Determination of pharmaceuticals in freshwater sediments using ultrasonic-assisted extraction with SPE clean-up and HPLC–DAD or LC-ESI-MS/MS detection

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Abstract

A robust and sensitive analytical method is presented for the extraction and determination of six pharmaceuticals in freshwater sediments. The pharmaceuticals were extracted from sediment using ultrasonic extraction with 2% NH_4OH in methanol (MeOH), followed by extraction into 2% formic acid in MeOH and then MeOH only. The resulting extracts were then analysed, after clean-up on HLB solid phase extraction cartridges, using a single gradient run using a Zorbax Eclipse XDB-C18 (150 × 4.6 mm, 5μm) column and a mobile phase consisting of 10 mM ammonium acetate and MeOH (pH=4.8). Analytes were detected and quantified using either Diode Array Detector (DAD) or tandem mass spectrometry (MS/MS) with electrospray ionization in positive mode. Validation studies were carried out using ten sediments sampled from the UK and Iraq with a wide range of characteristics. The mean calculated recoveries for fortified samples in all studied sediments ranged from 74.5 to 114.6% for atenolol, 72.3 to 124.9% for amitriptyline, 76.5 to 105% for mefenamic acid and 70.1 to 102% for diltiazem. Cimetidine and ranitidine showed lower recoveries which ranged from 40.2 to 68.4% and 30.4 to 55.2% respectively. Relative standard deviations (RSD) of recoveries for all sediment–pharmaceutical combinations ranged from 1.6 to 15.8%. The detection limits in sediments for the six analytes ranged from 15 to 58.5 ng g^{-1} and 0.03 to 3.5 ng g^{-1}, dry weight, for HPLC-UV and LC-ESI-MS/MS respectively. Overall the results indicate that the developed method is effective for use in monitoring the concentrations of pharmaceuticals in aquatic sediments in different regions.

Keywords: Pharmaceuticals, Ultrasonic extraction, Freshwater sediment, LC-ESI-MS/MS, HPLC-DAD

1. Introduction

In the last decade, the analysis of pharmaceutical residues in the environment has attracted significant scientific attention due to the potential risks that these compounds pose to ecosystems and human health.[1–3] Pharmaceuticals can enter wastewater systems and pass through wastewater treatment plants (WWTPs) into the natural environment where they can reach detectable concentrations.[4] Some of these compounds have the ability to partition to environmental solid phases (e.g. sediment and soil).[5] In order to understand the occurrence, fate and effects of these trace level contaminants, multi-residue, accurate, sensitive and powerful techniques such as liquid chromatography (LC) and gas chromatography (GC) are needed.[6,7] However, most of the available analytical techniques have been developed for the determination of these trace of contaminants in the dissolved phase[5] and most monitoring studies have focused on detecting pharmaceuticals in surface water[8–10] and wastewater.[11–13] Fewer analytical methods are available for soil[14–16] and sediment.[17,18]

The complexity of an environmental matrix can deeply affect the analysis of a pharmaceutical. Up to 90% of the analysis time can be spent on sample preparation and thus, great effort goes into the development of reliable sample preparation procedures which are as simple as possible in terms of operation and which minimise the number of steps in the analytical process.[19] For the analysis of pharmaceuticals in environmental solids, sample pre-treatment steps typically include extraction and analyte enrichment and clean-up, as these processes are essential and provide the opportunity to quantify many pharmaceutical compounds down to ng L^{-1} or ng Kg^{-1} concentrations.[20,21]

A variety of extraction procedures has been reported for organic pollutants, including pharmaceuticals, from solid environmental matrices such as sediment including methods based on microwave assisted extraction (MAE)[18,22] and pressurized liquid extraction (PLE - which is also commonly known as accelerated solvent extraction (ASE)).[19] For example, the use of ASE for extraction of pharmaceuticals from sediment results in recoveries greater than 116%.[23–25] Although these methods are comprehensive, use minimal amounts of solvent and reduce the processing time,[26] they are considered less popular because the instruments themselves may be complicated to use and expensive.[14,27] Therefore, ultrasonic-assisted extraction (UAE) is a frequently applied alternative technique for the extraction of pharmaceuticals from sediment.[19] The short extraction time and low solvent consumption of UAE, as well as its robustness, lower cost and ease of use, are some of the advantages of this extraction technique.[16,27–29]

The United States Environmental Agency (USEPA) published an analytical reference method (1694) based on UAE involving two different extraction methods under acidic and basic conditions for the determination of pharmaceuticals in environmental compartment including sediment.[30] Recently, Chen and Zhou[31] applied the UAE technique prior to UHPLC–MS/MS to investigate the occurrence and behaviour of 20 antibiotics from five classes in sediments from the Huangpu River, China. The method produced recoveries ranging from 44% to 141% for the targeted compounds. Lei et al.[32] determined the concentrations of six estrogens in sediment and generally showed recoveries higher than 79%, the exception being estriol (E3) which showed a recovery of 66%. Martín et al.[33] investigated the occurrence of pharmaceuticals in sediment and sludge using UAE prior to HPLC–DAD and fluorescence (Fl) analysis. The pharmaceutical compounds evaluated were nonsteroidal anti-inflammatory drugs, antibiotics, an anti-epileptic drug, a β-blocker, a nervous stimulant, estrogens and lipid regulators with recoveries ranging from 58.4 to 103% except for acetaminophen which showed a recovery <15%. More recently, de Sousa et al.[34] developed a UAE method for the simultaneous determination of hormones and pharmaceuticals from different therapeutic classes in sediment; the highest recovery was 120% for 17-β-estradiol at a concentration of 5 ng g^{-1} while the lowest recovery was 54% for propranolol at a concentration of 50 ng g^{-1}.

The use of solid-phase extraction (SPE) as a clean-up and analyte enrichment step prior to analysis also has a positive influence on the recovery of targeted compounds since the extraction steps described above are not selective.[19,27] SPE cartridges such as hydrophilic–lipophilic balance (HLB) and Strong anion exchange (SAX) cartridges have been used extensively in the clean-up of extracts from sediment samples. For instance, Zhou et al.[35] and Vazquez-Roig et al.[36] used tandem SAX–HLB cartridges to reduce the matrix effects of complex co-extracted components for pharmaceutical determination in sediment samples. The SAX column retained humic material and the HLB column retained the pharmaceuticals. SPE using HLB cartridges was employed by Chen and Zhou[31] when they studied the occurrence and behaviour of pharmaceuticals in sediment. HLB cartridges were also used by de Sousa et al.[34] for the clean-up and pre-concentration of pharmaceuticals extracted from sediment. Maximum obtained recoveries (54.1-156.0%) were seen at pH=9 and using 2 × 3 mL of methanol (MeOH) and 3mL of acetone for elution. Use of a tandem moderate anion exchange cartridge (MAX) and HLB was found to be the optimum method for pre-concentration and purification of 32 pharmaceuticals in sediment extracts.[26] Highest recoveries were obtained with ethyl acetate, MeOH and MeOH containing 2% acetic acid as elution solvent for MAX cartridges while ethyl acetate and MeOH were found to be the most effective eluents for the HLB cartridges.

