Development of a multi-residue method for the determination of human and veterinary pharmaceuticals and some of their metabolites in aqueous environmental matrices by SPE-UHPLC–MS/MS

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a b s t r a c t

The aim of the present work was to develop and validate a multi-residue method for the analysis of 33 human and veterinary pharmaceuticals (non-steroidal anti-inflammatory drugs (NSAIDs)/analgesics, antibiotics and psychiatric drugs), including some of their metabolites, in several aqueous environmental matrices: drinking water, surface water and wastewaters. The method is based on solid phase extraction (SPE) followed by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) and it was validated for different aqueous matrices, namely bottled water, tap water, seawater, river water and wastewaters, showing recoveries between 50% and 112% for the majority of the target analytes.

The developed analytical methodology allowed method detection limits in the low nanograms per liter level. Method intra- and inter-day precision was under 8% and 11%, respectively, expressed as relative standard deviation. The developed method was applied to the analysis of drinking water (bottled and tap water), surface waters (seawater and river water) and wastewaters (wastewater treatment plant (WWTP) influent and effluent). Due to the selectivity and sensitivity of the optimized method, it was possible to detect pharmaceuticals in all the aqueous environmental matrices considered, including in bottled water at concentrations up to 31 ng L^{-1} (salicylic acid). In general, non-steroidal anti-inflammatory drugs/analgesics was the therapeutic group most frequently detected, with the highest concentrations found in wastewaters (acetaminophen and the metabolite carboxyibuprofen at levels up to 615 and 120 μg L^{-1}, respectively).

1. Introduction

The consumption of pharmaceuticals may vary considerably from country to country [1], and no information for the total use of pharmaceuticals is available. They can be sold as prescription or over-the-counter medicines [2] and pharmaceuticals have an important role in the treatment and prevention of diseases in both human and veterinary medicine [3]. Additionally, they can be also used as growth promoters in animals [4].

Pharmaceuticals are continually released into the environment mainly as a result of their excretion in urine and/or feces, manufacturing processes, and disposal of unused or expired products [5]. Besides that, pharmaceuticals can also directly enter the terrestrial environment via different types of manure, slurries or other types of biosolids [4] and, after that, reach the aquatic environment due to soils run-off.

The pharmaceutical market has been growing over the last decades as well as the knowledge on the environmental impact of pharmaceuticals to ecosystems [6]. Once released into the environment, pharmaceuticals and their bioactive metabolites can be transported and distributed to water, soil or sediments, due to different factors, such as the physicochemical properties of the compounds and the characteristics of the receiving environment. Pharmaceuticals can accumulate in biota and induce adverse effects in aquatic or terrestrial organisms as well [5].

Wastewaters generated by hospital, industrial and domestic sources are pointed out as one of the most important source of pharmaceuticals to the aquatic environment [7]. Many pharmaceuticals, their metabolites and transformation products are incompletely removed in conventional wastewater treatment plants (WWTP)

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and thus discharged into the environment [8]. Therefore, the presence of pharmaceuticals at trace levels (nanograms to low micrograms per liter) has been reported in wastewaters, surface waters, groundwater's and, to a lesser extent, drinking waters [9–13]. In this way, sensitive and reliable analytical methods must be developed for the detection and quantification of pharmaceuticals in the aquatic environment.

Sample preparation is considered to be a fundamental step in environmental analytical procedures [14] and has to be selective, cheap, quick, and environmentally friendly [15]. The use of solid phase extraction (SPE) for the sample preparation has increased over the last years, because it is easy to operate, has increased selectivity with many new sorbents, and has the possibility to interface for automation and robotics [14]. Its versatility (purification, trace enrichment, desalting, derivatization, and class fractionation) allows SPE to be the first choice to be used in sample preparation of liquid samples.

Tandem mass spectrometry (MS) is considered to be a high resolution and sensitive detection technique due to its specificity and low limits of detection [16]. Each year, LC–MS/MS become even more sensitive and is an important detection method in environmental analysis [9]. Numerous studies have demonstrated its distinct advantages for trace analysis of pharmaceuticals in environmental samples [9,10,12].

The development of accurate multi-residue analytical methodologies for the simultaneous analyses of trace levels of human and veterinary pharmaceuticals in a wide range of aquatic environmental matrices is useful and necessary, in order to be possible to gather data on different ways of entrance of pharmaceuticals into the environment and, at the same time, evaluate the impact and distribution of either human and veterinary medicines in the aquatic environment. In the present study, an automated off-line SPE procedure followed by UHPLC coupled to triple quadrupole tandem MS with electrospray ionization source (ESI) was developed for the determination of 33 human and veterinary pharmaceuticals and some of their main metabolites, including non-steroidal anti-inflammatory drugs (NSAIDs)/analgesics, antibiotics, and psychiatric drugs. Different chromatographic and mass spectrometry parameters were optimized. A versatile SPE protocol was also developed in order to be applied to a great diversity of aquatic environmental matrices (e.g. wastewater, surface water, drinking water). Finally, the optimized method was successfully applied to sixteen samples embracing drinking water (bottled and tap water), surface water (seawater and river water) and wastewater (WWTP influent and effluent), allowing the evaluation of the distribution of the selected human and veterinary pharmaceuticals through the aquatic environment.

2. Materials and methods

2.1. Reagents, solvents and materials

All pharmaceuticals and isotopically labelled standards were of high purity grade (≥98%) and their physicochemical characteristics and suppliers are listed in Table SM1 of Supplementary Material.

Stock standard solutions (at a concentration of 1000 mg L^{-1}) were prepared on a weight basis in acetonitrile, with exception of naproxen and diclofenac that were prepared in acetonitrile:methanol (50:50, v/v), since these substances are very slightly soluble in pure acetonitrile and freely soluble in methanol [17]; and psychiatric drugs that were prepared in methanol. The antibiotics ofloxacin, ciprofloxacin, and enrofloxacin as well as the isotopically labelled standard ciprofloxacin-d8 were prepared in methanol adding NaOH 1 M as described by Ibáñez, M. et al. [18]. Carbamazepine-d10 (100 mg L^{-1}), venlafaxine-d6 (100 mg L^{-1}), diazepam-d5 (1000 mg L^{-1}), and norsertraline (100 mg L^{-1}) were purchased as free base in methanol.

All stock standard solutions were stored at −20 °C and renewed every 3 months, with the exception of antibiotics that were prepared monthly because of their limited stability. Working standard solutions containing all pharmaceuticals were prepared in acetonitrile:ultrapure water (30:70, v/v) by mixing appropriate amounts of each stock standard solution. These solutions were prepared before each analytical run.

A mixture with all isotopically labelled standards was prepared to be used for internal standard calibration.

Acetonitrile LC–MS grade was supplied by Biosolve (Valkenswaard, Netherland) and methanol LC–MS Ultra CHROMASOLV® was purchased from Sigma-Aldrich. Hydrochloric acid 37% was obtained from Carlo Erba (Rodano, Italy), and formic acid 98% PA-ACS and ethylenediaminetetraacetic acid disodium salt 2-hydrate (Na_2EDTA) (assay 99.9–101.0%) were obtained from Panreac (Barcelona, Spain). Ultrapure water (resistivity of 18.2 MΩ cm) was produced using a Simplicity 185 system (Millipore, Molsheim, France).

All chromatographic solvents were filtered through a 0.22 μm nylon membrane filter (Fioroni Filters, Ingré, France) using a vacuum pump (Dinko D-95, Barcelona, Spain) and degassed for 15 min in an ultrasonic bath (Sonorex Digital 10P, Bandelin DK 255P, Germany). Before the UHPLC–MS/MS analysis, sample extracts were filtered through 0.22 μm PTFE syringe filters (Specanalitica, Carcavelos, Portugal). SPE cartridges Strata-X (200 mg, 3 mL) from Phenomenex (California, USA) and Oasis MCX (150 mg, 6 mL) from Waters (Milford, Massachusets, USA) were used in the study of the SPE sorbent.

2.2. Sampling sites and sample collection

Sixteen samples were analyzed using the optimized analytical methodology. Two samples were collected for each type of water with the exception of seawater for which six samples were collected. Tap water samples were obtained from the tap of laboratory and in a private house located in the Porto area, whilst bottled waters were purchased in the local market. River samples were taken from Lis river, which crosses the city of Leiria in the center region of Portugal. Seawater samples from the Atlantic Ocean were collected in Porto coastal area from beaches with different bathing water quality (excellent, good and sufficient) [19]. Two beaches for each classification were selected. Wastewater samples (influent and effluent) were collected in two WWTPs located in the center region of Portugal (Leiria, Portugal). WWTP influent and effluent were 24 h composite samples, whereas the other types of waters were grab samples. Samples were collected in 2015.

Amber glass bottles pre-rinsed with ultrapure water were used for sample collection. After reception in the laboratory, wastewaters and river water were filtered through 0.45 μm nylon membrane filters (Fioroni Filters, Ingré, France) followed by 0.22 μm nylon membrane filters (Fioroni Filters, Ingré, France), while tap water and seawater were only filtered through 0.22 μm nylon membrane filters (Fioroni Filters, Ingré, France).

