Journal of Chromatography A, 1217 (2010) 2471–2483

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of pharmaceuticals in soils and sediments by pressurized liquid extraction and liquid chromatography tandem mass spectrometry

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ARTICLE INFO

Article history:
Available online 1 December 2009

Keywords:
Pharmaceuticals
Antibiotics
Environmental analysis
Liquid chromatography
Tandem mass spectrometry
Soil
Sediment
Solid-phase extraction
Multi-residue method

ABSTRACT

The present work describes the development of a sensitive analytical method based on pressurized liquid extraction (PLE) and pre-concentration by solid-phase extraction (SPE), followed by liquid chromatography–electrospray tandem mass spectrometry (LC–ESI-MS/MS) for the determination of seventeen pharmaceuticals in soils and sediments. The method is based on sample homogenisation using Na_2–EDTA washed sand and extraction with water at 90 °C. Special emphasis was placed on the optimization of the extraction procedure to develop a green method that reduces, at a maximum, the use of organic solvents in order to eliminate matrix components during the clean-up. The proposed method was linear in a concentration range from 0.3 to 333 ng g^{-1}, with correlation coefficients higher than 0.993. Method detection (MDLs) and quantification (MQLs) limits ranged from 0.1 to 6.8 ng g^{-1} and from 0.25 to 23 ng g^{-1}, respectively. Absolute recoveries were analyte dependent, varying between 50% and 105% at the MQL level, except for fenofibrate (40%) and diclofenac (34%). The intra-day and inter-day precision was given by RSD values from 0.7% to 7.9% and from 1.6% to 14.5%, respectively. Acetaminophen, carbamazepine, ciprofloxacin, clofibric acid, codeine, diazepam, fenofibrate, metropolol, ofloxacin and propanolol were detected at concentrations from MDL to 35.62 ng g^{-1} in soils and sediments from marsh areas. Due to the low recoveries, results for fenofibrate and diclofenac can only be considered as semi-quantitative. The method was fully suitable for the other 15 pharmaceuticals.

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1. Introduction

Thousands of tonnes of pharmacologically active substances are annually used in human medicine for treatment and/or prevention of illness. As an example, in Spain during 2006, a total of 30 tonnes of quinolones were consumed, which is equivalent to 3.2 defined daily doses per 1.000 inhabitants and day (DHD) [1–3]. In veterinary, there is also a great dependence on drugs—e.g. hormones, non-steroidal anti-inflammatories and antibiotics used as feed additives or as preventives [4].

Pharmaceutical residues in the environment, and their potential toxic effects, already have been recognized as an emerging research area in environmental chemistry [5]. A better knowledge of the occurrence and fate of pharmaceuticals release to the environment will attain a proper risk assessment for river basins, wetlands and others related ecosystems [6]. It is now well established that pharmaceuticals are widespread contaminants of wastewater effluents [7–10], surface and drinking waters [11,12], but limited publications treat their occurrence in terrestrial ecosystems [5]. To the best of our knowledge, up to now, the great majority of authors have focused their attention on antibiotics such as tetracyclines [13–16], macrolides [14,17], quinolones [15,18,19], sulfonamides [14,15] and β-lactames [15]. Only few studies deal with the analysis of pharmaceuticals other than antibiotics. Carbamazepine, clofibric acid, ibuprofen, salicylic acid, gemfibrozil, naproxen, ketoprofen, diphenhydramine and diclofenac have been analyzed by gas chromatography–mass spectrometry (GC–MS) [20–22] together with endocrine disrupting compounds or personal care products. Furthermore, Cuevas-Mestanza et al. [23] determined phenazone, carbamazepine, clofibric acid, ibuprofen, naproxen, ketoprofen, bezafibrate and propanolol in sediments by liquid chromatography with ultraviolet detection (LC–UV). Löffler and Ternes [24] simultaneously detected bezafibrate, clofibric acid, diclofenac, fenoprofen, gemfibrozil, ibuprofen, indomethacin, naproxen, ketoprofen, antibiotics and the antiparasitic ivermectin, in river sediment by LC with electrospray ionization (ESI) and tandem mass spectrometry (MS/MS). Radenović et al. [25] established a method for analyzing 31 pharmaceuticals (i.e. eight analgesics and anti-inflammatory drugs, five antibiotics, two psychiatric drugs, one antiulcer agent, one antiepileptic drug, four β-blockers, one diuretic, one hypoglycemic agent, five lipid regulator and cholesterol lowering statin drugs, and three antihistamines) from sewage

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0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.11.033

sludge by LC–ESI-MS/MS. This method was the starting point adopted by the same research group to obtain optimum extraction conditions for analysis of 42 pharmaceuticals in sewage sludges and sediments [26].

Extraction procedures for pharmaceuticals from soils and sediments commonly involve ultrasonication [22,24,27], ultracentrifugation [22,24,28,29], microwave assisted micellar extraction (MAME) [21,23] and/or pressurized liquid extraction (PLE) [13–17,20,25,26]. The extraction is followed by a clean-up step with solid-phase extraction (SPE), mainly using reversed phases such as Oasis Hydrophilic Lipophilic Balance (HLB) [20,25,26], LiChrolute EN (Merck) [13], Isolute ENV+ (Separtis) [29], polymeric phase (SDB-2) [15] or C_{18} [22,28]. Some procedures combine two cartridges in tandem, one containing a strong anion exchange phase (SAX) [14] to remove organic matter, and the other one of the previously mentioned reversed phases. Determination of these pollutants not only in soils but also in other environmental matrices has been carried out by GC–MS [20,21,30], but preferably by LC, since no time consuming derivatization is needed, either with photodiode array detection (DAD) [23,28,29] or MS [13–15,17,24–26]. As DAD is a non-specific detector, interferences of other matrix components may cause false positives detection at ultra trace levels, because of this LC–MS and, particularly LC–MS/MS, is considered the best choice.

In this paper, a sensitive multi-residue method is proposed for the simultaneous extraction of seventeen commonly used pharmaceuticals from soils and sediments, with many different polarities and pK_a's (acids, basics and neutrals). We opted for these compounds on the basis of levels of use in Spain and reported aquatic toxicity effects [1–3,24,25]. Table 1

shows their chemical structures and lists some relevant physicochemical properties. The selected pharmaceuticals belong to a great variety of different therapeutical classes: analgesics, β-blockers, antibiotics, anti-inflammatories, anticonvulsants, antidepressants and lipid regulators. The developed analytical method combines PLE using water as extractant, clean-up with SPE and determination by LC–ESI-MS/MS. The advantage of this method over the few reported applications is its suitability for a wide range of compounds and the reduction of the use of organic solvents, which results in a decrease of the analysis cost and safeguards the integrity of the analyst and the environment.

2. Materials and methods

2.1. Chemicals and materials

Acetaminophen, codeine, carbamazepine, ciprofloxacin, clofibric acid, diazepam, diclofenac, fenofibrate, ibuprofen, metoprolol, norfloxacin, ofloxacin, oxytetracycline, sulfamethoxazole, tetracycline, propanolol, trimethoprim and 4-epitetracycline hydrochloride were purchased from Sigma–Aldrich (Steinheim, Germany). 4-Epioxytetracycline was from Acros Organics (Morris Plains, NJ, USA). Ibuprofen-d_3, acetaminophen-d_3 and carbamazepine-d_2 (internal standards, ISs) were from CDN Isotopes (Quebec, Canada). All standards were of analytical grade (purity >95%). Stock solutions ($1000\,mg\,L^{-1}$) of each pharmaceutical were prepared in methanol with the exception of ciprofloxacin, which was prepared in water. Stocks solutions were stored at $-20\,^{\circ}C$. Working solutions, at different concentrations, were prepared monthly by dilution of the standard stock solutions in methanol–water (25:75, v/v). A mixture of the ISs at concentrations of $10\,ng\,\mu L^{-1}$ each was prepared in methanol and $10\,\mu L$ were added in soil and sediment samples to obtain concentrations of $33\,ng\,g^{-1}$. Formic acid (reagent grade), acetonitrile and methanol (gradient grade for liquid chromatography), were purchased from Merck (Darmstadt, Germany). High purity water was prepared using a Milli-Q water purification system (Millipore, Milford, MA, USA). Citric acid, ethylenediaminetetraacetic disodium salt dihydrate (Na_2–EDTA), di-potassium hydrogen phosphate (K_2HPO_4) and hydrochloric acid (37%), all reagent grades were purchased from Scharlau (Ferosa, Barcelona, Spain). The 0.1 M Na_2–EDTA–McIlvaine buffer solution was obtained mixing 61.45 mL of 0.1 M citric acid and 38.55 mL 0.2 M K_2HPO_4 and adding 3.36 g of Na_2–EDTA, to prevent the pharmaceuticals from complexing with Ca^{2+} and Mg^{2+} ions. The pH of the solution was fixed at pH 4 with hydrochloric acid (37%).

