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ORIGINAL PAPER

Assessment of the occurrence and distribution of pharmaceuticals in a Mediterranean wetland (L'Albufera, Valencia, Spain) by LC-MS/MS

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Abstract The distribution of 17 pharmaceuticals between water and the solid phase (sediments and soils) was studied by utilizing solid-phase extraction (SPE) and liquid chromatography–tandem mass spectrometry (LC-MS/MS). Two extraction procedures for soils and sediments, prior to the SPE, one based on pressurized liquid extraction (PLE) with hot water and the other on methanol/water ultrasonic extraction, were compared. Absolute recoveries were 71.2–99.3% [relative standard deviation (RSD) <21.4%)] for water, and the method detection limits (MDLs) ranged from 0.3 to 10 ng L^{-1}. Recoveries were 35.4–105.3% (RSDs <19.1%) and 42.1–97.8% (RSDs <14%) for soil and sediment samples, respectively, using PLE and 20.2–86.5% (RSDs <25.1%) and 30.3–97.4% (RSDs <19.1%) using ultrasonic extraction. Fifteen of the 17 pharmaceuticals were present in the L'Albufera water at concentrations up to 17 μg L^{-1}. Oxytetracycline and tetracycline were not detected. In sediments, only tetracycline, norfloxacin and diclofenac were not found. The other studied pharmaceuticals were present in the range from less than the method quantification limit (MQL) to 35.83 ng g^{-1}. Among the 17 target compounds, ofloxacin, ciprofloxacin, norfloxacin, trimethoprim, clofibric acid and diclofenac were not detected in soil samples. The average concentrations ranged from less than the MQL for ibuprofen to 34.91 ng g^{-1} for tetracycline. These results indicate that pharmaceuticals could survive the wastewater treatment processes, which could lead to their dissemination in water environments.

Keywords Pharmaceutical products · Surface water · Soil · Sediments · Wetlands · LC-MS/MS · Pressurized liquid extraction · Ultrasonic extraction · SPE

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Introduction

Numerous studies have evidenced the ubiquitous presence of pharmaceuticals in natural waters [1–11]. As a result of the distribution of this water between the aquatic and solid phase, the sediment and soil can be contaminated and their further migration can even lead to their infiltration into the drinking water sources [4, 5]. This phenomenon is particularly interesting in cases when the biologically treated or untreated wastewater is introduced into the aquatic ecosystems, because pharmaceuticals mainly come from human activities [12–15]. The pharmaceuticals are not necessarily persistent but they are hydrosoluble and their dumping can cause health and environmental problems that require further study [16]. This type of contamination is a growing problem that must be tackled to meet the Water Framework Directive of the European Union [17]. Thus, a better knowledge of the occurrence and fate of pharmaceuticals released into the environment will allow a proper risk assessment to be conducted for river

basins, wetlands and other related ecosystems [6, 12, 18]. In spite of the relatively high number of literature reports, the simultaneous investigation of these drugs in water, sediment and soil samples is scarce [19, 20].

Wetlands are amongst the Earth's most productive ecosystems, providing a diverse array of important ecological functions. Wetlands are fundamental to the maintenance of the water cycle because they purify and recycle water and, at the same time, capture and retain it from the rain. Wetlands are also important in the control of floods and flows, in the recharge of aquifers, in carbon sequestration, etc. [21, 22]. Among these ecosystems, the coastal wetlands present a great dynamism and biodiversity [9, 23]. Because of their open structure and relationship with the environment, coastal wetlands are usually eutrophic and rich in nutrients. These ecosystems are very fragile and are particularly sensitive to alterations in their water regime [23, 24]. In this sense, their situation becomes more critical in the Mediterranean area where the predicted climate evolution indicates a clear tendency toward rain shortage and increased temperatures [22]. Even though the importance of wetlands and the essential features that sustain have been recognized, wetland loss and degradation continues in Europe. The Water Framework Directive [17] clearly identifies the protection, recovery and conservation of these wetland zones as priority actions.

There are several methods to determine pharmaceuticals in the aquatic environment. They mainly consist of solid-phase extraction (SPE) or solid-phase microextraction (SPME) for isolation and enrichment, and liquid chromatography–mass spectrometry (LC-MS/MS) or gas chromatography–mass spectrometry (GC-MS) following derivatization for quantification [1, 7, 9–14, 25]. However, there are much fewer methods available for the extraction and quantification of pharmaceuticals at trace levels in solid matrices. The analytical procedures include extraction of the contaminants from the solid surfaces using ultrasonication, pressurized liquid extraction (PLE) and microwave-assisted solvent extraction (MAE) [2, 4, 5, 8, 24, 26, 27]. The next steps of the analytical procedure are the purification of the extracts and their determination using the same techniques described for water samples.

In the light of the above concerns, the aim of this work was to develop an analytical protocol for the sensitive determination of 17 pharmaceuticals and to apply it to the study of their spatial distribution between water, soil and sediment in water courses and channels of L'Albufera Natural Park. In this protocol, SPE was used to isolate and concentrate the chemicals from the water, followed by LC-MS/MS. For sediment and soil samples, the extraction results obtained by PLEs and ultrasonic shaking were compared. PLE conditions (dispersing agent, elution solvent, static time, number of cycles) were based on those used in a previous study [26]. The Valencia Community can be an adequate study case, where the scarcity of water and the human activity endanger the integrity and future of L'Albufera Natural Park, which is the most important wetland ecosystem of the Xuquer River Basin and one of the most significant in Spain [28, 29]. The Albufera was formed after an ancient gulf was closed off through the emergence of a coastline strip. The sandbar started to form roughly 6,000 years ago from the sediment brought down by the Turia and Xuquer rivers [18, 29–31]. The waves and the coastal currents brought the sediment along until it formed the sandbar which separated the Albufera from the Mediterranean Sea. This coastal lake is the morphological model of coastal wetland most common in the Mediterranean [18, 30]. The 17 pharmaceuticals chosen for the validation were selected as model substances based on their occurrence in wastewater and their distribution along the logarithmic octanol/water partition coefficient (log K_{ow}) scale, from log K_{ow} −0.13 to 5.19 [22]. The analytes were selected from different therapeutic classes: β-blockers (metoprolol and propanolol), antidepressants (diazepam), anti-epilectic drugs (carbamazepine), analgesics (acetaminophen and codeine), nonsteroidal anti-inflammatory drugs (ibuprofen and diclofenac), and lipid regulators (clofibric acid and fenofibrate) in addition to seven antibacterials (ciprofloxacin, norfloxacin, ofloxacin, oxytetracycline, sulfamethoxazole, tetracycline and trimethoprim).

Experimental

Chemicals and materials

All pharmaceutical standards were purchased from Sigma–Aldrich (Steinheim, Germany), except 4-epioxytetracycline that was from Acros Organics (Morris Plains, NJ, USA) and ibuprofen-d_3, acetaminophen-d_3 and carbamazepine-d_2 (internal standards, ISs) that were from CDN Isotopes (Quebec, Canada). All standards were of analytical grade (purity >97%). Stock solutions (1,000 mg L^{-1}) of each pharmaceutical were prepared in methanol with the exception of ciprofloxacin, which was prepared at 500 mg L^{-1} in water acidified with formic acid. Stock solutions were stored at −20 °C. Working solutions, at different concentrations, were prepared each 3 months by dilution of the standard stock solutions in methanol/water (25:75, v/v). A mixture of the ISs at concentrations of 10 μg mL^{-1} each was prepared in methanol and the corresponding quantity was added to water, soil and sediment samples to obtain concentrations of 50 μg L^{-1} or 50 ng g^{-1} in the final extract. Formic acid (reagent grade), acetone and dichloromethane (residue analysis) as well as 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). Sea sand was from Panreac (Barcelona, Spain). Ethylenediaminetetraacetic disodium salt dihydrate (Na_2EDTA), citric acid and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Scharlau (Ferosa, Barcelona, Spain).

Na_2EDTA-washed sea sand was prepared by placing 60 g of sand into a Buchner funnel and passing 120 mL of 0.1 M Na_2EDTA through it using a vacuum. Partial drying of the sand was carried out under vacuum. Thereafter, sand was completely dried in an oven at 100 °C. pH 7.4 McIlvaine buffer was obtained by mixing 9.15 mL 0.1 M citric acid with 90.85 mL 0.2 M Na_2HPO_4.

Oasis HLB 200 mg sorbent/6 mL cartridge (Waters Corp., Milford, MA, USA) and Isolute SAX 500 mg (Symta, Madrid, Spain) were used for SPE.