2.3. Analytical method

SPE cartridges were conditioned with 5 mL of methanol followed by 5 mL of ultrapure water and 5 mL of ultrapure water at pH 2 using a vacuum system manifold (Chromabond, Düren, Germany). A suitable volume of a 0.1 M Na_2EDTA solution was added to the water samples to achieve a final concentration of 0.1% (g solute/g solution). 250 mL of tap water, bottled water, seawater, and river water, 100 mL of WWTP effluent or 50 mL of WWTP influ-

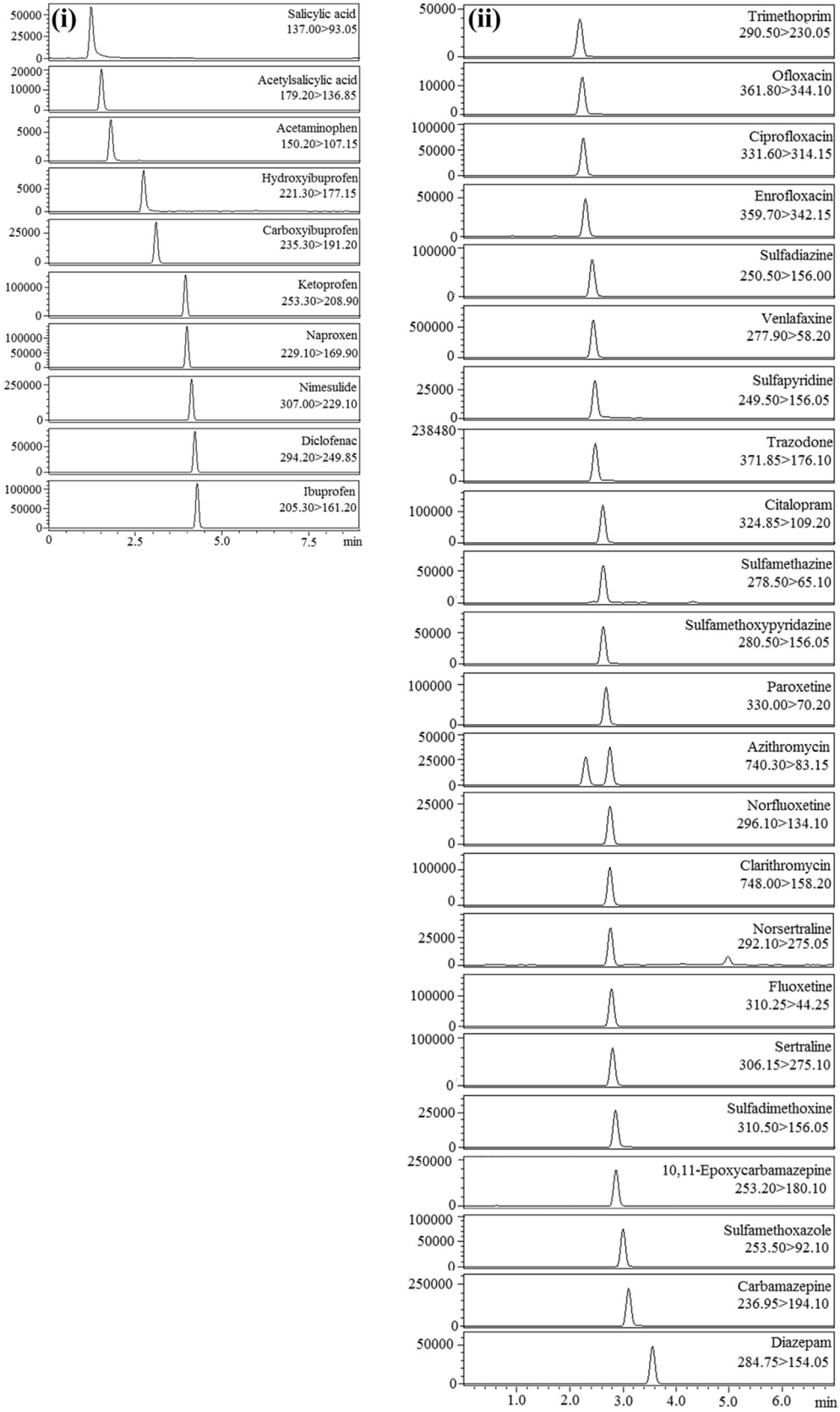

Fig. 1. MRM chromatogram of a standard mixture at 100 $\mu g\,L^{-1}$ of the selected pharmaceuticals analyzed in the negative (i) and positive (ii) ionization mode.

ent with pH adjusted to 2 with concentrated HCl were percolated through the cartridge. Then, the cartridges were rinsed with 5 mL of ultrapure water and dried under vacuum for 60 min to remove the excess of water. Finally, the elution was performed with 10 mL of methanol. Extracts were evaporated under a gentle stream of nitrogen and reconstituted with 500 μL of acetonitrile:ultrapure water (30:70, v/v). Lastly, 5 μL of a mixture of isotopically labelled standards was added in order to obtain a final concentration of 7.5 $\mu g\,L^{-1}$ for salicylic acid-d4, 150 $\mu g\,L^{-1}$ for acetaminophen-d4, 75 $\mu g\,L^{-1}$ for ibuprofen-d3, 10 $\mu g\,L^{-1}$ for carbamazepine-d10 and for fluoxetine-d5, 20 $\mu g\,L^{-1}$ for venlafaxine-d6, 40 $\mu g\,L^{-1}$ for diazepam-d5, 100 $\mu g\,L^{-1}$ for ciprofloxacin-d8 and azithromycin-d3, and 50 $\mu g\,L^{-1}$ for sulfamethoxazole-d4.

Quantification of pharmaceuticals was performed in a Nexera Ultra-High Performance Liquid Chromatography system (Shimadzu Corporation, Kyoto, Japan) equipped with two solvent delivery modules, a degasser, an autosampler, a column oven, and coupled to a triple-quadupole mass spectrometer detector LCMS-8030 with an electrospray ionization source (ESI). NSAIDs/analgesics were analysed in negative ionization mode, while antibiotics and psychiatric drugs were determined in the positive ionization mode. Different chromatographic conditions were used for each ionization mode. NSAIDs/analgesics were analysed using previously optimized conditions [20]. Briefly, chromatographic separation was achieved using a Kinetex C18 column (150 × 2.1 mm i.d., 1.7 μm particle size) from Phenomenex (California, USA), using ultrapure water as eluent A and acetonitrile as eluent B at a flow rate of 0.22 mL/min. The gradient elution was: 0–1.0 min, 30%–35.6% B; 1.0–2.0 min, 35.6%–100% B; 2.0–6.0 min, 100% B; 6.0–6.5 min, return to initial conditions; 6.5–10.5 min, re-equilibration of the column.

For positive ionization mode, the chromatographic conditions were optimized and chromatographic separation was carried out in a Cortecs™ UPLC® C18+ column (100 × 2.1 mm i.d.; 1.6 μm particle size) from Waters (Milford, Massachusets, USA). Eluent A was 0.1% formic acid in ultrapure water and eluent B was acetonitrile at a flow rate of 0.3 $mL\,min^{-1}$. The gradient elution started with 5% of eluent B, increasing to 100% B in 3 min, maintained 100% B during 0.5 min and then, returned to initial conditions within 0.5 min. The column was re-equilibrated for 3 min before the next injection. In both modes, the autosampler was operated at 4 °C, an injection volume of 5 μL was used and column temperature was kept at 30 °C. Lab Solutions LC–MS software (Shimadzu Corporation, Kyoto, Japan) was used for system control and data processing.

Source dependent parameters were optimized by the direct injection of a standard mixture solution 10 $mg\,L^{-1}$ for positive ionization mode, whereas for negative mode the MS/MS conditions are described in a previous work [20] and are the following: desolvation line temperature (DLT) was set at 250 °C and heat block temperature (HBT) at 300 °C; interface voltage (IV) at 5.0 kV; nebulizing gas (NGF) and drying gas (DGF) at a flow rate of 2.6 and 12.5 $L\,min^{-1}$, respectively. For positive ionization mode, the optimized conditions (see subsection 3.1.1) were as follows: DLT: 300 °C; HBT: 425 °C; IV: 5.0 kV; NGF: 2.6 $L\,min^{-1}$ and DGF:15.0 $L\,min^{-1}$. In both ionization modes, nitrogen was used as nebulizing and drying gas, and argon was used as collision induced dissociation gas at a pressure of 230 kPa. A dwell time of 25 ms and of 15 ms was used in negative and positive ionization, respectively.

Multiple reaction monitoring (MRM) is a method used in MS/MS in which an ion of a particular mass is selected in the first stage of a tandem mass spectrometer and an ion product of a fragmentation reaction of the precursor ion is selected in the second mass spectrometer stage, formed by collision-induced dissociation, for detection. The signal represents the precursor-to-product ion transition for a specific ion pair. The most intense product was set as quantifier ion whereas the second most intense was used as qualifier ion. Detailed data on the optimized mass spectrometry parameters (precursor ions, quantifier and qualifier ions, and ion ratio) as well as the corresponding internal standard used for quantification purposes is given in Table SM2 (Supplementary material).

2.4. Method validation

The performance of the method was evaluated through the estimation of the linearity, extraction recoveries, method detection limits (MDLs), method quantification limits (MQLs), precision as repeatability expressed as relative standard deviation (%RSD), reproducibility and matrix effects (ME) for each type of water sample.