Aluminium oxide 90 (neutral, acidic and basic) was from Merck, silica gel 60 (0.04–0.06 mm) from Scharlau, Florisil® 60–100 mesh from Sigma–Aldrich and sea sand for Panreac (Barcelona, Spain). EDTA and EDTA–McIlvaine washed sea sand were prepared by placing 60 g of sand into a Buchner funnel and passing 120 mL of the selected solution through it using vacuum. Partial drying of the sand was carried out by vacuum. Thereafter, sand was completely dried in an oven at $100\,^{\circ}C$.

Oasis HLB 60 mg sorbent/6 mL cartridge (Waters Corp., Milford, MA, USA), Strata-X 33 μm Polymeric Reversed Phase 200 mg (Phenomenex, Torrance, CA, USA) and Isolute SAX 500 mg (Symta, Madrid, Spain) were used for SPE.

2.2. Sampling and sample preparation

Sediments and soils were collected in sixteen points of five marsh areas of the Valencian Community (Spain), L'Albufera Natural Park, Prat Torreblanca-Cabanes and the marshes of Oliva-Pego, Silla and Moros. The main physical and chemical properties of typical soil and sediment of these areas are given in Table S1, Supplementary material. These soils are characterized by pH > 7, loamy texture, high calcium carbonate content (>30%) and low levels of organic matter (≈2%).

Soil samples of the upper 20 cm horizon layer were collected. From each sampling point, of $1\,m^2$, two sub-samples were taken. Once in the laboratory, samples were dried and passed through a 2 mm Ø sieve, and then, the sub-samples of each sampling point were homogenised to create a composite one. The composite soil samples were extended in a layer of approximately 1 cm thickness on polypropylene trays and air-dried in darkness at $20\,^{\circ}C$ to moisture content of approximately 3% water. Then, samples were stored in sealed plastic bag at $4\,^{\circ}C$.

Sediment samples, taken from irrigation channels and marshes, were of pH > 7.4, sandy loam texture, and with high content in calcium carbonate (>30%) and organic matter (>15%). These samples were weighed and approximately 800 g of each sample were placed in a polypropylene pot, frozen at $-80\,^{\circ}C$, lyophilised (Hetosicc CD4, Birkerod, Denmark), passed through a 2 mm Ø sieve, and homogenised. The process of lyophilisation was carried out at $-90\,^{\circ}C$ and with 0.440 bar vacuum over 7 days for each sediment sample to water content <1%. Finally the lyophilised samples were stored in sealed plastic bags at $-20\,^{\circ}C$ until the extraction.

Soil and sediment samples that do not show pharmaceuticals after a preliminary analysis were used as control blank and for the optimization and validation of the method.

2.3. Pressurized liquid extraction (PLE)

The soil and sediment samples were extracted by PLE using an ASE 200 system (Dionex, Sunnyvale, CA, USA). The selected sorbent was sea sand washed with Na_2–EDTA. Soil or sediment were weighed (3 g) into a mortar and added $10\,\mu L$ of a $10\,ng\,\mu L^{-1}$ mixture of the ISs. The sample was then mixed with approximately 25 g of Na_2–EDTA washed sea sand in the mortar. This mixture was put into a 22 mL extraction cell, then the cell was filled up with Na_2-EDTA washed sea sand. Whatman glass fiber filters were placed at

Table 1
Therapeutical classes, chemical structures and relevant physicochemical properties of the studied compounds.

Compound	Therapeutical class	CAS no.	Molecular weight	Structure	pK_a	log K_{ow}
Acetaminophen	Analgesic	103-90-2	151.17		9.38	0.46
Carbamazepine	Anticonvulsant	298-46-4	236.27		13.9	2.45
Ciprofloxacin	Antibiotic	85721-33-1	331.35		5.9/8.9	0.28
Clofibric acid	Lipid regulator	882-09-7	214.65		3.46	2.58
Codeine	Analgesic	76-57-3	299.36		8.2	1.52
Diazepam	Antidepressant	439-14-5	284.75		3.3	2.8
Diclofenac	Analgesic	15307-86-5	296.15		4.15	4.51
Fenofibrate	Lipid regulator	49562-28-9	360.83		–	5.19
Ibuprofen	Anti-inflammatory	15687-27-1	206.29		4.5	3.97
Metoprolol	β-Blocker	37350-58-6	267.36		9.7	1.88
Norfloxacin	Antibiotic	70458-96-7	319.33		6.22/8.51	−1.0

Table 1 (*Continued*)

Compound	Therapeutical class	CAS no.	Molecular weight	Structure	pK_a	log K_{ow}
Ofloxacin	Antibiotic	82419-36-1	361.37		6.05/8.22	−0.4
Oxytetracycline	Antibiotic	79-57-2	460.43		3.2/7.5/8.9	−1.3
Propanolol	β-Blocker	525-66-6	259.34		9.5	3.48
Sulfamethoxazole	Antibiotic	723-46-6	253.28		5.7	0.89
Tetracycline	Antibiotic	60-54-8	444.43		3.3/7.8/9.6	−1.2
Trimethoprim	Antibiotic	738-70-5	290.32		6.6	0.91

the bottom and top of the extraction cell to avoid the obstruction of the end caps by the soil or sediment particles. In the final method, the sample was heated to 90 °C with a static period of 7 min and extracted by a flush volume of 100% in three cycles using water. Pressure was set to 500 psi and a purge time to 1 min. The water volume ending up in the glass vial was approximately 30 mL, using a cell size of 22 mL.

2.4. SPE/clean-up

The process SPE/clean-up used in this work was based on that reported by Petrovic et al. [31] for the analysis of pharmaceuticals in water samples with slight modifications. SPE extraction was performed using a combination of SAX cartridge (strong anion exchange) and Oasis HLB cartridges [poly(divinylbenzene-co-*N*-pyrrolidone)]. The SAX cartridge was placed on top of the HLB cartridge. The conditioning of the SPE cartridges was performed with 5 mL of methanol followed by 5 mL of Milli-Q water at a flow rate of 1 mL min^{-1} through the cartridges using a vacuum system. The 40 mL of aqueous PLE extracts were loaded into the cartridges, the glass vials were rinse with 10 mL of distilled water that were also load into the cartridges. Samples were passed through the cartridges at a flow rate of 10 mL min^{-1}. The cartridges were rinsed with 5 mL of Milli-Q water and dried under vacuum for 15 min, to remove excess of water. Then, the SAX cartridge was removed and the analytes retained were eluted from the HLB sorbent with 6 mL of methanol at 1 mL min^{-1}. The extract was evaporated under a gentle stream of nitrogen and reconstituted with 1 mL methanol–water (25:75, v/v). Prior to injection, soil and sediment extracts were filtered using syringe PTFE filters (0.22 μm, Analisis Vinicos, Tomelloso, Spain).