Sampling area

L'Albufera was declared a Natural Park in 1986, covers an area of 210 km^2 and is located 12 km south of the city of Valencia (Spain). The park is part of the hydrographic Xuquer basin, which consists of the large (around 23 km^2) shallow (1- to 2-m depth) lagoon surrounded by rice fields (140 km^2), pine groves and dunes. This park is a place of high economic, tourist and scientific interest and it is included in the Ramsar Convention on Wetlands. The lagoon is also very important in regulating the water flow in the rice fields. The lagoon is freshwater-fed by a number of channels associated with the agricultural land uses, as well as springs, located either within the lagoon or in the surrounding marshland. At present, the water flow is controlled by a system of pumps and sluice gates at three artificial water outlets that link the lagoon to the sea, because the whole lagoon acts as a regulation reservoir in accordance with rice cultivation periods. Other serious effects are occurring in response to the industrialization of the neighbouring areas, demographic expansion in outskirt villages, tourist urbanization in coastal areas, and construction of a dense road network, which takes up over 40 ha. Wastewater from human activities is also dumped into the irrigation channels and the lagoon; the channels are designed to reuse reclaimed water for supplying the ecological flow in the wetland and the irrigation of farm areas.

Sampling was carried out in April and October 2008 at the points marked in Fig. 1. Sampling points were georeferenced (UTM D50). Water and sediment samples were mainly from irrigation channels, whereas soil samples were taken in the neighbouring area from the superficial horizon.

Water samples were taken from the same irrigation channels as sediments. They were obtained from the channel back or from bridges at a depth of less than 1 m (mostly 30 cm). Grab water samples (2.5 L) were collected in clean amber glass bottles. Before sample collection, each bottle was pre-rinsed with sample three times. Samples were transported in boxes packed with ice and were stored at 4 °C in a cold room upon arrival at the laboratory. They were treated within 48 h. Water samples were filtered through a Whatman GF/F glass microfiber membrane filter of 0.7 μm. Water samples had a pH ranging from 7.2 to 7.4.

Sediment samples (250 mL) were taken from irrigation channels and marshes using a Van Veen grab sampler, and transferred to polypropylene bags. Sediment samples were of pH>7.4, sandy loam texture, and with high content of calcium carbonate (>30%) and organic matter (>15%). These samples, once in the laboratory, were lyophilised (Hetosicc CD4, Birkerod, Denmark) and passed through a 2-mm-Ø sieve. The process of lyophilisation was carried out over 7 days for each sediment sample until the water content was less than 1%. Finally the lyophilised samples were stored in sealed plastic bags at 4 °C until the extraction.

Soils of this zone are developed on black and grey silts, affected intensely at the surface by the agricultural practices. The most important physical and chemical characteristics of these soils are an impermeable profile, carbonated, with hydromorphic features, and high salinity level. According to the Food and Agriculture Organization of the United Nations (FAO) classification [32], the soil comprises Calcareous gleic Fluvisol type in the saline phase, and Aplic Fluvisols. Soil samples were collected at the upper 20 cm horizon layer. Once in the laboratory, samples were dried and passed through a 2-mm-Ø sieve. The soil samples were extended in a layer of approximately 1-cm thickness on polypropylene trays and air-dried in darkness at 20 °C. Dried samples were stored in sealed plastic bags at 4 °C.

Pressurized liquid extraction of soil and sediment

The extraction method was based on a previous one developed in our laboratory [26]. Soil or sediment (3 g) were added 10 μL of a 10 ng μL^{-1} mixture of the ISs, and mixed with approximately 25 g of Na_2EDTA-washed sea sand in a mortar. This mixture was put into a 22-mL extraction cell and extracted by PLE using an ASE 200 system (Dionex, Sunnyvale, CA, USA) with hot water (90 °C) as extractant, a static period of 7 min, and a flush volume of 100% in three cycles. Pressure was set to 500 psi and 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.

Ultrasonication extraction of soil and sediment

The PLE method was compared with an ultrasonic extraction one published previously by Blackwell et al. [33]. Briefly, soil or sediment samples (3 g) and the corresponding amount of

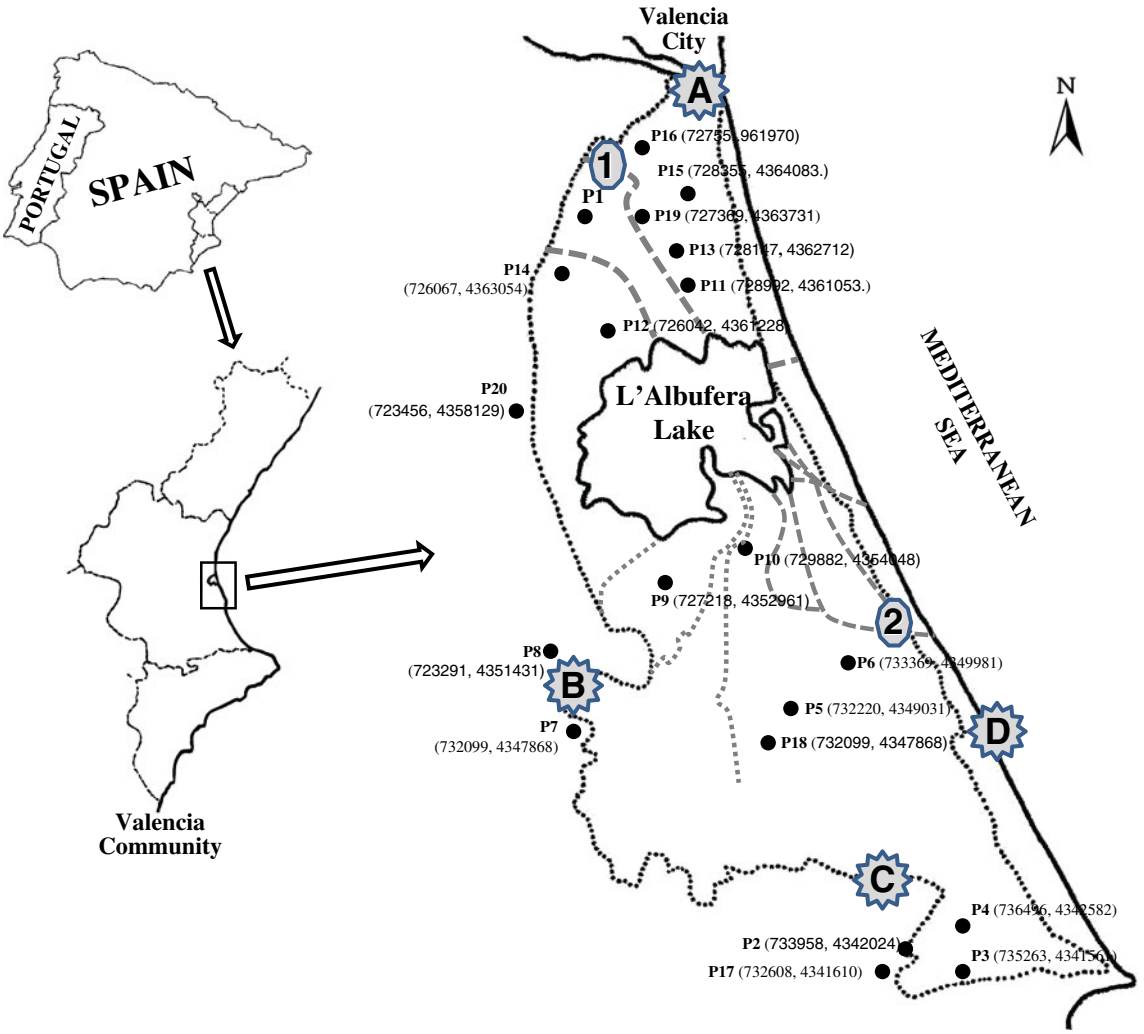


Fig. 1 Map with the location and georeferences of the sampling points. *1* Poyo Gully, *2* Plana Pound and Perello outflow channel. **A** Pinedo wastewater treatment plant (WWTP), **B** Albufera South WWTP, **C** Sueca WWTP and **D** El Mareny WWTP

ISs were placed into a 15-mL centrifuge tube and 5 mL extraction buffer (methanol/0.1 M Na_2EDTA/McIlvaine buffer, 50:25:25) was added. The tubes were vortex for 30 s and placed into an ultrasonic bath for 10 min before being centrifuged at approximately 1,200 *g* for 15 min. The supernatant was then combined and diluted to approximately 400 mL with distilled water to reduce the methanol content below 2%.

