09	3.13	4.69	3.21
Venlafaxine		$C_{17}H_{27}NO_2$	277.20	8.91; 14.42	2.74	3.17	3.28
Anxiolytic, hypnotic, sedative							
Diazepam		$C_{16}H_{13}ClN_2O$	284.07	2.92	3.08	4.05	2.82

[a] Metabolite.
[b] www.chemicalize.org.
[c] www.chemspider.com.

10,11-epoxycarbamazepine extracted from river sediments. Pharmaceuticals were chosen based on the high consumption of antidepressants worldwide [33]; their resistance to biodegradation and the low removal efficiency in WWTPs [34]. The quantification of these pharmaceuticals and metabolites was achieved by LC-ESI-MS and CID-MS/MS analyses.

2. Experimental

2.1. Chemicals and materials

All standards used were of high purity grade (≥97%). Carbamazepine, 10,11-epoxycarbamazepine, fluoxetine, norfluoxetine, sertraline, citalopram, venlafaxine, paroxetine, trazodone and diazepam were purchased from Sigma–Aldrich (Spain). Norsertraline was purchased as a methanolic solution from Cerilliant-Certified Reference Materials (Canada, USA). Fluoxetine, norfluoxetine, sertraline, venlafaxine, paroxetine, and trazodone were acquired in the form of hydrochloride salt. Isotopically labeled compounds, used as internal standards, were fluoxetine-d5 purchased from Sigma–Aldrich (Madrid, Spain), carbamazepine-d10 and venlafaxine-d6 from Cerilliant-Certified Reference Materials (Canada, USA), and diazepam-d5 from Lipomed AG, (Arlesheim, Switzerland).

Individual stock standard solutions were prepared on a weight basis in methanol (at a concentration of 1000 mg L^{-1}), with the exception of norsertraline that was acquired in a methanolic solution at a concentration of 100 mg L^{-1}. Isotopically labeled

standards were purchased as methanolic solutions at a concentration of 100 mg L^{-1} for carbamazepine-d10 and venlafaxine-d6 and 1000 mg L^{-1} for diazepam-d5. Only fluoxetine-d5 had to be prepared on a weight basis in methanol (at a final concentration of 1000 mg L^{-1}). After preparation, standards were stored at −20 °C. Fresh stock solutions of pharmaceuticals were renewed every six months.

Working standard solutions, containing all pharmaceuticals, were prepared in a mixture of acetonitrile/water (50:50, v/v) by appropriate dilution of individual stock solutions and were renewed before each analytical run by mixing appropriate amounts of the intermediate solutions. A separate mixture of isotopically labeled internal standards, used for internal standard calibration, was prepared in acetonitrile and further dilutions were also prepared in the same organic solvent.

Acetonitrile LC–MS grade was supplied by Biosolve (Valkenswaard, Netherland), methanol LC–MS Ultra CHROMASOLV® and ammonium hydroxide solution (NH_3 aq, 28.0–30.0%) were obtained from Sigma–Aldrich, tetrahydrofuran Lichrosolv® was purchased from Merck (Darmstadt, Germany), formic acid (98%, PA-ACS) was obtained from Panreac (Barcelona, Spain), and acetic acid glacial (ACS grade) was supplied by VWR Chemicals (Fontenay-sous-Bois, France). Ultra-pure water (resistivity of 18.2 MΩ cm^{-1}) was produced using a Simplicity 185 system manufactured by Millipore (Molsheim, France).

QuEChERS extract tubes were obtained from Agilent Technologies (Original, AOAC and EN methods) (Lake Forest, CA, USA). The Original salts contained 1 g NaCl and 4 g $MgSO_4$, while the acetate buffer (AOAC method) contained 1.5 g sodium acetate and 6 g $MgSO_4$, and the citrate buffer (EN method) contained 0.5 g sodium hydrogencitrate sesquihydrate, 1 g sodium citrate and 4 g $MgSO_4$.

Different dispersive SPE materials commercially available were tested, namely PSA (primary and secondary amine exchange), which contained 150 mg PSA and 900 mg $MgSO_4$; C18, containing 150 mg CEC18 and 900 mg $MgSO_4$; and mixtures of these two sorbents PSA/C18 (which contained 150 mg PSA, 150 mg CEC18 and 900 mg $MgSO_4$, or 300 mg PSA, 150 mg CEC18 and 900 mg $MgSO_4$ (EnviroClean®)). All the dispersive SPE materials were purchased from Agilent Technologies (Lake Forest, CA, USA), except for the last one (EnviroClean®) that was supplied by UCT (Bristol, PA, USA).

2.2. Sample collection and pretreatment

Six sediment samples from two rivers (Douro and Lima rivers), located in the north of Portugal, were collected in the middle course of the river, in May 2013. In Douro river samples were collected along the river, including a sediment sample collected in the nearby of a harbor. Samples were kept refrigerated (±4 °C) during the transport to the laboratory. Herein, sediment samples were frozen at −20 °C and lyophilized. After that, they were ground in a grinder and passed through a 0.25 mm sieve to obtain a homogeneous sample, before being extracted and analyzed.