2.5. LC–ESI-MS/MS

The LC separation was performed using an Alliance 2695 HPLC separation module (Waters). In positive ion (PI) mode, a column Sunfire C_{18} (4.6 mm × 150 mm, 3.5 μm, from Waters) and a Gemini C_{18} (4.0 mm × 2.0 mm) guard cartridge (Phenomenex) were used. The mobile phase combines eluent A (formic acid 0.1% in methanol) and eluent B (formic acid 0.1% in water) in a gradient programme that started at 20% A for 0.1 min, increased linearly to 90% A in 15 min, then increase to 98% A in 15 min, hold for 8 min, and returned to initial conditions after 1 min followed by 11 min of equilibration time. The flow rate was 0.2 mL min^{-1}. In NI mode, a column Luna C_{18} (2) 100 Å (2.0 mm × 150 mm particle size 3 μm) and Gemini C_{18} (4.0 × 2.0 mm) guard cartridge both from Phenomenex were used. The mobile phase was composite of acetonitrile/methanol (60:40, v/v) as eluent A and ammonium acetate 10 mM in water as eluent B, at a flow rate of 0.2 mL min^{-1}. The analytical column was preconditioned using 15% of acetonitrile and 85% of eluent B at the same flow rate for 11 min. A gradient programme was used as follows: 15% of eluent A for 0.1 min, followed by a linear increase to 98% in 5 min, held for 7 min. Then, a 3 min gradient returned to the preconditioning conditions 15%

of acetonitrile and 85% of eluent B. The injection volume was 20 μL.

The tandem MS analyses were performed on a Micromass Quattro triple quadrupole mass spectrometer (Manchester, UK). Instrument control, data acquisition and evaluation were done with the Masslynx NT software (v. 3.4).

In both PI and NI mode, the applied parameters were: radio frequency lens, 0.5 V; electrospray source block, 125 °C; low mass (LM) 1 resolution, 12.0; high mass (HM) 1 resolution, 12.0; LM 2 resolution, 12.0; HM 2 resolution, 12.0; multiplier 650 V; desolvation temperature: 350 °C; argon collision gas 2.5×10^{-3} mbar; cone nitrogen gas flow, 50 L h^{-1}; desolvation gas: 600 L h^{-1}. In PI mode, the extractor voltage was 2.0 V and capillary voltage 4.0 kV. In NI mode the parameters for the analysis were: extractor voltage, 1.0 V and capillary voltage 3.2 kV. The optimal quantification and confirmation transitions and their respective cone voltages and collision energies are listed in Table 2.

2.6. Validation of the analytical procedure

The criteria applied to confirm the identity of a suspected pharmaceutical were: (i) the ratio of the relative (to the I.S.) retention time of the analyte to that of the same analyte in standard solution should be within ±2.5% tolerance; (ii) the presence of a signal at each of the two SRM transitions for the analyte; (iii) the peak area ratio of the confirmation transition against the quantification one should be within the tolerance fixed by the EU criteria [32].

Linearity was studied using standard solutions and matrix-matched calibrations by analyzing in triplicate eight concentration levels, between 1 and 1000 μg L^{-1} in the final extract, equivalent to 0.3 and 333 ng g^{-1} in soil.

The matrix effects were studied by the evaluation of signal suppression or enhancement for each pharmaceutical. The signal suppression was calculated as a percentage of the decrease or increase in signal intensity in a sample matrix versus in methanol–water (25:75, v/v). The equation used for the signal suppression calculation was (Eq. (1)):

$$\text{signal suppression (\%)} = \left[1 - \frac{Sm}{Ss}\right] \times 100 \quad (1)$$

where *Sm* is the slope of the calibration curve for each analyte in the sample extract (soils or sediments) spiked after extraction, and *Ss* is the slope in solution standard (methanol–water, 25:75, v/v) at the same concentration than the spiked sample. No pharmaceuticals were previously detected in the samples.

The extraction recoveries of the different compounds for the entire PLE–SPE–LC–ESI-MS/MS procedures were determined for soil and sediments. Soil and sediment samples were spiked with the analytes at three different concentrations: (MQL, 50 and 100 ng g^{-1}) and 33 ng g^{-1} of each ISs (volume varied between 5 and 100 μL). The solvent was removed by evaporation in a fume cupboard for 10 min. Then, the spiked samples were stirred vigorously for 30 min in order to enable better contact of analytes with the matrix. After 24-h equilibration, these samples together with the correspondent blank samples were extracted and treated by the previously described protocol. Some soil and sediment samples were left to age in the dark, at room temperature, for a period of 3 months.

The precision of the method was determined by the repeated analysis of samples of soils and sediments spiked at concentrations of 50 ng g^{-1} and calculated as the relative standard deviation (RSD, %) of measurements in quintuplicate carried out in the same day and in five non-consecutive days.

Instrumental detection limits (IDLs) and instrumental quantification limits (IQLs) were estimated by direct injection of decreasing concentrations of the standard mixture, as the amount of analyte that gave a signal-to-noise ratio of 3:1 and 10:1, respectively, in SRM mode. Method detection limits (MDLs) were confirmed by injecting seven replicated extracts of samples spiked at the estimated concentrations. Method quantification limits (MQLs) were the lower concentration that provided acceptable recovery (relative recoveries ≥70%, excepting fenofibrate and diclofenac) and precision (<20%) was tested by analyzing spiked soil and sediment samples in quintuplicate.

3. Results and discussion

3.1. Optimization of the PLE procedure

All experiments for optimizing the different steps of the method were carried out by spiking a soil sample free of contamination with a mixture of pharmaceuticals at 50 ng g^{-1}. The choice of the extraction solvent is one of the most critical parameters. Methanol (MeOH), water, combinations of both solvents at different ratios (80:20 and 50:50, v/v), acetonitrile/water 50:50 (v/v), MeOH/57 mM citric acid 50:50 (v/v) and MeOH/0.1 M Na_2–EDTA 50:50 (v/v) were tested for the optimization of the PLE at different temperatures and with different sorbents. Ion complexing agent solutions, as citric acid or EDTA, frequently block the conductions and valves of the PLE system and do not improve significantly the recoveries of the analytes. Fig. 1A shows the recoveries obtained by extracting soil dispersed in Na_2–EDTA washed sea sand with water and mixtures of methanol–water and acetonitrile–water at 90 °C for 7 min at 500 psi and flush 100%. No great differences were observed in the recoveries provided by the different solvents. Water was selected as the best choice for its compatibility with SPE, and because it is an interesting solvent for ecological considerations.

The type of sorbent to disperse soil and sediment samples prior PLE (aluminium oxide 90, silica gel, Florisil® and sea sand) was studied. Although sea sand clearly provided the best recoveries and the coarse size of the sand grains favour the dispersion, values obtained were much lower than those reported in Fig. 1A (ranging from 25% to 60%). An explanation for these poor recoveries is that antibacterials significantly bind to matrix components, specifically organic matter and metals. According to the literature on the subject, complexes formed between antibacterials and divalent and trivalent cations present in soil or sediment can be displaced using complexing agents [3,5,18]. As the addition of a complexing agent to the water was quite incompatible with the instruments making the method less robust and did not yield better recoveries, the washing of sea sand with it was tested.

Sea sand washed with Na_2–EDTA 0.1 M and sea sand washed with 0.1 M Na_2–EDTA–McIlvaine buffer solution (pH 4) were selected since they are the most reported complexing extractant solutions [13,16,33]. Recoveries achieved using Na_2–EDTA were slightly superior or comparables to recoveries for the McIlvaine + EDTA combination.

Other PLE extraction parameters, such as the extraction temperature (50–110 °C), number of extraction cycles (1–5), pressure (500–2500 psi), flush volume (60–120% of the extraction cell volume) and static time (3–15 min) were studied in order to select the best conditions for the analysis of the selected pharmaceuticals. Recoveries obtained are shown in Fig. 1S, Supplementary material. The cell size of 22 and 11 mL were tested, giving best recoveries and clean extracts the 22 mL cell. The extraction temperature and the number of cycles applied were critical for improving recoveries, while pressure had no significant influence. The increase in the flush volume and static time got better recoveries to reach their maximum at the selected values. However their effect is not as accentuated as for the other parameters. Temperature presents the most erratic effect on the analyte recoveries as previously discussed [24,25]. The increase of temperature decreases significantly the dielectric constant of the water increasing the solubility of

Table 2
Conditions of MS/MS in PI and NI modes.