SPE for extraction of water samples and soil extracts clean-up

The process SPE/clean-up used for water samples was based on that reported by Petrovic et al. [14]. Water samples (250 mL, pH neutral) were spiked with 50 ng of surrogate/internal standards (acetaminophen-d_3, carbamazepine-d_2 and ibuprofen-d_3) and isolated using an Oasis HLB cartridge [poly(divinylbenzene-*co*-*N*-pyrrolidone)] preconditioned with 5 mL of methanol and 5 mL of Milli-Q water. Samples were passed through the cartridges at a flow rate of 10 mL min^{-1} and the cartridges were then rinsed with 5 mL of Milli-Q water and dried under vacuum for 15 min. The analytes retained were eluted with 6 mL of methanol. The extract was evaporated under a gentle stream of nitrogen and reconstituted with 1 mL methanol/water (25:75, v/v), filtered using syringe poly(tetrafluoroethylene) (PTFE) filters (0.22 μm, Analisis Vinicos, Tomelloso, Spain) and injected into the HPLC-MS/MS for analysis.

In the case of the aqueous PLE or ultrasonic extracts, obtained from soils and sediments, clean-up was performed in the same manner as for water samples, but instead of using a unique cartridge, a SAX one (strong anion exchange medium) was placed on top of the Oasis HLB cartridge and the former was removed just before the elution of the analytes.

LC-ESI-MS/MS

In accordance with our previous study [26], the LC separation was performed using an Alliance 2695 HPLC module (Waters). In positive ion (PI) mode, a Sunfire C18 column (4.6 mm×150 mm, 3.5 μm, from Waters) and a Gemini C18 (4.0 mm×2.0 mm) guard cartridge (Phenomenex) were used. The mobile phase was 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 increased to 98% A in 15 min, hold for 8 min, and returned to the initial conditions after 1 min followed by 11 min of equilibration time. The flow rate was 0.2 mL min^{-1}. In negative ion (NI) mode, a Luna C18 (2) column 100 Å (2.0 mm×150 mm, particle size 3 μm) and Gemini C18 (4.0×2.0 mm) guard cartridge, both from Phenomenex, were used. The mobile phase was 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). The optimal quantification and confirmation transitions, and their respective cone voltages (CV) and collision energies (CE) are listed in Table 1.

Method validation

Linearity was studied using standard solutions and matrix-matched calibrations by analysing in triplicate seven

Table 1 Retention time (T_r) of the pharmaceuticals, LC-MS/MS conditions in positive and negative ion mode and some physicochemical characteristics

Compound	T_r (min)	CV (eV)	Quantification transition	CE (eV)	Confirmation transition	CE (eV)	Log *P*	Log D_{ow} (pH 7.4)	pK_a
PI mode									
Acetaminophen[a]	16.50	25	152→110	15	152→92.5	25	0.46	0.34	9.38
Acetaminophen-d_3	16.40	20	155→111	15	155→92.5	20			
Carbamazepine[b]	25.92	30	237→193	35	237→192	40	2.45	2.67	13.9
Carbamazepine-d_2	25.92	35	239→195	20	239→194	30			
Ciprofloxacin[b]	14.51	30	332→314	20	332→231	35	0.28	−1.11	5.9/8.9
Codeine[a]	7.39	35	300→215	25	300→199	30	1.52	0.47	8.2
Diazepam[a]	28.88	40	285→154	25	285→193	30	2.8	2.79	3.3
Fenofibrate[b]	36.22	25	361→233	15	361→139	30	5.19	4.80	–
Metoprolol[a]	15.17	30	268→116	20	268→98	20	1.88	−0.1	9.7
Norfloxacin[a]	14.37	30	320→276	15	320→302	20	−1.0	−1.03	6.2/8.5
Ofloxacin[a]	13.77	30	362→318	20	362→261	25	−0.4	−0.44	6.05/8.2
4-Epioxytetracycline[a]	14.29	25	461→426	20	461→443	10	−1.3		
Oxytetracycline[a]	15.66	25	461→426	20	461→443	10	−1.3	−1.55	3.2/7.5/9.2
Propanolol[a]	18.20	30	260→116	18	260→183	20	3.48	1.2	9.5
Sulfamethoxazole[a]	20.00	25	254→92	25	254→156	15	0.89	0.89	1.85/5.7
4-Epitetracycline[a]	14.54	24	445→410	20	445→427	15	−1.2		
Tetracycline[a]	15.02	24	445→410	20	445→427	15	−1.2	−1.35	3.3/7.8/9.6
Trimethoprim[a]	11.81	40	291→123	25	291→230	25	0.91	0.05	6.6
NI mode									
Clofibric acid[c]	7.97	20	213→127	18	213→84.5	10	2.58	−1.36	3.46
Diclofenac[c]	9.57	20	294→250	15	294→214	25	4.51	1.26	4.15
Ibuprofen[c]	10.22	15	205→161	10	–	–	3.97	1.16	4.5
Ibuprofen-d_3	10.22	15	208→164	10	208→162	15			

[a] Related to acetamidophen-d_3 as internal standard when used

[b] Related to cabamazepine-d_2 as internal standard when used

[c] Related to ibuprofen-d_3 as internal standard when used

concentration levels, between 7.5 and 7,500 ng mL^{-1} in the final extract, equivalent to 2.5 and 2,500 ng g^{-1} in soil, and between 0.030 and 30 μg L^{-1} in water. The matrix effects were studied by comparison of the slopes of both regression equations.

The extraction recoveries of the different compounds for the entire procedures were determined for waters, soils and sediments. Samples were spiked with the analytes at three different concentrations; method quantification limits (MQLs), 0.05 and 0.5 μg L^{-1} for water; and MQLs, 5 and 50 ng g^{-1} for soils and sediments. For calculation of recoveries, the average concentrations measured in the non-spiked water samples were subtracted from the concentration values obtained for the spiked ones.

Method detection limits (MDLs) were confirmed by injecting seven replicated extracts of samples spiked at the estimated concentrations. MQLs were the lower concentration that provided acceptable recoveries (relative recoveries≥70%, excepting fenofibrate and diclofenac) and precision (<20%). It was tested by analysing spiked soil and sediment samples in quintuplicate.

Each sample was analysed in triplicate. Prior to sample analysis several tests were carried out to ensure system and laboratory performance. A calibration standard solution was used to validate calibration accuracy. The retention times of both native and labelled compounds were required to be within ±15 s of the respective retention times determined during the initial calibration. Throughout the analysis, precision and recovery were ensured. Laboratory blanks were analysed prior to each batch sample analysis consisting of 7 to 20 samples.

Results and discussion

Optimization and/or validation of the sample pre-treatment

Water samples

Oasis HLB SPE cartridges are commonly used for the analysis of pharmaceuticals in environmental matrices [1, 3, 7, 9–15]. Three parameters were optimized for the performance of the method in environmental waters: the sample extraction volume, wash volume after extraction and the elution solvent. SPE recoveries and MDLs were the criteria used to make the most appropriate choice for every parameter.

Three extraction water volumes were checked (100 mL, 250 mL and 500 mL). The extraction yield of the studied compounds is shown in the Electronic Supplementary Material Figure S1-A. In general, 100 and 250 mL were the volumes that provided the best recoveries with no big differences between them; 250 mL was therefore selected as the sample extraction volume because it yielded better MDLs.

Two cartridge wash volumes of water were tested (5 mL and 10 mL). Polar compounds gave better SPE recoveries with 5 mL (see Electronic Supplementary Material Figure S1-B). This is consistent with the fact that the solvent used for washing is water, which will elute some of the polar compounds with it. For polar compounds, the lower washing volume used was the better. For the other compounds this parameter is not so critical. Washing with 5 mL of water resulted in the best recovery for a larger number of compounds and was chosen for further analyses.

The recovery of the target compounds by SPE is highly dependent on the polarity of the eluent. Acetone, dichloromethane, acetonitrile and methanol were tested. The results (see Electronic Supplementary Material Figure S1-C) show that dichloromethane produced the lowest recovery for most compounds (<50%). Better recoveries were obtained with acetone and acetonitrile as the elution solvents, with most varying between 60 and 105%. The best recoveries (80–100%) were achieved eluting with methanol. Accordingly, it was chosen as the solvent for the simultaneous extraction of the studied pharmaceuticals from water.