It is noteworthy that the published methods for analysis of pharmaceuticals in sediments have dealt with compounds from only a limited number of classes with a limited range of physicochemical properties. Moreover, individual methods have tended to focus on only a few sediment types so the applicability of the methods to sediments more generally is unknown. The development of a robust, low cost and easy to use method like UAE capable of simultaneously extracting pharmaceuticals from different classes from sediments with varying characteristics is, therefore, highly warranted.

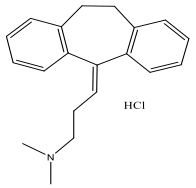
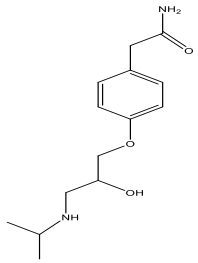
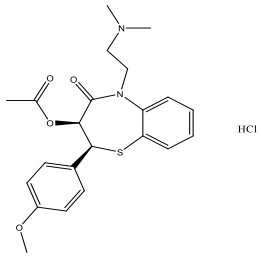
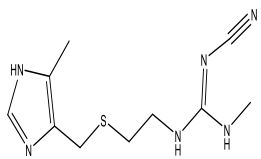
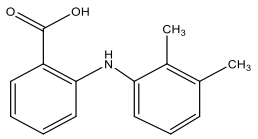
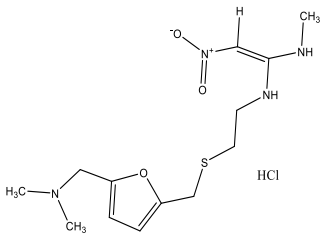
In this paper, we describe a rapid and simple method to simultaneously extract pharmaceuticals from different pharmaceutical classes (anti-depressants, anti-ulcer medicines, β-blockers, calcium channel blockers and nonsteroidal anti-inflammatory drugs (NSAID)) with different physicochemical characteristics (such as polarity and pKa) from a range of sediments. We believe that this method will be invaluable for use in future laboratory fate studies and environmental monitoring programmes. The developed methods combine the simplicity of UAE and the efficiency of clean-up and sample enrichment of SPE followed by detection and quantification using either the highly available HPLC–DAD technique or the highly sensitive LC-MS/MS method. The influences of sonication time, shaking time, solvent type and pH on extraction efficiency were evaluated as was the type of solvent used in the SPE/clean-up steps (conditioning, washing and sample elution).

2. Materials and Methods

2.1 Chemicals and reagents

Pharmaceutical standards (amitriptyline hydrochloride, atenolol, cimetidine, diltiazem hydrochloride, mefenamic acid and ranitidine) were purchased from Sigma–Aldrich (UK). All pharmaceutical standards were 98–99% pure. CAS registry numbers, therapeutic class and physico-chemical properties of the study compounds are listed in Table 1. The solvents used (acetonitrile (ACN), MeOH and acetone) were of high-performance liquid chromatography (HPLC) grade and were obtained from Fisher Scientific (UK). Ammonium solution (NH_4OH, 35%), ammonium acetate and citric acid were also obtained from Fisher Scientific. Formic acid (96 %) was obtained from Sigma-Aldrich (UK). Nitrogen, 99.9% pure, used for drying, was supplied by the University of Leeds (UK). Ultrapure water (18.2 MΩ.cm) was obtained from a Milli-Q device manufactured by ELGALabWater (UK). Stock solutions of 1000 mg L^{-1} were prepared in MeOH for each pharmaceutical. Working standards solutions were then prepared from the stock solution by serial dilution with MeOH and water (20:80) and kept in the dark at 4 °C until use.

Table 1 Structures, physico-chemical properties and therapeutic classes of the pharmaceuticals compounds used in the analytical development studies

Compound	Amitriptyline HCl	Atenolol	Diltiazem HCl	Cimetidine	Mefenamic acid	Ranitidine HCl
CAS No.	549-18-8	29122-68-7	33286-22-5	51481-61-9	61-68-7	66357-35-5
Therapeutic class	Anti-depressant	β- Blocker	Calcium channel blocker	Anti-histamine	Non-steroidal anti-inflammatory drug (NSAID)	Anti-Ulcer
Structure						
Formula	$C_{20}H_{24}ClN$	$C_{14}H_{22}N_2O_3$	$C_{22}H_{27}ClN_2O_4S$	$C_{10}H_{16}N_6S$	$C_{15}H_{15}NO_2$	$C_{13}H_{23}N_4O_3SCl$
pKa	9.4	9.6	8.06	6.8	3.73	8.08
Water solubility mg L^{-1}	9.71	1.33E+4	465	9380	20	24.7
Log Kow	4.92	1.37	2.8	0.4	2.42	0.27

2.2. Sediment collection and characterisation

Samples of river sediment were collected from different sampling sites: two from Iraq (Baghdad and Karbala) and eight from around the Yorkshire and Leicestershire regions in England. The sediments had different textures and organic carbon content (OC) and were selected in order to provide real environmental matrices for method development and validation. Sediments were sampled from the top 5 cm surface layer. Sediments were then transferred to the laboratory, where plant residues and debris were removed manually and the wet slurry was then sieved to 2 mm, homogenized and stored at 4±1 ^{o}C prior to use. Particle size analysis was performed using a Malvern laser granulometer (Hydro 2000MU, UK). The OC was determined using a total carbon content analyser (Viro Macro Elemental (CN) Analyser, Germany) and found to range from 0.98 to 9.9%. Cation exchange capacity was measured following the ISO 11260 & 14254 protocols using a dual view ICP-OES (Thermo iCAP 6500 duo) by Forest Research, UK. Selected physico-chemical properties of the sediments are given in Table S1 (Supporting information).

2.3. Extraction of pharmaceuticals from sediment

Ultrasonic extraction was used for the extraction of the study pharmaceuticals from sediment using a Grant XUBA3 ultrasonic water bath (65 W, 35 kHz) using three extraction cycles. A mass of 5 g of sediment (dry weight) was weighed into a 50 mL centrifuge tube. In the first cycle, 10 mL of 2% NH_4OH in MeOH was added and the mixture was then vortexed for 15 seconds. The slurry was then ultra-sonicated for 15 min and then agitated at 250 rpm for 10 minutes (Grant bio PSU-20i, UK). Afterward, the slurry sample was centrifuged at 4500 rpm for 10 min. The supernatant was filtered through a 0.45 μm nylon filter and then decanted into a 500-mL Erlenmeyer flask. The sediment residue was then further extracted with 10 mL of 2% formic acid in MeOH in the second cycle and then with only 5 mL of MeOH in the final cycle. The supernatants from the three steps were then combined. The MeOH was allowed to evaporate overnight, after which the extracts were filtered through GF/F glass microfiber filters from Whatman Int. (Maidstone, UK) by suction into Erlenmeyer flasks and diluted with Milli-Q water to give a total volume of 400 mL (MeOH < 5%).