Linearity was established by setting calibration curves (solvent and matrix matched) using linear regression analysis with concentrations in the range of 5–250 $\mu g\,L^{-1}$. Quantification of the analytes was performed by the internal standard approach. Recoveries were determined by comparing concentrations obtained after the SPE procedure, calculated by internal standard calibration, with the initial fortification levels. Since water samples can contain target compounds, blanks (non-spiked samples) were also analyzed and the levels found subtracted from those obtained for spiked samples. MDLs and MQLs were determined as the minimum amount of analyte detectable with a signal-to-noise ratio of 3 and 10, respectively. The limits were determined using real samples whenever possible, otherwise spiked samples were used. Method precision was determined by intra- and inter-day analysis (%RSD). Three standards mixtures containing all the analytes at a final concentration of 25, 50, and 100 $\mu g\,L^{-1}$ were used and six successive injections in one day and six consecutive days (triplicate injections) was performed, respectively. To assess the ME in all types of waters, the slope of the matrix matched calibration curve was compared with the slope of the calibration curve prepared in solvent (acetonitrile:ultrapure water (30:70, v/v)). A blank (sample without addition of standards) was simultaneously assayed in order to subtract the concentration of the target analytes present in the sample. ME was calculated according to Eq. (1) [9,21], respectively. A value of zero indicates that there is no ME, while for a positive value there is an ion enhancement signal and for a negative value an ion suppression signal.

$$\text{Signal supression}(\%) = \left(\frac{\text{slope}_{\text{matrix-matched}}}{\text{slope}_{\text{solvent}}} - 1\right) \times 100 \qquad (1)$$

3. Results and discussion

3.1. UHPLC–MS/MS

3.1.1. Ion source parameters

Source-dependent parameters such as DLT, HBT, IV, NGF, and DGF are key parameters to enrich the instrumental sensitivity [22] and, therefore they were studied. For NSAIDs/analgesics pharmaceuticals the source-dependent parameters were used as described in Paíga, P. et al. [20], while for pharmaceuticals analyzed in positive ionization mode the parameters were optimized. In the case of DLT, temperatures from 200 to 300 °C were tested and it was observed an increase of the signal from 220 to 225 °C, being constant therefore for psychiatric drugs. However, for antibiotics the signal increased with the increment of the temperature. Thus, a temperature of 300 °C was selected, since antibiotics showed lower sensitivity when compared with psychiatric drugs. For HBT, temperatures between 200 and 500 °C were studied and, in general, high sensitivity was observed for all the compounds using a temperature of 425 °C. The influence of IV in the analytical signal was evaluated between 0 and 5 kV and it was noticed an increase in the signal with an increment in IV. A maximum signal was achieved

for 5 kV, which was selected. NGF was studied between 0.5 and 3.0 L min^{-1} and it was observed an increase of the analytical signal from 0.5 to 2.6 L min^{-1} and after that a decrease. The highest signal intensity was obtained using 2.6 L min^{-1}. Lastly, DGF was varied between 10 and 20 L min^{-1} and a maximum response was obtained for a flow rate of 15 L min^{-1}.

3.1.2. Dwell time optimization

Using MRM for quantification Q1 and Q3 are set to filter specific ions as they pass through the ion optics. It is the dwell and the pause times together with the number of MRM transitions monitored that determine the cycle time and thus the number of data points obtained across a given peak [23]. While very short dwell times can be used for extended compound screening, higher dwell times are desirable for better Signal-to-Noise ratio. Thus, setting proper dwell time is necessary to achieve the best quality and quantity of data.

Different dwell times were studied in the interval of 1.0–100 ms. For antibiotics and psychiatric drugs, it was observed a constant analytical signal and a good reproducibility (RSD <5%) in the range of dwell time between 1.0 (RSD = 4.54%) and 15 ms (RSD = 3.09%). After that, an increase of the dwell time led to a decrease of the analytical signal with RSD values reaching 28%. Therefore, the best compromise between the analytical signal and the reproducibility was achieved using 15 ms for antibiotics and psychiatric drugs.

3.1.3. Chromatographic separation

Thirty-three human and veterinary pharmaceuticals (NSAIDs/analgesics, antibiotics, and psychiatric drugs) were studied in the present work. For NSAIDs/analgesics (negative ionization mode), chromatographic analysis was performed as described in Paíga, P. et al. [20]. For the remaining pharmaceuticals (positive ionization mode), the chromatographic conditions were optimized by evaluating different chromatographic columns, mobile phases, modes of elution, flow rates and column temperatures. The complexity of some compounds may cause problems during LC analysis, which often results in broad or tailing peaks, poor resolution or some shape issues due to an increased retention [24]. It was observed that for psychiatric drugs, 25% of acetonitrile was the initial maximum amount of organic solvent allowed for a good peak resolution, otherwise fronting and tailing peaks were obtained. In the other hand, for antibiotics at least 50% of organic solvent (acetonitrile) in the initial conditions was needed. It should be noted that even with that initial proportion of eluents, the tailing and fronting is not observed but large peaks were achieved.

The performance of two chromatographic columns was evaluated (Fig. SM1, Supplementary material). Using Kinetex C18 column, the best peak resolution was achieved with 10% of ACN in the initial conditions of the gradient elution. Peaks well defined were obtained for psychiatric drugs, however almost all antibiotics showed a bad peak resolution (Fig. SM1-A, Supplementary material). Replacing the stationary phase by the column Cortecs™ UPLC® C18+ (100 × 2.1 mm, 1.6 μm) and using the best chromatographic conditions achieved for Kinetex C18 column, in general, a much better peak resolution was observed for all pharmaceuticals. Therefore, the column Cortecs™ UPLC® C18+ was used for the chromatographic analysis in the positive ionization mode. Different mobile phases comprising several combinations of aqueous and organic eluents were tested in order to provide a better peak resolution and a good sensitivity as well. Both elution modes (isocratic and gradient) were tested. The best performance for all the pharmaceuticals was obtained using 0.1% formic acid in ultrapure water (A) and acetonitrile (B) in the mobile phase and a gradient elution as follows, initial conditions: 5% B; 0.0–3.0 min, 5–100% B; 3.0–3.5 min, 100% B; 3.5–4.0 min, return to initial conditions; 4.0-7.0 min, re-equilibration of the column. The flow rate was optimized to 0.3 mL min^{-1}. A representative chromatogram of a 100 μg L^{-1} standard mixture of the selected pharmaceuticals is present in Fig. 1.

3.2. Solid phase extraction optimization

3.2.1. Optimization of the SPE sorbent, sample pH adjustment and addition of Na_2EDTA

The performance of the polymeric sorbent Strata-X and of the mixed mode polymeric and cation exchange sorbent Oasis MCX on the extraction of the selected human and veterinary pharmaceuticals was evaluated. The effect of sample's pH in the recoveries of the selected pharmaceuticals was assessed for pH 2, 9, and without pH adjustment. Concentrated HCl or NaOH was used to adjust sample's pH. Different sample volumes (25, 50, 100, 250, and 500 mL) were also studied. Extraction efficiency of certain pharmaceuticals, like antibiotics, can be improved by adding Na_2EDTA to the samples, because soluble metals bound to the chelating agent, releasing the analyte and increasing the extraction efficiency [25]. Thus, the effect of adding Na_2EDTA, prior to extraction, in the recovery of the selected pharmaceuticals was also evaluated. Reconstitution solvent using different organic solvents (acetonitrile and methanol) and mixtures of solvents (methanol:ultrapure water (1:1, v/v), acetonitrile:ultrapure water (1:1, v/v), methanol:ultrapure water (3:7, v/v), acetonitrile:ultrapure water (3:7, v/v), methanol:ultrapure water with 0.1% formic acid (3:7, v/v), and acetonitrile:ultrapure water with 0.1% formic acid (3:7, v/v)) was also studied. All the optimizations were performed using 250 mL of ultrapure water and the extracts were reconstituted with 1 mL of acetonitrile. The obtained recoveries for the different tested conditions are present in the radar charts shown in Fig. 2.

Using Strata-X, recoveries increased for carboxyibuprofen, ibuprofen, salicylic acid, clarithromycin, azithromycin, norsertraline, and sertraline at sample pH 2, acetylsalicylic acid, salicylic acid, trimethoprim, citalopram, and venlafaxine without sample pH adjustment, and trimethoprim, sulfadimethoxine, norfluoxetine and venlafaxine at sample pH 9. A decrease of recoveries was obtained for sulfapyridine, norfluoxetine, and trazodone at sample pH 2, hydroxyibuprofen, sulfadimethoxine, azithromycin, and 10,11-epoxycarbamazepine without sample pH adjustment, and diclofenac, ibuprofen, naproxen, clarithromycin, azithromycin, norsertraline, sertraline, paroxetine, and trazodone at sample pH 9, when Na_2EDTA was added. Recoveries were constant for the remaining pharmaceuticals.

On the other hand, for Oasis MCX it was observed that, in all pH range, low recoveries were obtained for almost all NSAIDs/analgesics and antibiotics when Na_2EDTA was added to the sample, with the exception of acetaminophen that had the highest recovery (between 81.6 to 96.3%), whereas carboxyibuprofen was not detected (Fig. 2). In the case of psychiatric drugs, the behaviour observed was opposite and high recoveries were obtained with the addition of Na_2EDTA. Low recoveries were only obtained for norfluoxetine at sample pH 2 and 10,11-epoxycarbamazepine without sample pH adjustment. For ciprofloxacin, enrofloxacin, and ofloxacin, low recoveries (<10%) were achieved using both types of sorbents in all tested conditions. For trimethoprim, a lower recovery (in all pH range) was achieved (<30%) using Oasis MCX without Na_2EDTA addition than when the chelating agent was added. Using Strata-X, trimethoprim showed a good recovery in all experiments. For all the studied pH's the same behaviour was obtained for sulfonamide antibiotics and the high recoveries were achieved using Strata-X. After the addition of Na_2EDTA, sulfonamides' recoveries remained constant using Strata-X, but decreased when Oasis MCX was used. Carbamazepine, citalopram, and venlafaxine had constant recoveries in all studied conditions and no influence of