The method was validated and data are presented in Table 2. Linearity was determined using regression analysis between the area ratios and concentrations. Correlations of R^2>0.99, with the exception of ibuprofen, were obtained over a concentration range 30–30,000 ng L^{-1}. MDLs and MQLs ranged from 0.3 to 10.0 ng L^{-1} and 0.9 to 36 ng L^{-1}, respectively. The precision of the overall method was determined from five replicates. At low level, it varied by less than 20% in most cases with the exception of ibuprofen and, in high level spiked samples, it was always lower than 13%. Recoveries achieved for all target compounds ranged from 71.2 to 97.8% and from 85.2 to 98.5% at MLQs and 10 times MLQs, respectively. Oxytetracycline, 4-epioxytetracycline, tetracycline, 4-epitetracycline, metoprolol, propanol, acetaminophen and clofibric acid showed the lowest recovery rates (between 70 and 80%).

ESI-MS analysis may be subject to signal suppression or enhancement as a result of other components in the sample. Signal suppression was observed for all analytes detected. The level of suppression was greater than 10% for oxytetracycline, ofloxacin, fenofibrate, ciprofloxacin, norfloxacin, propanolol, sulfamethoxazole, carbamazepine, ibuprofen and clofibric acid, and greater than 20% for metoprolol and clofibric acid.

Soil and sediment samples

As was mentioned in the "Introduction," fewer methods have been developed for the determination of pharmaceuticals in soils and sediments [8, 24, 27, 33]

Table 2 Linearity and detection and quantitation limits, absolute recovery, reproducibility and matrix effect of the method used to determine pharmaceuticals in water

	MDLs	MQLs	Recoveries, % (RSD, %)		Linearity (R^2)	Matrix effect (%)
	ng/L	ng/L	At LOQ	At 10×LOQ		
Oxytetracycline	9.4	28.2	71.2 (14.1)	85.2 (10.4)	0.9987	−10.7
4-Epioxytetracycline	9.8	29.4	72.7 (12.7)	88.2 (9.7)	0.9989	−9.4
Tetracycline	10.0	30	73.5 (12.9)	85.3 (11.2)	0.9991	−8.6
4-Epitetracycline	9.8	29.4	75.6 (11.5)	87.7 (9.5)	0.9986	−7.8
Ofloxacin	8.1	24.3	86.4 (13.2)	92.5 (8.8)	0.9996	−11.9
Fenofibrate	1.8	5.4	90.3 (12.6)	97.4 (8.3)	0.9989	−12.8
Ciprofloxacin	12	36	91.2 (10.2)	93.6 (7.5)	0.9992	−12.4
Norfloxacin	9.6	28.8	90.4 (11.8)	98.5 (8.0)	0.9994	−10.5
Codeine	1.2	3.6	92.5 (13.2)	99.3 (7.9)	0.9997	−9.6
Trimethoprim	0.9	2.7	90.7 (14.9)	96.7 (8.2)	0.9997	−8.7
Diazepam	0.3	0.9	94.4 (12.8)	99.2 (7.7)	0.9996	−5.4
Metoprolol	1.2	3.6	80.1 (18.7)	93.2 (7.8)	0.9993	−25.2
Propanolol	0.6	1.8	75.8 (19.6)	94.2 (9.1)	0.9989	−18.2
Sulfamethoxazole	0.9	2.7	74.9 (10.3)	95.6 (8.9)	0.9992	−15.4
Carbamazepine	0.6	1.8	92.5 (12.3	99.3 (9.2)	0.9994	−18.9
Acetaminophen	0.9	2.7	72.8 (16.2)	81.7 (12.5)	0.9924	−4.2
Ibuprofen	4.8	14.4	97.8 (21.4)	98.3 (7.5)	0.9896	−15.4
Clofibric acid	1.5	4.5	79.2 (13.2)	85.2 (10.3)	0.9994	−12.1
Diclofenac	2.5	7.5	92.6 (11.8)	96.2 (10.9)	0.9989	−23.0

In this study, an ultrasonic-based extraction method was compared with an previously developed, very fast and simple, one-step PLE extraction with hot water (90 °C) [26], using a common clean-up procedure based on that developed to extract water samples. The performance characteristics for the majority of the 17 pharmaceuticals studied were acceptable for both methods. A comparison between the PLE and ultrasonic extraction method is illustrated in Fig. 2 via bar graphs for the obtained recovery data and in Table 3 via tabulated results for the MDLs, MQLs, matrix interferences and linearity.

Recoveries achieved at the MQLs were 35.4–105.3% for soil and 42.1–97.8% for sediments using PLE and 20.2–86.5% and 30.3–97.4% using ultrasonic extraction. These results showed a better performance of PLE in the extraction of pharmaceuticals than that of the ultrasonic extraction. Precision obtained was better using PLE method (between 5 and 19%) than using ultrasonic extraction (between 12 and 25%). MQLs of the target pharmaceuticals ranged from 0.1 ng g^{-1} (acetaminophen, carbamazepine and diazepam) to 5.3 ng g^{-1} (oxytetracycline) by PLE, and from 0.1 ng g^{-1} (acetaminophen) to 15.9 ng g^{-1} (tetracycline) by ultrasonic extraction. Limits of quantification (LOQs) were between 0.3 (trimethoprim) and 18.1 ng g^{-1} (oxytetracycline) by PLE, and between 0.5 (acetaminophen) and 48.2 ng g^{-1} (tetracycline) by ultrasonic extraction. Overall, the analytical method provided a higher LOQ for the ultrasonic extraction than for the PLE.

Matrix effects were observed and assessed for the spiked soil and sediment extracts. Soil and sediment matrix components decreased signal responses for all pharmaceuticals, excepting acetaminophen. The absolute matrix effects were −2.6 to 54.6% (suppression) for the PLE and −6.8–69.3% for the ultrasonic extraction. Relative recoveries with regards to an internal standard diminish the ion suppression. Results suggest that use of isotope-labelled internal standards is very important in reducing the matrix effects.

Summarizing, the number of pharmaceuticals with MLQs lower than 10 ng $g^{-1}$and acceptable RSDs (<20%) is slightly higher for the PLE method than the ultrasonic one. It should be kept in mind, however, that this is not only caused by the sensitivity of the LC-MS/MS detection method, which is little better for the PLE method because of the lower percentage of matrix effects, but also by the differences in recoveries and RSDs at the lowest spiked level. On the other hand, the speed and user-friendliness of the ultrasonic extraction in real practice are slightly better than for the PLE method, despite the extra centrifugation step, which can be easily performed in batch. Both methods were successfully applied in the L'Albufera Natural Park study during 2008. Figure 3 shows the LC-MS/MS