Compound	T_r (min)	CV[a] (eV)	Quantification transition[b]	CE[c] (eV)	Confirmation transition[b]	CE[c] (eV)
PI mode						
Acetaminophen	16.4	25	152 → 110	15	152 → 92.5	25
Acetaminophen-d_3	16.4	20	155 → 111	15	155 → 92.5	20
Carbamazepine	25.9	30	237 → 193	35	237 → 192	40
Carbamazepine-d_2	25.9	35	239 → 195	20	239 → 194	30
Ciprofloxacin	14.5	30	332 → 314	20	332 → 231	35
Codeine	7.4	35	300 → 215	25	300 → 199	30
Diazepam	28.9	40	285 → 154	25	285 → 193	30
Fenofibrate	36.2	25	361 → 233	15	361 → 139	30
Metoprolol	15.2	30	268 → 116	20	268 → 98	20
Norfloxacin	14.4	30	320 → 276	15	320 → 302	20
Ofloxacin	13.8	30	362 → 318	20	362 → 261	25
Oxytetracycline	15.7	25	461 → 426	20	461 → 443	10
Propanolol	18.2	30	260 → 116	18	260 → 183	20
Sulfamethoxazole	20.0	25	254 → 92	25	254 → 156	15
Tetracycline	15.0	24	445 → 410	20	445 → 427	15
Trimethoprim	11.8	40	291 → 123	25	291 → 230	25
NI mode						
Clofibric acid	8.0	20	213 → 127	18	213 → 84.5	10
Diclofenac	9.6	20	294 → 250	15	294 → 214	25
Ibuprofen	10.2	15	205 → 161	10	–	–
Ibuprofen-d_3	10.2	15	208 → 164	10	208 → 162	15

[a] Cone voltage.
[b] Transition = precursor ion → product ion.
[c] Collision energy.

non-polar analytes. The recoveries obtained at 70 °C were appropriate for most of analyzed compounds. The increase flush volume and the duration of the static cycles improved the recoveries. Ofloxacin, fenofibrate, codeine, trimethoprim, diazepam, metoprolol, propanolol, ibuprofen and clofibric acid gave better recoveries at temperature of 90 °C. The temperature of 110 °C provided slightly improved recoveries for ofloxacin, ciprofloxacin, metoprolol, propanolol and diclofenac but thermal degradation of some compounds can occur. The best results were obtained with the conditions reported in Section 2.3.

3.2. Optimization of isolation and pre-concentration using SPE

The performance of different types of SPE cartridges was tested, including two polymeric sorbents (Oasis HLB and Strata-X) and a strong anion exchange sorbent (Isolute SAX). The Isolute SAX car-

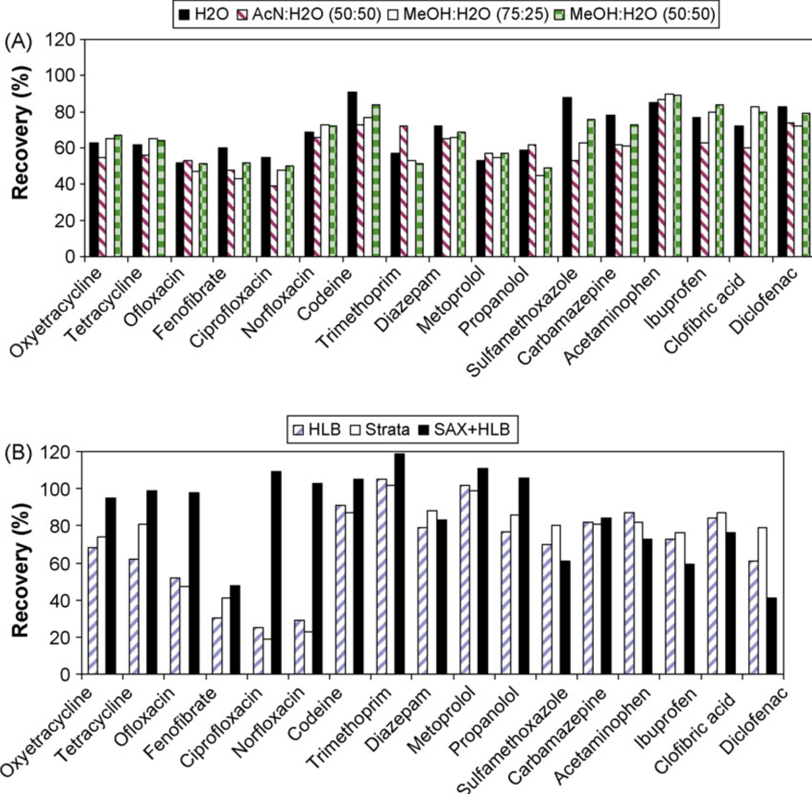

Fig. 1. Comparison of recoveries obtained using different (A) extraction solvents and (B) SPE cartridges. Concentration level 50 ng g^{-1}.

tridge was used combined in tandem with the OASIS HLB cartridges. The SAX column reduces matrix interferences by adsorbing anionic humic and fulvic acids from the soil extracts, avoiding contamination, blocking and overloading of the HLB sorbent. Aqueous extracts obtained from PLE have a pH of 7 and, in these conditions, pharmaceuticals are in their neutral or cationic form (see pK_a values in Table 1) and, consequently, they are not retained on the SAX cartridge.

The effect of the PLE extract acidification prior pass it through SPE cartridge provided recoveries between 5% and 20% lower for basic and neutral pharmaceuticals than those obtained without acidification. Nevertheless, for acidic compounds results were very similar.

As it is shown in Fig. 1B, the behaviour of Strata-X and Oasis HLB was very similar, achieving both recoveries better than 70%, except for ofloxacin, ciprofloxacin, norfloxacin and fenofibrate. The coupling of a previous SAX cartridge, to eliminate interfering and matrix compounds, increased the analyte's recoveries to values higher than 70%, except for fenofibrate, the recovery of which remains unchanged. This effect may due to its relatively non-polar character (it has the lowest polarity of all selected pharmaceuticals), therefore Oasis HLB and Strata-X cartridges were not able to retain this compound at all [34]. On the other compounds, the inclusion of the SAX cartridge only causes a marked decrease in the recovery of diclofenac (see Fig. 1B). This could be because at pH 7, diclofenac is in zwitterionic form keeping partly retained to the negatively charged SAX cartridge or forming complexes with the organic matter that are retained in the SAX cartridge. Between Oasis HLB and Strata-X, the former was finally selected just because the higher availability of these cartridges in the laboratory.

3.3. Optimization of LC–MS/MS

Most of the compounds showed maximum sensitivity operating in the PI mode excepting ibuprofen, diclofenac and clofibric acid that only give response in NI mode.

In PI mode, three columns (Waters Sunfire C_{18}, Waters Xterra C_{18} and Phenomenex Luna C_{18} (2)) were tested using mobile phases composed of different proportions of methanol or acetonitrile and water with different additives, such as ammonium acetate and formic acid, at various concentrations. The optimal separation of 14 compounds detected in PI mode was achieved using the Waters Sunfire column, and methanol and water, both with 0.1% formic acid, as mobile phase. Acid was used to improve ionization and sensitivity of MS detection. Fig. 2 depicts SRM chromatograms for the spiked soil at level 25 ng g^{-1}, illustrating the good separation and narrow peak shape obtained for the selected compounds in the PI mode. Signals of matrix components (marked in Fig. 2) were only observed for the transitions corresponding to acetaminophen and trimethoprim. Those matrix compounds gave peaks that were well separated from the analytes peak and did are not present in the confirmatory transition. The peak corresponding to the epimer of the tetracycline was also visible in the chromatogram. Some transformation of tetracycline to its epimer was always observed. This compound was quantified as the sum of both isomers. On the contrary, oxytetracycline did not show epimerization. These results agree with previous studies [16].

