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RESEARCH PAPER

Combining cationic and anionic mixed-mode sorbents in a single cartridge to extract basic and acidic pharmaceuticals simultaneously from environmental waters

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Abstract The aim of the present study is to broaden the applications of mixed-mode ion-exchange solid-phase extraction sorbents to extract both basic and acidic compounds simultaneously by combining the sorbents in a single cartridge and developing a simplified extraction procedure. Four different cartridges containing negative and positive charges in the same configuration were evaluated and compared to extract a group of basic, neutral, and acidic pharmaceuticals selected as model compounds. After a thoroughly optimization of the extraction conditions, the four different cartridges showed to be capable of retaining basic and acidic pharmaceuticals simultaneously through ionic interactions, allowing the introduction of a washing step with 15 mL methanol to eliminate interferences retained by hydrophobic interactions. Using the best combined cartridge, a method was developed, validated, and further applied to environmental waters to demonstrate that the method is promising for the extraction of basic and acidic compounds from very complex samples.

Keywords Cationic mixed-mode solid-phase extraction · Anionic mixed-mode solid-phase extraction · Sorbent combination · High-resolution mass spectrometry · Environmental waters

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Introduction

Solid-phase extraction (SPE) remains the most commonly used sample preparation technique for liquid samples in several analytical methods. Its wide acceptance is the result of the great advantages that it provides, such as the enrichment of the analytes with high recoveries and the enhanced selectivity thanks to the availability of different types of sorbents [1]. SPE is often combined with chromatographic techniques such as liquid chromatography (LC) and gas chromatography (GC) coupled with mass spectrometry (MS) detection, obtaining methods with high sensitivity and selectivity to determine several target compounds in complex matrices at trace levels [2–5].

Among the different sorbents available for SPE, mixed-mode sorbents combine a polymeric structure with one type of ionic functional groups (cationic or anionic) in each cartridge, giving them the capability of retaining compounds through reversed-phase and ion-exchange interactions [6]. They were developed to promote selectivity for ionic compounds, broadening the groups of analytes that can be retained by a single sorbent [7–9]. The most important feature of mixed-mode sorbents is the possibility of including a washing step with organic solvents in the SPE procedure, which allows the elimination of interferences retained by hydrophobic interactions. In several studies, the potential of these sorbents has been proven to reduce the matrix effect (ME) in LC-MS-based methods, by eliminating interferences during washing steps in the SPE procedure [10–13].

There are four types of mixed-mode sorbents depending on the functional groups attached to the polymer particles: strong or weak cationic-exchangers (SCX or WCX) and strong or weak anionic-exchangers (SAX or WAX). The sorbents with strong properties include functional groups that are charged in the entire pH range (such as sulfonic acid or quaternary

ammonium groups), while those with weak properties have groups with a more reduced working pH range depending on their pK_a values (carboxylic acid or tertiary or secondary amine groups). When working with any of these sorbents, the selection of the SPE conditions is very important, as parameters such as pH or type of clean-up and elution solvent have a great impact on the performance of the sorbents [6]. By fine-tuning the SPE protocol, these sorbents can extract an extensive range of compounds with different properties (non-polar, polar, or ionic) or they can be selective towards ionic compounds if appropriate washing steps are applied.

Successful applications of mixed-mode sorbents commercially available or prepared in-house with strong or weak properties have been reported, describing the extraction of different groups of compounds from complex matrices [14–19]. Analytes such as pharmaceuticals, drugs of abuse, and compounds of biological interest have been extracted from environmental waters, foodstuff, and biologic fluids [20–23]. In most of the studies published to date, the potential of mixed-mode sorbents is limited to basic or acidic analytes depending on the type of ion-exchanger selected. Only a few studies have explored the result of combining both types of sorbents to extract a whole list of target compounds with both basic and acidic properties. Lavén et al. [24] developed an SPE procedure in which a SCX sorbent was placed in tandem with an SAX sorbent to extract 15 basic, neutral, and acidic pharmaceuticals simultaneously. More recently, Deeb et al. [25] proved that a tandem combination of SCX and SAX sorbents gave the highest recoveries in ultrapure water when compared to several SPE sorbents. However, the procedures of these methods are more complicated than those using a single cartridge. Besides, in cartridges coupled in tandem, the ionic interactions of cations and anions are not established simultaneously but firstly in the first cartridge and later in the second cartridge during the wash. Therefore, pH control of both processes is more difficult. The aim of the present study is to broaden the use of mixed-mode sorbents by combining for the first time mixed-mode sorbents with strong or weak cationic and anionic properties to obtain single SPE cartridges with the purpose of extracting selectively and simultaneously basic and acidic compounds. Cation- and anion-exchangers have been combined in the same cartridge before [26] but with the sole purpose of cleaning interferences from the sample and not ionically retaining basic and acidic analytes at the same time. When combining the sorbents in a single cartridge, the SPE procedure is simplified as long as the SPE conditions are correctly selected. With this in mind, a systematic evaluation was performed of combinations of the four types of mixed-mode sorbents (SCX, WCX, SAX, and WAX) by pairs with opposite charges for the selective extraction of ionizable pharmaceuticals. The influence of strong or weak functional groups in the sorbents was observed, as well as the effect of changing the pH and other parameters on the performance of the extraction. This is the first time that the four possible combinations of available mixed-mode ion-exchangers are studied in detail for different parameters in order to design a simple load-wash-elution SPE protocol for cartridges with zwitterionic character. Subsequently, the best combination of sorbents was evaluated in environmental waters in terms of recoveries and ME using LC-high-resolution mass spectrometry (HRMS).