2.4. SPE/clean-up

The diluted sediment extracts were adjusted to pH=10 using NH_4OH solution prior to solid phase extraction (SPE). The SPE was conducted on 6-mL (200 mg) Oasis HLB SPE cartridges (Waters, UK). The SPE cartridges were preconditioned using 5 mL of MeOH followed by 10 mL of Milli-Q water. Diluted aqueous extract

samples were loaded onto the SPE cartridge at a rate of 10-20 mL min^{-1} and passed through the cartridges under vacuum (Supelco VisiprepTM, UK). Cartridges were then rinsed with 10 mL of 5% MeOH in Milli-Q water and then dried under air for 30 minutes. Finally, cartridges were eluted with 2×2.5 mL MeOH followed by 1 mL of 2% NH_4OH in MeOH. The eluates were dried under a gentle nitrogen stream using a DB-3A, TECHNE (UK) concentrator at 30 ^oC. The extract was reconstituted into 1.0 mL of water: MeOH (20:80) and then sonicated for 1 minute and filtered through a 0.22 µm nylon filter. The samples were then stored in a freezer at -20 ^oC prior to HPLC-DAD or LC/MS/MS analysis.

2.5. Instrumental analysis

2.5.1 HPLC-DAD analysis

HPLC, coupled with diode array detector (DAD), analysis of cleaned up extracts was performed using a Perkin Elmer, Flexar system. A reversed phase C18 analytical column of 150 mm × 4.6 mm, 5.0 µm (Zorbax Eclipse XDB-C18) was used for separation and quantification. A Zorbax Eclipse XDB-C18 4.6 mm × 12.5 mm, 5.0 µm guard column was also used. The column temperature was maintained at 35 °C and the injected sample volume was 10 µL. Two mobile phases were used (A and B) comprising 10 mM ammonium acetate/acetic acid buffer (pH 4.8) and ACN respectively and the flow rate was 1.0 mL min^{-1}. The gradient elution program was as follows: 90% of A for the equilibration and sample holding steps which lasted for 1 min each, mobile phase B then increased to 25% from 1-11 min and then rapidly increased to 90 % from 11-13 min. This composition was held for a further 5 min before the mobile phase composition then returned to the initial condition. The column was re-equilibrated for 6 min at the initial mobile phase composition. The use of a step function rather than a smooth gradient reduced the retention times of the more strongly retained compounds so that all analytes were eluted in less than 25 min, which was also the total run time. The detection wavelength was 225 nm. Quantification was achieved based on peak area using calibration curves developed from known standards.

2.5.2 LC-ESI-MS/MS analysis

LC-MS/MS was performed using the same chromatographic conditions as the HPLC-DAD using an Applied Biosystems/MDS Sciex API 3000 triple quadrupole mass spectrometer interfaced with a Dionex UltiMate® 3000 LCi. The tandem MS was performed using a triple quadrupole (TQD) mass spectrometer equipped with an electro spray ionization (ESI) source. All compounds were analysed in positive ionization mode. For MS detection, the instrument was operated in multiple reaction monitoring (MRM) mode and identifications were

made by comparing retention times and substance specific mass spectra. All data were acquired and processed using Analyst 1.4.2 software. Instrumental conditions are listed in Table S2 (supporting information).

2.6. Method characterization

Before the validation of the extraction methods, analytical methods were validated in terms of instrumental linearity, sensitivity (instrumental detection limit IDL and instrumental quantification limit IQL) and precision using standard solutions of the pharmaceuticals. The calibration curves were constructed by analysing at least five concentration levels (in triplicate) in the ranges of 0.1- 10.0 μg mL^{-1} for HPLC-DAD method and from 0.01 to 2.0 μg mL^{-1} for LC-MS/MS method to confirm linearity. IDLs and IQLs were calculated by using the signal-to-noise ratio of 3 and 10, respectively. The precision of the method was determined by repeated analysis of samples at three concentrations. The precision of the methods was expressed as the relative standard deviation (% RSD).

Matrix-matched calibration curves (6 points) were prepared by spiking the target pharmaceuticals into an extract of 5.0 g of sediment. The extraction recoveries of the different pharmaceuticals for the entire procedure (RECtotal), SPE/clean-up step (REC_{SPE}) were determined using BTC sediment. Triplicate samples of sediment (5g) were spiked with 0.2, 0.5 and 1.0 μg g^{-1} of a mixture of the study pharmaceuticals and were then extracted using the methods described above. Extracts were analysed in duplicate to allow calculation of method uncertainty. Validation of the method was performed for different parameters such as linearity, accuracy, precision and sensitivity. Limit of detection (LOD) and limit of quantification (LOQ) were estimated at a signal to noise (S/N) ratio of 3 and 10, respectively using the lowest spiked concentration into the sediment. Blank samples were used to determine the specificity and selectivity of the method.

Recoveries for the SPE/clean-up step (REC_{SPE}) were determined by spiking extract samples (400 mL) containing <5% of MeOH with a mixture of the pharmaceutical analytes. In another tube, sediment samples were extracted without spiking. Target compounds were added just in the reconstitution step. All recoveries were calculated in comparison to a standard sample. The differences in recoveries between spiked samples in the extraction step, prior to clean-up and the standard was helpful to distinguish between recoveries for each step. The detailed validation procedure used and equations to calculate each extraction step recoveries are provided in the Supporting Information (Section S1). The matrix effects were studied by the evaluation of signal suppression or enhancement for each pharmaceutical when LC-ESI-MS/MS analysis was used and was calculated according to equation S2 (more details in section S2, Supporting Information).

3. Result and discussion

3.1. Development of chromatographic methodology

The main objective of the chromatographic method was to analyse six pharmaceuticals with different physicochemical characteristics. Preliminary experiments were carried out to optimize the instrumental conditions for the detection of target compounds. Parameters, such as column type, mobile phase, optimum pH, flow rate, and column oven temperature were carefully studied. First, a Supelco 516 C-18-DB reverse-phase (150 x 4.6 mm, 5 μm) column was tested with a variety of mobile phases but was found to be inadequate. Analyte peaks showed significant tailing and reproducibility and resolution was not acceptable. These problems were overcome when a Zorbax Eclipse XDB-C18 reverse-phase column (150x 4.6 mm i.d., 5μm) was used.

A variety of mobile phases was investigated for optimization of the chromatographic conditions. The challenge was to optimize the mobile phase conditions for a series of compounds with a wide range of retention factors while providing an acceptable analysis time. The use of mobile phases consisting of formic acid and ammonium acetate with MeOH and/ or ACN were explored. The suitability of each mobile phase was determined on the basis of the sensitivity, stability and run time required for the analysis. The pH adjustment of the mobile phase played an important role in optimizing the chromatographic separation of ionisable chemicals. Different pH values were tested and the highest resolution with good retention times was achieved with 10 mM ammonium acetate at a pH adjusted to 4.8 as the aqueous mobile phase. At this pH value, all compounds were in the protonated form and retention was at maximum and constant. Lower pH of the mobile phase resulted in peak tailing. The best wavelength obtained to show best peak shapes and higher response was at 225nm for the HPLC-DAD method. Several gradient elution programs were tested to achieve the optimal separation of the analytes. For example, one and two segment linear gradient programmes were tested to improve the resolution for gradient separations. The first segment was optimized to achieve the desired separation for poor retention pharmaceuticals (atenolol, cimetidine and ranitidine) by the column. This segment was slow due to the narrow range of elution which was found to be affected by a rapid increase of solvent B% and consequently resulted in poor resolution. On the other hand, a scouting gradient method was used to optimize parameters such as initial and final % of mobile phase B.