In NI mode, the chromatographic separation was very troublesome, even though there were only three compounds to be detected. Six analytical columns were tested (Waters Sunfire C_{18}, Waters Xterra C_{18}, Phenomenex Luna C_{18} (2), Luna C_8, Phenomenex Gemini C_{18} and C_6-Phenyl) with different mobile phases. To illustrate the problems, Fig. 3 shows SRM chromatograms from an extract of a spiked sediment at 20 ng g^{-1} analyzed by NI mode, using different LC columns. In many proofs, the three analytes were too much separated even using a high percentage of acetonitrile in the mobile phase and their elution order was inverted. The most apolar compound – ibuprofen – eluted first and the most polar one – diclofenac – was the longest retained requiring more than 20 min to elute from the column and presented with broad shape unacceptable to quantify (Fig. 3A). This can be related to the formation of zwitterionic forms as discussed for diclofenac in the SPE optimization. In other cases, chromatographic separation was achieved, but peaks appeared with "crown" (Fig. 3B). The shape of these peaks did not improve significantly even adding ammonium acetate as mobile phase additive. Finally, separation was achieved (Fig. 3C) on the Luna C_{18} (2) with a mixture of acetonitrile/methanol (60:40, v/v), and preconditioning the column prior the next injection with acetonitrile instead of acetonitrile/methanol (60:40).

The acquisition of, at least, two transitions for reliable confirmation is possible for all pharmaceuticals, except ibuprofen that gives only one fragment with reasonable sensitivity (Table 2). In the PI mode, the confirmation of the compound identity by a second transition requires an additional injection due to the high number of transitions needed for the simultaneous quantification and confirmation. The acquisition of two transitions for each compound would entail to monitor more than 28 transitions, which would reduce the number of point per peak leading to unsatisfactory peak shapes. The IDL ranged from 2 pg injected for carbamazepine, trimethoprim and fenofibrate to 34 pg for acetaminophen. The IQLs were between 7 and 114 pg injected. The second injection monitoring a fewer number of transitions allows to confirm the identity of pharmaceuticals at these low levels.

3.4. Validation of the method

3.4.1. Specificity and selectivity of the method

The specificity and selectivity of the method were established by the analysis of blank samples. The absence of any chromatographic peak in soil and sediment extracts, at the same retention times as target pharmaceuticals, indicated that there were not matrix compounds that might give a false positive signal in these blank samples.

3.4.2. Linearity and matrix effects

Matrix-matched calibration curves prepared in every type of sample showed good linearity between 1 and 1000 ng mL^{-1}, with a correlation coefficient $\geq$0.993 (Table 3). Absolute signal suppression measured for compounds analyzed under PI conditions varied from 3% to 54% in sediments and from 0.6% to 56% in soils, as it can be seen in Table 4. A slight signal suppression was observed for metoprolol, codeine, trimethoprim and fenofibrate (<15% calculated using the absolute recovery). In the case of acetaminophen a little enhancement of signal was observed ($\approx$3%) as already reported [25]. For the other compounds, higher suppression (up to 55%) was observed. For the compounds analyzed under NI conditions, suppression ranged from 19% to 34% in sediments and from 15% to 31% in sediments. The impact of the matrix effect was almost equivalent in both matrices but different for each compound. The suppression effect was only partly corrected by the addition of internal standards since matrix effects are compound dependent. However, the use of matrix-matched standards compensated quite well for the suppression effect achieving accurate quantification.

3.4.3. MDLs and MQLs

Table 3 also outlines MDLs for soil and sediment samples that were in the range 6–408 pg injected or 0.2–6.8 ng g^{-1} in sediments and 5–311 pg injected or 0.1–5.3 in soils. The sensitivity for sediment and soil samples was comparable. These MDLs were of the same order than those reported by Cuevas-Mestanza et al. [23] using GC–MS and Radjenović et al. [25] using LC–MS/MS and better than others reported in previous studies by LC [23,24]. Cuevas-

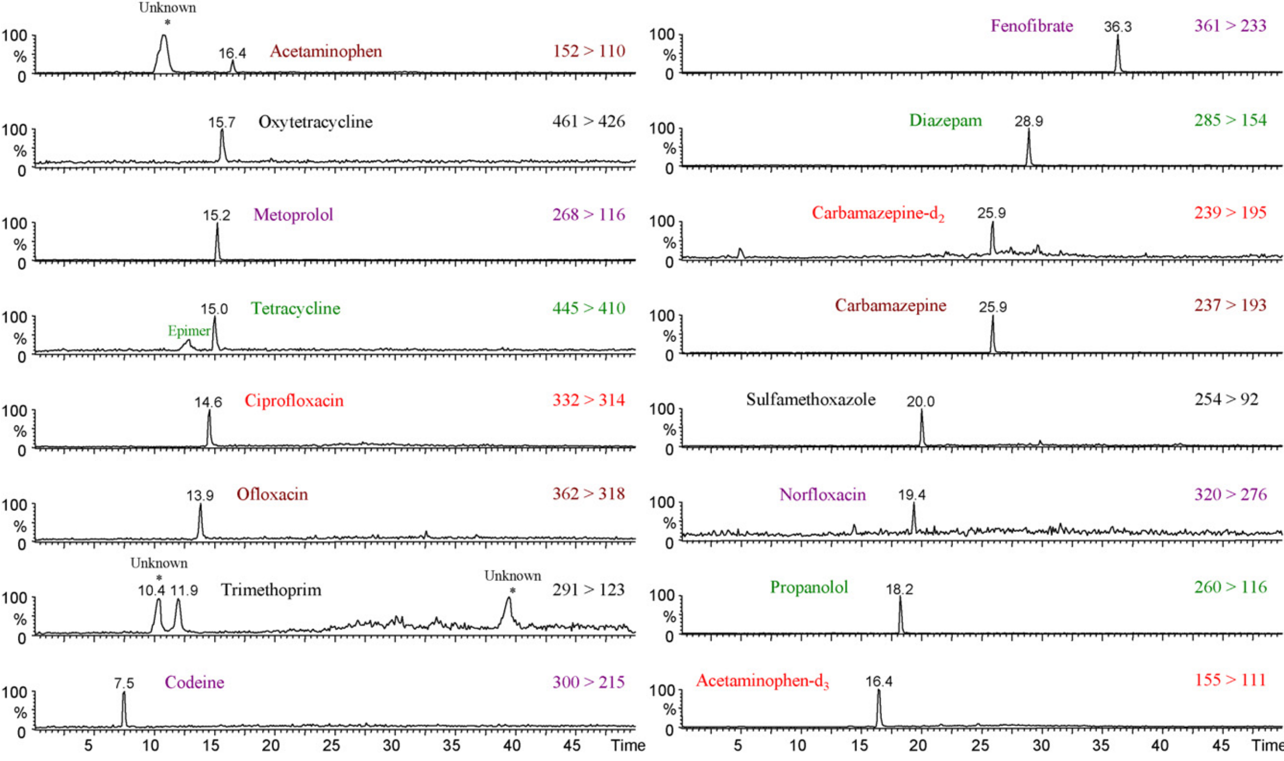

Fig. 2. LC–MS/MS chromatogram in PI mode obtained from an extract of soil spiked at 25 ng g^{-1} of each compound.