Experimental

Reagents and standards

The pharmaceuticals or metabolites with basic properties, atenolol (ATE), ranitidine (RAN), trimethoprim (TRI), metoprolol (MET) and propranolol (PROP); as well as the neutral pharmaceuticals, caffeine (CAFF), antipyrine (ANTI) and carbamazepine (CBZ); and those with acidic properties, salicylic (SAL AC) and clofibric acid (CLO AC), fenoprofen (FEN), diclofenac (DICLO) and ibuprofen (IBP), were purchased as pure standards from Sigma-Aldrich (St. Louis, MO, USA). The structures, pK_a values, and exact masses of all of the analytes are shown in Table S1 in the Electronic Supplementary Material (ESM). Solid standards were dissolved in methanol (MeOH) to prepare stock solutions of 1 mg/mL which were stored at − 20 °C. Working solutions with the mixture of all the pharmaceuticals were prepared in ultrapure water every week and stored at 4 °C.

Ultra-gradient HPLC-grade MeOH and acetonitrile (ACN) were obtained from J.T. Baker (Deventer, the Netherlands), while ultrapure water was obtained from a water purification system (Veolia, Sant Cugat del Vallès, Spain). Acetic acid (CH_3COOH) and formic acid (HCOOH) were purchased from SDS (Peypin, France) and Sigma-Aldrich, respectively. Ammonium hydroxide (NH_4OH) was obtained from Panreac (Barcelona, Spain).

Sampling

River water samples were collected from the River Ebre in Catalonia, while influent and effluent wastewater samples were collected from sewage treatment plants located in Tarragona and Reus. Both treatment plants include primary and secondary treatments in their processes. Once the samples were collected in pre-cleaned bottles, they were stored at − 20 °C until the day of analysis. Before any SPE procedure, the samples were filtered through a 1.2-μm glass-fiber membrane filter (Fisherbrand, Loughborough, UK) and then through a 0.22-μm nylon membrane filter (Scharlab, Barcelona, Spain).

Solid-phase extraction procedure

Four different 100 mg cartridges containing combinations of individual sorbents with different functional groups (Table 1) were evaluated to extract basic and acidic pharmaceuticals simultaneously. The sorbents combined in the cartridges were the commercially available Oasis MCX, Oasis MAX, Oasis WCX, and Oasis WAX from Waters Corporation (Milford, MA, USA). For simplicity, they will be referred to as SCX, SAX, WCX, and WAX from now on. The amount in grams of each individual sorbent used for each cartridge was established to obtain balanced cationic and anionic moieties. The configuration that gave the best results was the strong cationic/strong anionic one (SCX/SAX). The protocol was then transferred to 500 mg cartridges (110 mg of SCX and 390 mg of SAX) of this combination to extract the environmental waters. These cartridges were conditioned with 10 mL of MeOH, followed by 10 mL of ultrapure water adjusted to pH 7. The selected loading volumes were 100 mL for river water and effluent wastewater samples and 50 mL for influent wastewater samples. All samples were adjusted to pH 7 using either NH_4OH or HCOOH depending on the original pH of the raw water. After loading the samples, a washing step was introduced consisting of 15 mL of MeOH. The elution was performed in two subsequent steps: (1) 5 mL of a 10% HCOOH in MeOH solution; and (2) 5 mL of a 5% NH_4OH in MeOH solution. Both fractions were collected in the same vial and the extract was evaporated to dryness using a centrifugal evaporator miVac Duo (Genevac, Ipswich, UK) and later reconstituted with 1 mL of ultrapure water for river water samples and 2 mL for effluent and influent wastewater samples.

LC-HRMS

A Thermo Scientific Accela 1250 UHPLC system (Bremen, Germany) equipped with an Accela Autosampler automatic injector and an Accela 1250 pump was coupled with a Thermo Scientific Exactive Orbitrap™ mass spectrometer for the chromatographic analysis. The mass spectrometer worked with a heated electrospray ionization (HESI) source and a higher energy collisional dissociation (HCD) cell. The chromatographic column used was the Ascentis Express C_{18} (100 mm × 2.1 mm i.d., 2.7 μm particle size) supplied by Supelco (Sigma-Aldrich), and the mobile phase was a mixture of solvent A (0.5% CH_3COOH in H_2O) and solvent B (MeOH). The optimal pH for the separation of the analytes was 2.8. The column was kept at 25 °C and the mobile phase was pumped at 400 μL/min. The injection volume used was 25 μL and the tray of vials inside the automatic injector was kept at 10 °C. The optimal gradient profile started with 2% of solvent B which was increased to 30% within 6 min and then increased again to 80% within 6 min and held for a further 1 min. After this, solvent B was increased to 100% within 2 min and left isocratic for 3 min and later returned to the initial conditions within 2 min.