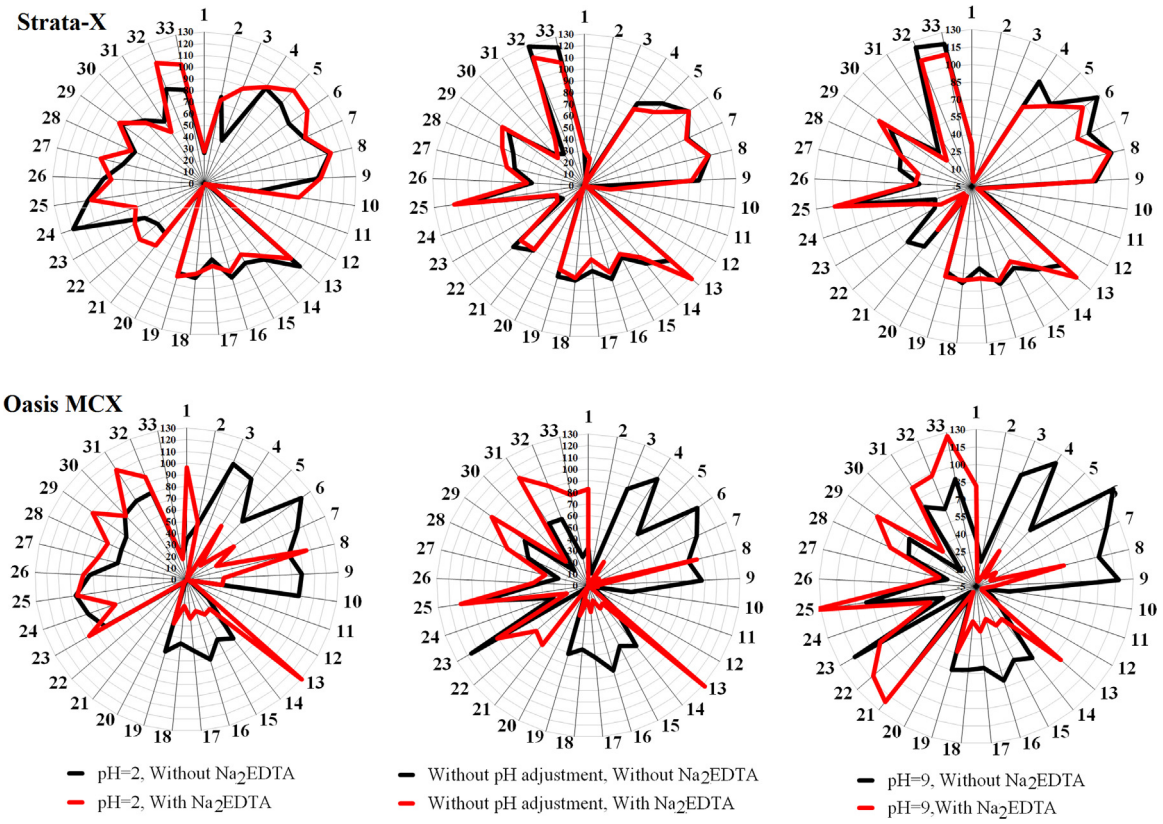

Fig. 2. Recoveries obtained in the study of solid phase extraction sorbent, sample pH adjustment (pH 2, without pH adjustment, and sample pH 9), and addition or not addition of Na_2EDTA (**NSAIDs/analgesics**: 1- Acetaminophen, 2- Acetylsalicylic acid, 3- Carboxyibuprofen, 4- Diclofenac, 5- Hydroxyibuprofen, 6- Ibuprofen, 7- Naproxen, 8- Nimesulide, 9- Ketoprofen, 10- Salicylic acid; **Antibiotics**: 11- Ciprofloxacin, 12- Enrofloxacin, 13- Trimethoprim, 14- Sulfamethoxypiridine, 15- Sulfapyridine, 16- Sulfamethazine, 17- Sulfadimethoxine, 18- Sulfadiazine, 19- Sulfamethoxazole, 20- Ofloxacin, 21- Clarithromycin, 22- Azithromycin; **Psychiatric drugs**: 23- Norsertraline, 24- Norfluoxetine, 25- Carbamazepine, 26- Fluoxetine, 27- Sertraline, 28- Citalopram, 29- Venlafaxine, 30- Paroxetine, 31- Trazodone, 32- Diazepam, 33- 10,11-Epoxycarbamazepine).

sorbent, sample's pH or addition of Na_2EDTA was verified in the recovery results. In the case of paroxetine, for both types of SPE sorbents at pH 2, no difference was observed by the addition of Na_2EDTA. For sertraline and trazodone recoveries were high using Oasis MCX with the addition of Na_2EDTA.

A marked decrease in the recovery of NSAIDs/analgesics and antibiotics using Oasis MCX was observed when Na_2EDTA was added (Fig. 2). Although several studies mentioned the importance of adding a cation complexing agent (Na_2EDTA) to chelate metals and to minimize interferences for some pharmaceuticals [9,26], our results are in agreement with literature [27,28], where a decrease of recoveries for some pharmaceuticals was also observed when Na_2EDTA was added. A possible justification for this is that when the Na_2EDTA is present in excess, it chelates not only metals but also organic compounds [27].

Comparing the performance of both sorbents could be concluded that Strata-X allowed higher recoveries for the majority of the analysed pharmaceuticals. This could be due to a low retention of target compounds in Oasis MCX, since this mixed sorbent was a lower amount of reversed-phase sorbent comparatively to Strata-X.

For almost all pharmaceuticals, the highest recoveries were obtained when sample pH was adjusted to 2. These results can be explained by the presence of acidic functional groups in the molecular structure of many pharmaceuticals, therefore lowering pH under their pKa values enhances the presence of neutral forms and their interaction with the reversed-phase sorbent.

Despite of the good recoveries found for some pharmaceuticals using Oasis MCX, for the majority of the selected compounds the best recoveries were achieved using Strata-X cartridges, with sample pH adjusted to 2 and adding Na_2EDTA to the sample. The recoveries of NSAIDs/analgesics ranged from 72.5% for acetylsalicylic acid to 111.5% for nimesulide with exception of acetaminophen (recovery of 27.8%). For most of pharmaceuticals, our recoveries were in accordance with results obtained by Gros, M. et al. [9] and Weigel, S. et al. [29] using polymeric sorbents. A similar recovery for acetaminophen was obtained by Weigel, S. et al. [29] and the low recovery obtained might be justified by its ready water solubility limiting the retention of acetaminophen. For antibiotics, recoveries were between 67.2% for clarithromycin and 97.8% for trimethoprim, with the exception of ciprofloxacin, enrofloxacin, and ofloxacin, with recoveries lower than 11%. Low recoveries (about 30%) for ciprofloxacin and ofloxacin were also obtained by S. Castiglioni et al. [30], which pointed out the step of evaporation to dryness as the reason for the low recoveries for these antibiotics. For psychiatric drugs, recoveries ranged from 52.7% to 111.5% for trazodone and diazepam, respectively.

3.2.2. Reconstitution solvent after SPE extraction

None of the studied conditions previously described in subsection 3.2.1., allowed obtaining good recoveries for the fluoroquinolone antibiotics ciprofloxacin, enrofloxacin, and ofloxacin. Fluoroquinolones have an amphoteric behaviour (pKa = 5 and pKa = 8–9) and their solubility depend of the pH [31]. Thus, different reconstitution solvents were tested, embracing 100% of organic solvents or mixtures of solvents. The obtained results are present in Fig. SM2 (Supplementary material). Using 100% of acetonitrile or 100% of methanol low recoveries were achieved, namely: 1.8, 17.1, and 13.5% for 100% of acetonitrile and 11.9, 53.2, and 50.1% for 100% methanol, for ciprofloxacin, enrofloxacin, and ofloxacin, respec-