For the development of the modified QuEChERS extraction procedure, 5 g of sieved sediment were weighted into a 50 mL polypropylene extract tube and 1 mL of a standard mixture, containing all pharmaceuticals, prepared in acetonitrile was added, so as to have a final concentration of 100 ng g^{-1}. Then, the tube was vortexed to allow a uniform distribution of the analytes, and afterwards the sediment was dried under a gentle stream of nitrogen to evaporate the solvent and left overnight to better incorporated the target analytes.

2.3. Modified QuEChERS extraction procedure

Five grams of sieved sediment were weighted into a 50 mL polypropylene extract tube and 10 mL of ultra-pure water was added. The tube was vortexed for 30 s and after that 15 mL of 2% ammonium hydroxide in acetonitrile was added to the tube. Again, this was vortexed for 30 s and then QuEChERS original salts were added. The mixture was immediately manually shaken and afterwards vortexed for 1 min. The sample was centrifuged at 4000 rpm for 5 min, and after that 12 mL of the acetonitrile layer was transferred into a 15 mL dispersive SPE tube (containing 150 mg PSA and 900 mg $MgSO_4$), vortexed for 1 min and then centrifuged at 4000 rpm for 5 min. Finally, 9 mL of the extract were transferred to a glass tube, evaporated to dryness under a gentle stream of nitrogen and reconstituted with 500 μL of a mixture water/acetonitrile (50:50, v/v). Lastly, 5 μL of a standard mixture containing all isotopically labeled compounds (1 mg L^{-1} for carbamazepine-d10 and fluoxetine-d5, and 2 mg L^{-1} for venlafaxine-d6 and diazepam-d5) were added in the extract as internal standards for further UHPLC–MS/MS analysis.

2.4. UHPLC–MS/MS analysis

Chromatographic analysis was performed on a Nexera Ultra-High Performance Liquid Chromatography system (Shimadzu Corporation, Kyoto, Japan), equipped with two solvent delivery modules, a degasser, an autosampler, and a column oven, and coupled to a triple-quadupole mass spectrometer detector LCMS-8030 with an electrospray ionization source (ESI). The chromatographic separation was carried out in a Cortecs® UPLC® C18+ column (100 × 2.1 mm i.d.; 1.6 μm particle size) from Waters (Milford, MA, USA). The mobile phase was constituted by 0.1% formic acid in water as eluent A and acetonitrile as eluent B, at a flow rate of 0.3 mL min^{-1}. The gradient elution was performed as follow: 0–3.0 min, 5–100% B; 3.0–3.5 min maintain 100% B; 3.5–4.0 min return to initial conditions; 4.0–7.0 min re-equilibration of the column. The column oven was kept at 30 °C and the autosampler was operated at 15 °C. An injection volume of 5 μL was used. Lab Solutions LCMS software (Shimadzu Corporation, Kyoto, Japan) was used for system control and data processing.

The ESI source was operated in the positive ionization mode. An individual standard solution at a concentration of 1 mg L^{-1} was directly injected to select the precursor ion through full scan mode, to choose the most abundant fragments and to optimize the mass spectrometer parameters such as declustering potential, collision energy and collision cell exit potential. More detailed information (Full scan-spectra and its respective ion product spectra) is given in Supplementary material. Quantification of analytes was performed on multiple reaction monitoring (MRM) mode by monitoring two MRM transitions between the precursor ion and the two most abundant fragment ions for each compound. The first MRM transition (MRM 1) was used for quantification and the second one (MRM 2) for identification purposes. A dwell time of 15 ms was set for all the compounds. The optimized MS/MS parameters are shown in Table 2.

MS/MS conditions were optimized by the direct injection of a standard mixture solution 1 mg L^{-1} and are the following: nebulizing gas (nitrogen) and drying gas (nitrogen) flow: 2.6 and 15.0 L min^{-1}, respectively; dessolvation temperature: 300 °C; heat block temperature: 425 °C; interface voltage: 5.0 kV; collision induced dissociation gas (argon): 230 kPa.

2.5. Method validation

Method validation was done using a sediment sample from Douro River. All the validation studies were performed using the same sediment sample, which was previously analyzed to determine the presence of the selected psychiatric drugs. Since it was not possible to get a sediment sample without psychiatric drugs, blanks (no-spiked samples) were always analyzed and the concen-

Table 2
MS/MS parameters for the analysis of the selected pharmaceuticals.

