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# Development of sol-gel silica-based mixed-mode zwitterionic sorbents for determining drugs in environmental water samples

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ABSTRACT

Four novel mixed-mode zwitterionic silica-based functionalized with strong moieties sorbents were synthesized and evaluated through solid-phase extraction (SPE) to determine acidic and basic drugs in environmental water samples. All sorbents had the same functionalization: quaternary amine and sulfonic groups and $C_{18}$ chains so that hydrophobic and strong cationic exchange (SCX) and strong anionic exchange (SAX) interactions could be exploited, in addition, two of them had carbon microparticles embedded.

All sorbents retained both acidic and basic compounds in the preliminary assays but only the basic compounds were retained selectively through ionic exchange interactions when a clean-up step was introduced. The SPE method was therefore optimized to promote the selective retention of the basic compounds, initially with the two best-performing sorbents.

After optimization of the SPE protocol, these sorbents were evaluated for the analysis of environmental water samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method with the best-performing sorbent was then validated with 100 mL of river samples and 50 mL of effluent wastewater samples in terms of apparent recoveries (%$R_{app}$) spiking samples at 50 ng/L (river) and 200 ng/L (river and effluent), matrix effect, linear range, method quantification and detection limits, repeatability, and reproducibility. It should be highlighted that %$R_{app}$ ranged from 40 to 85% and matrix effects ranged from -17 to -4% for spiked river samples. When the method was applied to river and effluent wastewater samples, most compounds were found in the range from 24 to 1233 ng/L with detection limits from 1 to 5 ng/L.

## 1. Introduction

Complex samples require selective sample treatments to separate the analytes from the interferences that may cause matrix effect, mainly in liquid chromatography-mass spectrometry (LC-MS). One way to achieve this is to use selective materials in sorptive extraction techniques, the most representative of which is solid phase extraction (SPE) [1,2]. Variants of this technique are also used, such as microsolid-phase extraction (µSPE) [3], dispersive solid-phase extraction (dSPE) [4], on-line SPE [5] and pipette tip solid-phase extraction (PT-SPE) [6], as well as other sorptive extraction techniques such as stir bar sorptive extraction (SBSE) [7] or fabric phase sorptive extraction (FPSE) [8].

In recent years, research has focused on developing new sorbents [9] that can improve the sensitivity and selectivity of the methods in which they are applied, through the decrease of the interferences and the matrix effect.

Mixed-mode ion-exchange sorbents are an example of these new types of sorbents [10,11]. These sorbents can retain non-charged compounds through hydrophobic interactions and charged compounds through ion-exchange interactions, thus enabling them to interact with a wide range of compounds. The compounds retained by hydrophobic interactions are eluted with an organic eluent. Those retained by ion-exchange interactions, on the other hand, require an acidic or basic eluent to disrupt the interactions with the sorbent. This duality affords great flexibility. For instance, if the target compounds are in the ionic state (e.g. acidic or basic

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compounds), a clean-up step with an organic solvent can remove the hydrophobic compounds attached to the sorbent. The acidic or basic compounds can then be eluted selectively with an acidic or basic solvent [10–12], which neutralizes the compounds and enables the ionic interactions to be disrupted.

These sorbents can be classified according to the type of ion-exchange interaction established. On the one hand, the sorbents are anionic exchangers if they retain anionic compounds, being strong exchangers (SAX) or weak exchangers (WAX) depending on the functionalization. On the other hand, the sorbents are cationic exchangers if they retain cationic compounds. In this case, they can also be strong (SCX) or weak (WCX) depending on the functionalization.

The pH in the different steps of the extraction protocol is therefore the key parameter when this kind of sorbent is used. To select the pH to promote high retention of the analytes, the $pK_a$ of each compound must be taken into account to ensure that, at that pH, the analytes are charged and can interact with the sorbent, which is also charged at the working pH.

The most common mixed-mode ion-exchange sorbents are polymeric sorbents, and they are available commercially as, for example, Oasis (from Waters) or Strata X (from Phenomenex). Another interesting group are silica-based sorbents, though these are less stable at extreme pH than polymeric sorbents. They also usually present low retention of polar compounds, though this may be beneficial since they have fewer unspecific interactions than polymeric-based sorbents [12]. However, silica-based sorbents have high organic resistance and good mechanical stability. Moreover, the silanol groups present in the silica network are easy to modify, which enables a wide range of functionalization [13–19]. Liu et al. [14], for example, developed two sorbents when functionalizing mesoporous silica with octadecylsilane or octylsilane and sulfonic acid to obtain a mixed-mode sorbent based on reversed-phase and SCX interactions. These sorbents were satisfactorily evaluated for determining veterinary drug residues.

One of the main problems with mixed-mode ion-exchange sorbents is that most of them are only based on one type of ionic interaction (as occurs with the commercial sorbents [12]), which means that they are selective for only one type of compound (basic or acidic). One approach to extract both acidic and basic compounds could be the combination of commercial polymeric anionic and cationic mixed-mode ion-exchange sorbents in a single cartridge [20] or in series [21,22] to determine acidic and basic compounds in one extraction. For instance, commercial anionic and cationic Oasis sorbents were combined in a single cartridge to selectively extract acidic and basic compounds from water samples [20]. Another approach is the development of sorbents that combine anionic and cationic interactions, i.e. zwitterionic exchangers. One of the developments in the field of new sorbents is the study of materials that can simultaneously retain cationic and anionic compounds through zwitterionic-exchange interactions. One example is the microporous polymer developed by Nadal et al. [23], which was used to determine a mixture of drugs, pharmaceuticals and sweeteners with acidic and basic character in water. In this study, polymeric-based microspheres were developed for SPE based on weak anionic and cationic interactions that were controlled using the pH of the loading solution. By loading samples at pH 6, it was possible to retain acidic and basic compounds to determine those compounds in river and effluent wastewater samples through liquid chromatography-mass spectrometry in tandem LC-MS/MS.

Some silica-based [16,18] and polymer-based [23–25] zwitterionic sorbents have already been developed, though research is still needed. The silica-based sorbents reported [16,18] are based on weak ionic interactions since they are functionalized with carboxylic groups and primary amines, in both cases the chargeability of the sorbents depended on the pH along the SPE protocol.

In our study, we present a series of zwitterionic silica-based sorbents based on the functionalization of a silica network. Two of these sorbents were based on silica without modification and two were based on silica with carbon microparticles embedded. All sorbents were functionalized with quaternary amines and sulfonic acid groups, therefore the novelty of the sorbents arise in the functionalization of silica with strong ionic moieties, so that, the sorbent will be always charged at any pH. Once the sorbents were synthesized, they were evaluated using SPE and the best-performing sorbent was used to selectively determine basic drugs in river and effluent wastewater water samples through LC-MS/MS.