In the ion source, basic pharmaceuticals (ESM Table S1) were ionized in the positive mode using the following optimal parameters: spray voltage, 2 kV; skimmer voltage, 25 V; capillary voltage, 40 V; and tube lens voltage, 80 V. In the case of acidic pharmaceuticals, the negative ionization mode was used and the optimal parameters were as follows: spray voltage, 3.5 kV; skimmer voltage, − 15 V; capillary voltage, − 15 V; and tube lens voltage, − 80 V. Gas flow rates and temperatures were the same for both ionization modes: sheath gas, 40 AU (adimensional units); auxiliary gas, 5 AU; heater and capillary temperature, 350 °C; and probe position adjustment: side to side, 0, vertical C and micrometer, 0.5.

Four time windows were used to acquire the data: the first and third (0 to 7.5 min and 9.5 to 11 min) were set in positive mode, the second (7.5 to 9.5 min) in both modes, and the last (11 to 20 min) in negative mode. In all of the windows, two scan events were used for each ionization mode, corresponding to a full scan (at 50,000 FWHM with 250 ms of injection time), which was alternated with a fragmentation scan (at 10,000 FWHM with 50 ms of injection time). Because the second window operated in both positive and negative modes, four scan events were used. The optimal voltage in the HCD cell selected in all the fragmentation scans was 25 eV. For quantification, the response of the molecular ions was used and, for confirmation, the presence of the most abundant fragment ions and the corresponding ion ratios were considered.

Results and discussion

Optimization of LC-HRMS conditions

There are several studies in the literature describing the chromatographic separation of the group of pharmaceuticals selected in the present study, where the use of a C_{18} stationary phase is quite common [27–29]. For this study, the Ascentis Express C_{18} (100 mm × 2.1 mm i.d., 2.7 μm particle size) was compared to the Ascentis RP-Amide (100 mm × 2.1 mm i.d., 2.7 μm particle size) which has proven to offer better retention for polar compounds [30]. The mobile phase was optimized with respect to the organic solvent (ACN or MeOH) in each stationary phase and the type of acid added to the aqueous phase (HCOOH or CH_3COOH). As relevant observations, it can be said that CH_3COOH was chosen over HCOOH because it offered better ionization for FEN and IBP in the ion source. The best results were obtained using ACN in the RP-Amide phase and MeOH in the C_{18} phase. However, in the RP-Amide phase, the first eluting compounds eluted near the void volume, for which the C_{18} column was selected, using MeOH as the organic solvent of the mobile phase ("LC-HRMS" section).

Table 1 Configurations of the four cartridges prepared from the individual mixed-mode sorbents

Individual sorbent	Ionic functional group	meq/g	Configuration	mg	meq
SCX (Oasis MCX)	Sulfonic acid	1	(1) SCX/SAX	22 SCX	0.0220 SCX
				78 SAX	0.0195 SAX
SAX (Oasis MAX)	Dimethylbutylamine	0.25	(2) SAX/WCX	76 SAX	0.0190 SAX
				24 WCX	0.0180 WCX
WCX (Oasis WCX)	Carboxylic acid	0.75	(3) SCX/WAX	38 SCX	0.0380 SCX
				62 WAX	0.0372 WAX
WAX (Oasis WAX)	Piperazine	0.6	(4) WCX/WAX	44 WCX	0.0330 WCX
				56 WAX	0.0336 WAX

For optimization of the HRMS conditions, the voltages, gas flow rates, temperatures, and the position of the ionization probe were varied until obtaining a compromise of the highest response for all the compounds. As expected, basic and neutral pharmaceuticals (ATE, RAN, TRI, CAFF, MET, ANTI, PROP, and CBZ) showed the highest response in the positive mode, while acidic pharmaceuticals (SAL AC, CLO AC, FEN, DICLO, and IBP) were better ionized in the negative mode.

Optimum fragmentation energy was selected as a compromise of the voltage at which the highest response was obtained for all fragments. The exact mass of the molecular ions and the selected fragments for each analyte are shown in Table S1 (see ESM) and they are according to the literature [31–34]. The fragments that displayed the highest response were considered to determine the instrumental limits of detection (ILODs), which were the concentrations at which the peak corresponding to the fragment showed a signal to noise ratio (S/N) of 3, or the signal was higher than 1×10^3 for the analytes with no noise. The instrumental limit of quantification (ILOQ) was the concentration corresponding to the first point of the calibration curve. The observed ILODs ranged between 0.05 and 2.5 μg/L, while the ILOQs ranged between 0.2 and 2.5 μg/L, with FEN, DICLO, and IBP being the analytes with higher limits, due to their lower response. Linearity was evaluated from 0.2 to 1000 μg/L, with lack of linearity being observed for the entire range. Therefore, low level and high level calibration curves were constructed for most of the analytes. Each analyte showed different ranges of linearity but, in general, low level calibration curves were between 0.2 and 100 μg/L, while high level calibration curves were between 25 and 1000 μg/L.

Solid-phase extraction

Behavior of the four cationic/anionic combinations

The individual commercial sorbents Oasis MCX, Oasis MAX, Oasis WCX, and Oasis WAX were combined to obtain four configurations such that the ratio of cationic-exchanger to anionic-exchanger was 1:1 in terms of their ion-exchange capacity. As can be seen in Table 1, the result was four different types of cartridges with cationic/anionic functionalities and strong/strong, strong/weak, or weak/weak properties (SCX/SAX, SCX/WAX, WCX/SAX, and WCX/WAX).