Compound	Internal standard	Rt (min)	Precursor ion (*m*/*z*)	MRM 1 (Quantification)				MRM 2 (Identification)				Ion ratio (±SD) (*n* = 5)
		Product ion (*m*/*z*)	DP (V)	CE (V)	CXP (V)	Product ion (m/z)	DP (V)	CE (V)	CXP (V)			
Venlafaxine	Venlafaxine-d6	2.44	277.90 $[M+H]^+$	58.20	−30	−21	−10	260.30	−30	−13	−18	4.66 (±0.29)
Venlafaxine-d6	–	2.44	283.50 $[M+H]^+$	64.05	−30	−21	−11	–	–	–	–	–
Trazodone	Fluoxetine-d5	2.48	371.85 $[M+H]^+$	176.10	−30	−26	−18	148.15	−30	−36	−15	1.29 (±0.14)
Citalopram	Fluoxetine-d5	2.61	324.85 $[M+H]^+$	109.20	−30	−26	−22	262.20	−30	−19	−18	3.49 (±0.18)
Paroxetine	Fluoxetine-d5	2.68	330.00 $[M+H]^+$	70.20	−22	−33	−13	44.15	−22	−26	−17	1.58 (±0.11)
Norfluoxetine	Fluoxetine-d5	2.75	296.10 $[M+H]^+$	134.10	−14	−8	−13	30.20	−14	−12	−30	1.41 (±0.15)
Norsertraline	Fluoxetine-d5	2.77	292.10 $[M+H]^+$	275.05	−14	−10	−19	–	–	–	–	–
Fluoxetine-d5	–	2.77	315.15 $[M+H]^+$	44.25	−11	−14	−16	–	–	–	–	–
Fluoxetine	Fluoxetine-d5	2.78	310.25 $[M+H]^+$	44.25	−11	−14	−16	148.15	−11	−10	−15	10.92 (±0.82)
Sertraline	Fluoxetine-d5	2.80	306.15 $[M+H]^+$	275.10	−28	−13	−19	159.05	−28	−29	−16	0.89 (±0.08)
10,11-Epoxycarbamazepine	Carbamazepine-d10	2.86	253.20 $[M+H]^+$	180.10	−12	−25	−12	236.15	−12	−11	−16	1.30 (±0.03)
Carbamazepine-d10	–	3.08	247.15 $[M+H]^+$	204.15	−23	−22	−14	–	–	–	–	–
Carbamazepine	Carbamazepine-d10	3.09	236.95 $[M+H]^+$	194.10	−21	−20	−13	193.10	−21	−35	−13	4.94 (±0.21)
Diazepam-d5	–	3.54	290.10 $[M+H]^+$	154.05	−13	−29	−10	–	–	–	–	–
Diazepam	Diazepam-d5	3.55	284.75 $[M+H]^+$	154.05	−27	−29	−15	193.05	−27	−33	−13	1.15 (±0.04)

Rt—retention time; DP—declustering potential; CE—collision energy; CXP—collision cell exit potential.

tration of target analytes was determined and subtracted to the spiked sediment samples.

The identification and confirmation of the selected pharmaceuticals was made in agreement with EU Commission Decision 2002/657/EC [35], using as criteria two MRM transitions between the precursor ion and the two most abundant product ions; the MRM ratio, which was calculated as the ratio between the abundance of the two transitions considered; and the retention time of the analyte. For the quantification of the analytes in real samples, matrix-matched calibration method was used together with isotopically labeled standards in order to correct the matrix effects.

The performance of the method was evaluated in terms of linearity, extraction recoveries, method detection (MDL) and quantification (MQL) limits, precision (intra- and inter-day), and matrix effects.

Linearity of the method was settled for each compound by analyzing spiked sediment samples over a range of concentrations between 0.08 ng g^{-1} and 100 ng g^{-1}.

Method detection limit (MDL) and method quantification limit (MQL) were determined as the minimum amount detectable of analyte with a signal-to-noise ratio of 3 and 10, respectively. Whenever possible, MDL and MQL were calculated as the average of those estimated in real samples, otherwise spiked samples were used.

Extraction recoveries of target analytes were determined using sediment samples spiked at three different levels (low: 5 ng g^{-1}; medium: 50 ng g^{-1}; and high: 100 ng g^{-1}). Recoveries were determined by comparing the concentrations obtained, calculated by internal standard method using matrix-matched calibration curves, with those of the initial spiking levels. For each concentration, samples were analyzed in triplicate ($n = 3$). As sediment samples may contain the target analytes, blanks (no-spiked samples) were analyzed to determine their concentrations and afterwards subtracted to the spiked sediment samples.

Method precision was determined by repeated intra- and inter-day analysis for three concentrations (5 ng g^{-1}, 50 ng g^{-1} and 100 ng g^{-1}). Intra-day precision was analyzed by ten successive injections for each concentration in one day, whereas inter-day precision was assessed for the same concentrations in three successive days. Precision was expressed as the relative standard deviation (RSD) of the replicate measurements.