## 2. Experimental

### 2.1. Reagents and standards

Chemicals and reagents for sol-gel mixed-mode zwitterionic sorbents include methyl trimethoxysilane (MTMS), tetramethyl orthosilicate (TMOS), activated carbon, trifluoroacetic acid (TFA), isopropanol (IPA), methylene chloride, methanol (MeOH), and ammonium hydroxide purchased from Sigma-Aldrich (St. Louis, MO, USA). Octadecyl trimethoxysilane ($C_{18}$-TMS), 3-mercaptopropyl trimethoxysilane (3-MPTMS), N-Trimethoxysilylpropyl-N,N,N-trimethyl ammonium chloride and 4-(Trimethoxysilylethyl) benzyltrimethyl ammonium chloride were obtained from Gelest Inc. (Morrisville, WI, USA).

Thirteen drugs were selected for the sorbent evaluation. Six of these were basic, atenolol (ATE), trimethoprim (TRI), metoprolol (MTO), venlafaxine (VEN), ranitidine (RAN) and propranolol, while seven were acidic, bezafibrate (BEZ), clofibric acid (CLO), diclofenac (DICLO), fenoprofen (FEN), flurbiprofen (FLB), naproxen (NPX) and valsartan (VAL). All these drugs were purchased as pure standards from Sigma-Aldrich (purity >96%).

Stock solutions of individual standards were prepared in methanol (MeOH) at a concentration of 1000 mg/L and stored at −20 °C. Working solutions of a mixture of all compounds were prepared weekly in a mixture of ultrapure water and MeOH (80/20 v/v) and stored at 4 °C in brown bottles in the dark. Ultrapure water was provided by a water purification system (Millipore, Burlington, United States), while “HPLC grade” MeOH and acetonitrile (ACN) were purchased from J. T. Baker (Deventer, The Netherlands). “MS grade” ACN and water were purchased from Scharlab (Barcelona, Spain). Formic acid (HCOOH), acetic acid (AcOH) and HCl were acquired from Sigma-Aldrich.

### 2.2. Synthesis of sol-gel mixed-mode zwitterionic sorbents

Sol solutions to create the sol-gel mixed-mode zwitterionic sorbents were obtained by sequential addition and subsequent vortexing of methyl trimethoxysilane (MTMS), tetramethyl orthosilicate (TMOS), octadecyl trimethoxysilane ($C_{18}$-TMS), 3-mercaptopropyl trimethoxysilane (3-MPTMS), N-trimethoxysilyl propyl N,N,N-trimethyl ammonium chloride (N-TMPTMAC), isopropanol (IPA) and trifluoroacetic acid (TFA, 0.1M) in a 50 mL centrifuge tube. The relative ratios of the various ingredients (MTMS, TMOS, $C_{18}$-TMS, 3-MPTMS, N-TMPTMAC, IPA, and TFA were 1: 1: 0.1: 0.1: 0.2: 3.8: 3, respectively. To introduce phenylethyl linker connected to trimethyl ammonium chloride, N-trimethoxysilyl propyl N,N,N-trimethyl ammonium chloride was replaced with 4-(trimethoxysilylethyl) benzyl trimethyl ammonium chloride in another set of sol-gel sorbents. The mixture was vortexed for 5 min and then sonicated for 15 min to remove any trapped air bubbles from the sol solution. The sol solution was kept at room temperature for 8 h to allow the sol-gel precursors to be hydrolysed.

Freshly prepared ammonium hydroxide (1 M) was then added in droplets to the sol solution under continuous stirring in a magnetic stirrer. The solution slowly became viscous before turning into solid gel. To produce activated carbon impregnated sol-gel sorbent, 0.5 g of activated carbon was added to the sol solution before ammonium hydroxide solution was added.

The solid gel was thermally conditioned and aged at 60 °C for 48 h. The monolithic bed of the sol-gel network was then crushed and dried at 80 °C for 24 h and the sol-gel sorbent was crushed into fine particles in a ball mill and rinsed with MeOH: methylene chloride (50:50, v/v) under sonication for 30 min. The particles were air-dried and treated with 30% $H_2O_2$ (with 0.1 M sulphuric acid) for 4 h. The particles were rinsed with deionized water several times and then dried at 80 °C for 12 h. The sol-gel mixed-mode zwitterionic sorbents were then ready for loading into the SPE cartridges.

### 2.3. Structure of sol-gel mixed-mode zwitterionic sorbents

The characterization of the sorbents was performed with a Cary 670 FTIR, Agilent Technologies Cary 600 Series FTIR Spectrometer (Agilent Technologies, Santa Clara, CA, USA) for the Fourier Transform Infrared Spectroscopy (FT-IR) and with a JEOL JSM 5900LV Scanning Electron Microscope (SEM) equipped with EDS-UTW detector, JEOL USA, Inc. (Peabody, MA, USA) for recording SEM images.

The four sorbents (Fig. 1) tested in this study were based on a silica skeleton functionalized with $C_{18}$ to perform hydrophobic interactions; quaternary amines to perform SAX interactions; and sulfonic groups to perform SCX interactions.

All sorbents were functionalized with the same groups to perform SAX and SCX interactions. Two of them ($SiO_2$-SAX/SCX - $SiO_2$-SAX/SCX(Ph)) were based on a silica network (S-type) and two ($SiO_2$-C-SAX/SCX - $SiO_2$-C-SAX/SCX(Ph)) were based on a silica network with activated carbon embedded (C-type). Fig. 1 shows the structure of the four sorbents tested. $SiO_2$-SAX/SCX and $SiO_2$-C-SAX/SCX had the same functionalization, with propyl groups between the network and the quaternary amine. $SiO_2$-SAX/SCX(Ph) and $SiO_2$-C-SAX/SCX(Ph) also had the same functionalization, though in this case with a phenylethyl group in the anionic exchange chain.

### 2.4. Solid-phase extraction procedure

An empty 6 mL SPE cartridge (Symta, Madrid, Spain) was fitted with a 10 µm polyethylene frit (Symta) and filled with 200 mg of sorbents. A 10 µm polyethylene frit was then placed above the sorbent bed.

The SPE procedure was performed in an SPE manifold (Teknokroma, Barcelona, Spain) connected to a vacuum pump. The first step was to condition the sorbents with 5 mL of MeOH and 5 mL of ultrapure water adjusted at pH 3. 100 mL of sample adjusted at pH 3 with HCl were loaded into the cartridge. For the effluent wastewater samples, the volume was 50 mL. After the loading step, the washing step was performed with 5 mL of MeOH. Finally, the elution step involved 5 mL of MeOH containing 5% of $NH_4OH$. The eluted volume was evaporated with a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) to complete dryness and then reconstituted with 1 mL of initial mobile phase solution ($H_2O$/ACN, 95/5, v/v). The reconstituted extracts were filtered using 0.45 µm polytetrafluoroethylene (PTFE) syringe filters (Scharlab) before analysis. To reuse the SPE cartridges a washing step with MeOH was performed and then, it was completely dried by applying vacuum for 10 min.

Samples from river and effluent wastewater treatment plants were filtered through a 0.45 µm Nylon membrane filter (Scharlab). The effluent samples were previously filtered using a 1.2 µm glass-fibre membrane filter (Fisherbrand, Loughborough, UK).