Careful attention was paid to the pH values of the loading and the elution step, taking into account the pK_a of both the functional groups of the sorbents and the pK_a of the analytes. In this sense, when loading the sample, both the functional groups of the sorbents and the analytes must be in their ionic form in order to establish ionic interactions [6, 35, 36]. In contrast, in the elution step, either the functional groups of the sorbents and/or the analytes must be in their neutral form to disrupt retention and favor the elution of the analytes.

For instance, a sample can be loaded in the SAX/SCX configuration using a pH value between 5 and 8 (Fig. 1) because basic analytes are charged up to ~ pH 8 and acidic analytes are charged from ~ pH 3 or 5 (see pK_a values in ESM Table S1). The pK_a values of the moieties of the sorbents were not taken into account in this case because strong functionalities are always charged throughout the pH range. Therefore, the pH value for the elution was selected such that the analytes are converted into their neutral form. Acidic pharmaceuticals were eluted using an acidic solution while basic pharmaceuticals were collected using a basic solution.

When weak moieties are included in the configuration of the cartridges, the pK_a of the functional groups attached to the sorbents must be considered. In the case of the SCX/WAX configuration, the pK_a of the piperazine group attached to the polymer (WAX) is ~ 6, as stated by the manufacturer. Therefore, the optimal loading pH range is between 5 and 6 (Fig. 1), considering the pK_a values of the analytes and the sorbents. Furthermore, all of the analytes should elute in a single fraction when using a basic solution because basic pharmaceuticals would convert into their neutral form, as well as the piperazine groups in the polymer, disrupting their interactions with the acidic pharmaceuticals.

As a result of the previous considerations, the SPE procedure initially used for the four configurations is described below. A volume of 50 mL of ultrapure water adjusted to pH 5 using HCOOH spiked with a mixture of the analytes

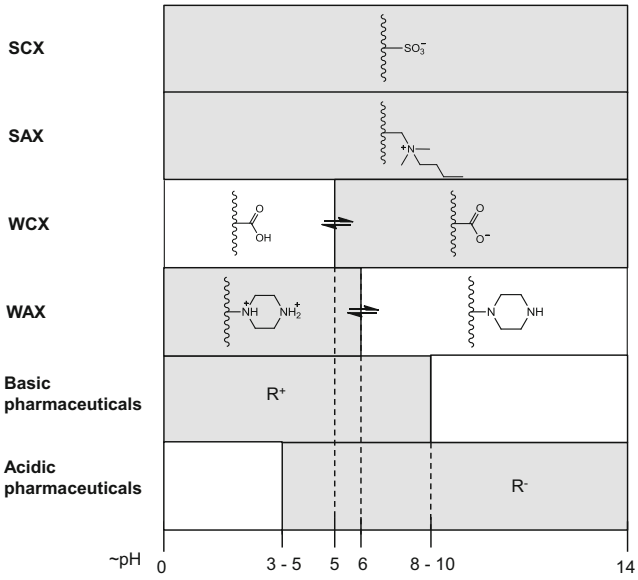

Fig. 1 Charge state of functional groups of the sorbents and the analytes along the pH range (indicated in gray when the charge state is ionic)

(75 μg/L) was percolated through the cartridges after conditioning. The washing step introduced consisted of two fractions of 1 mL of MeOH each. Finally, the elution step was performed in two steps: (1) 5 mL of a 5% HCOOH solution and, (2) 5 mL of a 5% NH_4OH solution for all the cartridges, except for the SCX/WAX configuration, for which the elution steps were interchanged (the basic elution was performed before the acidic elution). In this case, the elution steps were interchanged to promote the elution of all the charged analytes in the first fraction. During the present evaluation of the cartridges, all SPE fractions were diluted with ultrapure water (to 5 mL the washing fraction and to 25 mL the elution one) and analyzed separately in order to evaluate possible losses of the analytes.

From the results detailed in Table 2, it can be seen that all of the analytes were retained by the cartridges during the loading step, which was expected, as the combinations of the sorbents should have the capability of retaining basic, acidic, and neutral compounds. During the washing step, only CAFF, ANTI, and CBZ were completely rinsed, which is in line with their pK_a values, as they are neutral compounds. Their retention in cation-exchangers has been proven [37], behavior that could be explained by their ability to develop partial charges through electron delocalization. However, in a cartridge in which negative charges are coexisting with positive charges, this delocalization might be compromised and so the analytes end up behaving as neutral compounds rather than weak bases.

The rest of the pharmaceuticals were isolated during the elution step, suggesting that these compounds with basic and acidic properties establish strong ionic interactions with the sorbents in the prepared cartridges. Furthermore, the analytes were isolated in the acidic or the basic elution fractions exactly as predicted by their pK_a values (Table 2). For instance, in the SAX/SCX configuration, the acidic pharmaceuticals eluted in the acidic elution, while the basic pharmaceuticals eluted in the basic elution. In the case of the SCX/WAX configuration, all of the analytes were isolated during the basic elution. No differences were observed in the retention of the selected analytes with regard to the strong and weak character of the sorbents included in the cartridges, showing that all of the combinations of the sorbents work well as long as the SPE conditions are properly chosen.