Matrix effects were evaluated in order to assess the potential interference of the matrix in the measurement of the analytical signal, which can promote either ion suppression or enhancement. Therefore, to evaluate the interference of matrix components in the compounds ionization, and how each pharmaceutical is affected by them, matrix effects were assessed by comparing the analytical signals obtained with sediment extract spiked with a standard mixture solution containing all the target analytes and the analytical signals of the standards prepared in acetonitrile/water (50:50, v/v) with the same concentration. Matrix effect was evaluated using Eq. (1).

$$\text{Matrix effect (\%)} = \left(\frac{\text{Area matrix} - \text{Area blank}}{\text{Area solvent}} - 1\right) \times 100 \quad (1)$$

where Area matrix is the peak area of the spiked extract sediment, Area blank is the peak area of the non-spiked sediment, and Area solvent is the peak area of the standard solution prepared in acetonitrile/water (50:50, v/v).

3. Results and discussion

3.1. Development of a modified QuEChERS extraction method

To determine the best conditions for the QuEChERS extraction method, different parameters were optimized, namely: choice of the QuEChERS salts, selection of the organic solvent modifier used in the extraction solvent, volume of water, volume of extraction solvent, and selection of the dispersive SPE sorbent.

In each optimized step, recoveries were evaluated using Eq. (2) by comparing the peak area for sediment samples spiked at 100 ng g^{-1} (Area spiked) with the peak area obtained for sediment extracts spiked after sample preparation at the same concentration (Area reference). A blank (sediment extract without spike) (Area blank) was always analyzed in each condition and the peak areas of the pharmaceuticals found subtracted to the peak area of the spiked sediment.

$$\text{Recoveries (\%)} = \left(\frac{\text{Area spiked} - \text{Area blank}}{\text{Area reference}}\right) \times 100 \quad (2)$$

3.1.1. Optimization of the QuEChERS extraction procedure

The first optimized parameter was the QuEChERS salts. The efficiency obtained for three commercial available mixtures of QuEChERS salts (original, acetate buffer and citrate buffer) was compared for the extraction of the selected pharmaceuticals. For that, 10 mL of water and 10 mL of acetonitrile were used. For most of the selected pharmaceuticals, original QuEChERS salts gave best recoveries (Fig. S12, Supplementary material), so they were chosen for the extraction. However the use of buffer salts for the extraction of pharmaceuticals from soil, sediment or sludge matrices is a most common approach described in literature [27,28,30,32].

Different organic solvents have been used in QuEChERS extraction, being the most common acetonitrile. In this way, for the optimization of the extraction solvent acetonitrile was tested alone and in the presence of different organic solvent modifiers, namely: 1% acetic acid, 2% ammonium hydroxide, and 10% tetrahydrofuran. Organic solvent modifiers were selected based on previously published methods. Acetic acid was chosen due to the modification of the AOAC method [36], tetrahydrofuran had shown to improve the extraction of antidepressants, such as fluoxetine [30], and ammonium hydroxide was a method adaptation based on the good recoveries obtained in the extraction of antidepressants from waters when this organic solvent modifier was used in the elution step of SPE [37]. The extractions were performed with 10 mL of water and 10 mL of the different organic solvent. Nevertheless acetonitrile and 2% ammonium hydroxide gave similar recoveries for almost all the selected pharmaceuticals, the addition of this organic solvent modifier showed better recoveries, especially for the antidepressants (fluoxetine, sertraline and paroxetine) and their metabolites (norfluoxetine and norsertraline) (Table 3). This could be justified by the physical-chemical properties of these compounds, which have high pKa values (around 9) (Table 1). In this way, at higher pH values, antidepressants are in their neutral form, having more affinity for the extraction solvent. Therefore, based on the results, ammonium hydroxide was chosen as the organic solvent modifier of the extraction solvent. To the best of our knowledge is the first time that basic acetonitrile was used as extraction solvent in the QuEChERS method for the extraction of pharmaceuticals from solid environmental matrices, since the most common organic solvents used were acetonitrile [27,32] or acidified acetonitrile [29–31]. It is worth highlighting that both approaches showed lower recoveries for the extraction of antidepressants [27,30].

The influence of the percentage of ammonium hydroxide in the organic solvent was also studied. For that, percentages of 1, 2, 5 and 10% ammonium hydroxide in acetonitrile were tested. In general, an increase in the percentage of ammonium hydroxide until 5% did not show significant differences in the obtained recoveries, with the exception of carbamazepine, diazepam and 10,11-epoxycarbamazepine that showed higher recoveries for 2% ammonium hydroxide in acetonitrile, while for a highest percentage of ammonium hydroxide (10%) all the compounds showed