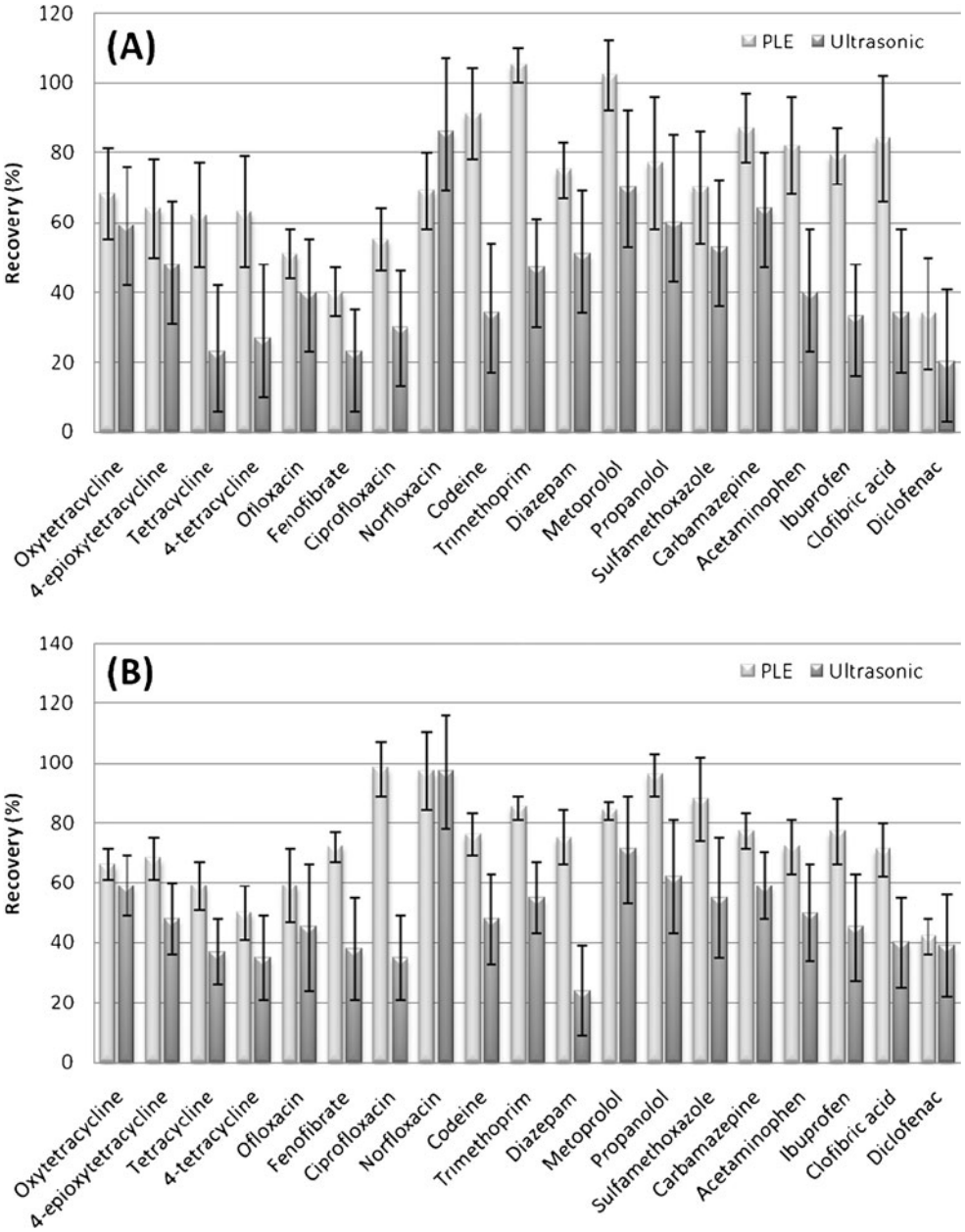


Fig. 2 Recoveries and RSDs obtained by ultrasonic and PLE methods at the MQLs for (**A**) soil samples and (**B**) sediment samples

chromatograms obtained from a soil sample (P10) extracted using PLE (Fig. 3a) or ultrasonic extraction (Fig. 3b). As can be observed, acetaminophen and carbamazepine were not detected using ultrasonic extraction because of the higher MDLs of this method.

Occurrence and distribution of pharmaceuticals in L'Albufera Natural Park

Occurrence of pharmaceuticals in the water samples is shown in Table 4, and analysis of sediments and soils extracted by PLE is shown in Tables 5 and 6. Concentrations of carbamazepine and ibuprofen in water, soil and sediment are compared in the Electronic Supplementary Material, Figures S3 and S4. Water samples taken at points P3, P6, P9, P10 and P14 suffered an unfortunate accident and could not be analysed. Among the 17 pharmaceuticals screened in surface waters from the L'Albufera Natural Park, 13 (acetaminophen, carbamazepine, ciprofloxacin, codeine, diazepam, diclofenac, metoprolol, ofloxacin, propanolol, sulfamethoxazole, ibuprofen, clofibric acid and trimethoprim) were detected (Table 4). Tetracycline, oxytetracycline and fenofibrate were not present in water samples but they were in soil or sediment samples, and norfloxacin was not detected in any of the samples (Tables 4, 5, 6).

The 15 water samples analysed were contaminated by pharmaceuticals. In these samples, carbamazepine was the

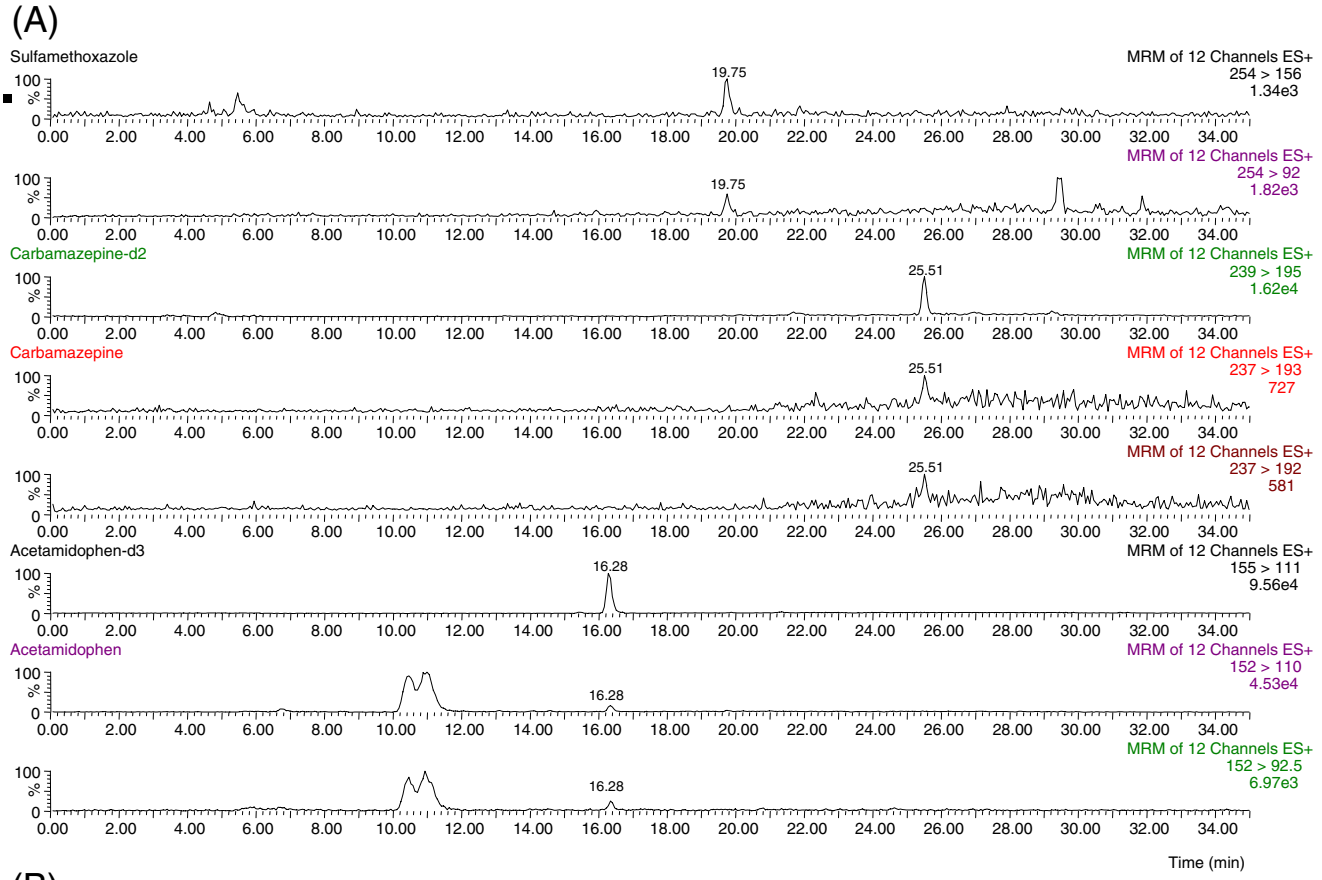
Table 3 MDLs, MQLs and % of matrix effect obtained using PLE and ultrasonic extraction methods for soil samples