In the MS/MS analysis, a standard solution (10 μg mL^{-1}) of each pharmaceutical was directly infused along with the mobile phase into the mass spectrometer with ESI, as the ionization source. The mass spectrometer was tuned in positive ionization mode and full scan mode was used for the identification of precursor ions. MRM

mode was used for monitoring and ESI source temperature, capillary and cone voltage and flow rate of desolvation gas were optimized to obtain the highest intensity of precursor molecules of the six analytes. The collision gas pressure and collision energy of collision were optimized for maximum response of the fragment ions obtained. Precursor ions and product ions for MS detection and their respective collision energies are listed in the supporting information (Table S2) together with typical retention times of all target analytes. MRM and UV chromatograms of standard solutions are shown in Figure 1.

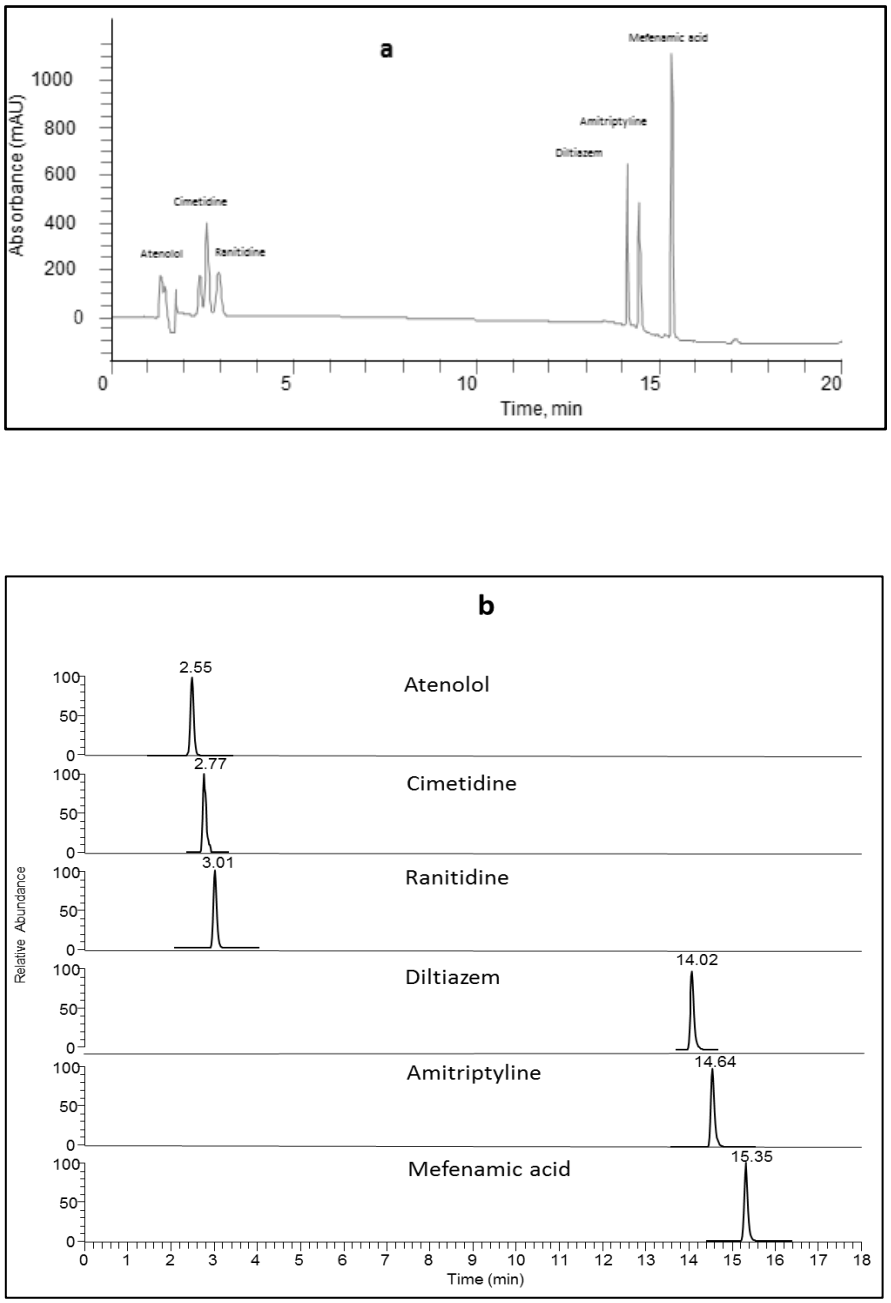


Figure 1 Chromatogram of a mixture of the pharmaceuticals in MeOH. Chromatographic conditions: Zorbax Eclipse XDB-C18 reverse-phase column (250 mm × 4 mm i.d., 5 µm); Mobile phase of 10mM ammonium acetate with acetic acid and ACN at pH= 4.8; flow rate 1.0 mL min^{-1}; (a) at λmax = 225 nm for HPLC; (b) MRM chromatogram for LC-ESI-MS/MS.

3.2 Optimization of sediment extraction method

The study pharmaceuticals covered a diverse range of pharmaceutical classes which were considerably different in polarity and acid–base properties. To extract the selected pharmaceuticals, it was important to consider the likely degree of binding of the compounds to sediment and organic matter and how these factors affect the efficiency of UAE of the organic contaminants.[4,5] Partitioning data for sediment/water systems have been previously reported.[37] Diltiazem and Amitriptyline exhibit moderately strong adsorption to sediment while atenolol, mefenamic and cimetidine show weak affinity to sediment. No partitioning coefficient values were available in the literature for ranitidine in sediment or soil. The optimization of extraction parameters were performed on BTC sediment. The variables optimized were solvent type, pH and sonication and shaking time.

Method optimisation was done using the BTC sediment spiked with the study pharmaceuticals at a concentration of 1 μg g^{-1}. A number of extraction solvents were tested in order to identify the optimum solvent (Table 2). Test solvents (ACN, MeOH and acetone) were chosen according to literature data as these solvents have commonly been used in previous studies.[12,15,38] Two extraction cycles involving 15 min sonication using 10 mL of solvent were used in the solvent optimization procedures. An initial experiment using only ACN was conducted and resulted in mean recoveries of around 65% for amitriptyline and less than 40% for the other compounds. Better recoveries were achieved for atenolol, ranitidine and mefenamic acid when MeOH was used as the extraction solvent while amitriptyline and diltiazem showed a slight decline in recoveries when MeOH was used. Overall, acetone showed poor extraction recoveries for all pharmaceuticals (Table 2). These finding indicated the need for a combination of solvents since the compounds have different physico-chemical properties and using a single solvent resulted in low extraction efficiencies.