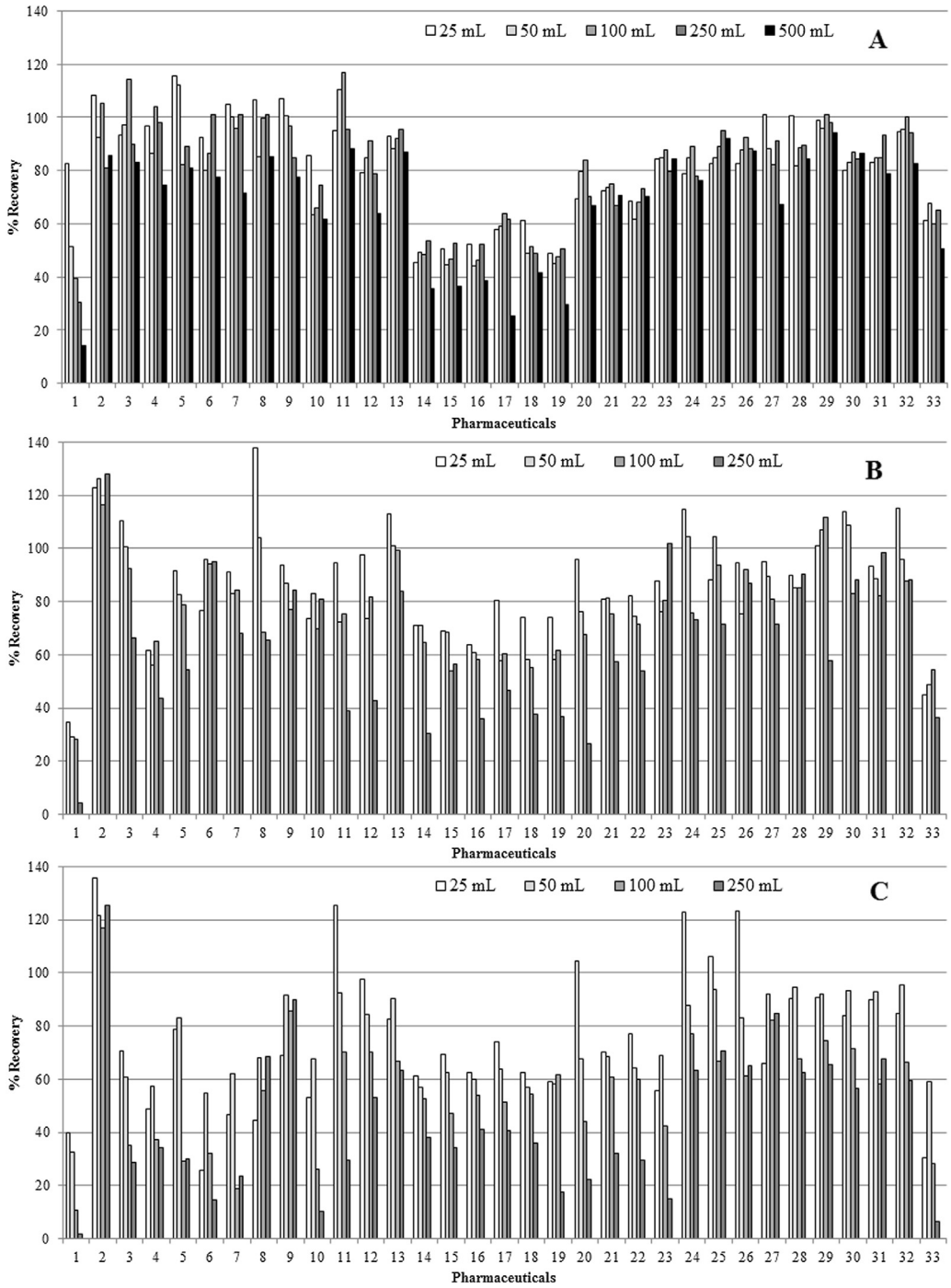

Fig. 3. Sample volume breakthrough in (A) river water; (B) WWTP effluent, and (C) WWTP influent (1- Acetaminophen, 2- Acetylsalicylic acid, 3- Carboxyibuprofen, 4- Diclofenac, 5- Hydroxyibuprofen, 6- Ibuprofen, 7- Naproxen, 8- Nimesulide, 9- Ketoprofen, 10- Salicylic acid,11- Ciprofloxacin, 12- Enrofloxacin, 13- Trimethoprim, 14- Sulfamethoxypiridine, 15- Sulfapyridine, 16- Sulfamethazine, 17- Sulfadimethoxine, 18- Sulfadiazine, 19- Sulfamethoxazole, 20- Ofloxacin, 21- Clarithromycin, 22- Azithromycin, 23- Norsertraline, 24- Norfluoxetine, 25- Carbamazepine, 26- Fluoxetine, 27- Sertraline, 28- Citalopram, 29- Venlafaxine, 30- Paroxetine, 31- Trazodone, 32- Diazepam, 33- 10,11-Epoxycarbamazepine).

tively. Therefore, mixtures of organic solvents with ultrapure water at different proportions (1:1 and 3:7 (v/v)) were tested. The mixture of ultrapure water and acetonitrile improved the recoveries of all fluoroquinolones (>80%) and recoveries remained constants for both tested proportions (1:1 and 3:7 (v/v)). When ultrapure water was mixed with methanol in the proportion (1:1, v/v) recoveries were only higher for ciprofloxacin, while for the proportion 3:7 (v/v), recoveries increased for all fluoroquinolones with 49.1, 58.2, and 65.4% for ciprofloxacin, enrofloxacin, and ofloxacin, respectively.

Since the chromatographic separation of antibiotics and psychiatric drugs embracing the use of 0.1% of formic acid in the aqueous eluent, it is important to check the recoveries with the acidification of the aqueous phase in the reconstitution solvent. Thus, mixtures of 0.1% of formic acid in ultrapure water with acetonitrile or methanol were also tested, using the proportion 3:7 (v/v), due to the high recoveries obtained with this proportion. No signifi-

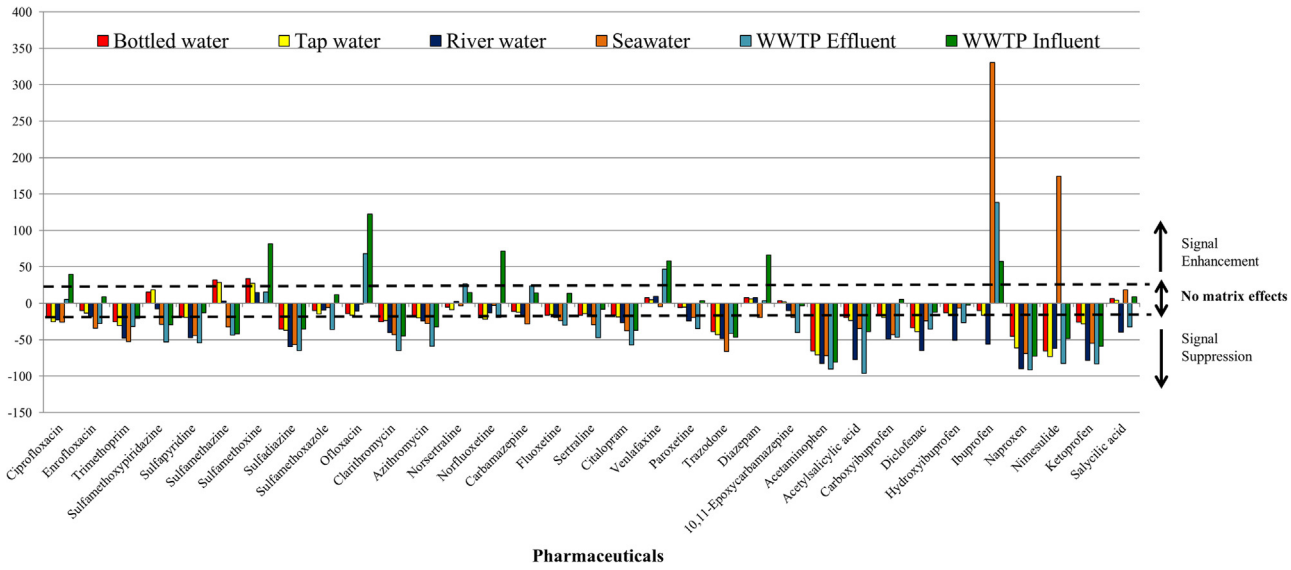

Fig. 4. Matrix effect for the selected pharmaceuticals in the different aqueous environmental matrices.

cant improvements were noticed with the acidification of ultrapure water and recoveries between 85 and 102% and from 52 to 61% were obtained using acetonitrile and methanol as organic solvent, respectively. Therefore, the mixture of acetonitrile and ultrapure water in the proportion of 3:7 (v/v) was selected as reconstitution solvent.

3.2.3. *Sample volume breakthrough*

The study of the breakthrough volume was performed using surface water (river water) and wastewaters (WWTP influent and effluent). Volumes between 25 and 500 mL (river water) and from 25 to 250 mL (wastewaters) were tested. Results of recoveries are shown in Fig. 3.

The mean recoveries obtained were: 82.9 (25 mL), 78.7 (50 mL), 81.3 (100 mL), 79.0 (250 mL), and 68.2% (500 mL) for river water; 91.0 (25 mL), 81.0 (50 mL), 76.6 (100 mL), and 69.0% (250 mL) for WWTP effluent; and 76.8 (25 mL), 75.1 (50 mL), 53.7 (100 mL), and 44.0% (250 mL) for WWTP influent, with RSD values lower than 10%.

The recovery of acetaminophen decreased with the increase of the sample volume, which is in agreement with previous works [20,32]. Acetaminophen showed the same behaviour in all types of water tested. Even with a recovery lower than 50%, acetaminophen is detected in environment due to its high consumption. Therefore 250, 100, and 50 mL were the sample volumes selected for surfaces waters, WWTP effluent and WWTP influent, and recoveries between 30.3% (acetaminophen) and 101% (ibuprofen, naproxen, and nimesulide) for river water, 28.4 (acetaminophen) to 116% (acetylsalicylic acid) for WWTP effluent, and 32.6 (acetaminophen) to 122% (acetylsalicylic acid) for WWTP influent were achieved.

3.3. *Method performance*

Linearity, MDLs, MQLs, repeatability, reproducibility, recoveries and ME were performed. The linearity of the method was established by setting calibration curves (solvent and matrix matched) using linear regression analysis with concentrations in the range of 5–250 $\mu g\,L^{-1}$. All pharmaceuticals gave good fits ($R > 0.994$). Calibration standards were measured at the beginning and at the end of each sequence. To check the signal stability, one calibration standard (100 $\mu g\,L^{-1}$) was injected repeatedly throughout the sequence. Solvent blanks consisting of acetonitrile were prepared to run after every five samples for monitoring the instrumental background.

Recoveries were evaluated at three levels of fortification for each type of water sample. The obtained results are summarized in tables SM3a and SM3b (Supplementary material). RSD values lower than 10% were achieved for all water samples. Generally, good recoveries were obtained for all the studied pharmaceuticals (>50%), except for acetaminophen in all type of water samples and for sulfapyridine, sulfamethazine, sulfamethoxypiridazine and sulfadimethoxine in seawater. Recoveries ranged from 50.5 (sulfamethoxazole) to 100% (ketoprofen, nimesulide, and paroxetine) for bottled water, 50.1 (sulfamethoxazole) to 98.0% (ciprofloxacin) for tap water, 56.6 (sulfadiazine) to 109% (naproxen) in seawater, 47.8 (sulfamethoxazole) to 102% (ciprofloxacin) for river water, 50.8 (10,11-epoxycarbamazepine) to 129% (acetylsalicylic acid) in WWTP effluent, and 51.6 (10,11-epoxycarbamazepine) to 122% (acetylsalicylic acid) in WWTP influent, respectively. The developed method showed good reproducibility, with RSD values ranging from 0.05 to 10% in all matrices (Table SM3, Supplementary material). Similar recoveries were achieved by M. Gros et al. [33] for surface water, WWTP effluent, and influent, ranging from 60 to 142%, from 62 to 121%, and from 50 to 151%, respectively, and by Cai et al. [11] for drinking water, showing recoveries between 61.4-124.3% for most of the selected pharmaceuticals. The obtained recoveries for seawaters are also in agreement with literature, where recoveries from 26.6 to 229% were reported [34].