### 2.5. Instrumentation and chromatographic conditions

The initial tests and the optimization of the SPE conditions were performed with an Agilent 1200 UHPLC equipped with a binary pump, an autosampler, an automatic injector, and a diode array detector (DAD) (Agilent, Waldbronn, Germany). The chromatographic column used was a Luna® Omega 5 µm Polar C18 100 (150 × 3.0 mm, 5 µm particle size) supplied by Phenomenex (Torrance, CA, United States). The mobile phase was a mixture of ultra-

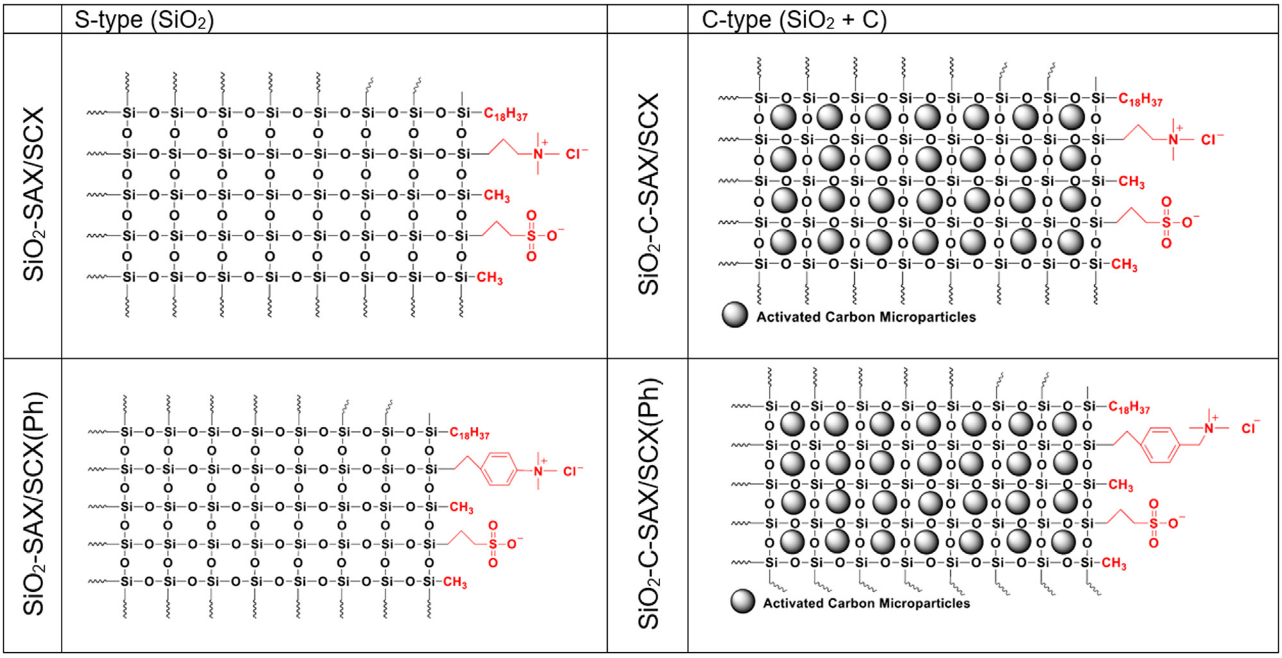

**Fig. 1.** Structure of the sol-gel mixed mode zwitterionic sorbents.

pure water adjusted to pH 3 with HCl (solvent A) and ACN (solvent B). The gradient profile began with 5% of B. The % of B was then increased to 40% within 10 min, then to 45% within 4 min, and finally to 100% within 1 min. It was then held at 100% for 3 min before returning to the initial conditions in 1 min, where it was held for 3 min to stabilize the column. The column temperature was 30 °C and the flow rate was 0.4 mL/min. The injection volume was 20 µL. ATE, TRI, MTO, PRO, BEZ, VAL, FEN, FLB and CLO were measured at 210 nm, while RAN, VEN, DICLO, and NPX were measured at 230 nm.

Once the SPE conditions were optimized, the method was validated for the basic compounds analysing real samples with LC-MS/MS using an Agilent 1260 Infinity 2 connected to a triple quadrupole mass detector Agilent 6460 and electrospray ionization (ESI) interface. The chromatographic conditions were the same as in LC-DAD, except that the injection volume was 10 µL and the pH of solvent A was adjusted with HCOOH rather than HCl. The optimized parameters in the (ESI) MS/MS were gas temperature 320 °C, gas flow rate 10 mL/min, nebulizer pressure 35 psi and the capillary voltage 3000 V. The fragmentor potential for all transitions was 100 V. For each compound, the diagnostic ion was $[M+H]^+$. One of the transitions was used as quantifier and at least one more was used as qualifier. Table S1 shows the MRM transitions selected and their collision energies.

### 2.6. Validation parameters

The method was validated in terms of recovery, matrix effect, linear range, method quantification and detection limits, repeatability and reproducibility.

Recovery (%R) and apparent recovery ($\%R_{app}$) were used to evaluate the yield of the extraction. %R was obtained with LC-DAD, being the ratio of the concentration obtained after the SPE of a spiked sample and the concentration expected. $\%R_{app}$ was obtained in the same way that %R but the analysis was performed with LC-MS/MS, and it considers the extraction recovery and the matrix effect.

The matrix effect (%ME) was calculated from the formula: $\%ME = (C_{Exp}/C_{Theo} \times 100) - 100$, where "$C_{Exp}$" is the concentration obtained by spiking a blank sample after SPE and "$C_{theo}$" is the expected concentration. A negative value indicates suppression of the signal, while a positive value indicates enhancement.

The instrumental linear range was evaluated with external calibration curves analysing in triplicate seven solutions with different concentrations. Matrix matched calibration curves were obtained spiking river samples at seven different concentrations.

Method quantification limit (MQL) was obtained from the matrix-matched calibration curves, being the lowest concentration from the curve and method detection limit (MDL) was calculated as the concentration that provided a signal-to-noise ratio of 3.

Repeatability was obtained as the % relative standar deviation (%RSD) intra-day ($n = 3$) analysing by triplicate samples spiked at the same concentration the same day. The reproducibility between days was obtained as the %RSD inter-day ($n=3$) analysing samples ($n=3$) spiked at the same concentration during different days ($n=3$).

## 3. Results and discussion

### 3.1. Synthesis of the sol-gel mixed-mode zwitterionic sorbents

Many environmental and biological samples simultaneously contain neutral, acidic and basic analytes. If all the analytes are of interest, the separation and preconcentration of these compounds pose serious analytical challenges. One way to solve this analytical challenge is to create a mixed-mode zwitterionic sorbent by incorporating a neutral carbon chain, a cation exchanger, and an anion exchanger into a single sorbent. To maintain the cations and anions in their charged state at full pH range, the cation exchanger and anion exchanger should be strong so that they maintain their ionic state at all pH levels. Octadecyl silane is the most prevalent sorbent in SPE. Octadecyl trimethoxysilane was therefore chosen as the neutral sorbent. To include a SCX in the sorbents, 3-mercaptopropyl trimethoxysilane, which generates propyl sulfonic acid after oxidation, was used. N-trimethoxysilyl propyl N,N,N-trimethyl ammonium chloride and 4-(trimethoxysilylethyl) benzyl trimethyl ammonium chloride were used as SAX. To incorporate these functional groups into the silica network, sol-gel synthesis, which is considered a popular, environment-friendly and facile synthesis approach, was used. Sol-gel synthesis can be performed under acidic or basic catalysis or acidic hydrolysis followed by condensation in basic environment. Acidic hydrolysis followed by basic condensation renders the sol-gel network stronger and more porous [26]. Moreover, to facilitate synthesis, the sol-gel process enables the creation of sol-gel sorbent particles or surface coating in situ at room temperature. Propyl sulfonic acid was obtained after post-gelation treatment of the sorbent with 30% hydrogen peroxide (impregnated with 0.1 M sulphuric acid). The creation of sol-gel mixed-mode zwitterionic sorbents is a new milestone in separation science.