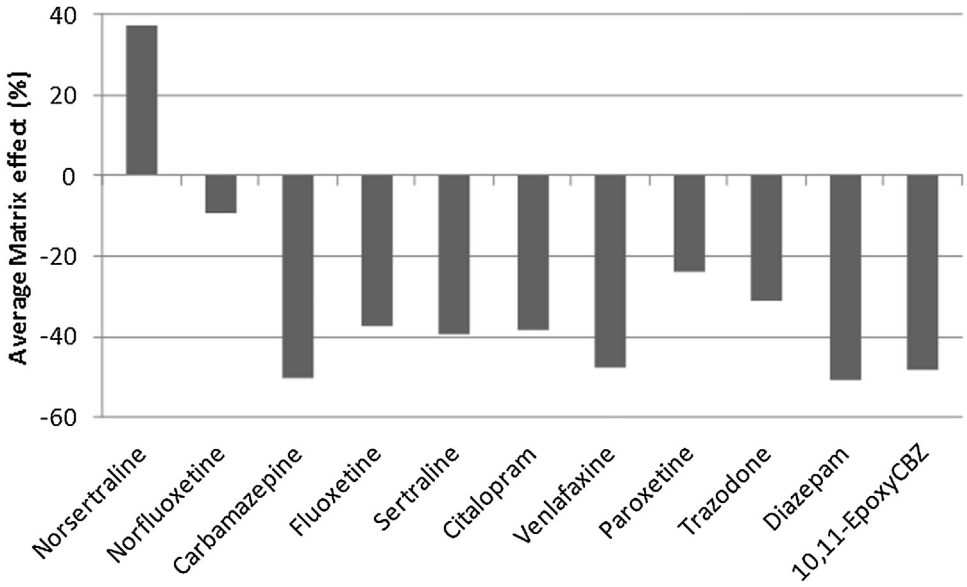


Fig. 2. Matrix effects, expressed in percentage, obtained for the selected pharmaceuticals using the developed QuEChERS-UHPLC–MS/MS method.

Table 3
Influence of the presence of organic solvent modifiers in the extraction solvent (acetonitrile) in the recovery of the selected pharmaceuticals.

Pharmaceutical	Acetonitrile	1% CH_3COOH in ACN	2% NH_4OH in ACN	10% THF in ACN
	Mean recovery (%) ± SD	Mean recovery (%) ± SD	Mean recovery (%) ± SD	Mean recovery (%) ± SD
Venlafaxine	84.8 ± 0.1	77.8 ± 0.1	87.9 ± 2.0	63.8 ± 0.1
Trazodone	91.4 ± 1.7	56.3 ± 1.5	84.2 ± 1.0	48.5 ± 1.0
Citalopram	84.5 ± 0.1	62.6 ± 4.8	89.1 ± 3.0	40.2 ± 0.2
Paroxetine	69.9 ± 0.9	33.9 ± 0.6	79.4 ± 1.7	17.8 ± 0.2
Norfluoxetine[a]	69.5 ± 2.1	44.0 ± 2.8	77.0 ± 1.3	17.1 ± 0.2
Norsertraline[a]	71.7 ± 0.8	38.4 ± 0.9	107.7 ± 9.1	18.5 ± 0.2
Fluoxetine	74.3 ± 0.7	31.0 ± 1.3	82.0 ± 0.9	17.2 ± 0.2
Sertraline	75.3 ± 1.1	29.0 ± 0.8	85.6 ± 0.6	17.4 ± 0.2
10,11-Epoxycarbamazepine[a]	84.8 ± 0.4	76.8 ± 1.4	94.0 ± 0.5	79.0 ± 0.1
Carbamazepine	97.8 ± 1.0	92.4 ± 0.8	96.5 ± 3.0	83.3 ± 3.2
Diazepam	86.9 ± 2.6	63.3 ± 1.1	91.2 ± 1.6	42.8 ± 0.7

[a] Metabolite.

a decrease in recoveries. Thus, 2% ammonium hydroxide in acetonitrile was chosen as organic solvent modifier of the extraction solvent.

After the selection of the organic solvent modifier, the volume of extraction solvent was optimized. Several volumes of basic acetonitrile (10, 15, 20, 25 and 30 mL) were tested. In general, an increase in the volume of extraction solvent led to a slight improvement in the recoveries until 25 mL, whereas for higher volumes, the obtained recoveries decreased. Hence, a final volume of 15 mL was chosen, since the recoveries were similar to those obtained with higher volumes of extraction solvent, reducing in this way the consumption of organic solvent and also the co-extraction of interfering compounds (Fig. S13, Supplementary material).

Since QuEChERS extraction method was initially developed for matrices with a higher content of water than sediments or soils, before their analysis the matrix has to be reconstituted [38]. With that purpose the need of a rehydration step was studied by evaluating the recovery of pharmaceuticals without water and with different volumes of water (5, 10 and 15 mL). For almost all the compounds, the rehydration of the matrix is desirable, since higher recoveries were achieved using 10 mL of water. Although the differences were not significant among the volumes tested. Only the metabolites norfluoxetine and norsertraline showed higher recoveries without addition of water. The use of 10 mL of water also showed a better separation between the aqueous and organic phases, allowing the collection of a higher volume of organic phase to concentrate. The improvement in the extraction recoveries could be explained by a competition between water and pharmaceuticals for the adsorption sites of humic substances present in the sediment, promoting their desorption. Additionally, the rehydration of the sediment also allows a better access of the extraction solvent into the sediment pores, improving the liquid partition of pharmaceuticals between aqueous and organic phases [31].