It must be highlighted that, when combining sorbents using the same weight rather than the same ion exchange capacity (meq), the charge in excess had an effect on the analytes with the same charge, showing, for instance, how acids were partially lost in the washing fraction (data not shown) when the negative charge was predominant in the cartridge. In summary, the potential of applying combinations of mixed-mode sorbents to extract basic and acidic analytes simultaneously was demonstrated, using a simple SPE procedure with the advantage of eliminating neutral interferences in the washing step.

Because in the SAX/WCX cartridge sometimes the basic pharmaceuticals were partially lost in the wash, and the WAX/WCX cartridge has a very narrow range of pH at which both the analytes and sorbents are charged, the most promising combinations were the SCX/SAX and SCX/WAX cartridges. These two configurations were used to evaluate if the preliminary conditions predicted as most favorable were in fact optimal, by studying the effect of changing the SPE parameters in the performance of the extraction. In addition, the use of conditions that, in theory, should not work properly will further confirm the presence of ionic interactions as the driving force of the retention of the charged analytes.

Influence of pH on retention

The effect of changing the pH of the loading was studied in both the SCX/SAX and the SCX/WAX cartridges, as these parameters are very important in the overall procedure. Initially, different pH values (2, 7, 10, and 12) were tested in the loading step, following the same SPE procedure described in the previous section, for both the SCX/SAX and SCX/WAX cartridges. In theory, for the SCX/SAX cartridge, the optimal loading pH range is ~ 5 to 8, because acidic pharmaceuticals are in their neutral form at pH 2 and basic analytes are uncharged at pH 10 and 12 (Fig. 1). Table 3 shows how acidic pharmaceuticals were lost in the washing step when loading at pH 2, while basic ones were lost in the washing step when loading at pH 12, as expected. In this table, the recoveries obtained for pH 2 and 12 are shown, as these were the pH conditions under which ionic interactions were weakened. It was also expected that, when loading at pH 5 and 7, all pharmaceuticals (with the exception of the neutral ones) were

Table 2 Recoveries (%) obtained when 50 mL of ultrapure water adjusted at pH 5 was percolated through the four cartridge combinations

	Analyte	SCX/SAX					SAX/WCX					SCX/WAX					WCX/WAX				
		w1	w2	e_a	e_b		w1	w2	e_a	e_b		w1	w2	e_b	e_a		w1	w2	e_a	e_b	
Basic	ATE				76	76			85		85			81		81			82		82
	RAN				79	79			64		64			80		80			56		56
	TRI				82	82			85		85			81		81			85		85
	MET				76	76			85		85			80		80		2	83		85
	PROP				82	82			92		92			86		86			91		91
Neutral	CAFF	76	19			94	88	11			100	60	35	5		100	70	30			100
	ANTI	63	19			82	79	10			89	18	23	50		92	59	28			87
	CBZ	65	21			86	70	20			90	50	35			85	52	33	2		87
Acidic	SAL AC			35	25	59			4	17	21			90		90				19	19
	CLO AC			88	19	107			91		91			85		85			85		85
	FEN			91		91			91		91			89		89			91		91
	DICLO			80		80			80		80			83		83			73		73
	IBP			92		92			97		97	1	2	83		86		6	93		99

%RDS lower than 15% in all cases

w1 and w2 wash fraction 1 and 2, e_a acidic elution, e_b basic elution, *w1* 1 mL MeOH, *w2* 1 mL MeOH, e_a 5 mL 5% HCOOH in MeOH, e_b 5 mL 5% NH_4OH in MeOH, Σ total sum of the recoveries of the four fractions

retained through ionic interactions. At pH 10, basic pharmaceuticals were partially lost in the washing step (7 to 23%) rather than being completely lost, as expected. This was not surprising, as pH 10 is around the pK_a values of these analytes, so the test was performed at the limit of the conversion between the ionic and the neutral form.

For the SCX/WAX cartridge, the optimal loading pH range is ~ 5 to 6, because acidic pharmaceuticals are in their neutral form at pH 2 and the piperazine group of the WAX sorbent is in its neutral form from pH ~ 6 upwards, as stated by the manufacturer of the sorbents (Fig. 1). In Table 3, it can be seen that both acidic and basic pharmaceuticals were lost in the washing step when loading the sample at pH 12, while, at pH 2, it was the acidic analytes that were isolated in this step, just as expected. As anticipated at pH 7 (data not shown), the acidic pharmaceuticals were strongly retained during the MeOH wash, rather than losses being observed. Actually, acidic pharmaceuticals are partially lost starting from pH 10, suggesting that the piperazine group might have a higher pK_a within the polymeric network. At this pH, basic pharmaceuticals were also ionically retained, which is explained by the fact that these conditions are at the limit of the pK_a values of these analytes, just as observed before for the SCX/SAX cartridge. Low recoveries observed for SAL AC during these tests was explained by excessive retention on the cartridges, which was resolved by raising the % HCOOH in MeOH from 5 to 10% for the elution in further tests.

In this section, it was confirmed that the strong retention observed for charged pharmaceuticals was due to the establishment of ionic interactions between the analytes and the charged functional groups of the sorbents. These interactions are only possible in the range of pH values at which both the analytes and sorbents are in their charged form. When these conditions are not met, the ionic interactions are weakened and retention is driven only by hydrophobic interactions.