	PLE				Ultrasonic			
	MDL ng g^{-1}	MQL ng g^{-1}	Matrix effect (%)	Linearity (R^2)	MDL ng g^{-1}	MQL ng g^{-1}	Matrix effect (%)	Linearity (R^2)
Oxytetracycline	5.3	18.1	18.8	0.9972	8.1	24.3	23.3	0.9963
4-Epioxytetracycline	5.2	15.6	17.2	0.9985	7.9	23.9	24.2	0.9962
Tetracycline	4.8	16.3	16.7	0.9992	15.9	48.2	30.4	0.9990
4-Epitetracycline	5.1	15.3	17.1	0.9990	13.2	39.0	32.9	0.9991
Ofloxacin	1.0	4.0	41.3	0.9994	4.3	13.1	57.2	0.9996
Fenofibrate	0.3	0.6	4.5	0.9990	2.0	6.0	12.1	0.9992
Ciprofloxacin	4.1	10.4	52.0	0.9987	10.5	31.5	62.5	0.9989
Norfloxacin	4.7	15.1	54.6	0.9991	5.6	17.4	69.3	0.9987
Codeine	0.3	1.3	9.5	0.9995	0.8	2.5	14.6	0.9991
Trimethoprim	0.2	0.9	11.3	0.9993	0.4	1.2	19.8	0.9990
Diazepam	0.1	0.3	21.0	0.9996	0.2	0.6	29.4	0.9996
Metoprolol	0.5	1.2	3.1	0.9954	1.2	3.8	12.9	0.9968
Propanolol	0.4	0.5	21.8	0.9969	0.8	1.6	32.4	0.9990
Sulfamethoxazole	0.3	0.9	16.3	0.9991	1.1	3.3	28.3	0.9993
Carbamazepine	0.1	0.5	14.8	0.9992	0.3	0.9	25.6	0.9994
Acetaminophen	0.1	0.8	−2.6	0.9944	0.1	0.5	−6.8	0.9942
Ibuprofen	1.8	4.3	22.9	0.9958	8.6	26.8	49.4	0.9964
Clofibric acid	0.3	1.6	33.7	0.9985	1.4	4.3	52.6	0.9989
Diclofenac	0.6	3.2	19.3	0.9990	3.0	9.2	30.6	0.9992

substance most frequently detected (14 samples, 93% of the samples) with concentrations ranging up to 31.0 ng L^{-1}. The mean concentration calculated by considering the non-detected values as zero and those of samples less than the MQL as the MQL was 9.6 ng L^{-1}. A high presence of this drug was reported by other researchers too, with mean concentrations between 1 and 794 ng L^{-1} [34–37]. Some studies confirmed that carbamazepine is not sorbed to sediments in an appreciable degree, thus it is not significantly biodegraded in wastewater treatment plants (WWTPs), and that it enters the environment in considerable amounts [38]. Acetaminophen and ibuprofen were detected with frequency lower than 66%, but at higher mean concentrations of 1,204.4 ng L^{-1} and 289.9 ng L^{-1}, respectively. For ibuprofen, a significant removal in WWTPs is reported in the literature [38], and as a result of its low distribution constant value, the removal should be based on biodegradation. Sulfamethoxazole was detected in 60% of the samples at lower mean concentration of 27.3 ng L^{-1}. This frequency of positive samples and mean concentration is similar to those reported by other authors [34], but differs from a few studies that only found some traces below the MLQ [35, 39]. Of the other pharmaceuticals, diclofenac was found in 6 samples (40%), codeine, ofloxacin and propanolol in 5 (33%), ciprofloxacin, diazepam and clofibric acid in 4 (27%), trimethoprim in 3 (20%) and metoprolol in 2 (13%) with lower mean concentrations (Table 4). Figure S2 in the Electronic Supplementary Material shows the LC-MS/MS chromatogram obtained for water sample P14 and illustrates the good performance of the analytical method for different pharmaceuticals.

These target compounds varied spatially, being detected at higher concentrations at P1, P2, P8, P11, P13 and P19. The highest concentrations for 5 out of the 12 detected compounds in surface water were found at site P8. This sample point is located near of the Albufera South WWTP (Fig. 1). However, this point is not connected to the irrigation channels that gather in the wastewater coming out of the WWTP. This point is near of an industrial area with different nightclubs, and the high level of pharmaceuticals could be due to the direct spillage of sewage water in the small irrigation channels. In contrast, samples from P7, which is close to the system that drives the wastewater to the lake to maintain the ecological flow, do not show high concentrations of pharmaceuticals.

The second group of points, with high concentrations and frequency of pharmaceuticals, were the sites P1, P19, P13 and P11 (Tables 4, 5 and 6). These sites are mainly located parallel to the Poyo Gully, just where the pipes that carry purified water from the Pinedo WWTP to the little port of Catarroja (Portet de Catarroja) flow. This WWTP provides a constant flow of 1 m^3/s of treated water that


(A)
Sulfamethoxazole
MRM of 12 Channels ES+
254 > 156
1.34e3
19.75
MRM of 12 Channels ES+
254 > 92
1.82e3
19.75
Carbamazepine-d2
MRM of 12 Channels ES+
239 > 195
1.62e4
25.51
Carbamazepine
MRM of 12 Channels ES+
237 > 193
727
25.51
MRM of 12 Channels ES+
237 > 192
581
25.51
Acetamidophen-d3
MRM of 12 Channels ES+
155 > 111
9.56e4
16.28
Acetamidophen
MRM of 12 Channels ES+
152 > 110
4.53e4
16.28
MRM of 12 Channels ES+
152 > 92.5
6.97e3
16.28
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00
Time (min)

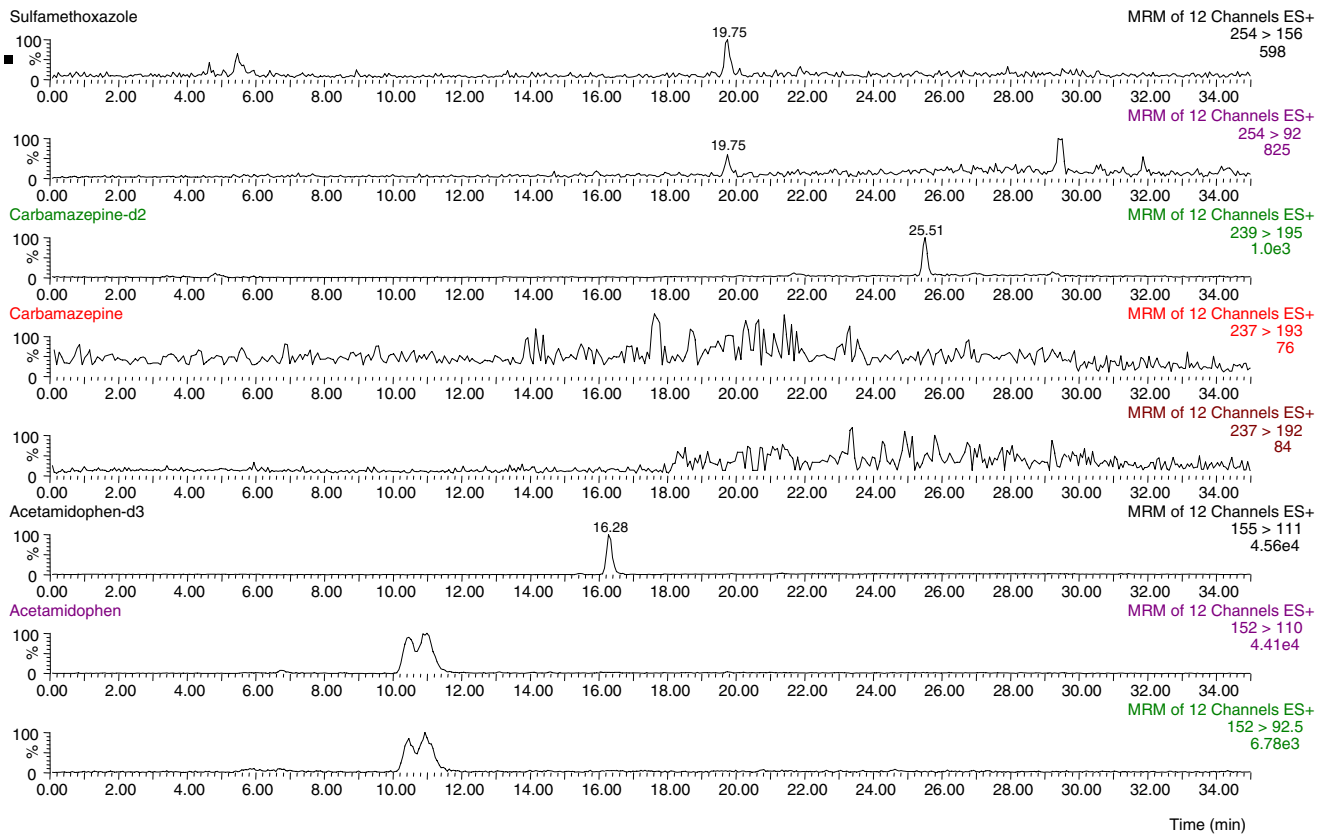

(B)
Sulfamethoxazole
MRM of 12 Channels ES+
254 > 156
598
19.75
MRM of 12 Channels ES+
254 > 92
825
19.75
Carbamazepine-d2
MRM of 12 Channels ES+
239 > 195
1.0e3
25.51
Carbamazepine
MRM of 12 Channels ES+
237 > 193
76
MRM of 12 Channels ES+
237 > 192
84
Acetamidophen-d3
MRM of 12 Channels ES+
155 > 111
4.56e4
16.28
Acetamidophen
MRM of 12 Channels ES+
152 > 110
4.41e4
MRM of 12 Channels ES+
152 > 92.5
6.78e3
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00
Time (min)

Fig. 3 LC-MS/MS chromatograms obtained by injecting extracts of soil sample P10 (**A**) using PLE method and (**B**) ultrasonic extraction. For concentrations see Table 6

arrives into L'Albufera lake through this point. L'Albufera Natural Park is the main recipient of water from the Pinedo WWTP. In particular, this WWTP injects 73 hm^3/year into the water system of L'Albufera Park irrigation network. This is the greatest single contribution to flows that the wetland receives, and it is fundamentally important for the natural ecosystem and irrigation of crops. The dissipation of pharmaceuticals through these points from the point P1, where the wastewater flows to the lake, was observed.