Several reported studies have indicated that the use of a mixture of polar organic solvents in water results in superior extraction of pharmaceuticals from solid environmental samples.[35,39,40] Mixtures of ACN: 0.2M citric acid (50:50) and MeOH: 0.2M citric acid (50:50) were therefore tested. Significant improvements were observed for diltiazem and mefenamic acid compared to the single solvent evaluations with mean recovery percentages of >50% been obtained when citric acid was combined with ACN or MeOH. However, the recoveries of cimetidine and ranitidine were poor (<20%) while atenolol showed lower recovery when a MeOH: citric acid mixture was used (Table 2). The method proposed by Li et al.[41] using ACN with 2% NH_4OH was used and resulted in very good recoveries for amitriptyline, atenolol, mefenamic acid with mean recoveries of just over 50% being seen for diltiazem. However, the presence of interfering compounds in the chromatograms

was found to be more significant when this method was used. When 2% NH_4OH in MeOH was tested, higher recoveries were found to be comparable to those obtained using ACN except for amitriptyline which showed a lower mean recovery (86.3%).

The obtained results led us to incorporate another extraction step instead of the second extraction cycle using 2% NH_4OH in MeOH, to improve the recovery of acidic compounds by using 2% formic acid in MeOH. The acidification of the extraction solvent protonates the acidic functional groups (e.g. carboxylic acid, phenol groups) in the organic fractions of solid matrix.[40] This step improved the overall recoveries of pharmaceuticals even though the obtained recovery of amitriptyline was lower than seen in the earlier work but still greater than 90%. A final step, using 5 mL of pure MeOH, was then added and showed improvement in the recoveries (>50%) for ranitidine and cimetidine without significantly affecting the recoveries of other pharmaceuticals in the mixture.

Table 2 Recovery of selected pharmaceuticals using single solvent and mixtures (2 cycles) and ultrasonic extraction of sediment spiked at 1µg g^{-1} (BTC sediment)

Pharmaceutical	Extraction solvent						
	ACN	MeOH	Acetone	ACN:0.2Mcitric acid	MeOH:0.2Mcitric acid	ACN:2%NH_4OH	MeOH:2%NH_4OH
Amitriptyline	65.0±4.2	51.0±7.6	18.0±4.8	68.0±5.2	59.0±5.5	105.2±11.4	86.3±5.2
Atenolol	10.5±1.2	44.3±3.5	12.2±1.2	19.5±1.2	49.0±6.2	80.1±9.6	88±4.6
Cimetidine	14.3±2.3	12.5±4.6	8.1±1.5	14.3±2.3	12.5±4.6	43.1±2.4	46.2±2.0
Diltiazem	39.0±5.3	30.0±4.1	22.0±2.5	57.0±2.9	55.5±3.5	54.3±2.1	78.2±3.1
Mefenamic acid	29.5±4.9	41.9±4.9	22.3±6.2	52.5±2.2	56.0±4.1	74.2±7.9	75.12±2.5
Ranitidine	10.8±1.0	18.2±2.5	11.0±1.7	10.8±0.9	18.2±2.5	31.2±4.3	35.2±1.2

The effects of the sonication time and shaking step were also examined. Short sonication times (5 and 10 min) were tested. Using a 5 min sonication time, a significant decrease in recovery of pharmaceuticals was observed while cimetidine and ranitidine were not detected. Slightly better recoveries were observed at 10 minutes sonication for all pharmaceuticals except diltiazem which showed a lower recovery percentage (74.2%). More efficient extraction was achieved at 15 min so this was selected as final sonication time (Figure 2). Although UAE efficiency increases with time, sonication for more than 15 minutes did not improve the recoveries of selected pharmaceuticals. Using an extraction slurry shaking step at 250 rpm for 10 min was found to increase

pharmaceutical recoveries in sediment by up to 8.5% compared to no shaking. Recoveries obtained after shaking extraction slurry for 5min showed no significant increase in recoveries from optimized shaking time (10 min). Longer shaking (15min) showed no improvements in recoveries (data not shown). Therefore, a 10 min shaking time was chosen to decrease the total extraction time (Figure 2).

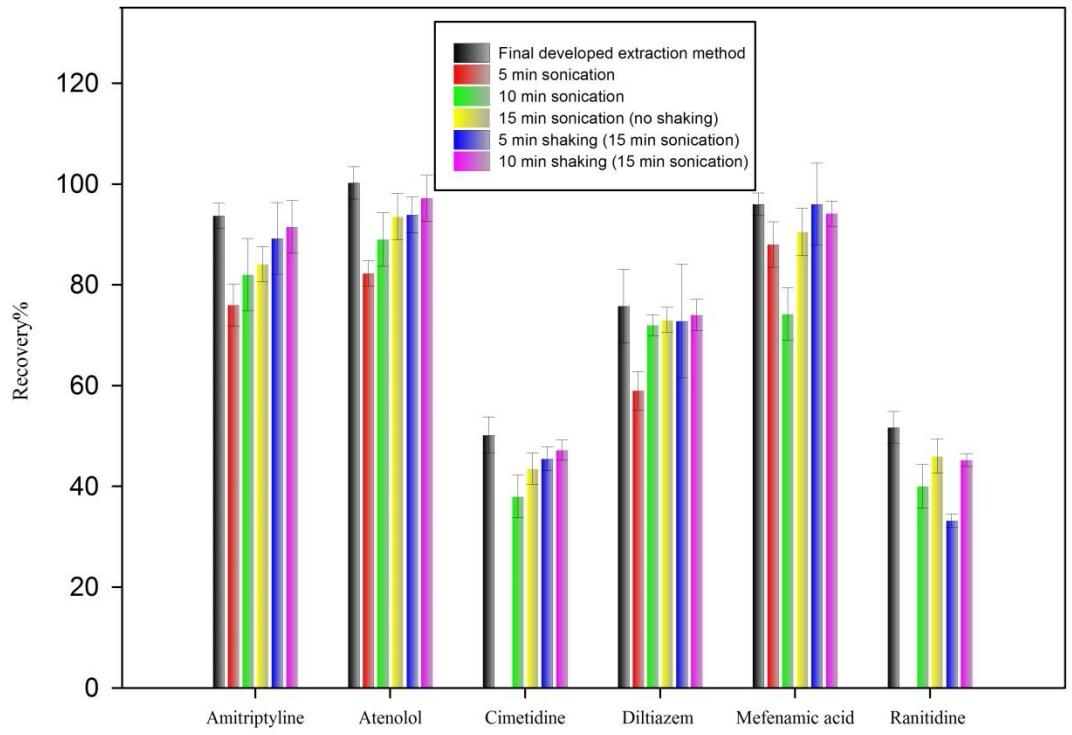


Figure 2 Recovery of selected pharmaceuticals using the optimized method at different sonication and shaking times (BTC sediment)