Table 3 Recoveries (%) obtained in ultrapure water when the SCX/SAX and the SCX/WAX configurations were loaded at pH 2 and 12

	Analyte	pH 2					pH 12						
		SCX/SAX			SCX/WAX		SCX/SAX			SCX/WAX			
		w	e_a	e_b	w	e_b	w	e_a	e_b	L	w	e_b	
Basic	ATE			79		85	63		12		54	30	
	RAN			84		86	77		2		78	6	
	TRI			82		84	82				83		
	MET			82		83	59		15		53	30	
	PROP	1		89		90	48	1	36		44	46	
Neutral	CAFF	56			67		69				84		
	ANTI	15	19	37	3	78	79				87		
	CBZ	86			85		87				89		
Acidic	SAL AC	9	28	12		93		16	9	69	1		
	CLO AC	44	39		12	71		86		78	11		
	FEN	81	6		75	16		85			91		
	DICLO	55	25		22	58		83			92		
	IBP	84			84			87			81		

%RSD lower than 15% in all cases

L loading, *w* w1 + w2 (1 mL MeOH + 1 mL MeOH), e_a 5 mL 5% HCOOH in MeOH, e_b 5 mL 5% NH_4OH in MeOH

Optimization of other SPE conditions

Using the same cartridges selected previously, SCX/SAX and SCX/WAX, other parameters of the SPE procedure were evaluated, such as volume in the different steps and washing solvent. To optimize the washing volume, 50 mL of ultrapure water adjusted to pH 5 was loaded in both cartridges, which were then washed with subsequent fractions of 1 mL of MeOH up to 15 mL. It was observed that the neutral pharmaceuticals were lost completely in the first 1 mL washing fraction in both types of cartridges, while no losses were recorded for the rest of the analytes in any of the fractions. Only IBP was partially lost (40%) starting from the seventh fraction. After ascertaining that this loss was due to the selection of a pH value too close to the pK_a of IBP (4.85), and confirming that, at pH 7, the compound was not lost, the loading pH was set at this value from this point onwards. Thus, the washing volume was set at 15 mL of MeOH because no losses were observed for any of the target analytes and a volume higher than this was considered excessive. Actually, this volume of 15 mL is already high for the amount of sorbent used (100 mg) and, as such, it was expected to eliminate a large number of interferences in complex matrices.

In subsequent tests, 50 mL of ultrapure water adjusted to pH 7 was loaded in both cartridges, which were further washed with ACN instead of MeOH, to evaluate the influence of a different organic modifier. No differences were observed between the two organic solvents in any of the cartridges. As MeOH is most commonly used for the washing steps in mixed-mode SPE procedures, it was used for further extractions. The elution volume was optimized by passing 1 mL fractions up to 5 mL of the elution solvents as follows, for SCX/SAX: (1) 10% HCOOH in MeOH solution followed by (2) 5% NH_4OH in MeOH solution; as for SCX/WAX: only 5% NH_4OH in MeOH. For both cartridges, the volume initially selected of 5 mL of each eluting solution proved to be enough to completely elute all of the analytes, so it was considered optimal.

For both configurations, SCX/SAX and SCX/WAX, the SPE protocol established to this point was transferred to 500 mg cartridges, which allowed the loading of up to 500 mL of ultrapure water (adjusted to pH 7) to then be washed with 15 mL of MeOH. As a result, up to 500 mL could be passed through the cartridges without observing losses of any of the analytes during the loading or washing step, obtaining recoveries that ranged from 84 to 97%. It can be seen that the retention of the combined sorbents for ionic pharmaceuticals is good and it is comparable or better to the results obtained in individual separated cartridges. For example, in studies where cation-exchangers were used to extract the same basic pharmaceuticals, recoveries were between 63 and 114% even when only aqueous washing steps were used [23, 38]. When acidic pharmaceuticals were extracted in an in-house anion-exchanger, recoveries ranged between 91 and 98% when introducing a washing step with 10 mL of MeOH [16]. Compared to cartridges combined in tandem [24], the present results were also similar or better and the protocol was significantly simpler.

Environmental water samples

The optimized SPE procedures for each type of cartridge, SCX/SAX and SCX/WAX, were evaluated in river water and wastewaters to observe the performance of the combinations of the sorbents when dealing with complex matrices. The neutral analytes (CAFF, ANTI, and CBZ) will not be discussed below, as it was proven that they are lost during the washing step fraction. A considerable ME is commonly observed when determining pharmaceuticals in environmental waters, which increases when using high volumes of sample [39]. Thus, the first parameter evaluated when testing the optimized method in these types of matrices was the sample volume. The optimal volumes are indicated in "Solid-phase extraction procedure" section, selected according to the breakthrough volume of the analytes and the ME observed.

At this point, both the SCX/SAX and SCX/WAX cartridges were compared with regard to their performance in environmental waters to select a single configuration for subsequent tests. The optimal procedure described in "Solid-phase extraction procedure" section was applied to analyze 100 mL of effluent wastewater sample (spiked at 2.5 μg/L) using the two types of cartridges. The results are shown in Table 4. The $\%R_{SPE}$ was defined as the recovery obtained only in the SPE procedure and it was calculated as the ratio between the concentrations obtained from a sample spiked before and after the SPE procedure. The ME was calculated from the concentration obtained when the extract of the sample was spiked just before injection into the LC-HRMS. This concentration (C_{exp}) was introduced in the formula $\%ME = [(C_{exp}/C_{theo}) \times 100] - 100$, where C_{theo} is the theoretical concentration in the final volume of sample injected in the LC-HRMS instrument. The $\%R_{apparent}$ was defined as the recovery of the whole method and it was calculated from the concentration obtained from a sample spiked at the beginning of the complete analysis. All of the experimental concentrations mentioned were calculated using a calibration curve prepared in pure standard.