### 3.2. Characterization of sol-gel silica based mixed mode zwitterionic sorbents

All the sorbents were subjected to characterization using Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). However, as the results provided were quite similar, we only present the results of the tests performed with $SiO_2$-SAX/SCX. FT-IR spectra reveal valuable information regarding the functional composition of the building blocks and their successful integration into the final composite material. SEM images, on the other hand, shed light on the surface morphology of the composite material.

#### 3.2.1. Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra of the individual building blocks, methyl trimethoxysilane (MTMS), octadecyl trimethoxysilane ($C_{18}$-TMS), 3-mercaptopropyl trimethoxysilane (3-MPTMS), N-trimethoxysilyl N,N,N-trimethyl ammonium chloride (TMTAMC) and the sol-gel mixed mode zwitterionic sorbent are presented in Fig. 2(a–e), respectively. All FT-IR spectra were collected over a range between 3000 and 700 $cm^{-1}$ at a resolution 4 $cm^{-1}$.

FT-IR spectra of MTMS (Fig. 2a) displays several signature bands at 1266 and 789 $cm^{-1}$ which are attributed to the vibration of $CH_3$ group connected to Si on the precursor molecule. The peaks at 1077 and 1189 $cm^{-1}$ are attributed to C-O stretching vibration Si-O-$CH_3$. The peaks at 2842 and 1464 $cm^{-1}$ are attributed to C-H stretching and bending vibration of Si-O-$CH_3$, respectively [27]. The noteworthy peaks in the C18-TMS spectra 2922 $cm^{-1}$ and 2852 $cm^{-1}$ which can be assigned to antisymmetric [$\nu_a$ ($CH_2$)] and symmetric [$\nu_s$ ($CH_2$)] bands for the alkene chains of $C_{18}$-TMS. The FT-IR spectra of 3-mercaptopropyl trimethoxysilane demonstrate signature band at 2560 $cm^{-1}$ that can be attributed to S-H stretching [28]. The bands at 1187 and 1080 $cm^{-1}$ are related to $-CH_3OCH_3$ [28]. The signature band in N-trimethoxysilyl N,N,N-trimethyl ammonium chloride FT-IR spectra includes 1480 $cm^{-1}$ that can be attributed to N-$CH_3$ bending vibration [29]. It is important to note that all precursors have a common end consisting of -Si $(CH_3)_3$. As a result, many spectral bands are common. The FT-IR spectra of sol-gel $SiO_2$-SAX/SCX include many bands such as 1505, 1441, 1314, 1061, and 778 $cm^{-1}$ that also appeared in the FT-

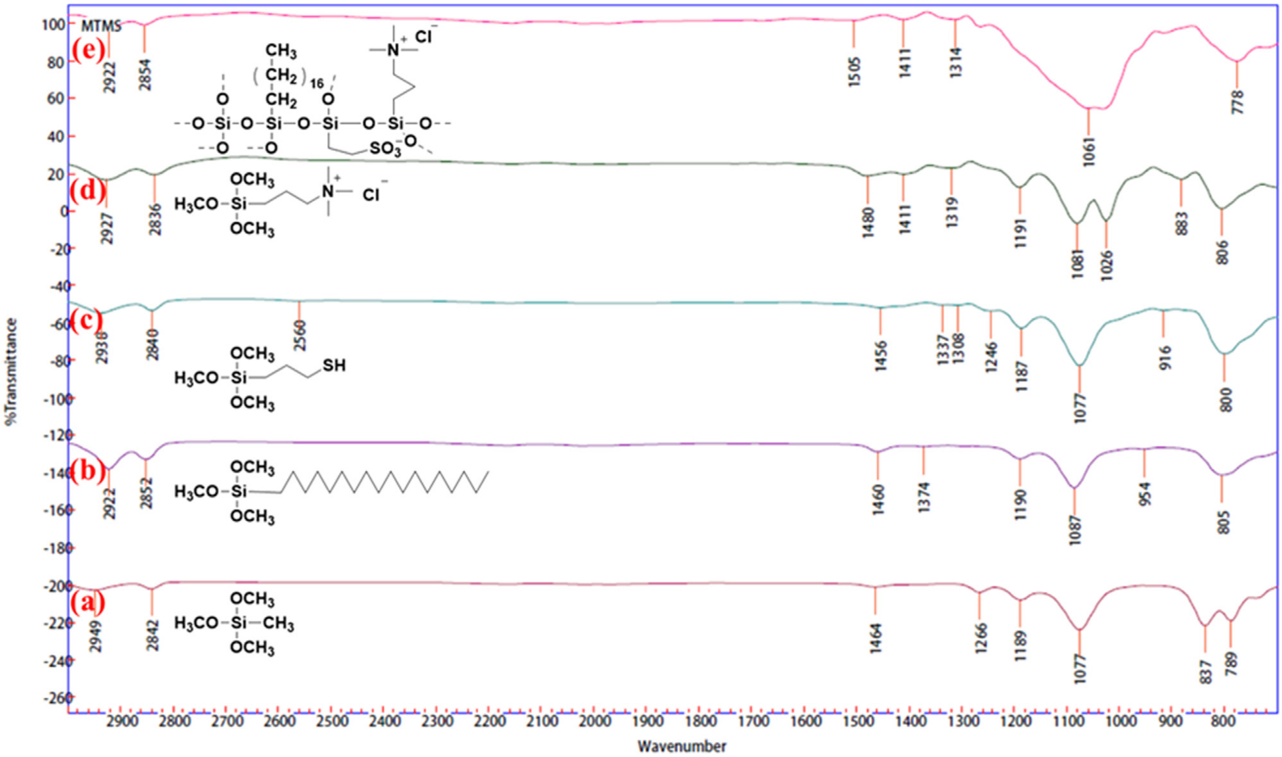

**Fig. 2.** FT-IR spectra of (a) methyl trimethoxysilane; (b) octadecyl trimethoxysilane; (c) 3-mercaptopropyl trimethoxysilane; (d) N-trimethoxysilyl N,N,N-trimethyl ammonium chloride; (e) sol-gel mixed-mode zwitterionic sorbent.