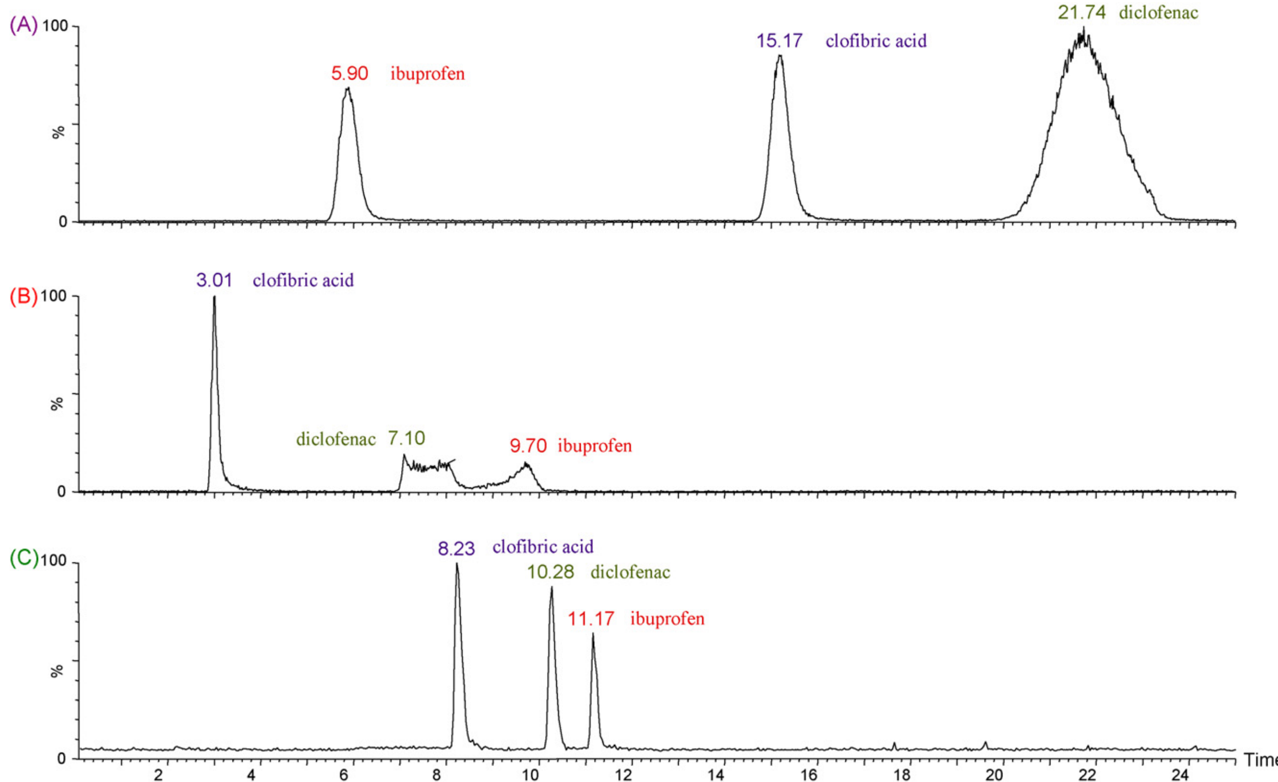

Fig. 3. LC–MS/MS chromatograms in NI mode obtained from an extract of soil spiked at 25 ng g^{-1} of each compound using (A) analytical column Phenomenex Luna C_{18} (2) (150 mm × 4.6 mm, 5 μm), mobile phase: water (a) and MeOH (b) and gradient from 15% to 98% b in 5 min and hold 8 min. (B) Analytical column Phenomenex C_6-Phenyl (150 mm × 2.0 mm, 3 μm), mobile phase: water (a), acetonitrile (b) and gradient from 25% to 98% b in 10 min, and (C) analytical column Phenomenex Luna C_{18} (2) (150 mm × 2.0 mm, 3 μm), mobile phase: water 5 mM ammonium formiate (a), acetonitrile/MeOH (60:40, v/v) (b), gradient from 15% to 98% b in 5 min and hold 7 min, preconditioning 15% of acetonitrile and 85% of a, 15 min.

Table 3
Linearity equation, method detection limits (MDL), repetitivity and reproducibility.

Compound	Equation[a]	Linearity (r^2)	MDL		Repetitivity RSD (%) (n = 5)	Reproducibility RSD (%) (n = 5)
			(pg injected)	(ng g^{-1})		
Sediments						
Oxytetracycline	$y = 63.655x - 383.79$	0.9998	408	6.8	7.4	13.2
Tetracycline	$y = 149.19x - 1284.2$	0.9996	354	5.9	7.9	14.5
Ofloxacin	$y = 109.52x - 1419.29$	0.9998	162	2.7	4.3	11.9
Fenofibrate	$y = 804.32x - 3202.5$	0.9994	36	0.6	3.1	4.4
Ciprofloxacin	$y = 178.92x - 993.45$	0.993	240	4.0	7.6	12.8
Norfloxacin	$y = 16.50x - 207.52$	0.9996	312	5.2	3.2	9.1
Codeine	$y = 36.841x - 85.117$	0.9991	24	0.4	3.6	5.9
Trimethoprim	$y = 197.03x - 1043$	0.998	18	0.3	6.1	8.0
Diazepam	$y = 300.58x - 631.71$	0.998	6	0.8	2.3	2.4
Metoprolol	$y = 223.06x + 2050.22$	0.9994	24	0.4	1.1	2.0
Propanolol	$y = 267.52x - 217.44$	0.998	12	1.2	0.9	2.3
Sulfamethoxazole	$y = 106.84x - 279.86$	0.998	18	0.3	1.0	1.6
Carbamazepine	$y = 218.38x - 48.63$	0.9995	12	0.2	1.2	2.8
Acetaminophen	$y = 137.23x + 241.15$	0.9993	18	0.3	1.2	3.5
Ibuprofen	$y = 48.708x - 302.94$	0.996	96	1.6	2.4	3.9
Clofibric acid	$y = 75.863x - 202.68$	0.998	30	1.5	1.7	3.2
Diclofenac	$y = 63.037x - 52.366$	0.9990	60	1.0	3.1	7.8
Soils						
Oxytetracycline	$y = 86.964x - 452.45$	0.997	311	5.3	6.9	14.1
Tetracycline	$y = 314.60x - 1322.4$	0.9992	296	4.8	7.5	14.3
Ofloxacin	$y = 197.48x - 1574.5$	0.9994	181	2.9	3.7	9.4
Fenofibrate	$y = 874.56x - 234.5$	0.9990	38	0.6	3.8	5.2
Ciprofloxacin	$y = 156.45x - 345.29$	0.998	272	4.1	7.8	13.2
Norfloxacin	$y = 34.645x - 234.25$	0.9991	280	4.7	4.2	8.0
Codeine	$y = 90.83x - 74.498$	0.9995	24	0.3	2.1	3.3
Trimethoprim	$y = 234.10x - 897.3$	0.9993	13	0.2	3.6	4.7
Diazepam	$y = 323.08x - 324.63$	0.9996	5	0.1	2.9	3.9
Metoprolol	$y = 564.30x + 434.92$	0.995	27	0.5	1.8	5.3
Propanolol	$y = 894.36x - 582.34$	0.997	22	0.4	1.2	2.1
Sulfamethoxazole	$y = 183.90x - 327.74$	0.9991	33	0.6	1.1	2.7
Carbamazepine	$y = 423.21x - 89.07$	0.9992	8	0.1	0.7	3.0
Acetaminophen	$y = 283.41x + 113.76$	0.994	12	0.1	0.8	2.8
Ibuprofen	$y = 76.740x - 45.92$	0.996	114	1.8	3.3	4.5
Clofibric acid	$y = 86.353x - 129.63$	0.998	20	0.3	1.2	2.9
Diclofenac	$y = 156.45x - 84.53$	0.9990	35	0.6	2.4	4.6

[a] Calculated as peak areas versus concentration.

Table 4
Percent of signal suppression of pharmaceuticals in sediments and soils spiked after extraction.