The other point that presents a remarkable concentration of pharmaceuticals was P2. This point is located near of Sueca WWTP. However, it is not clear why this point shows such a distribution pattern. Summarizing, higher concentrations of the detected pharmaceuticals were mainly found in the sites located near the WWTP outflows. This distribution is reasonable because the main sources of these pharmaceuticals are effluents of sewage treatment plants.

Table 5 outlines the concentration of pharmaceuticals in sediments. Sediments were not available at the sampling points P4, P10, P17, P18 and P20. Pharmaceuticals were not detected in the sediments taken from points P3, P5 and P7. Fourteen pharmaceuticals (acetaminophen, carbamazepine, ciprofloxacin, clofibric acid, codeine, diazepam, fenofibrate, ibuprofen, metoprolol, ofloxacin, oxytetracycline, propanolol, sulfamethoxazole, trimethoprim) were detected in the sediment samples collected from the L'Albufera Natural Park, whereas the remaining three compounds were not detected in any sample (Table 5). Carbamazepine was detected in 73% of the samples, followed by ofloxacin and codeine (53%), propanolol and acetaminophen (47%), and ibuprofen (40%) (Table 5). When considering mean concentrations in sediment (15 samples), ibuprofen was the dominating compound (6.73 ng g^{-1}), followed by ofloxacin (2.56 ng g^{-1}), codeine (2.36 ng g^{-1}) and oxytetracycline (1.88 ng g^{-1}). Oxytetracycline can bind to humic acids, proteins and organic matter as well as anionic groups in sand and soil. As a result of its high distribution constant in both sandy and loam soils, oxytetracycline is expected to show strong sorption [40].

Mean concentrations of the pharmaceuticals in sediment samples were about one thousand times lower than in water for all the target compounds, but concentration patterns remained the same, i.e. carbamazepine was the dominating

Table 4 Concentration of pharmaceuticals (ng L^{-1}) in waters samples from L'Albufera, Valencia, Spain

Pharmaceuticals	P1	P2	P4	P5	P7	P8	P11	P12	P13	P14	P16	P17	P18	P19	P20	Mean value[c]
Oxytetracycline[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0
Tetracycline[b]	n.d.	n.d.	n.d	n.d	n.d	n.d.	n.d	n.d	n.d	n.d	n.d	n.d	n.d.	n.d.	n.d	0.0
Ofloxacin	49.3	n.d.	n.d.	n.d.	n.d.	43.9	<MQL	n.d.	n.d.	n.d.	n.d.	n.d.	<MQL	34.3	n.d.	11.7
Fenofibrate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	0.0
Ciprofloxacin	14.1	n.d.	n.d.	n.d.	n.d.	30.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	20.0	6.1	4.7
Norfloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	0.0
Codeine	68.1	n.d.	n.d.	n.d.	n.d.	434.0	34.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	27.1	<MQL	37.8
Trimethoprim	53.6	n.d.	n.d.	n.d.	n.d.	32.5	40.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	8.4
Diazepam	5.6	n.d.	n.d.	n.d.	n.d.	6.3	6.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.6	n.d.	1.5
Metoprolol	5.8	n.d.	n.d.	n.d.	n.d.	n.d	5.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	0.8
Propanolol	6.8	n.d.	n.d.	n.d.	n.d.	8.4	5.9	n.d.	1.5	n.d.	n.d.	n.d.	n.d.	3.3	n.d.	1.7
Sulfamethoxazole	139.0	24.1	17.4	n.d.	n.d.	44.8	144.0	n.d.	n.d.	4.1	10.7	6.3	n.d.	18.9	n.d.	27.3
Carbamazepine	24.1	11.4	3.1	<MQL	<MQL	21.9	31.0	n.d	15.3	2.2	6.8	2.4	3.7	16.0	<MQL	9.6
Acetaminophen	n.d.	13.9	n.d.	<MQL	n.d	17,699.4	23.1	<MQL	n.d.	14.9	26.2	15.1	18.9	n.d	249.2	1,204.4
Ibuprofen	131.2	n.d.	n.d.	25.3	<MQL	3,913.7	84.3	n.d.	<MQL	n.d.	<MQL	101.4	<MQL	34.2	n.d.	289.9
Clofibric acid	n.d.	n.d.	<MQL	21.7	n.d	71.4	n.d	n.d.	n.d.	n.d.	n.d.	42.3	n.d.	n.d	n.d.	9.3
Diclofenac	125.6	n.d.	n.d.	42.6	n.d	260.9	57.6	n.d.	n.d	n.d.	n.d.	73.2	n.d.	25.3	n.d.	39.0

The most contaminated samples were P1, P8, P11 and P19

n.d. not detected

[a] Sum of oxytetracycline and 4-epioxytetracycline

[b] Sum of tetracycline and 4-epitetracycline

[c] Mean values were calculated by considering n.d. as zero and values less than MQL as the MQL

Table 5 Concentration of pharmaceuticals (ng g^{-1}) in sediment samples from L'Albufera, Valencia Spain

Pharmaceuticals	P1	P2	P6	P8	P9	P11	P12	P13	P14	P15	P16	P19	Mean values[c]
Oxytetracycline[a]	<MQL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.88
Tetracycline[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00
Ofloxacin	6.53	n.d.	4.12	n.d.	<MQL	3.98	n.d.	4.07	n.d.	7.05	4.25	4.36	3.91
Fenofibrate	0.81	<MQL	n.d.	n.d.	n.d.	n.d.	n.d.	<MQL	n.d.	n.d.	n.d.	n.d.	0.76
Ciprofloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.21	n.d.	n.d.	0.01
Norfloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00
Codeine	6.18	n.d.	n.d.	5.96	n.d.	1.59	n.d.	5.08	<MQL	<MQL	3.38	5.96	2.36
Trimethoprim	<MQL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.09	0.45
Diazepam	1.18	n.d.	n.d.	1.43	0.95	n.d.	n.d.	1.08	<MQL	1.33	1.27	n.d.	0.54
Metoprolol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.36	n.d.	<MQL	n.d.	2.04	0.41
Propanolol	1.23	0.68	n.d.	0.90	n.d.	n.d.	n.d.	0.84	n.d.	1.64	0.65	1.11	0.47
Sulfamethoxazole	n.d.	n.d.	n.d.	n.d.	n.d.	1.59	n.d.	2.73	n.d.	n.d.	n.d.	n.d.	0.29
Carbamazepine	<MQL	0.75	0.62	1.36	n.d.	0.94	<MQL	1.14	<MQL	2.07	2.12	1.29	0.87
Acetaminophen	3.98	<MQL	n.d.	<MQL	<MQL	<MQL	1.97	<MQL	n.d.	n.d.	n.d.	n.d.	0.66
Ibuprofen	35.83	n.d.	n.d.	4.42	n.d.	n.d.	n.d.	15.57	n.d.	23.57	4.93	16.56	6.73
Clofibric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.12	n.d.	n.d.	0.07
Diclofenac	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00

The most contaminated samples were P1, P8, P13 and P19

n.d. not detected

[a] Sum of oxytetracycline and 4-epioxytetracycline

[b] Sum of tetracycline and 4-epitetracycline

[c] Mean values were calculated by considering n.d. as zero and values less than MQL as the MQL. Samples P3, P4 and P7 were taken into account for the calculations

compound (Tables 4 and 5). However, diazepam and codeine occur mainly in sediments. Because biodegradation (or phototransformation) is incomplete in solid samples, important residues of fluoroquinolones can persist in agricultural soils several months after application [41]. Diclofenac, despite its high hydrophobicity, is not present in sediment and was only found in a water samples. This fact was stated by other researchers [4, 42] and occurs because diclofenac is rapidly metabolized by photodegradation and microflora of river sediments to its major metabolite 5-hydroxy-diclofenac [20, 42, 43]. Besides, in our case, this observation could also be explained because diclofenac is the compound worst extracted by the method.