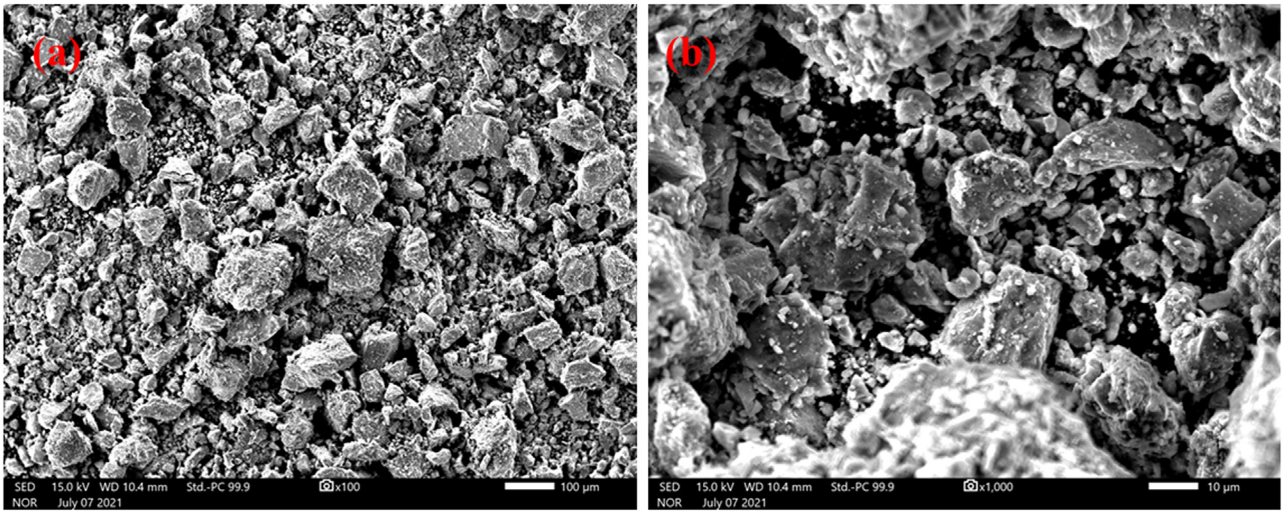

**Fig. 3.** Scanning electron microscopy of sol-gel $SiO_2$-SAX/SCX sorbent at (a) 100x magnifications ; (b) 1000x magnifications.

IR spectra of individual building blocks which manifests successful integration of the building blocks into sol-gel $SiO_2$-SAX/SCX.

### 3.2.2. Scanning Electron Microscopy (SEM)

The surface morphology of the sol-gel $SiO_2$-SAX/SCX was investigated using a Scanning Electron Microscope. The SEM images are presented in Fig. 3(a, b) at 100x and 1,000x magnifications, respectively. The SEM images revealed that the particle sizes are not homogeneously distributed and possess irregular shapes. Some particles of the $SiO_2$-SAX/SCX are in sub-micron size while others are bigger, in the range of 50-60 micron (gross estimation). The surface of the particles apparently look rough that should enhance the interaction between the particles and the analytes during the extraction process. The broad range of particle size distribution also helps reducing the void volume due to the close packing of the sorbents.

## 3.3. Optimization of the SPE procedure

The SPE procedure was optimized using a mixture solution of standards prepared in ultrapure water. The analysis was performed using LC-DAD.

### 3.3.1. Extraction performance evaluation of the sorbents

Since the functionalization of the sorbents evaluated was based on strong ionic interactions, they will always be charged at any pH. To select the initial pH, the $pK_a$ of the compounds was therefore considered (Table 1) and it was set at 5 (pH at which the acidic

**Table 1**
$pK_a$ of the compounds and recoveries performed by each sorbent at initial conditions (see text).

| Compounds | | $pK_a$[a] | S-type | | C-type | |
|---|---|---|---|---|---|---|
| | | | $SiO_2$-SAX/SCX | $SiO_2$-SAX/SCX(Ph) | $SiO_2$-C-SAX/SCX | $SiO_2$-C-SAX/SCX(Ph) |
| Bases | ATE | 9.6 | 94 | 72 | 96 | 79 |
| | RAN | 8.2 | 93 | 87 | 90 | 54 |
| | TRI | 7.1 | 97 | 82 | 89 | 68 |
| | MTO | 9.7 | 96 | 81 | 87 | 50 |
| | VEN | 10.1 | 92 | 84 | 83 | 38 |
| | PRO | 9.4 | 98 | 70 | 94 | 57 |
| Acids | BEZ | 3.8 | 98 | 86 | 80 | 20 |
| | VAL | 3.6 | 87 | 90 | 74 | 30 |
| | FEN | 4.5 | 100 | 74 | 88 | 37 |
| | FLB | 4.4 | 101 | 45 | 92 | 0 |
| | CLO | 3.2 | 91 | 71 | 82 | 29 |
| | DICLO | 4.1 | 82 | 43 | 53 | 8 |
| | NPX | 4.1 | 103 | 89 | 86 | 16 |

*RSD (%) $< 10\%$ ($n = 3$).

[a] $pK_a$ values obtained from PubChem for all compounds except for BEZ, FLB and CLO (values obtained from Drugbank).

and basic compounds were charged). The conditions of loading volume and elution were based on a previous study reported by our group [23] that analyzed acidic and basic compounds using a weak zwitterionic sorbent. These conditions were: 25 mL of loading volume and an elution step with 5 mL of 5% AcOH in MeOH to elute the acidic compounds; and 5 mL of 5% $NH_4OH$ in MeOH to elute the basic compounds.

The four sorbents were initially tested to discern which ones provided the highest recoveries. The four sorbents had the same functionalization, with sulfonic groups to perform SCX interactions and quaternary amines to perform SAX interactions. The difference between these sorbents was the support since some were based on the silica network (S-type), while for others the silica network was embedded with activated carbon microparticles (C-type). Each group had two variants: one in which the SAX groups were bonded through a propyl group to the silica network ($SiO_2$-SAX/SCX and $SiO_2$-C-SAX/SCX), and another which had a phenylethyl group between the silica network and the SAX groups ($SiO_2$-SAX/SCX(Ph) and $SiO_2$-C-SAX/SCX(Ph)).

As Table 1 shows, the sorbents that provided the greatest recoveries were $SiO_2$-SAX/SCX and $SiO_2$-C-SAX/SCX. The recoveries of sorbents $SiO_2$-SAX/SCX(Ph) and $SiO_2$-C-SAX/SCX(Ph) were significantly lower than those of $SiO_2$-SAX/SCX and $SiO_2$-C-SAX/SCX. Adding the aromatic ring seemed to hamper interactions between the compounds and the ionic exchange groups, thus resulting in lower recoveries. Sorbents $SiO_2$-SAX/SCX(Ph) and $SiO_2$-C-SAX/SCX(Ph) were therefore discarded, and the subsequent tests were performed with $SiO_2$-SAX/SCX and $SiO_2$-C-SAX/SCX.

Moreover, by comparing the S-type and C-type sorbents it can be observed that the S-type sorbents presented higher recoveries than the C-type sorbents. For example, DICLO presented a %R of 82% with $SiO_2$-SAX/SCX and 53% with $SiO_2$-C-SAX/SCX.