The influence of the solvent used in the reconstitution of the extract was tested using several solutions with different compositions of water/acetonitrile. The percentage of acetonitrile was varied from 10% to 100% for a final volume of 500 μL. The results indicated that, in general, there was an increase in the signal intensity of the analytes with the increment of acetonitrile in the reconstitution solvent, stabilizing the signal intensity, for some of the compounds, for percentages of acetonitrile equal or above 50% (Fig. S14, Supplementary material). It should be noticed that the reconstitution in 100% acetonitrile led to a worst chromatographic performance for some of the selected pharmaceuticals, which exhibited tailing in the peaks (data not shown). Therefore, a mixture of water/acetonitrile (50:50, v/v) was chosen for reconstitution solvent.

3.1.2. Optimization of the dispersive SPE clean-up

Dispersive SPE (dSPE) is the most common clean-up approach used together with QuEChERS extraction method to overcome

matrix effects of the analysis of solid environmental matrices [29–32]. Therefore, different dSPE sorbents commercially available were tested, namely PSA, C18 and mixtures PSA/C18, by evaluating both the recovery of the selected pharmaceuticals and the matrix effects. For that purpose Eq. (2) (Section 3.1) and Eq. (1) (Section 2.5) were used. The results obtained are presented in Figs. 1a and 1b, where the different dSPE sorbents are also compared with an extraction protocol without clean-up step. The introduction of a clean-up step allowed getting better recoveries, principally when PSA or a mixture PSA/C18 sorbents was used (Fig. 1a). Given that both sorbents showed a similar matrix effect, PSA was chosen for clean-up, because the recoveries with this sorbent were slightly higher for most of the selected pharmaceuticals. PSA was also used as dSPE sorbent in the extraction of pharmaceuticals from drinking water treatment sludge [29] and sewage sludge [30], since the primary and secondary amines present in its chemical structure enabled a better retention of polar interfering compounds co-extracted [29]. Besides PSA, it is also described the use of C18 and a mixture of PSA/GCB (graphitized carbon black) as dSPE sorbents for the analysis of pharmaceuticals in soil [31] and sediment [32], respectively.

3.2. UHPLC–MS/MS optimization

All the compounds were analyzed in the positive ionization mode and precursor ions corresponded to the protonated molecules $[M+H]^+$ (Table 2). Two MRM transitions between the precursor ion and the two most abundant product ions were selected for each compound. In the case of the metabolite norsertraline, only one MRM transition was monitored, because it showed a poor fragmentation. For internal standards, just one transition was selected, since they are isotopically labeled compounds that are not suitable to be found in environmental samples. For all the compounds cone voltages and collision energies were also optimized in order to have the best signal intensity (Table 2).

MS/MS conditions in the electrospray ionization source were also studied, by optimizing the nebulizing and drying gas flow rates, the desolvation and heat block temperatures, and the interface voltage. Nebulizing gas flow rate was studied from 0.5 L to 3.0 L min^{-1} while drying gas flow rate varied between 10.0 and 17.0 L min^{-1}. The best conditions were fixed at 2.6 and 15.0 L min^{-1} for nebulizing and drying gases, respectively. Several temperatures were tested for the desolvation (ranged from 200 to 300 °C) and heat block temperatures (varied from 200 to 500 °C). Higher sensitivity was obtained when 300 and 425 °C were used as desolvation and heat block temperatures, respectively. At last, interface voltage was tested from 0.5 kV to 5.0 kV and it was observed that an increment in interface voltage led to an increase in the sensitivity of pharmaceuticals. Hence, an interface voltage of 5.0 kV was selected.

Chromatographic conditions were optimized in terms of mobile phase, flow rate, gradient and column temperature. Regarding the mobile phase, methanol and acetonitrile were tested as organic solvent while ultra-pure water with formic acid was defined as aqueous phase, in order to enhance the ionization of the compounds in the positive ionization mode. Better sensitivity and peak shape were obtained using 0.1% formic acid in ultra-pure water and acetonitrile. After that, the reminding chromatographic parameters were also optimized in order to improve chromatographic resolution and peaks shape, and to get the shortest analysis time. Flow rates between 0.2 and 0.35 mL min^{-1} were studied and the optimum flow rate was set at 0.3 mL min^{-1}. The elution of the analytes was performed on a 7 min gradient. Different column temperatures were also evaluated (30 °C, 35 °C and 40 °C) and the column temperature was set at 30 °C. All the analytes exhibited a better peak shape and resolution under the optimized conditions.

3.3. Method validation

The developed analytical methodology was validated taking into account the following criteria: linearity, sensitivity (in terms of method detection and quantification limits), recoveries, precision (intra- and inter-day), and matrix effects. Detailed analytical quality assurance data is given in Table 4. Calibration curves were set using linear regression analysis over the established concentration range of 0.08 ng g^{-1} to 100 ng g^{-1} (dry weight, dw), with all compounds giving good fits ($r^2 > 0.998$). In order to check possible fluctuation in analytical signals, a standard mixture solution was injected in the beginning and in the end of each sequence as well as throughout the sequence.