In order to predict the distribution of a drug between a solid phase (sediment) and water, a number of different mechanisms involved in drug sorption have to be taken into account, the most importants are being sorption to organic matter, surface adsorption to mineral constituents, ion exchange, complex formation with metal ions such as Ca^{2+}, Mg^{2+}, Fe^{3+} or Al^{3+} and hydrogen bonding. Most of these mechanisms are hard to calculate under the variable conditions of each particular environment; usually, only the octanol/water partition coefficient (K_{ow}) is utilized to predict the behaviour of drugs in water [44]. In this way, a compound with a high value of K_{ow} tends to accumulate in soil or sediment. By contrast, those with a low K_{ow} will tend to remain in water. Fenofibrate, the compound with the highest K_{ow} (out of those studied), is only found in sediment and soils, and not in water. But this behaviour is not replicated in the case of other compounds with high K_{ow} like diclofenac (that was only found in water), ibuprofen and propanolol. This highlights the large quantity of chemical interactions that take place, and the difficulty in establishing those most important in the behaviour of contaminants in the environment.

Table 6 shows that soil samples from P7, P8, P17 and P20 were not contaminated by the studied pharmaceuticals. Highest mean concentrations of the pharmaceuticals in the soil were observed in sample P13. The mean concentrations of the pharmaceuticals in soil were between 0.06 for propanolol and 2.5 ng g^{-1} for tetracycline (Table 6). Of all compounds, acetaminophen showed the highest concentrations (16.05 ng g^{-1}), and carbamazepine the highest prevalence over soil locations, which reflects its high resistance to natural transformation processes such as adsorption and phototransformation. Diazepam was in six soils and in seven sediment samples. It is a lipophilic substance and showed a very low mobility in all types of soil. It can be expected that its leaching behaviour was

Table 6 Concentration of pharmaceuticals (ng g^{-1}) in soil samples from L'Albufera, Valencia, Spain

Pharmaceuticals	P1	P2	P3	P4	P5	P6	P9	P10	P11	P12	P13	P14	P15	P16	P18	P19	Mean values[c]
Oxytetracycline[a]	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.30
Tetracycline[b]	n.d.	n.d.	<MQL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	34.91	2.50
Ofloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00
Fenofibrate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.83	n.d.	n.d.	n.d.	0.81	n.d.	0.08
Ciprofloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00
Norfloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00
Codeine	<MQL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.07
Trimethoprim	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00
Diazepam	1.09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.73	0.90	n.d.	1.19	<MQL	0.95	0.34
Metoprolol	1.66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.75	n.d.	n.d.	n.d.	n.d.	n.d.	0.17
Propanolol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.78	n.d.	n.d.	n.d.	n.d.	0.48	0.06
Sulfamethoxazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.63	n.d.	n.d.	4.17	n.d.	n.d.	n.d.	n.d.	n.d.	0.29
Carbamazepine	n.d.	0.77	0.58	0.87	0.69	0.52	0.65	0.52	1.38	<MQL	3.81	0.64	1.91	1.99	0.80	1.14	0.84
Acetaminophen	n.d.	<MQL	n.d.	n.d.	n.d.	n.d.	0.42	<MQL	<MQL	n.d.	16.05	0.24	n.d.	n.d.	<MQL	n.d.	1.00
Ibuprofen	<MQL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.22
Clofibric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00
Diclofenac	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00

The most contaminated samples were P10, P13 and P19

n.d. not detected

[a] Sum of oxytetracycline and 4-epioxytetracycline

[b] Sum of tetracycline and 4-epitetracycline

[c] Mean values were calculated by considering n.d. as zero and values less than MQL as the MQL. Samples P7, P8, P17 and P20 were taken into account for the calculations

mainly determined by the organic carbon content of the soils. An extensive transformation of diazepam in the soil is unlikely, because diazepam was widely stable in a water/sediment test under aerobic conditions, and transformation products might have shown certain mobility in the soil due their increased polarity [45].

Environmental implications

The environmental risks to aquatic organisms were assessed by using the mean values and worst case scenario in L'Albufera Natural Park on the basis of the risk quotients (RQ) calculated using maximum measured environmental concentrations (MECs) and predicted non-effect concentrations (PNECs) collected from the literature [46–49] (Table 7). It should be taken into account that the choice of data can obviously affect the outcome. Ecotoxicity data can be provided by the open scientific literature or by pharmaceutical companies. The former source is preferred because it offers lower effect values (indicating a higher risk) than the latter in a majority of the risk assessments. Only for clofibric acid and ibuprofen were the PNECs based on company-owned data. For the other PNEC values, data originating from standard tests were preferred, with long-term studies being prioritized over short-term ones. Although data from algae and fish species were indistinctly used, data from the base-set species, i.e. algae, crustaceans (*Daphnia magna* or *Ceriodaphnia dubia*) and fish, were prioritized. According to the RQ classification scheme from Hernando et al. [50], mean concentrations of ciprofloxacin, propanolol and ibuprofen and a high concentration of diclofenac could pose a low risk to the aquatic organisms (RQ between 0.1 and 1) and high concentrations of ciprofloxacin, propanolol, sulfamethoxazole and ibuprofen could pose a medium risk to the aquatic organisms (RQ between 1 and 10). These results are a good example of the interest in monitoring pharmaceuticals in the environment.

Conclusions

Two fast and efficient extraction methods for the determination of 17 pharmaceuticals in soils and sediments by LC-MS/MS analysis were optimized and validated. An ultrasonic extraction method was compared with a previously developed PLE one. The performance characteristics for the majority of the pharmaceuticals studied were acceptable in both methods. The number of pharmaceuticals with lower MQLs, higher recovery and acceptable RSDs is slightly higher for the PLE method than for the ultrasonic one. However, the speed and user-friendliness of the ultrasonic extraction method in real practice are slightly better than for the PLE. Both methods proved to be successful as a quantitative, multi-residue method for pharmaceutical residues analysis in real soil and sediment samples.

The application of the method to real samples provided evidence that L'Albufera Natural Park was contaminated by significant amounts of pharmaceuticals. Higher levels and frequency of these compounds appear in the north area of the lagoon, which is consistent with the utilization of wastewater from the Pinedo WWTP. Tetracyclines and fenofibrate are mainly accumulated in soil and sediments, and diclofenac only appears in water samples. Concentrations of pharmaceuticals in water samples are higher than those in sediment and soil samples. In the water samples, all sampling points analysed contain some of the studied drugs, with values between 2.2 ng L^{-1} and 17.7 μg L^{-1}. In sediment samples, 12 of the 16 samples have some of the studied substances, with values ranging from 0.21 to 35.8 ng g^{-1}, and in soils between 0.24 and 16.05 ng g^{-1}. The results confirmed that the method is suitable for screening

Table 7 Predicted no effect concentrations (PNECs), measured environmental concentrations (MECs) and risk quotients (RQs) (maximum MEC/ PNEC) of the detected pharmaceuticals in the L'Albufera Natural Park water samples

Compound	$PNEC_{water}$ (μg L^{-1})	MEC_{mean} (μg L^{-1})	$MEC_{maximum}$ (μg L^{-1})	RQ (MEC_{mean}/ PNEC)	RQ ($MEC_{maximum}$/ PNEC)
Acetaminophen	9.2[a]	1.204	17.699	0.131	1.924
Carbamazepine	0.42[b]	0.010	0.031	0.023	0.073
Ciprofloxacin	0.005[a]	0.005	0.038	0.940	7.620
Metoprolol	31[a]	0.001	0.006	0.000	0.000
Propanolol	0.005[c]	0.002	0.008	0.340	1.680
Sulfamethoxazole	0.118[a]	0.003	0.144	0.023	1.220
Tetracycline	0.09[a]	0.008	0.054	0.001	0.003
Trimethoprim	16[c]	0.009	0.071	0.002	0.017
Clofibric acid	4.2[b]	0.039	0.261	0.000	0.002
Diclofenac	0.1[d]	0.290	3.913	2.900	39.000
Ibuprofen	7.1[c]	1.204	17.699	0.170	2.492

[a] PNEC data obtained from [47]
[b] PNEC data obtained from [48]
[c] PNEC data obtained from [49]
[d] PNEC data obtained form [51]

these compounds in waters, and highlight the necessity of eliminating these pollutants in the wastewater treatment plants before their discharge into the environment.

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