### 3.3.2. Optimization of the loading pH

As we explained in the Introduction, the control of pH is important when evaluating these sorbents, thus, the first parameter to be evaluated was the pH of the loading solution, which governs the retention of the compounds. Since the sorbents were based on strong ion-exchange interactions, they were charged at any pH. The loading pH was therefore used to control the chargeability of the analytes.

As has been highlighted in Section 3.3.1., pH 5 was initially selected since in this range all compounds were charged considering the $pK_a$ of the analytes. Moreover, a cleaning step of 2 mL was also introduced to check whether the compounds were being retained through ionic interactions. As can be observed in Fig. 4, where results of $SiO_2$-SAX/SCX are presented, good recoveries are obtained for basic compounds, only ATE and RAN provided %R below 80%. However, the acidic compounds provided low recoveries.

Then, pH 3 and 9 were evaluated to promote the specific ionic interactions in each range; at pH 3, the cationic interactions displayed by the basic compounds and at pH 9, the anionic interactions by the acidic compounds. Attending to Fig. 4, it can be observed that the recoveries of the basic compounds improved with pH 3, achieving recoveries higher than 80% for the six compounds. However, at pH 9, the recoveries of the acidic compounds did not improve. pH 4 was evaluated since the best results were obtained at pH 3 and we considered interesting to test this pH. As can be observed in Fig. 4, the results for basic compounds were slightly better than pH 5 and slightly worse than pH 3. The good recoveries were explained since at these pHs, the analytes were protonated and therefore able to interact with the sorbent through ionic interactions. The low recoveries obtained for the acidic compounds suggested that retention occurred only via hydrophobic interactions since these compounds were eluted from the sorbent when MeOH was applied, meaning that the SAX interactions did not work.

The optimization of the pH was performed with $SiO_2$-SAX/SCX and $SiO_2$-C-SAX/SCX. Although Fig. 4 shows the results obtained from the pH evaluation with $SiO_2$-SAX/SCX both sorbents provided similar results, being the %R of $SiO_2$-SAX/SCX slightly higher.

Jin et al. [16] also observed that only basic compounds were retained via ionic interactions. These authors evaluated a homemade mixed-mode zwitterionic sorbent based on weak interactions grounded in carboxylic acids and secondary amines to determine a group of acidic, basic and neutral compounds with a loading pH of 6.

Given the zwitterionic nature of the sorbents, the loading pH should have been closer to the neutral pH used by Jin et al. [16], who chose a loading pH of 6 to determine basic antidepressants in aquatic products using a homemade zwitterionic mixed-mode sorbent functionalized with carboxylic acids and secondary amines. The above authors observed that the acidic compounds were not retained through ionic exchange interactions [16]. A similar explanation can be adapted in our study, in which all the acidic compounds presented aromatic rings that tended to interact with the $C_{18}$ chains through hydrophobic interactions.

When the clean-up step was included, the behavior of the sorbents was therefore closer to a cationic exchanger than to a zwitterionic exchanger. As occurred in previous studies [30,31] that evaluated SCX sorbents to selectively determine basic compounds from aqueous samples and selected a pH in the acidic range, the loading pH for our study was acidic.

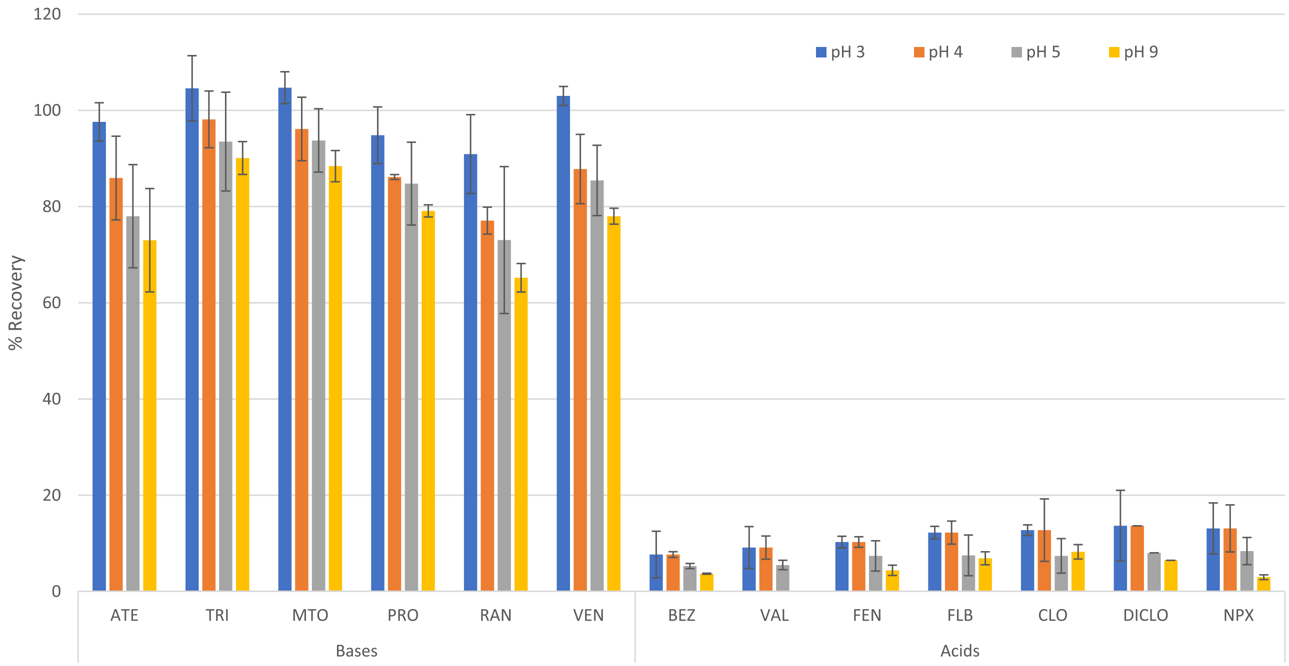

**Fig. 4.** Comparison of the recoveries obtained at pH 3, 4, 5 and 9 with the $SiO_2$- SAX/SCX sorbent.

### 3.3.3. *Optimization of the clean-up step*

A clean-up step is needed to remove the interferences and to increase the selectivity of the method. In the previous section, we introduced a clean-up with 2 mL of MeOH. We then used 5 mL of MeOH to test whether the cleaning volume could be increased without the recoveries being affected, thereby enhancing the selectivity of the extraction.

Table 2 shows results when 25 mL of sample was loaded at pH 3 with or without a clean-up step (2 or 5 mL). When this clean-up step (2 or 5 mL of MeOH) was applied, both sorbents showed the same performance, with recoveries for the basic compounds above 80% and those for the acidic compounds below 10%.

As we mentioned earlier, the results for acidic compounds proved that these compounds were retained through hydrophobic interactions since they were removed from the sorbent with MeOH. On the other hand, the basic compounds were retained through ionic exchange interactions since they were not eluted during the clean-up step.