3.3 Optimization of SPE/clean-up

Due to the complex nature of sediment components, the analytes can be masked in the chromatographic separation and in the final detection. Thus, the use of an SPE step was necessary for purification and for reducing the effects of the matrix interfering substances, resulting in sample enrichment.[26,42,43] The effect of pH manipulation of the diluted UAE extract on SPE recoveries using the optimal elution solvents was tested by adjusting the pH to 2, 4.6 and 10 (Table 3). The acidification of extracts prior to SPE clean-up increased the recoveries for all pharmaceuticals except cimetidine which showed a low SPE recovery (60.8%) and total

recovery of 46.2%. At pH 4.6, amitriptyline showed better SPE mean recovery (110.5%) than at pH 2.0 and a significant improvement was seen in the SPE recovery of cimetidine (75.2%) while total recoveries showed a slight decrease. On the other hand, significant improvements in recoveries were observed at pH 10 with overall SPE recoveries > 88% and total recoveries > 50%. Based on the obtained SPE results, the loss of analytes during the clean-up step appeared to be minimal and the low recoveries of the overall method for some pharmaceuticals (e.g. cimetidine and ranitidine) could be attributed to the inefficient extraction from sediment during the UAE step [40] or the presence of organic matter in sediment which may affect the sensitivity of the analysis.[44]

The elution solvent type is frequently the most important and frequently studied variable in the optimisation of an analytical method.[15] MeOH (2x2.5 mL) was selected as the best choice for the elution of all the analytes. To improve the elution of pharmaceuticals, a third step has been added by using MeOH and 2% formic acid in MeOH and MeOH and 2% NH_4OH in MeOH. The use of 1.0 mL of 2% formic acid in MeOH slightly enhanced the total recovery for most of the pharmaceuticals except atenolol and ranitidine (Figure 3). Consequently, 1 mL of 2% NH_4OH in MeOH was used.

Table 3 Recoveries of the optimized SPE (± SD) and corresponding total recoveries (± SD) and of pharmaceuticals at different pH values (n=3)

Compound	Total recovery at pH=2	REC_{SPE} pH=2	Total recovery at pH=4.6	REC_{SPE} pH= 4.6	Total recovery at pH=10	REC_{SPE} pH=10
Amitriptyline	86.3±5.2	95.5 ± 4.6	93.3±3.3	110.5 ± 4.2	93.7±2.5	102.0 ± 3.8
Atenolol	88.0±4.6	90.8 ± 5.2	91.0±6.0	90.0 ± 3.4	100.2±3.2	110 .0± 7.5
Cimetidine	46.2±2.0	60.8 ± 6.3	44.2±4.0	75.2 ± 7.0	50.18±3.6	88.5 ± 4.2
Diltiazem	78.2±3.1	95.5 ± 8.1	76.2±3.1	80.1 ± 5.8	75.8±7.3	90.8 ± 6.2
Mefenamic acid	86.1±2.5	110.4 ± 6.7	88.1±3.5	104.2 ± 3.8	96.0±2.2	99.8 ± 4.7
Ranitidine	35.2±1.2	78.5 ± 8.2	43.2±2.2	85.5 ± 4.2	51.7±3.2	90.5 ± 5.5

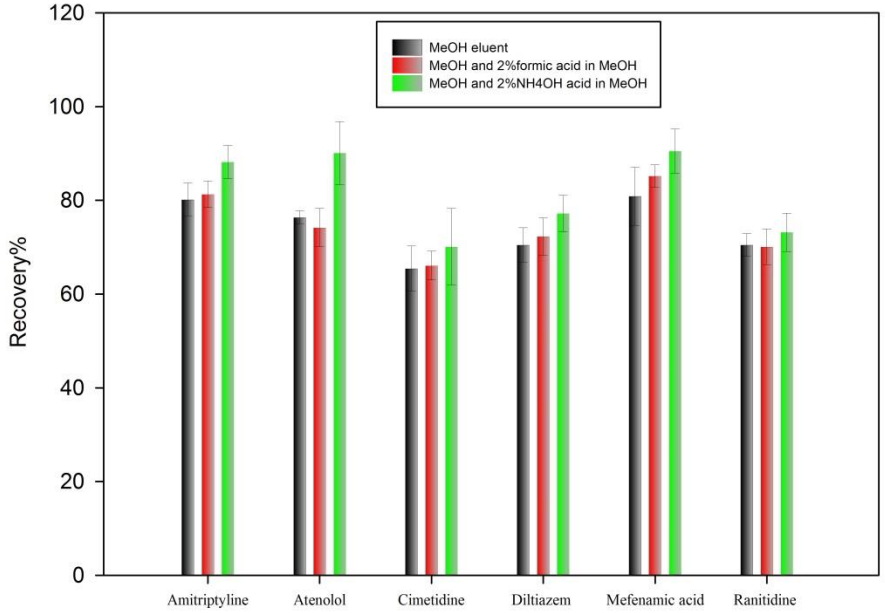


Figure 3 total recoveries of selected pharmaceuticals using different SPE elution solvents (BTC sediment).

4. Validation and method performance

The HPLC-DAD and LC-ESI-MS/MS methods were validated in terms of linearity, recovery, precision and potential for matrix effects. Chromatograms of blank sample extracts showed no interferences from the method. Table 4 and Figure 4 show the method validation data (more details in Supporting Information). The extraction method performance was validated using the optimized method in ten types of sediment in terms of recovery for three spiking levels of 0.2, 0.5 and 1.0 µg g^{-1} and 0.1, 0.2, 0.5 µg g^{-1} for HPLC-DAD and LC-MS/MS, respectively. For all sediments, relative standard deviation (RSD) values of the analytical method ranged from 1.6 to 15.8%. These RSD values demonstrate good precision since values up to 20.0% are accepted for pharmaceutical analysis in environmental samples considering the complexity of the matrix and the number of steps involved.[33,45,46] The obtained recovery values at the three concentration levels indicate the applicability of method to determine a wide range of concentrations. The performance of the methods for the studied pharmaceuticals in different sediments at different concentrations is shown in Tables S3 and S4 (Supporting Information). Generally, the results showed better recovery results for pharmaceuticals in sediments with low organic content (HLM and HAB) and lower recoveries in the sediments with higher organic matter content (BW and GER sediments). This behaviour might be explained by the presence of naturally occurring organic matter in these samples which may mask the analytes and diminish their recovery[4]; and/ or the higher affinity of some

selected pharmaceuticals (e.g. amitriptyline) to adsorb onto sediment via hydrophobic interactions.[37] For amitriptyline, the recoveries from all sediments using the proposed extraction method were good and ranged from 70.4 to 111.8% for the lowest spiked concentration (200 ng g^{-1}) using HPLC-DAD. The LODs ranged from 17.3 ng g^{-1} (GER sediment) to 56.9 ng g^{-1} (BW sediment) while the RSDs ranged from 3.7 to 12.1%. Using LC-MS/MS analysis, amitriptyline showed recoveries ranging from 96.0% (GER sediment) to 106.6% (BGD sediment) at the lowest concentration of 100 ng g^{-1} and showed a very low LOD (0.07 ng g^{-1}) (Table 4). For atenolol, recoveries were within the same range for amitriptyline and ranged from 75.0 (BW sediment) to 113.2% (HLM sediment) using HPLC-DAD while this compound showed lower recoveries at the lower concentrations determined by LC-MS/MS. Mefenamic acid showed recoveries for all pharmaceuticals ranging from 76.5 to 102.5% while the LODs were relatively low and ranged from 14.0 ng g^{-1} (SKF sediment) to 24.0 ng g^{-1} (HLM sediment) using HPLC-DAD. The highest recovery obtained for diltiazem was 99.35% (HAB) while the lowest was 58.8% (BW sediment). The LODs ranged from 12.6 ng g^{-1} (HLM sediment) to 45.2 ng g^{-1} (GER sediment) and 0.03 ng g^{-1} (MIL sediment) to 0.1 ng g^{-1} (BTC sediment) using UV and tandem MS detectors respectively. The efficiency of recovery for both cimetidine and ranitidine were low and ranged from 40.5 (BW sediment) to 67.3% (HAB sediment) and from 29.5 (MIL sediment) to 52.3% (MOR sediment) respectively. Ranitidine showed a low LOD of 0.2 ng g^{-1} (HLM sediment) using LC-MS/MS. Overall, the optimized method provided acceptable recoveries and sensitivities for most of the target compounds.