Compound	Sediments		Soils	
	Absolute	Relative[a]	Absolute	Relative[a]
Positive mode				
Oxytetracycline	18.8 ± 1.7	0.1 ± 2.3[b]	11.1 ± 1.9	0.4 ± 2.7[b]
Tetracycline	16.7 ± 5.1	−2.5 ± 1.8[b]	8.4 ± 2.9	0.5 ± 1.2[b]
Ofloxacin	41.3 ± 6.4	27.7 ± 3.1[b]	35.6 ± 4.0	26.5 ± 3.3[b]
Fenofibrate	4.5 ± 0.3	18.6 ± 1.9[c]	4.3 ± 0.5	13.7 ± 1.6[c]
Ciprofloxacin	52.0 ± 1.9	3.1 ± 0.4[c]	56.0 ± 3.3	6.2 ± 0.7[c]
Norfloxacin	54.6 ± 2.4	46.7 ± 0.7[b]	40.7 ± 3.2	38.6 ± 1.8[b]
Codeine	9.5 ± 1.0	4.9 ± 1.3[b]	9.3 ± 1.5	6.2 ± 2.1[b]
Trimethoprim	11.3 ± 4.8	6.2 ± 2.7[b]	10.1 ± 2.0	3.2 ± 1.3[b]
Diazepam	21.0 ± 0.8	7.2 ± 1.6[b]	25.4 ± 1.9	7.1 ± 3.0[b]
Metoprolol	3.1 ± 2.0	−13.8 ± 0.4[b]	0.6 ± 1.3	−5.1 ± 0.3[b]
Propanolol	21.8 ± 1.8	8.1 ± 4.5[b]	16.2 ± 1.1	7.5 ± 3.7[b]
Sulfamethoxazole	16.3 ± 0.9	1.6 ± 1.1[b]	14.8 ± 2.4	3.5 ± 2.1[b]
Carbamazepine	14.8 ± 1.7	0.0 ± 1.3[c]	8.8 ± 1.6	0.3 ± 1.9[c]
Acetaminophen	−2.6 ± 0.2	−0.5 ± 0.9[b]	−4.9 ± 1.6	0.1 ± 0.6[b]
Negative mode				
Ibuprofen	22.9 ± 1.4	−6.7 ± 0.6[d]	27.4 ± 1.0	−2.2 ± 0.9[d]
Clofibric acid	33.7 ± 1.9	12.4 ± 3.4[d]	31.4 ± 1.3	10.5 ± 2.1[d]
Diclofenac	19.3 ± 0.8	−26.8 ± 1.7[d]	15.3 ± 1.8	−13.4 ± 1.6[d]
Internal standards				
Acetaminophen-d_3	−17.2 ± 0.5	–	−15.2 ± 0.8	–
Carbamazepine-d_2	14.9 ± 1.1	–	17.6 ± 1.4	–
Ibuprofen-d_3	35.8 ± 1.7	–	29.9 ± 1.2	–

[a] Recovery relative to ISs.
[b] Acetaminophen-d_3.
[c] Carbamazepine-d_2.
[d] Ibuprofen-d_3.

Table 5
MQL (ng g^{-1}), recoveries (%) and RSDs at three spiking levels.

Pharmaceuticals	Soil							Sediment						
	MQL			50 ng/g		100 ng/g		MQL			50 ng/g		100 ng/g	
	MQL (ng/g)	Absolute recovery (%)	Relative recovery (%)[a]	Absolute recovery (%)	Relative recovery (%)[a]	Absolute recovery (%)	Relative recovery (%)[a]	MQL (ng/g)	Absolute recovery (%)	Relative recovery (%)[a]	Absolute recovery (%)	Relative recovery (%)[a]	Absolute recovery (%)	Relative recovery (%)[a]
Positive mode														
Oxytetracycline	18	68 ± 13	96 ± 6	63 ± 16	94 ± 13	59 ± 5	88 ± 9	23	66 ± 5	90 ± 10	63 ± 9	94 ± 6	64 ± 10	92 ± 8
Tetracycline	16	62 ± 15	98 ± 5	62 ± 10	99 ± 6	60 ± 10	95 ± 7	19	68 ± 8	88 ± 9	64 ± 8	92 ± 9	70 ± 13	97 ± 7
Ofloxacin	7	52 ± 7	100 ± 12	54 ± 17	99 ± 16	58 ± 12	99 ± 4	6	59 ± 12	96 ± 8	55 ± 10	91 ± 8	50 ± 12	101 ± 6
Fenofibrate	1.5	40 ± 7	66 ± 6	46 ± 15	64 ± 12	41 ± 9.2	59 ± 10	1.8	50 ± 5	66 ± 7	48 ± 5	67 ± 8	47 ± 14	64 ± 3
Ciprofloxacin	10	55 ± 9	86 ± 8	52 ± 16	87 ± 11	63 ± 13	91 ± 5	11	59 ± 9	84 ± 13	62 ± 11	88 ± 6	55 ± 12	83 ± 5
Norfloxacin	15	69 ± 11	71 ± 10	64 ± 12	77 ± 10	70 ± 11	83 ± 3	17	72 ± 13	74 ± 8	70 ± 6	73 ± 8	67 ± 8	74 ± 6
Codeine	1.3	91 ± 13	108 ± 6	90 ± 12	85 ± 7	94 ± 8.4	93 ± 7	1.5	98 ± 7	101 ± 4	95 ± 5	99 ± 5	99 ± 6	102 ± 4
Trimethoprim	0.9	105 ± 5	119 ± 4	95 ± 11	101 ± 4	91 ± 9.0	106 ± 8	1.2	97 ± 4	104 ± 7	95 ± 6	99 ± 11	93 ± 10	101 ± 7
Diazepam	0.25	79 ± 8	104 ± 1	79 ± 13	102 ± 10	78 ± 6.8	101 ± 6	2.3	76 ± 9	107 ± 12	79 ± 7	98 ± 5	77 ± 8	103 ± 5
Metoprolol	1	102 ± 10	81 ± 9	104 ± 12	87 ± 6	99 ± 7.5	85 ± 11	0.8	85 ± 3	92 ± 8	93 ± 8	95 ± 7	98 ± 6	99 ± 4
Propanolol	0.5	77 ± 19	96 ± 2	78 ± 16	99 ± 11	72 ± 5.1	102 ± 4	3.7	75 ± 7	90 ± 11	74 ± 11	88 ± 8	71 ± 17	81 ± 15
Sulfamethoxazole	0.9	70 ± 16	97 ± 3	76 ± 14	108 ± 9	79 ± 1.9	107 ± 13	0.9	84 ± 14	99 ± 4	87 ± 5	103 ± 14	85 ± 11	94 ± 9
Carbamazepine-d_2	–	103 ± 13	101 ± 5	102 ± 10	98 ± 4	94 ± 12	98 ± 7	–	96 ± 9	103 ± 7	91 ± 9	104 ± 17	98 ± 8	97 ± 5
Carbamazepine	0.5	82 ± 10	104 ± 6	86 ± 13	104 ± 7	85 ± 14	106 ± 12	0.5	88 ± 6	110 ± 5	88 ± 3	96 ± 7	91 ± 12	102 ± 10
Acetaminophen-d_3	–	84 ± 9	–	89 ± 11	–	87 ± 2.3	–	–	77 ± 11	–	79 ± 7	–	82 ± 8	–
Acetaminophen	0.8	87 ± 14	102 ± 9	74 ± 7	106 ± 7	82 ± 5.8	112 ± 7	0.5	72 ± 9	98 ± 8	74 ± 4	101 ± 5	72 ± 10	103 ± 6
Negative mode														
Ibuprofen	4	73 ± 8	84 ± 4	75 ± 7	86 ± 3	81 ± 9.9	89 ± 5	3.6	77 ± 11	85 ± 6	85 ± 9	93 ± 8	83 ± 18	92 ± 12
Ibuprofen-d_3	–	79 ± 13	–	61 ± 6	–	66 ± 7.2	–	–	74 ± 9	–	72 ± 6	–	70 ± 6	–
Clofibric acid	1.6	84 ± 18	102 ± 4	74 ± 11	80 ± 6	78 ± 11	91 ± 9	4.2	71 ± 9	97 ± 6	74 ± 5	95 ± 7	77 ± 10	110 ± 4
Diclofenac	3	34 ± 16	62 ± 6	37 ± 5	67 ± 2	35 ± 9.3	66 ± 12	3.7	42 ± 6	66 ± 7	37 ± 8	65 ± 12	39 ± 16	69 ± 10

[a] Recovery relative to ISs as reported in Table 4.

Table 6
Concentration (ng g^{-1}) of pharmaceuticals in different marsh areas of the Valencian Community.

Compound	Albufera sediment	Oliva-Pego sediment	Prat soil	Moros sediment	Silla soil
Oxytetracycline					
Tetracycline					
Ofloxacin	8.95	12.03		<MQL	
Fenofibrate	13.20			17.23	
Ciprofloxacin		5.95			
Norfloxacin					
Codeine	3.35				
Trimethoprim					
Diazepam	2.50	2.86	4.65	3.72	
Metoprolol	6.57				
Propanolol	1.51	2.60			
Sulfamethoxazole					
Carbamazepine	1.81	2.93	5.77	6.85	1.43
Acetaminophen	<MQL				<MQL
Ibuprofen					
Clofibric acid	<MQL	35.62			
Diclofenac					

Mestanza et al. [23] reported MDLs that varied between 4 ng g^{-1} for ibuprofen and 167 ng g^{-1} for fenofibrate. Löffler and Ternes [24] gave values of MDLs between 0.4 and 20 ng g^{-1}. As can be observed in Table 5, the MQLs were also at low nanogram per gram levels and ranged from 0.5 ng g^{-1} for carbamazepine acetaminophen and propanolol to 23 ng g^{-1} for oxytetracycline, which makes the method useful for the determination of low levels of pharmaceuticals in soils and sediments in real environmental samples.