**Table 2**
Recoveries obtained when 100 mL of ultrapure water were loaded without cleaning and cleaning with 2 and 5 mL of MeOH clean.

| | | $SiO_2$-SAX/SCX | | | $SiO_2$-C-SAX/SCX | | |
|---|---|---|---|---|---|---|---|
| | | No clean | 2 mL | 5 mL | No clean | 2 mL | 5 mL |
| Bases | ATE | 94 | 95 | 99 | 96 | 95 | 98 |
| | RAN | 93 | 84 | 84 | 90 | 100 | 86 |
| | TRI | 97 | 99 | 99 | 89 | 104 | 100 |
| | MTO | 96 | 104 | 101 | 87 | 92 | 100 |
| | VEN | 92 | 94 | 92 | 83 | 84 | 88 |
| | PRO | 98 | 92 | 89 | 94 | 94 | 86 |
| Acids | BEZ | 98 | 7 | 2 | 80 | 2 | 0 |
| | VAL | 87 | 3 | 4 | 74 | 1 | 0 |
| | FEN | 100 | 6 | 2 | 88 | 3 | 2 |
| | FLB | 101 | 8 | 4 | 92 | 6 | 5 |
| | CLO | 91 | 8 | 7 | 82 | 7 | 7 |
| | DICLO | 82 | 11 | 2 | 53 | 0 | 0 |
| | NPX | 103 | 10 | 4 | 86 | 4 | 2 |

* RSD (%) < 10%. ($n = 3$).

The clean-up step was set at 5 mL of MeOH since there was no evident decrease in the recoveries when the volume was increased from 2 mL to 5 mL. Moreover, this increase would help to increase selectivity. It is common to use MeOH to perform the clean-up step when working with mixed-mode ion-exchange sorbents to disrupt the hydrophobic interactions and promote selectivity. Using 5 mL has been reported in a study with a homemade mixed-mode SCX sorbent [32]. In another study [23], the volume was set at 1 mL to reduce the loss of analytes in the determination of illicit drugs, sweeteners and pharmaceuticals using a homemade mixed-mode ion-exchange zwitterionic sorbent based on weak ionic interactions.

Other studies, on the other hand, have reported a clean-up step not fully based on MeOH. Hu et al. [33], for example, performed this step with 2 mL of a mixture of water/MeOH (95/5, v/v) when using a modified silica sorbent with a triazine to determine anthraquinones in urine, which could not be enough to produce a remarkable clean-up effect. Therefore, an aqueous clean-up was not evaluated in this study and a clean-up step with 5 mL of MeOH was selected.

### 3.3.4. *Optimization of the elution*

Initially, the elution was conducted in two steps: an acidic step (5% AcOH in MeOH) to elute the acidic compounds and a basic step (5% $NH_4OH$ in MeOH) to elute the basic compounds. Since the acidic compounds are eluted just with MeOH, the AcOH was not needed, and the acidic step was then removed.

After testing 5% $NH_4OH$ in MeOH in a previous section, the two options tested were 5 mL of 10% $NH_4OH$ in MeOH and 10 mL of 5% $NH_4OH$ in MeOH. All three options provided similar results: 85-100% for the $SiO_2$-C-SAX/SCX sorbent and 90–105% for the $SiO_2$-SAX/SCX sorbent. The first option was therefore chosen since it is greener and generates a lower volume to evaporate.

This elution has previously been used in some studies to elute basic compounds [20,23,31] from mixed-mode ion-exchange sorbents. Moreover, when Salas et al. [20] studied combinations of commercial cation and anionic exchangers, the authors also began with elution in two steps, i.e. an acidic step based on 5% AcOH in MeOH and a basic step with 5% $NH_4OH$ in MeOH. However, during the optimization process it was observed that all compounds were eluted in the basic step.

### *3.3.5. Optimization of the loading volume*

The final step in the optimization process was the loading volume. The larger the loading volume, the higher the preconcentration factor, though the breakthrough volume should also be taken into account.

The initial volume was 25 mL, while 100, 250 and 500 mL were also tested with standard solutions. Every volume showed good recoveries for both sorbents (80–100% for $SiO_2$-C-SAX/SCX and 85–105% for $SiO_2$-SAX/SCX). The results were therefore good even with 500 mL with standard solutions.

Then, 100 and 250 mL were evaluated with spiked river samples to select the loading volume with river samples. As Fig. S1 shows, a significant decrease in the recoveries occurred when the volume was increased from 100 to 250 mL. The volume selected with river samples was therefore 100 mL. Klan et al. [30] tested 100, 200, 500 and 1000 mL and obtained satisfactory results for most of their analytes when analysing river water samples. However, a significant decrease in %R was observed in the most polar compounds. The authors considered the increase in time inherent to the increase in volume. They also considered the possibility that the cartridge would get clogged and decided to select 200 mL as the loading volume.

When working with effluent wastewater samples, recoveries were low with 100 mL. The loading volume was therefore reduced to 50 mL, which led to satisfactory recoveries (44–78%). Gilart et al. [32] also evaluated the loading volume and found that 500 mL presented good recoveries in standard solutions. For effluent wastewater samples, however, they also had to reduce the volume to 50 mL.

## *3.4. Validation of the method*

The method was validated for river water samples and effluent wastewater from treatment plants using LC-ESI-MS/MS to improve sensitivity and selectivity. The chromatographic method was transferred to LC-MS/MS, which enabled work at lower concentrations. The parameters of gas temperature, gas flow rate, nebulizer pressure and capillary voltage were optimized experimentally. Gas temperature was evaluated between 200 and 400 °C; gas flow rate between 6 and 14 mL/min; nebulizer pressure between 20 and 60 psi; and capillary voltage between 2500 and 5000 V. For each compound, the fragmentor potential was also evaluated between 50 and 200 V. The collision energy (CE) was evaluated between 0 and 30 eV. The conditions selected are shown in Section 2.5 and Table S1. For all fragments, the CE ranged from 15 to 25 eV, except for VEN, which ranged from 7 to 5 eV.

The instrumental linear range was 0.5–250 µg/L for most compounds. The $R^2$ was above 0.995 for all compounds except MTO, whose $R^2$ was 0.992. The instrumental LOD and LOQ were 0.1 µg/L and 0.5 µg/L, respectively for all compounds except VEN, whose limits were 0.05 and 0.1 µg/L, respectively.

Before validating the method, the best performing sorbent was selected. Since both sorbents provided good results during the optimization of the SPE procedure, to select one of them, they were tested in terms of apparent recovery (%$R_{app}$), when river samples were spiked at 200 ng/L. To calculate the apparent recovery correctly, a blank was measured to subtract the signal of the analytes naturally present from the signal of the spiked samples. As Table 3 shows (and as we highlighted during the optimization procedure), the $SiO_2$-SAX/SCX sorbent showed higher %$R_{app}$ for the spiked river samples (with values ranging from 60 to 78%), while the results for the $SiO_2$-C-SAX/SCX sorbent ranged from 47 to 62 % (except for ATE, whose %$R_{app}$ were 40 and 34%, respectively). The sorbent chosen to validate the method was therefore $SiO_2$-SAX/SCX.