When the impact of potential matrix effects was evaluated, most of pharmaceuticals were subjected to ion suppression at least in one sediment type. Atenolol exhibited signal suppression of up to 42.5% followed by cimetidine with signal suppression of 38.0% in the high organic content sediment (BW). On the other hand, amitriptyline and diltiazem showed signal enhancement of 12.4% in HLM and BGD sediments respectively (Table S5, Supporting Information). Many strategies to reduce matrix effects are suggested in the literature including dilution, clean-up steps after extraction, the use of isotopically labeled standards, preparation of a matrix-matched standard curve and single-point standard addition where the actual samples (hydrophilic/polar pharmaceuticals) are used to create a calibration plot individually. [47–51] In the current study, due to the clear effects of the co-eluting interferences during analysis by the mass spectrometry detector with electrospray interfaces and the different polarity of analytes, matrix-matched calibration was selected as an appropriate approach to compensate for the matrix effects during analysis. [47,51,52]

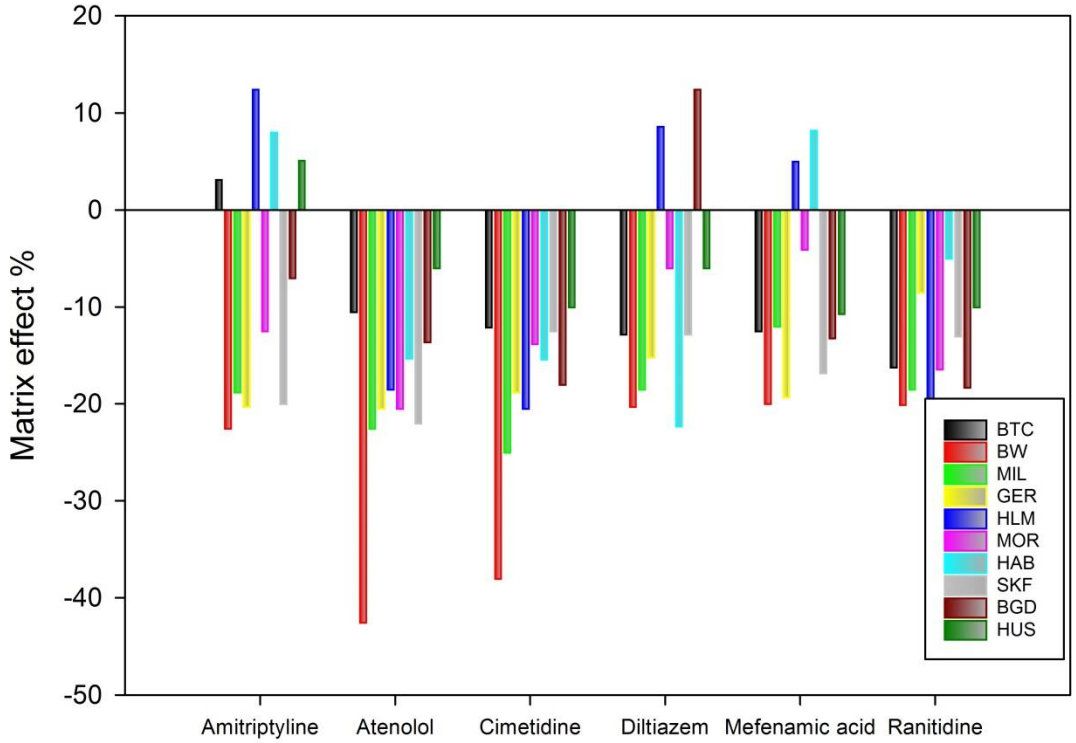


Figure 4 Characterisation of the impacts of matrix interferences on the analysis of the study pharmaceuticals in sediments using UAE extraction, SPE clean-up and LC-ESI-MS/MS detection. Data are shown for ten sediments spiked with pharmaceuticals at a concentration of 100 ng g^{-1}

A number of studies in the literature have reported methods for the successful extraction of the study pharmaceuticals from sediment although these studies used different solvents, clean-up steps, and vary in the complexity of the matrix tested and the detection technique. Our results are in line with other work for atenolol analysis in sediment using ASE extraction, MeOH as a solvent and UHPLC-MS for detection where recoveries ranged from 118-135%; [25] and higher than those reported (65.7-74.8%) using a UAE method, two cycles of MeOH and MeOH water (50:50) as solvents and using UHPLC-MS/MS analysis.[34] Diltiazem recoveries using UAE were comparable to a PLE method using 0.1 M ammonium solution and MeOH (50:50) as solvent while cimetidine showed recoveries similar to or better than what was obtained in this study ranging from 50to > 80%.[26] Amitriptyline and mefenamic acid showed better recoveries than results (39.3% and 28%, respectively) obtained by pressurized hot water extraction–stir bar sorptive extraction–derivatization.[53]

5. Conclusion

Sample preparation is a key prerequisite for the analysis of pharmaceuticals at trace levels in environmental media, and it is often the most labour-intensive, time-consuming and least sophisticated step of the analytical procedure. In this study, a simple, inexpensive, low solvent consumption and ambient temperature UAE method was developed and validated for a range of pharmaceuticals in sediment samples with different physico-chemical properties and produced reasonable recoveries and precisions. The obtained recoveries demonstrate that UAE is an attractive, affordable, and effective alternative to existing extraction methods (i.e. PLE, MAE) for organic contaminants from sediment. This work also provides evidence about the employability of UAE to extract pharmaceuticals or other organic contaminants with different properties. The more widely available analytical (HPLC-DAD) and the highly sensitive LC-ESI-MS/MS techniques were used to determine the analytes after SPE to clean-up the samples. The data on the performance of the proposed method demonstrate its suitability for use in future environmental fate and monitoring studies.