MDLs and MQLs varied depending on the aquatic environmental matrix considered and higher values were achieved for WWTP effluent and influent (Table SM4, Supplementary material). In general, MDLs ranged from 0.02 $ng\,L^{-1}$ for salicylic acid, naproxen, and nimesulide, in bottled water, to 185 $ng\,L^{-1}$ for norsertraline in WWTP influent, and MQLs varied between 0.04 $ng\,L^{-1}$ for salicylic acid, in bottled water, and 562 $ng\,L^{-1}$ for norsertraline, in WWTP influent. It should be highlighted that the highest MDLs and MQLs were obtained to the metabolites carboxyibuprofen, hydroxyibuprofen, and norsertraline and the pharmaceuticals ibuprofen and ciprofloxacin (up to 185 and 562 $ng\,L^{-1}$, respectively).

Three different concentrations (25, 50, and 100 $\mu g\,L^{-1}$) were used and twelve successive injections in one day and triplicate injections in six consecutive days were performed for the intra- and inter-day precision. The overall method precision was satisfactory, with RSD values ranging from 0.6 (hydroxyibuprofen) to

Table 1a
Concentration of pharmaceuticals, in ng L^{-1}, detected in bottled water, tap water, and seawater.

Pharmaceuticals	Concentration (ng L^{-1}) ± RSD(%)									
	Bottled water		Tap water		Seawater[a]					
	BW1	BW2	TW1	TW2	SW1-B	SW2-B	SW3-G	SW4-G	SW5-Y	SW6-Y
NSAIDs/analgesics										
Salicylic acid	**30.6 ± 5.12**	**21.2 ± 4.23**	**66.0 ± 5.10**	**39.4 ± 1.91**	**169.1 ± 4.28**	**91.3 ± 2.37**	**73.7 ± 5.90**	**92.6 ± 5.15**	**73.4 ± 4.18**	**137.5 ± 0.89**
Acetylsalicylic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**5.12 ± 7.13**	n.d.	n.d.
Acetaminophen	n.d.	n.d.	n.d.	n.d.	**224.6 ± 0.37**	**98.6 ± 3.81**	**53.2 ± 6.37**	**156.3 ± 6.04**	**269.7 ± 0.61**	**59.4 ± 3.06**
Hydroxyibuprofen	n.d.	n.d.	n.d.	n.d.	**29.0 ± 2.87**	**30.8 ± 7.71**	**27.3 ± 1.79**	**30.3 ± 8.12**	**98.9 ± 5.62**	**27.6 ± 8.57**
Carboxyibuprofen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**270.4 ± 3.97**	n.d.
Ketoprofen	n.d.	**< MDL**	**< MDL**	**< MDL**	**11.3 ± 6.41**	**17.7 ± 6.98**	**11.2 ± 0.52**	**11.9 ± 5.94**	**12.9 ± 4.97**	**12.3 ± 5.61**
Naproxen	n.d.	n.d.	n.d.	n.d.	**17.5 ± 1.05**	n.d.	n.d.	**23.9 ± 2.87**	**177.7 ± 6.59**	**17.4 ± 0.16**
Nimesulide	n.d.	n.d.	n.d.	n.d.	**5.2 ± 9.12**	n.d.	n.d.	n.d.	n.d.	n.d.
Diclofenac	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**2.36 ± 2.87**	n.d.	**3.99 ± 4.76**	n.d.
Ibuprofen	**<MDL**	**<MDL**	**<MDL**	**<MDL**	**27.6 ± 0.47**	**23.8 ± 0.63**	**32.7 ± 3.39**	**33.6 ± 7.73**	**40.6 ± 4.10**	**9.4 ± 4.3**
Psychiatric drugs										
Venlafaxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trazodone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Citalopram	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Paroxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Norfluoxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Norsertraline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fluoxetine	**0.27 ± 5.66**	n.d.	n.d.	**1.90 ± 1.24**	n.d.	**0.27 ± 5.96**	**0.25 ± 6.24**	**0.74 ± 5.16**	**0.27 ± 1.81**	n.d.
Sertraline	n.d.	n.d.	n.d.	n.d.	n.d.	**< MDL**	n.d.	n.d.	n.d.	**8.09 ± 9.18**
10,11-Epoxycarbamazepine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carbamazepine	**22.1 ± 2.32**	n.d.	**22.3 ± 1.52**	**20.0 ± 0.580**	n.d.	n.d.	n.d.	n.d.	n.d.	**28.3 ± 3.12**
Diazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antibiotics										
Trimethoprim	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ofloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ciprofloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Enrofloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfadiazine	n.d.	n.d.	n.d.	n.d.	**<MDL**	**<MDL**	**<MDL**	**<MDL**	**<MDL**	**<MDL**
Sulfapyridine	n.d.	n.d.	n.d.	n.d.	**<MDL**	**<MDL**	**<MDL**	**<MDL**	**<MDL**	n.d.
Sulfamethazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfamethoxypiridazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	**<MDL**	**<MDL**	**<MDL**
Azithromycin	n.d.	n.d.	n.d.	n.d.	**<MDL**	**<MDL**	n.d.	**<MDL**	**<MDL**	**<MDL**
Clarithromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfamethoxazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**1.65 ± 17.4**	**<MDL**	n.d.	n.d.
Sulfadimethoxine	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	**<MDL**	**<MDL**	**<MDL**	n.d.

[a]Beaches classified as: B-"excellent" with a blue flag, G-"good" with a green flag, and Y-"sufficient" with yellow flag.

Table 1b
Concentration of pharmaceuticals, in ng L^{-1}, detected in river water, WWTP influent and effluent.

Pharmaceuticals	Concentration (ng L^{-1}) ± RSD(%)					
	River		WWTP Influent		WWTP Effluent	
	RW1	RW2	IW1	IW2	EW1	EW2
NSAIDs/analgesics						
Salicylic acid	**89.2 ± 9.13**	**128.2 ± 8.93**	**6332.3 ± 3.37**	**33535.5 ± 5.15**	**186.7 ± 4.25**	**126.9 ± 7.90**
Acetylsalicylic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acetaminophen	**<MDL**	**4.9 ± 12.57**	**30030.4 ± 1.36**	**615134.9 ± 6.94**	**736.0 ± 6.80**	**2139.0 ± 7.79**
Hydroxyibuprofen	**<MDL**	n.d.	**190.2 ± 0.44**	**198.4 ± 1.27**	**284.7 ± 0.97**	**358.7 ± 1.23**
Carboxyibuprofen	n.d.	n.d.	**41554.0 ± 5.03**	**120365.0 ± 4.68**	n.d.	n.d.
Ketoprofen	**<MDL**	**<MDL**	**<MDL**	**<MDL**	**22.3 ± 0.21**	**55.9 ± 0.35**
Naproxen	n.d.	**<MDL**	**2078.7 ± 5.17**	**533.3 ± 2.89**	**<MDL**	**110.7 ± 8.82**
Nimesulide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diclofenac	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ibuprofen	**<MDL**	**<MDL**	**4389.3 ± 7.44**	**14124.8 ± 8.52**	**517.4 ± 8.23**	**323.7 ± 9.68**
Psychiatric drugs						
Venlafaxine	n.d.	n.d.	**<MDL**	**15.4 ± 4.19**	**91.9 ± 6.45**	**170.9 ± 0.19**
Trazodone	n.d.	n.d.	**<MDL**	**17.7 ± 8.96**	**7.5 ± 7.94**	**21.7 ± 11.7**
Citalopram	n.d.	n.d.	n.d.	**15.1 ± 1.37**	**26.1 ± 7.06**	**61.4 ± 7.98**
Paroxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Norfluoxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Norsertraline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fluoxetine	**3.3 ± 0.85**	**3.7 ± 3.92**	**5.2 ± 1.52**	**8.8 ± 1.15**	**12.9 ± 6.78**	**27.5 ± 4.93**
Sertraline	**<MDL**	**<MDL**	n.d.	n.d.	**<MDL**	n.d.
10,11-Epoxycarbamazepine	n.d.	n.d.	n.d.	n.d.	n.d.	**88.0 ± 3.36**
Carbamazepine	**32.9 ± 3.27**	**34.4 ± 0.16**	**66.2 ± 4.54**	**110.9 ± 6.12**	**98.5 ± 6.88**	**244.9 ± 3.34**
Diazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antibiotics						
Trimethoprim	n.d.	n.d.	n.d.	n.d.	n.d.	**59.3 ± 13.0**
Ofloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ciprofloxacin	n.d.	n.d.	**118.9 ± 0.30**	n.d.	**96.6 ± 9.29**	n.d.
Enrofloxacin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfadiazine	n.d.	n.d.	n.d.	**<MDL**	n.d.	n.d.
Sulfapyridine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfamethazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfamethoxypiridazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Azithromycin	n.d.	n.d.	n.d.	**67.0 ± 4.59**	n.d.	**11.4 ± 12.3**
Clarithromycin	n.d.	**<MDL**	n.d.	n.d.	n.d.	**70.4 ± 5.75**
Sulfamethoxazole	n.d.	n.d.	n.d.	**224.1 ± 4.30**	n.d.	**73.4 ± 9.42**
Sulfadimethoxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

7.73% (norsertraline) for intra-day and 4.29 (salicylic acid) to 11.1% (norsertraline) for inter-day precision (Table SM5, Supplementary material).