Recoveries of the selected pharmaceuticals were evaluated at three spiking levels (5 ng g^{-1}, 50 ng g^{-1} and 100 ng g^{-1}) by comparing concentrations obtained after all the QuEChERS extraction method, calculated by internal standard method using matrix-matched calibration curves, with those of the initial spiking level. For norsertraline, it was not considered the lowest spiking level (5 ng g^{-1}), because this was below its MQL (6.94 ng g^{-1}). Recoveries between 47 and 110% were obtained for all the compounds, being the human metabolite of carbamazepine, 10,11-epoxycarbamazepine, that showed the lowest values (47–61%) (Table 4). The recovery values obtained with the developed QuEChERS extraction method were compared to those previously published in literature; however, to the best of our knowledge, only the antiepileptic carbamazepine was extracted from sediments using QuEChERS extraction approach [32]. The recoveries obtained in the present work (91–99%) were slightly higher than those reported by Berlioz-Barrier et al. [32] (82–85%) for the analysis of carbamazepine in sediments. Comparatively to other extraction methods, the recoveries obtained with the developed QuEChERS extraction method were similar to those described in literature for carbamazepine and diazepam using PLE [19,20]. Similar recoveries for the extraction of carbamazepine from sediments were also reported using ultrasonic extraction [23]. Nevertheless, Cueva-Mestanza et al. [24] and Darwano et al. [22] had lower recoveries for this antiepileptic drug when using microwave assisted micellar extraction (78%) and ultrasonic extraction (72–80%), respectively.

Precision of the method was assessed in terms of repeatability (intra-day) and reproducibility (inter-day) for each pharmaceutical at three different concentrations, in order to ensure a correct quantification. Precision intra- and inter-day varied from 0.01 to 4.23% and from 1.02 to 17.6%, respectively (Table 4).

Method detection limits (MDL) and method quantification limits (MQL) ranged from 0.01 to 0.36 ng g^{-1} and from 0.04 to 1.20 ng g^{-1}, respectively, for all pharmaceuticals with the exception of norsertraline that showed high MDL and MQL (2.08 and 6.94 ng g^{-1}, respectively) (Table 4), showing that the developed analytical method as a good sensitivity.

3.3.1. Matrix effects

Sediment matrix effects were evaluated using Eq. (1) (Section 2.5) and considering three different concentrations. The average values are illustrated in Fig. 2, and as can be seen, matrix effects were observed for all the selected pharmaceuticals. Except for norsertraline, all the selected pharmaceuticals showed ion suppression, varying between −9% and −51%. In the case of norsertraline there was a signal enhancement (+37%). Since it was proved the presence of matrix effects, those have to be corrected in order to be possible an accurate quantification of the selected pharmaceuticals in sediment. A common approach is the addition to the extract of isotopically labeled compounds that are structurally similar to

Table 4
Method performance: method detection and quantification limits (MDL and MQL), recoveries, intra- and inter-day precision at three concentrations (5 ng g^{-1}, 50 ng g^{-1} and 100 ng g^{-1}).

Pharmaceutical	MDL (ng g^{-1})	MQL (ng g^{-1})	Recoveries (±RSD) (%) (n = 3)			Intra-day precision (RSD, %) (n = 10)			Inter-day precision (RSD, %) (n = 3)		
			5 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}	5 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}	5 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}
Venlafaxine	0.08	0.26	99 ± 1	87 ± 2	92 ± 3	1.38	1.23	3.05	7.00	1.02	3.02
Trazodone	0.04	0.14	91 ± 2	88 ± 1	95 ± 2	2.93	2.12	1.51	16.0	17.6	15.6
Citalopram	0.27	0.90	88 ± 4	94 ± 5	107 ± 3	3.06	2.10	0.94	13.8	17.0	15.6
Paroxetine	0.07	0.22	88 ± 1	71 ± 2	84 ± 1	4.23	1.96	1.65	4.54	13.6	9.88
Norfluoxetine[a]	0.33	1.11	86 ± 4	70 ± 2	76 ± 5	3.75	1.56	1.85	4.93	1.79	5.86
Norsertraline[a]	2.08	6.94	n.d.	69 ± 3	86 ± 4	n.d.	1.34	1.80	n.d.	5.24	12.1
Fluoxetine	0.03	0.09	78 ± 3	81 ± 2	92 ± 3	1.43	1.14	0.41	4.39	3.16	2.48
Sertraline	0.03	0.09	95 ± 1	95 ± 3	103 ± 4	0.24	0.64	0.41	7.65	6.88	6.17
10,11-Epoxycarbamazepine[a]	0.01	0.04	47 ± 1	55 ± 2	61 ± 2	1.66	0.01	1.91	7.11	6.80	16.5
Carbamazepine	0.36	1.20	91 ± 2	90 ± 3	99 ± 1	1.71	0.50	1.46	2.22	6.35	3.10
Diazepam	0.02	0.07	94 ± 1	104 ± 1	110 ± 3	1.23	2.15	1.11	5.49	3.14	3.09

[a] Metabolite; RSD—relative standard deviation; n.d.—not detected.