It can be observed in Table 4 that, for the SCX/SAX configuration, values of $\%R_{SPE}$ were between 83 and 104%, with the exception of RAN (60%) and SAL AC (62%), similar to the SCX/WAX configuration, which showed values between 79 and 109%, except for RAN (69%) and IBP (68%). $\%R_{apparent}$ values ranged from 45 to 88% and from 47 to 88% for the SCX/SAX and SCX/WAX cartridges, respectively. The ME was below − 28% in both cartridges, except for ATE and TRI, which showed values around − 48%. Clearly,

Table 4 Comparison of the performance between the SCX/SAX and SCX/WAX configurations for extracting the selected basic and acidic pharmaceuticals from 100 mL of effluent wastewater

	Analyte	SCX/SAX			SCX/WAX		
		$\%R_{apparent}$	$\%R_{SPE}$	%ME	$\%R_{apparent}$	$\%R_{SPE}$	%ME
Basic	ATE	53	104	− 49	55	105	− 48
	RAN	51	60	− 15	52	69	− 25
	TRI	50	97	− 48	54	97	− 44
	MET	70	84	− 17	73	91	− 20
	PROP	68	83	− 18	58	79	− 27
Acidic	SAL AC	45	62	− 28	79	109	− 27
	CLO AC	88	97	− 10	88	105	− 17
	FEN	72	95	− 24	74	102	− 28
	DICLO	74	101	− 27	76	104	− 27
	IBP	68	96	− 28	47	68	− 31

%RSD lower than 10% in all cases

no differences were observed between both types of configurations, suggesting that either of them can be equally used for simultaneously extracting the charged pharmaceuticals from the samples. For further tests, the SCX/SAX was selected from the two types of sorbents because it gave a higher $\%R_{apparent}$ for IBP, which is one of the analytes with a lower response in the LC-HRMS.

Using the SCX/SAX configuration, the ME was compared when extracting effluent and influent wastewaters (spiked at 5 and 10 μg/L, respectively), and the washing step was applied or omitted, to determine the efficiency of including this cleaning step in the SPE procedure. Figure 2 shows the results of this evaluation, demonstrating a decrease in the ME obtained for several of the analytes, especially basic pharmaceuticals, when the washing step was included. The ME obtained when applying the washing step ranged between − 49 and − 15% for effluent wastewater, and between − 51 and −20% in the case of influent wastewater, which was higher than expected considering that the volume used for the washing step (15 mL MeOH) was quite high. These results can be attributed to the presence of a high content of ionic interferences in the samples that contribute to the ME to a high degree.

The SPE procedure using the SCX/SAX configuration was further evaluated in river and wastewater samples in terms of $\%R_{SPE}$ and $\%R_{apparent}$. The results obtained for river waters and influent wastewaters spiked at 2.5 and 10 μg/L, respectively, are summarized in Table 5. Satisfactory $\%R_{SPE}$ values were obtained for all the matrices, being higher than 90% in 70% of the cases. Values of $\%R_{apparent}$ for river water samples were between 45 and 109%, with the exception of IBP (31%), while, for effluent and influent wastewater samples, values ranged from 52 to 83% (except for SAL AC, which were 30%) and from 34 to 76%, respectively. The ME obtained with the present method can be attributed only to ionic interferences. The method demonstrated the capability of simultaneously retaining acidic and basic analytes and the advantage of eliminating all neutral interferences, features that could be transferred to other groups of ionizable compounds and highly complex matrices.

Method validation and application to environmental samples

The optimized method using the SCX/SAX cartridge was validated in river water and effluent and influent wastewater samples to check its repeatability and detection and quantification limits. Repeatability was expressed as the relative standard deviation (%RSD, $n = 5$) and it was evaluated within the same day (results shown in Table 5) and on consecutive days. The method exhibited satisfactory precision, as %RSD values ranged between 0.4 and 24% for all of the pharmaceuticals in all matrices.

Because several of the target analytes were present in the non-spiked wastewater samples, the use of matrix-matched calibration curves to correct the ME was not possible for these matrices. In these cases, external calibration curves were used for quantification, taking $\%R_{apparent}$ values into consideration. In the case of river water, a matrix-matched calibration curve was prepared by spiking at different concentrations 100 mL volumes of river water, which were extracted in the SCX/SAX cartridges and subsequently injected into the LC-HRMS instrument. Linearity was good for all of the compounds ($R^2 \geq 0.9988$) between the MQLs (reported in Table 5) and 500 ng/L, except for RAN, SAL AC, and IBP, which showed poor linearity in the concentration range tested. In these cases, external calibration curves were also used.