Matrix effects (ME) is one significant drawback in ESI MS quantitative analysis, because the ESI source is highly susceptible to other components present in the matrix. The ME could be defined as the change in UHPLC–MS/MS response of an analyte, by suppression or enhancement of the signal, caused by coeluting matrix compounds, relative to an injection of a pure standard [35]. ME were evaluated for the different aquatic environmental matrices and results are shown in Fig. 4 and Fig. SM3 (Supplementary material). It was observed ME in all the studied matrices, which, in general, was expressed as an ion suppression for almost all the pharmaceuticals. Although compounds like ciprofloxacin, sulfamethazine, sulfamethoxine, ofloxacin, norsertraline, norfluoxetine, venlafaxine, diazepam, ibuprofen and nimesulide showed ion enhancement for different matrices, mainly wastewaters (WWTP influent and effluent). In the case of ibuprofen and nimesulide, the ion enhancement was more pronounced in seawater (Fig. 4). This could be justified by the high salt content of this matrix, suggesting that the salt residues might still be present in the sample extract, coeluting with the selected analytes [36–39]. On the other hand, ion suppression was usually more evident for matrices such as WWTP effluent and river water, being this effect more pronounced for NSAIDs/analgesics (Fig. 4). Nevertheless, antibiotics like clarithromycin, azithromycin and most of the sulfonamides as well as the antidepressants venlafaxine and citalopram and the metabolite norsertraline also had a marked ion suppression in WWTP effluent (Fig. 4).

Evaluating the general ME observed for the different aquatic environmental matrices, it was noticed that ME were less pronounced (<20%) in bottled water and tap water for the majority of the selected pharmaceuticals (67% and 58% of pharmaceuticals fit in this level, respectively), while for surface waters, WWTP influent and effluent, more than 50% of the pharmaceuticals had ME higher than 20%. In fact, due to the high complexity of wastewaters, approximately one-third of the compounds showed ME higher than 50% (Fig. SM3, Supplementary material).

3.4. Application to real samples

A total of sixteen samples embracing different types of water were analyzed using the developed analytical methodology. In Tables 1a and 1b, the concentration of the pharmaceuticals detected in each analyzed samples is in bold. It was possible to detect pharmaceuticals in all the considered aquatic environmental matrices, including drinking water. In fact, pharmaceuticals such as carbamazepine, fluoxetine, ibuprofen and ketoprofen, and the metabolite salicylic acid were found in bottled and tap waters, at concentrations up to 30.6 and 66.0 ng L^{-1}, respectively (Table 1a). Concentrations were higher in tap water with salicylic acid, carbamazepine, and fluoxetine reaching concentrations of 66.0, 22.3, and 1.97 ng L^{-1}, respectively. Similar concentrations of carbamazepine were detected in drinking water of China [11], while for salicylic

acid, carbamazepine and fluoxetine the levels found in France [40], Italy [41], and USA [42] were lower than those reported herein. Pharmaceuticals have already been detected in bottled water in Spain, though, contrarily to what happen in this study, always at concentrations below MQL [43].

Six seawater samples were collected in beaches with different bathing water quality (excellent, good and sufficient) [19] and it was possible to detect all the NSAIDs/analgesics, as well as six antibiotics, and three psychiatric drugs in at least one sample (Table 1a). In fact, the NSAIDs/analgesics acetaminophen, ibuprofen and ketoprofen and the metabolites salicylic acid and hydroxyibuprofen were detected in all the analyzed samples. NSAIDs/analgesics were found at concentrations ranging from 2.36 ng L^{-1} (diclofenac) to 270.4 ng L^{-1} (carboxyibuprofen), whereas the levels of psychiatric drugs varied between 0.25 ng L^{-1} and 28.3 ng L^{-1}, for fluoxetine and carbamazepine, respectively. Sulfonamides were the most detected antibiotics in seawater, but only sulfamethoxazole was quantified (1.65 ng L^{-1}). Similar findings were reported for antibiotics in the Mediterranean coast [44], while the NSAID ibuprofen was found at higher levels [45], and acetaminophen and salicylic acid at lower concentrations [9,46] than those reported herein. On the other hand, carbamazepine was detected at similar concentrations (4.0–26.3 ng L^{-1}) in seawater from Canada [47], while higher concentrations of fluoxetine were reported in Pacific Ocean [48].

The results obtained for river water and wastewaters (WWTP influent and effluent) are shown in Table 1b. In river water, the highest concentrations were reported for the metabolite salicylic acid (89.2–128.2 ng L^{-1}), followed by the antiepileptic carbamazepine (32.9–34.4 ng L^{-1}) and the analgesic acetaminophen (<MDL-4.90 ng L^{-1}). Clarithromycin was the only antibiotic detected in river water, but below MDL (Table 1b). Similar findings were reported for Spanish rivers [22] and in Jamaica Bay [49].

Regarding wastewaters, in WWTP influents, the highest concentrations were found for the NSAIDs/analgesics acetaminophen, naproxen, and ibuprofen, and for the metabolites carboxyibuprofen and salicylic acid, reaching levels up to 615 μg L^{-1} (acetaminophen) (Table 1b); while in WWTP effluents, pharmaceuticals like acetaminophen, ibuprofen and carbamazepine and the metabolites salicylic acid and hydroxyibuprofen showed the highest concentrations (up to 2139 ng L^{-1}). Similar levels of NSAIDs/analgesics were reported in WWTP effluents in Spain [35], while in Canada higher levels were found [50]. Identical concentrations of carbamazepine and fluoxetine were also found in Spanish [35] and Chinese wastewaters [51], respectively.

4. Conclusions

Pharmaceuticals are one of the major groups of emerging contaminants that are commonly found and targeted in environmental analysis. A proper sample preparation combined with LC–MS/MS analysis can ensure the sensitivity and accuracy required for the trace analysis of pharmaceuticals in the environment.

Gathering a SPE extraction protocol using a polymeric sorbent (Strata-X) with the UHPLC–MS/MS technology for chromatographic analysis allowed getting a fast, reliable, sensitive, robust and accurate multi-residue analytical method for the simultaneous analyses of trace levels of human and veterinary pharmaceuticals in a wide range of aquatic environmental matrices. The developed analytical method enabled the simultaneous analysis of a total of 33 pharmaceuticals and metabolites belonging to three therapeutic groups and having important differences in their chemical structures. Recoveries higher than 50% were obtained for the selected pharmaceuticals and metabolites for all the aqueous environmental matrices, with the exception of acetaminophen; and sulfapyridine, sulfamethazine, sulfamethoxypiridazine, and sulfadimethoxine in seawater. MDLs in the low ng L^{-1} range were achieved, allowing the application of the developed tool in the monitoring of trace levels of pharmaceuticals.

The optimized method was applied to six types of water embracing drinking water, surface water and wastewater, in a total of sixteen samples, proving to be an interesting tool to gather data on the different ways of entrance of pharmaceuticals into the environment as well as to evaluate the impact and distribution of either human and veterinary medicines in the aquatic environment, allowing to track the presence of pharmaceuticals and their metabolites among the water cycle. The obtained results showed a widespread occurrence of pharmaceuticals in the aquatic environment, being NSAIDs/analgesics the therapeutic group most frequently detected in all aqueous environmental matrices, followed by psychiatric drugs and antibiotics.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2016.12.013.

References

[1] H. Goossens, M. Ferech, R. Vander Stichele, M. Elseviers, Outpatient antibiotics use in Europe and association with resistance: a cross-national database study, Lancet 365 (2005) 579–587.
[2] J.R. Vane, R.M. Botting, Mechanism of action of nonsteroidal anti-inflammatory drugs, Am. J. Med. 104 (1998) 2–8.
[3] A.B.A. Boxall, The environmental side effects of medication. How are human and veterinary medicines in soils and water bodies affecting human and environmental helath? EMBO reports, Eur. Mol. Biol. Organ. 5 (12) (2004), http://dx.doi.org/10.1038/sj.embor.7400307 (Available in: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1299201/pdf/5-7400307.pdf, Retrieved on 21/07/2016.).
[4] B. Halling-Sørensen, S. Nors Nielsen, P.F. Lanzky, F. Ingerslev, H.C. HoltenLützhøft, S.E. Jørgensen, Occurrence, fate and effects of pharmaceutical substances in the environmentd a review, Chemosphere 36 (1998) 357–393.
[5] M.S. Diaz-Cruz, M.J.L. de Alda, D. Barcelo, Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge, TrAC-Trends Anal. Chem. 22 (2003) 340–351.
[6] A. Di Guardo, D. Calamari, E. Benfenati, B. Halling-Sørensen, E. Zuccato, R. Fanelli, Pharmaceuticals as Environmental Contaminants: Modeling Distribution and Fate, 3th ed., Springer, Germany, 2001.
[7] S.T. Glassmeyer, E.T. Furlong, D.W. Kolpin, J.D. Cahill, S.D. Zaugg, S.L. Werner, M.T. Meyer, D.D. Kryak, Transport of chemical and microbial compounds from known wastewater discharges: potential for use as indicators of human fecal contamination, Environ. Sci. Technol. 39 (2005) 5157–5169.
[8] W.C. Li, Occurrence sources, and fate of pharmaceuticals in aquatic environment and soil, Environ. Pollut. 187 (2014) 193–201.
[9] M. Gros, S. Rodríguez-Mozaz, D. Barceló, Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry, J. Chromatogr. A 1248 (2012) 104–121.
[10] A. Lolić, P. Paíga, L.H.M.L.M. Santos, S. Ramos, M. Correia, C. Delerue-Matos, Assessment of non-steroidal anti-inflammatory and analgesic pharmaceuticals in seawaters of North of Portugal: occurrence and environmental risk, Sci. Total Environ. 508 (2015) 240–250.
[11] M.-Q. Cai, R. Wang, L. Feng, L.-Q. Zhang, Determination of selected pharmaceuticals in tap water and drinking water treatment plant by high-performance liquid chromatography-triple quadrupole mass spectrometer in Beijing, China, Environ. Sci. Pollut. Res. 22 (2015) 1854–1867.