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Table 4 Method validation data for the complete UAE-SPE HPLC-DAD at 200 ng g^{-1} and UAE-SPE LC-MS/MS at 100 ng g^{-1} for the studied pharmaceuticals in different sediment

Sediment	Analysis method	Atenolol		Cimetidine		Amitriptyline		Diltiazem		Mefenamic acid		Ranitidine	
		Recovery% (± RSD%)	LOD (LOQ) ng g^{-1}	Recovery% (± RSD%)	LOD (LOQ) ng g^{-1}	Recovery% (± RSD%)	LOD (LOQ) ng g^{-1}	Recovery% (± RSD%)	LOD (LOQ) ng g^{-1}	Recovery% (± RSD%)	LOD (LOQ) ng g^{-1}	Recovery% (± RSD%)	LOD (LOQ) ng g^{-1}
***BTC**	HPLC-DAD	95.2 (6.2)	37.1 (122.5)	48.3 (10.4)	31.3 (103.3)	85.5 (6.3)	34.0 (112.2)	75.9 (4.3)	20.4 (67.3)	90.4 (4.1)	23.3 (76.9)	50.3 (6.4)	20.2 (66.7)
	LC-MS/MS	93.1 (5.9)	1.9 (6.0)	50.3 (2.1)	0.7 (2.3)	99.6 (10.3)	0.3 (1.0)	80.2 (10.2)	0.1 (0.4)	82.5 (2.3)	2.3 (8.0)	45.2 (7.8)	0.3 (1.0)
BGD	HPLC-DAD	79.2 (11.0)	55.6 (183.5)	45.22 (5.4)	15.4 (50.8)	72.5 (4.5)	20.5 (67.7)	77.30 (7.5)	36.8 (121.5)	99.2 (2.4)	15.0 (49.5)	33.4 (6.5)	13.2 (43.6)
	LC-MS/MS	75.2 (6.2)	1.5 (5.0)	42.5 (8.1)	0.5 (1.7)	106.6 (9.8)	0.2 (0.7)	75.6 (2.8)	0.07 (0.2)	75.8 (4.1)	0.1 (0.4)	50.3 (2.3)	0.8 (2.4)
HUS	HPLC-DAD	79.6 (6.2)	32.2 (106.0)	40.5 (9.9)	26.7 (88.1)	80.0 (5.5)	28.2 (93.1)	63.21 (8.3)	34.1 (112.5)	102.5 (3.2)	21.3 (70.3)	30.4 (15.8)	31.4 (103.6)
	LC-MS/MS	72.2 (10.5)	2.7 (9.0)	52.1 (3.6)	0.7 (2.4)	70.0 (9.3)	0.13 (0.5)	77.8 (7.2)	0.04 (0.13)	80.0 (3.6)	0.15 (0.5)	40.3 (7.2)	0.4 (1.3)
SKF1	HPLC-DAD	81.5 (4.5)	23.0 (76.0)	42.6 (5.7)	15.4 (50.8)	111.8 (3.7)	25.5 (84.2)	60.2 (9.3)	35.2 (116.2)	85.3 (2.5)	14.0 (46.2)	32.1 (6.3)	12.4 (40.9)
	LC-MS/MS	83.0 (9.8)	3.5 (12.0)	49.6 (2.8)	0.5 (1.7)	95.5 (5.7)	0.18 (0.6)	60.3 (5.3)	0.04 (0.13)	90.1 (5.3)	2.5 (8.0)	45.5 (4.5)	0.6 (1.8)
HAB2	HPLC-DAD	94.4 (4.7)	28.0 (92.5)	67.3 (4.5)	19.0 (62.7)	80.5 (4.1)	20.5 (67.7)	99.4 (4.9)	30.8 (101.6)	101.3 (3.0)	19.5 (64.4)	48.5 (9.7)	30.2 (99.7)
	LC-MS/MS	73.9 (5.2)	2.2 (7.0)	51.9 (5.5)	0.7 (2.3)	102 (7.3)	0.14 (0.5)	92.2 (9.1)	0.06 (0.2)	74.1 (8.0)	2.0 (6.0)	42.3 (6.6)	0.6 (1.9)
MIL3	HPLC-DAD	97.7 (5.6)	34.6 (114.2)	52.4 (5.4)	17.8 (58.7)	70.45 (5.6)	25.3 (84.0)	69.3 (9.5)	39.1 (129.0)	87.5 (3.7)	20.8 (68.6)	29.5 (10.8)	21.3 (70.3)
	LC-MS/MS	70.5 (6.4)	1.3 (5.0)	44.2 (8.2)	0.6 (1.9)	81.8 (5.6)	0.07 (0.25)	65.1 (5.2)	0.03 (0.1)	70.2 (5.1)	0.3 (1.0)	45.2 (8.1)	0.6 (1.9)
HLM	HPLC-DAD	113.2 (3.6)	25.5 (84.2)	49.3 (9.6)	30.2 (100.0)	101.7 (6.0)	38.0 (125.5)	69.5 (2.5)	12.6 (42.0)	96.5 (4.0)	24.0 (79.2)	45.5 (9.8)	27.8 (91.7)
	LC-MS/MS	73.2 (6.2)	1.8 (6.0)	48 .1 (14.3)	0.6 (1.9)	80.0 (5.9)	0.09 (0.3)	91.5 (8.6)	0.04 (0.16)	87.8 (5.9)	0.1 (0.3)	44.3 (5.1)	0.2 (0.6)
MOOR	HPLC-DAD	85.4 (4.9)	25.8 (85.1)	55.5 (8.2)	28.7 (94.7)	99.3 (4.1)	25.3 (83.5)	77.3 (4.1)	20.5 (67.7)	89.5 (4.1)	23.2 (106.3)	52.1 (9.5)	32.1 (105.9)
	LC-MS/MS	75.6 (12.3)	2.5 (8.0)	41.2 (6.5)	0.8 (2.5)	83.6 (9.1)	0.13 (0.5)	65.3 (7.7)	0.05 (0.17)	70.1 (5.1)	0.2 (0.6)	48.0 (5.8)	0.3 (0.9)
GER	HPLC-DAD	88.6 (5.1)	28.3 (93.3)	50.9 (6.1)	19.7 (65.0)	88.7 (3.1)	17.3 (57.1)	71.8 (11.6)	45.2 (149.1)	80.8 (3.5)	18.2 (60.1)	52.3 (7.5)	24.8 (81.8)
	LC-MS/MS	80.2 (6.8)	2.5 (8.0)	50.4 (15.5)	1.2 (4.0)	69.1 (10.4)	0.2 (0.7)	72.1 (3.4)	0.02 (0.07)	83.1 (5.8)	2.0 (6.0)	52.5 (6.9)	0.5 (1.6)
BW	HPLC-DAD	75.0 (12.4)	58.5 (193.1)	40.5 (12.4)	31.2 (103.0)	90.3 (10.1)	56.9 (187.8)	58.2 (5.9)	29.6 (97.7)	76.4 (3.4)	16.2 (53.5)	36.4 (12.4)	28.5 (94.1)
	LC-MS/MS	77.1 (6.1)	1.9 (6.0)	48.0 (4.6)	0.6 (1.8)	70.3 (8.1)	0.14 (0.5)	60.2 (6.3)	0.05 (0.17)	70.0 (4.4)	0.1 (0.4)	40.1 (9.1)	0.7 (2.2)

* Sediment used for method development

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