For the wastewater samples, method detection (MDLs) and quantification (MQLs) limits were estimated from the instrumental limits (ILODs and ILOQs), taking into account the $\%R_{apparent}$ values. For river water samples, MDLs were the spiked concentrations that showed a signal for the more

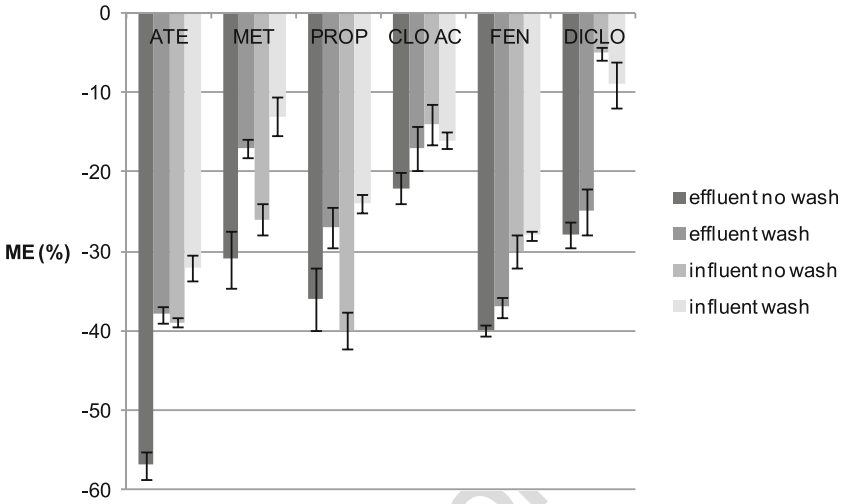

Fig. 2 ME (%) obtained for 100 mL of effluent (spiked at 5 μg/L) and 50 mL of influent (10 μg/L) wastewater samples when applying or not the washing step (15 mL of MeOH) of the optimized SPE procedure

abundant fragment around 1×10^3, while MQLs were the first points of the matrix-matched calibration curves. MDLs were between 0.5 and 5 ng/L for river water samples, while for effluent and influent wastewater samples, values were between 1 and 75 ng/L and between 3 and 260 ng/L, respectively. Table 5 shows the MQLs values, which ranged from 1 and 25 ng/L for river water samples, while, for effluent and influent wastewater samples, values ranged from 5 to 80 ng/L and from 15 to 265 ng/L, respectively.

Three different samples of effluent and influent wastewater and two different samples of river water were analyzed using the validated method for the SCX/SAX combination. The ranges of concentrations found are shown in Table S2 in the ESM. Moreover, Figs. S1–S3 in the ESM show the extract ion chromatograms of analyzed samples for each type of matrix studied. In river samples, several compounds were detected, but only ATE, MET, SAL AC, and CLO AC were quantified at concentrations between 1 and 50 ng/L. Most of the compounds were quantified in effluent and influent wastewater samples with levels ranging from 20 to 2500 ng/L in the case of effluent wastewater samples and from 40 to 50,000 ng/L in the case of influent wastewater. The pharmaceuticals found at the highest concentrations were ATE, SAL AC, DICLO, and IBP and levels found for all of the analytes were in line with those reported in the literature [4, 31, 40].

Conclusions

The four combinations tested SCX/SAX, SCX/WAX, WCX/SAX, and WCX/WAX, simultaneously and strongly retained basic and acidic pharmaceuticals, as long as the charges are balanced and the SPE conditions are carefully selected. No substantial differences were observed between the four combinations evaluated in ultrapure water apart from the optimum loading pH ranges, suggesting that they all could be potentially useful depending on the application. The correct selection

Table 5 $\%R_{apparent}$, $\%R_{SPE}$, ME, %RSD ($n = 5$), and MQLs values obtained when 100 mL of river water or 50 mL of influent wastewater sample was percolated through SCX/SAX cartridge

Analyte	$\%R_{apparent}$	River				$\%R_{apparent}$	Influent			
		%RSD	$\%R_{SPE}$	%ME	MQL (ng/L)		%RSD	$\%R_{SPE}$	%ME	MQL (ng/L)
ATE	109	3	104	− 5	1	61	1	102	− 42	15
RAN	68	11	83	− 25	20	71	1	79	10	110
TRI	98	5	94	− 4	2	67	1	98	− 35	120
MET	102	2	93	5	2	70	8	93	− 24	115
PROP	90	16	90	− 3	2	70	10	97	− 33	115
SAL AC	50	4	92	− 34	20	34	14	57	− 40	120
CLO AC	65	16	90	− 21	2	76	22	101	− 19	35
FEN	45	6	76	− 36	5	49	8	99	− 41	15
DICLO	48	18	74	− 32	2	62	4	107	− 24	15
IBP	31	14	60	− 41	25	38	2	116	− 61	265

of the pH value used to load the samples and elute the analytes was very important in terms of favoring the ionic interactions between the analytes and the sorbents.

The strong retention of the analytes on the sorbents allowed the introduction of a washing step with a high volume of MeOH (15 mL), which proved to eliminate neutral interferences present in the matrices. As indicated by the results obtained when extracting environmental waters using the present method, ionic interferences contribute to the matrix effect to a high degree. The performance of the method was comparable to other studies reported but the protocol was considerably simpler thanks to the combination of the cartridges. The method was validated in river and wastewater samples and several of the selected analytes were successfully quantified in the samples at levels that were similar to those reported in other studies.

The potential of combining sorbents to obtain positive and negative charges in the same SPE cartridge was confirmed and optimal extraction conditions were given to obtain the best performance. Promising results might be expected for other basic or acidic compounds and other samples with complex matrices.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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