[12] L.H.M.L.M. Santos, M. Gros, S. Rodriguez-Mozaz, C. Delerue-Matos, A. Pena, D. Barceló, M.C.B.S.M. Montenegro, Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: identification of ecologically relevant pharmaceuticals, Sci. Total Environ. 461–462 (2013) 302–316.
[13] A.M.P.T. Pereira, L.J.G. Silva, C.M. Lino, L.M. Meisel, A. Pena, Assessing environmental risk of pharmaceuticals in Portugal: an approach for the selection of the Portuguese monitoring stations in line with directive 2013/39/EU, Chemosphere 144 (2016) 2507–2515.
[14] N. Fontanals, R.M. Marcé, F. Borrull, New materials in sorptive extraction techniques for polar compounds, J. Chromatogr. A 1152 (2007) 14–31.
[15] A. Białk-Bielińska, J. Kumirska, M. Borecka, M. Caban, M. Paszkiewicz, K. Pazdro, P. Stepnowski, Selected analytical challenges in the determination of pharmaceuticals in drinking/marine waters and soil/sediment samples, J. Pharm. Biomed. Anal. 121 (2016) 271–296.
[16] R.E. Majors, D. Wilmington, Sample preparation fundamentals for chromatography. Agilent Tecnhologies.
[17] British Pharmacopeia on-line. http://bp2012.infostar.com.cn/ Retrieved on 27/01/2015.
[18] M. Ibáñez, C. Guerrero, J. Sancho, F. Hernández, Screening of antibiotics in surface and wastewater samples by ultra-high-pressure liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry, J. Chromatogr. A 20 (2009) 2529–2539.
[19] European Comission, in: E. Union (Ed.), Off. J. Europ. Union, 2006, pp. 37–51.
[20] P. Paíga, A. Lolić, F. Hellebuyck, L. Santos, M. Correia, C. Delerue-Matos, Development of a SPE-UHPLC-MS/MS methodology for the determination of non-steroidal anti-inflammatory and analgesic pharmaceuticals in seawater, J. Pharm. Biomed. Anal. 106 (2015) 61–70.
[21] B. Huerta, S. Rodriguez-Mozaz, C. Nannou, L. Nakis, A. Ruhí, V. Acuña, S. Sabater, D. Barcelo, Determination of a broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water treatment plant-impacted river, Sci. Total Environ. 540 (2016) 241–249.
[22] A. Kruve, K. Herodes, I. Leito, Optimization of electrospray interface and quadrupole ion trap mass spectrometer parameters in pesticides liquid chromatography/electrospray ionization mass spectrometry analysis, Rapid Commun. Mass Spectrom. 24 (2010) 919–926.
[23] Applied Biosystems Considerations when using LC/MS/MS Systems with Fast and High Resolution Liquid Chromatography, http://www3.appliedbiosystems.com/cms/groups/psm_marketing/documents/generaldocuments/cms_050558.pdf, Application note Fast and High Resolution HPLC, retrieved on 27/01/2016.
[24] B. Kinsella, J. O'Mahony, E. Malone, M. Moloney, H. Cantwell, A. Furey, M. Danaher, Current trends in sample preparation for growth promoter and veterinary drug residue analysis, J. Chromatogr. A 1216 (2009) 7977–8015.
[25] F. Hernández, J.V. Sancho, M. Ibánez, C. Guerrero, Antibiotics residue deterination in environmental waters by LC–MS, TrAC-Trends Anal. Chem. 26 (2007) 466–485.
[26] S. Yang, K.H. Carlson, Solid-phase extraction-high-performance liquid chromatography–ion trap mass spectrometry for analysis of trace concentrations of macrolide antibiotics in natural and waste water matrices, J. Chromatogr. A 1038 (2004) 141–155.
[27] S.-C. Kim, K. Carlson, Quantification of human and veterinary antibiotics in water and sediment using SPE/LC/MS/MS, Anal. Bioanal. Chem. 387 (2007) 1301–1315.
[28] R.M. Baena-Nogueras, M.G. Pintado-Herrera, E. González-Mazo, P.A. Lara-Martín, Determination of pharmaceuticals in coastal systems using solid phase extraction (SPE) followed by ultra performance liquid chromatography −tandem mass spectrometry (UPLC–MS/MS) curr, Anal. Chem. 11 (2015) 1–19.
[29] S. Weigel, R. Kallenborn, H. Hühnerfuss, Simultaneous solid-phase extraction of acidic, neutral and basic pharmaceuticals from aqueous samples at ambient (neutral) pH and their determination by gas chromatography–mass spectrometry, J. Chromatogr. A 1023 (2004) 183–195.
[30] S. Castiglioni, R. Bagnati, D. Calamari, R. Fanelli, E. Zuccato, A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewater, J. Chromatogr. A 1092 (2005) 206–215.
[31] V.T. Andriole, The Quinolones, third edition, Academic Press, San Diego, California, 2000.
[32] L.H.M.L.M. Santos, P. Paíga, A.N. Araújo, A. Pena, C. Delerue-Matos, M.C.B.S.M. Montenegro, Development of a simple analytical method for the simultaneous determination of paracetamol, paracetamol-glucuronide and p-aminophenol in river water, J. Chromatogr. B 930 (2013) 75–81.
[33] M. Gros, M. Petrovic, D. Barceló, Tracing pharmaceutical residues of different therapeutic classes in environmental waters by using liquid chromatography/quadrupole-linear ion trap mass spectrometry and automated library searching, Anal. Chem. 81 (2009) 898–912.
[34] R. Moreno-González, S. Rodriguez-Mozaz, M. Gros, D. Barceló, V.M. León, Seasonal distribution of pharmaceuticals in marine water and sediment from a mediterranean coastal lagoon (SE Spain), Environ. Res. 138 (2015) 326–344.
[35] M. Gros, M. Petrovic, D. Barcelo, Development of a multi-residue analytical methodology based on liquid chromatography-tandem mass spectrometry (LC–MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters, Talanta 70 (2006) 678–690.
[36] M. Borecka, A. Białk-Bielinska, G. Siedlewicz, K. Kornowska, J. Kumirska, P. Stepnowski, K. Pazdro, A new approach for the estimation of expanded uncertainty of resultsof an analytical method developed for determining antibiotics inseawater using solid-phase extraction disks and liquidchromatography coupled with tandem mass spectrometry technique, J. Chromatogr. A 1304 (2013) 138–146.
[37] D. Patel, Matrix effect in a view of LC–MS/MS: An overview, Int. J. Pharm. Biol. Sci. 2 (2011) 559–564.
[38] G. McEneff, L. Barron, B. Kelleher, B. Paull, B. Quinn, A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent receiving marine waters and marine bivalves, Sci. Total Environ. 476–477 (2014) 317–326.
[39] K. Wille, H. Noppe, K. Verheyden, J.V. Bussche, E. De Wulf, P.V. Caeter, C.R. Janssen, H.F. De Brabander, L. Vanhaecke, Validation and application of an LC–MS/MS method for the simultaneous quantification of 13 pharmaceuticals in seawater, Anal. Bioanal. Chem. 397 (2010) 1797–1808.
[40] E. Vulliet, C. Cren-Olivé, M.-F. Grenier-Loustalot, Occurrence of pharmaceuticals and hormones in drinking water treated from surface waters, Environ. Chem. Lett. 9 (2011) 103–114.
[41] R. Loos, J. Wollgast, T. Huber, G. Hanke, Polar herbicides pharmaceutical products, perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and nonylphenol and its carboxylates and ethoxylates in surface and tap waters around Lake Maggiore in Northern Italy, Anal. Bioanal. Chem. 387 (2007) 1469–1478.
[42] M.J. Benotti, R.A. Trenholm, B.J. Vanderford, J.C. Holady, B.D. Stanford, S.A. Snyder, Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water, Environ. Sci. Technol. 43 (2009) 597–603.
[43] S.G. Alonso, Y. Valcárcel, J.C. Montero, M. Catalá, Nicotine occurrence in bottled mineral water: analysis of 10 brands of water in Spain, Sci. Total Environ. 416 (2012) 527–531.
[44] P. Vazquez-Roig, V. Andreu, C. Blasco, Y. Picó, Risk assessment on the presence of pharmaceuticals in sediments, soils and waters of the Pego–Oliva Marshlands (Valencia, eastern Spain), Sci. Total Environ. 440 (2012) 24–32.
[45] A. Togola, H. Budzinski, Multi-residue analysis of pharmaceutical compounds in aqueous samples, J. Chromatogr. A 1177 (2008) 150–158.
[46] N.A. Alygizakis, P. Gago-Ferrero, V.L. Borova, A. Pavlidou, I. Hatzianestis, N.S. Thomaidis, Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater, Sci. Total Environ. 541 (2016) 1097–1105.
[47] J. Magner, M. Filipovic, T. Alsberg, Application of a novel solid-phase-extraction sampler and ultra-performance liquid chromatography quadrupole-time-of-flight mass spectrometry for determination of pharmaceutical residues in surface sea water, Chemosphere 80 (2010) 1255–1260.
[48] K. Nödler, D. Voutsa, T. Licha, Polar organic micropollutants in the coastal environment of different marine systems, Mar. Pollut. Bull. 85 (2014) 50–59.
[49] M.J. Benotti, B.J. Brownawell, Distributions of pharmaceuticals in an urban estuary during both dry- and wet-weather conditions, Environ. Sci. Technol. 41 (2007) 5795–5802.
[50] G.L. Brun, M. Bernier, R. Losier, K. Doe, P. Jackman, H.B. Lee, Pharmaceutically active compounds in Atlantic Canadian sewage treatment plant effluents and receiving waters, and potential for environmental effects as measured by acute and chronic aquatic toxicity, Environ. Toxicol. Chem. 25 (2006) 2163–2176.
[51] S.L. Yuan, X.M. Jiang, X.H. Xia, H.X. Zhang, S.K. Zheng, Detection, occurrence and fate of 22 psychiatric pharmaceuticals in psychiatric hospital and municipal wastewater treatment plants in Beijing China, Chemosphere 90 (2013) 2520–2525.