Table 5
Concentration of psychiatric drugs, expressed in ng g^{-1} (dry weight), in sediments from Douro and Lima rivers. Standard deviation (SD) values are presented in brackets.

Pharmaceutical	Concentration (ng g^{-1}, dry weight) (±SD					
	Douro river					Lima river
	D1	D2	D3	D4	D5	
Venlafaxine	26.4 (±0.21)	1.33 (±0.02)	1.32 (±0.01)	1.27 (±0.01)	1.26 (±0.02)	n.d.
Trazodone	5.60 (±0.34)	<MDL	<MDL	<MDL	n.d.	<MDL
Citalopram	14.4 (±1.82)	0.99 (±0.07)	<MQL	<MQL	<MQL	n.d.
Paroxetine	3.10 (±0.27)	1.52 (±0.07)	1.46 (±0.06)	n.d.	n.d.	n.d.
Norfluoxetine[a]	4.99 (±1.03)	1.15 (±0.02)	n.d.	n.d.	n.d.	n.d.
Norsertraline[a]	<MQL	n.d.	<MDL	<MDL	<MDL	n.d.
Fluoxetine	7.78 (±0.99)	0.72 (±0.01)	0.51 (±0.01)	0.42 (±0.01)	0.39 (±0.01)	n.d.
Sertraline	7.89 (±0.24)	0.40 (±0.07)	0.15 (±0.03)	0.15 (±0.01)	<MDL	<MQL
10,11-Epoxycarbamazepine[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carbamazepine	1.82 (±0.05)	<MDL	<MDL	<MDL	<MDL	n.d.
Diazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Metabolite; n.d.—not detected; <MDL—below method detection limit; <MQL—below method quantification limit.

the target analytes [27]. Other approach described for environmental matrices is the use of the matrix-matched calibration method [38]. In this work, the strategy to overcome matrix effects was to gather these two approaches and, therefore, the quantification of pharmaceuticals in sediments was made using internal standard calibration method together with matrix-matched calibration curves.

3.4. Application to real samples

The developed method was applied to the determination of psychiatric drugs in sediments from two rivers, located in the north of Portugal, which suffer different pressures from human activities. A total of six sediment samples were analyzed.

With the exception of diazepam and the metabolite 10,11-epoxycarbamazepine, all the others psychiatric drugs were detected in river sediments. A higher number of selected pharmaceuticals (7) were detected in sediments from Douro river comparatively to Lima river, where only two pharmaceuticals were detected (trazodone and sertraline) (Table 5). Among the selected psychiatric drugs, antidepressants were those present in higher concentrations in river sediments. For instance, in Douro river, concentrations of antidepressants ranged from not detected (paroxetine and trazodone) to 26.4 ng g^{-1} (venlafaxine). On the other hand, the antiepileptic carbamazepine that has low removal efficiency in WWTPs [34] and whose presence in Douro river water had been described at levels ranging from 0.37 ng L^{-1} to 178 ng L^{-1} [39], was found in Douro river sediment at a lower concentration (1.82 ng g^{-1}). Similar levels of carbamazepine were also reported in rivers from Spain [20] and South Africa [40].

Besides the quantification of parent compounds, in Douro river was also possible to detect the two main human metabolites of the antidepressants fluoxetine and sertraline (norfluoxetine and norsertraline, respectively). Nevertheless metabolites were at lower concentrations than the parent compounds (Table 5).

4. Conclusion

A new, simple, rapid and robust analytical method for the determination of eight psychiatric drugs and three metabolites in sediment was developed based on QuEChERS-UHPLC–MS/MS. This method uses a modified approach for the extraction of basic compounds, allowing the analysis of different families of psychiatric drugs, namely antidepressants, antiepileptics and anxiolytics, as well as some of their human metabolites. The QuEChERS extraction protocol was modified, by using basic acetonitrile as extraction solvent. By coupling with UHPLC–MS/MS analysis, detection limits in the low ng g^{-1} range (0.01–2.08 ng g^{-1}) were achieved. Thus, the developed method is both sensitive and selective and a reliable tool to monitor pharmaceuticals in sediment samples. Additionally, this is also an environmentally-friend approach, owing to the low consumption of organic solvents.

The developed method was successfully applied to the analysis of sediments of two Portuguese rivers, showing the occurrence of various antidepressants and the antiepileptic carbamazepine, at levels up to few nanograms per gram. Besides pharmaceuticals, the presence of human metabolites was also reported, pointing out the relevance of extending monitoring programmes to the analysis of these compounds as well.

Due to its simplicity and by allowing a faster extraction, the proposed methodology could be further applied to gather data on the presence of psychiatric drugs in sediments, which are considered an important environmental compartment for the accumulation of pollutants.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.01.079.

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