3.4.4. Recovery and precision

Precision data are also listed in Table 3 for soil and sediments. The repeatability values were in the range of 0.1–7.9% for sediments and 0.7–7.5% for soils and the reproducibility ones of 1.6–14.5% for sediments and of 2.1–14.3% for soils. These results did not show apparent differences between soil and sediment samples and are similar to those reported by other studies [14,16,23].

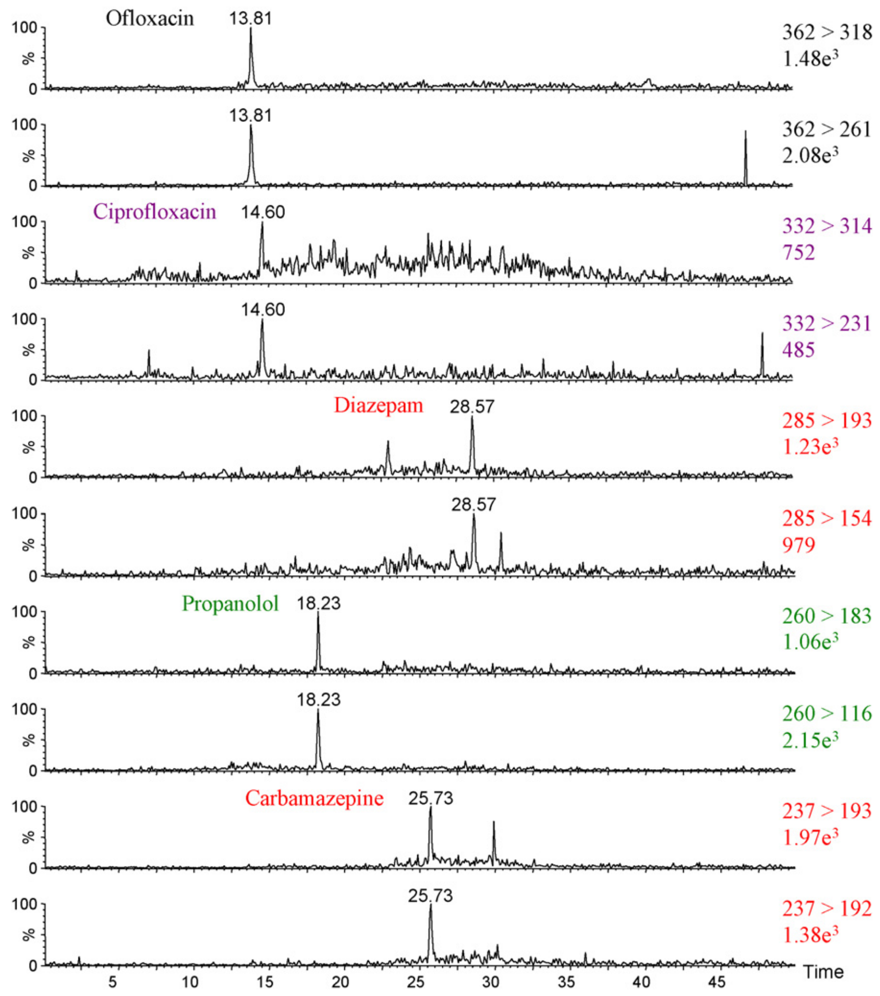

Fig. 4. Chromatograms showing the analysis of target pharmaceuticals in a Oliva-Pego sediment sample, including two SRM transitions for each analyte.

Due to lack of a reference material the recovery of the developed method was tested by spiking soil and sediments samples after 24 h of equilibration time. Absolute and relative recoveries were determined in soil and sediment at three concentration levels. The results of these experiments are summarized in Table 5. Absolute recoveries ranged from 34% to 105% with RSDs < 19% for soils and from 37% to 99% with RSDs < 17% for sediments. Relative recoveries ranged from 59% to 119% with RSDs lower than 16% for soils and from 64% to 110% with RSDs lower than 17% for sediments. The recoveries varied significantly depending on compound but not depending on the matrix. Decreasing recoveries from aged-spiked soils for sulfonamides were recently reported [14] indicating that spiked samples can be a poor indicator of incurred samples unless they are adequately aged. To check this point, several soil and sediment samples were aged for 3 months. Absolute recoveries ranging from 32% to 106% for soils and between 42% and 98% for sediments were obtained. Comparison of the recovery obtained for each pharmaceutical in freshly spiked and aged-soils and sediments are presented in Fig. 2S, Supplementary material. There is no evident difference between values obtained by both spiking procedures either in soil or sediment. A qualitative difference was only observed for tetracycline because the percentage of its epimer increased at the expenses of that of the tetracycline. These results demonstrated the performance of the developed extraction method to isolate analytes occurring in samples.

3.4.5. Application to samples

Table 6 shows the concentrations of the target pharmaceuticals detected in the contaminated samples. Of 16 samples analyzed, pharmaceuticals were detected in 5. Most detected compounds were carbamazepine (detected in all samples) and diazepam (in four samples) with concentrations between 1.4 and 6.8 ng g^{-1}. Ofloxacin, fenofibrate, ciprofloxacin, codeine, metoprolol, propanolol, acetaminophen and clofibric acid were less frequently present. Maximum concentrations were detected for clofibric acid, with average concentration of 35.6 ng g^{-1} and for fenofibrate at 17 ng g^{-1}, even through the results for fenofibrate can only be consider as semi-quantitative. A chromatogram obtained from a sediment sample taken at the Oliva-Pego marsh using both, quantification and confirmatory, SRMs for each detected analyte is shown in Fig. 4. These preliminary date indicate that pharmaceuticals may be discharged in large amounts through wastewater effluents from human origin, arriving into natural environments. To our knowledge, these results present the first evidence of contamination of marsh areas with pharmaceuticals.

4. Conclusions

The developed method attains simultaneous extraction by PLE and pre-concentration by SPE of seventeen pharmaceuticals with a great variety of polarities and pK_a's, from soils and sediments. The use of LC–MS/MS afforded high sensitivity (MQLs in the low ng g^{-1}) and achieves unequivocal identification of these compounds. PLE followed by SAX + Oasis HLB proved efficient clean-up, yielding recovery rates for the selected compounds generally over 70%. Fenofibrate and diclofenac were the exception with recoveries up to 34%. These low recoveries only allow to obtain semi-quantitative results for these compounds.

However, in comparison with other studies, the present method achieves a significant increase in sensitivity achieving a decrease of the quantity of pharmaceuticals that could be detected in soils and sediments, being a powerful protocol to highlight pollution of pharmaceuticals in the ecosystems. The proposed analytical method also consumed very small amount of toxic chemicals and reagents (less than 11 mL of methanol per sample), with minimum waste production. It is also simple and inexpensive. Hence, it is considered to be a green analytical technique and environmental friendly method.

The application of this method to environmental samples proofs that significant amounts of acetaminophen, carbamazepine, ciprofloxacin, clofibric acid, codeine, diazepam, fenofibrate, metoprolol, ofloxacin and propanolol contaminate soils and sediments of marsh areas. According to the detected concentrations of fenofibrate in samples, more selective conditions for the analysis of this compound could be of interest (considering its non-polar character).

These data show that the proposed method is suitable for environmental monitoring and could be useful to establish the occurrence of selected human pharmaceutical compounds in soils and sediments with high content of organic matter.

Acknowledgements

The authors thank the Spanish Ministry of Education and Science together the European Regional Development Funds (ERDF) (projects GCL2007-66687-C02-01/BOS and GLC2008-01693/BTE) for financial support. Pablo Vazquez-Roig is holder of FPI grant from the Ministry of Science and Innovation (MICINN, Spain).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2009.11.033.

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