**Table 3**
%$R_{app}$ obtained with each sorbent when 100 mL of river samples spiked at 200 ng/L was extracted.

| Compound | $SiO_2$-SAX/SCX | $SiO_2$-C-SAX/SCX |
|---|---|---|
| ATE | 40 | 34 |
| RAN | 78 | 62 |
| TRI | 71 | 58 |
| MTO | 66 | 55 |
| VEN | 73 | 36 |
| PRO | 60 | 47 |

[*] RSD (%) < 10%. ($n = 3$).

The addition of carbonaceous particles into the sol-gel composite sorbent increased the overall surface area of the composite sorbent but decreased the absolute loading of the sol-gel silica sorbent, and consequently, the overall interaction sites. It is evident from the recovery data that the sorption feature of the carbonaceous particles in the composite sol-gel sorbent played no role in the extraction process. In a future project, we intend to investigate the impact of carbonaceous particles on other type of molecules. After selecting the best sorbent, river samples were analyzed to perform the validation, according to the parameters described in Section 2.6, in terms of recovery at two concentrations (50 ng/L and 200 ng/L), matrix effect, linear range, method quantification and detection limits (MQL and MDL), repeatability (% RSD, $n$ = 3) and reproducibility between days (% RSD, $n = 3$).

As Table 4 shows, %$R_{app}$ spiking at 50 ng/L were good, i.e. 60–85% for all compounds except ATE, whose %$R_{app}$ were 40%, being similar results to the %$R_{app}$ spiking at 200 ng/L presented in Table 3. These recoveries were comparable to those obtained by Nadal et al. [23] (58%–87%) when determining TRI, MTO and PRO in 100 mL of river samples using a homemade mixed-mode zwitterionic sorbent based on weak ionic interactions. Zhu et al. [13], whose values ranged from 75 to 98%, also obtained slightly higher recoveries when analysing aromatic amines in environmental water samples with a WCX mixed-mode silica-based sorbent. Moreover, Afonso-Olivares et al. [34] obtained recoveries ranging from 78 to 98% when determining pharmaceuticals (ATE among others) in seawater with a commercial sorbent (Oasis HLB).

The %MEs (Table 4) were remarkably low (ranging from -17 to -4 %), which indicates low ion suppression due to the inclusion of a clean-up step with 5 mL of MeOH. These results are lower than those found in other studies, e.g. Krizman et al. [31], who used a commercial sorbent (Oasis MCX) and obtained matrix effects for opioids and their metabolites ranging from -38 to -7%. For their part, Nadal et al. [24] obtained matrix effects ranging from -30 to +5 when using a mixed-mode SAX/WCX sorbent to determine, for example, TRI, MTO, RAN, ATE and PRO.

The linear range was obtained from matrix-matched calibration curves and river samples were spiked from 1 to 500 ng/L. In all cases, determination coefficient ($R^2$) was above 0.99.

Attending to the method quantification and method detection limits (Table 4), in both cases, the values were in the ng/L range, which were comparable to those found in developed methods based on determining those analytes in river samples [23,30,32], whose limits were also in the ng/L range.

The values for repeatability (intra-day precision, $n = 3$) and reproducibility (inter-day precision, $n = 3$) were acceptable (as Table 4 shows, in all cases they were below 16%).

The method was also evaluated in terms of %$R_{app}$, %ME and %RSD with effluent wastewater samples. Since the analytes were present at high concentrations in the effluent wastewater samples, no matrix-matched calibration curves were done. To quantify the

**Fig. 5.** Chromatogram of an effluent wastewater sample when it was analyzed using the developed method.

**Table 4**
Validation parameters for $SiO_2$-SAX/SCX sorbent with river samples.

| Compound | %$R_{app}$ (50 ng/L) | %ME | Linear range (ng/L) | MQL (ng/L) | MDL (ng/L) | % RSD intra-day ($n = 3$) | % RSD inter-day ($n = 3$) |
|---|---|---|---|---|---|---|---|
| ATE | 48 | -16 | 2–500 | 2 | 1 | 8 | 13 |
| RAN | 72 | -9 | 5–500 | 5 | 2 | 9 | 11 |
| TRI | 60 | -14 | 2–500 | 2 | 1 | 7 | 9 |
| MTO | 66 | -14 | 2–500 | 2 | 1 | 10 | 15 |
| VEN | 85 | -17 | 2–500 | 2 | 1 | 9 | 13 |
| PRO | 63 | -4 | 10–500 | 10 | 5 | 11 | 16 |

analytes in real samples, external calibration curves and apparent recoveries were used. The %$R_{app}$ obtained spiking at 200 ng/L ranged from 40 to 71%. Lower concentrations were not evaluated due to the presence of the compounds in the sample. Moreover, spiking at higher concentration was neither evaluated since similar recoveries in river samples were obtained when spiking the samples both at 50 and 200 ng/L.These results are comparable to others reported, e.g. a combination of commercial cationic and anionic exchangers (where the %$R_{app}$ ranged from 50 to 73% [20]), and with a novel SCX sorbent (where the %$R_{app}$ ranged from 39 to 84% [32]). In both cases similar compounds were determined. The %ME obtained ranged from -25% to -18%. These results are comparable to those obtained by Gilart et al. [32] (ranging from -12 and +21%), who used a novel SCX sorbent when determining similar compounds (ATE, PRO, MET, RAN and TRI among others). Moreover, Jaukovic et al. [35] determined cardiovascular drugs (MTO among others) in effluent wastewater samples using a commercial sorbent (Oasis HLB), and obtained higher recoveries ranging from 84 to 106% and higher %ME ranging from -28 to +23%. In all cases, %RSD intraday ($n = 3$) was below 14%.

**Table 5**
Range of concentrations (ng/L) obtained after the analysis of river and effluent wastewater samples through SPE-LC-MS/MS method based on the $SiO_2$-SAX/SCX sorbent.

| Compound | River samples | Effluent wastewater samples |
|---|---|---|
| ATE | <MQL | 234–282 |
| RAN | 24–32 | 848–1233 |
| TRI | 31–39 | 59–642 |
| MTO | 29–67 | 29–113 |
| VEN | 32–72 | 653–970 |
| PRO | <MQL | 48–82 |

* RSD (%) < 15%. ($n = 3$).

### 3.5. Analysis of real samples

Samples from Ebro river and samples from effluent wastewater treatment plants from Reus and Tarragona, Spain were analyzed.

Table 5 shows the occurrence of the compounds in river and effluent wastewater samples. As can be observed, the concentrations of ATE and PRO were below the MQL in the river samples, while those of RAN, TRI, MTO and VEN were found at similar levels of concentration ranging from 24 to 72 ng/L. Nadal et al. [36] also analyzed Ebro river samples and found that the concentrations of ATE and PRO (ranging from 0.7 to 9.5 ng/L) were above their MQL. On the other hand, the concentration of TRI was below its MQL. The concentration of MTO found in the present study was one order of magnitude higher than that found in the study by Nadal et al.: 29–67 ng/L compared to 3–7 ng/L. When Klan et al. [30] determined MTO, PRO, RAN and VEN in river water samples from Slovenia, all compounds were below the MQL except for VEN, whose concentration ranged from 0.08 to 3.01 ng/L.

Regarding the effluent wastewater samples (Fig. 5), all compounds were quantified. RAN and VEN presented the highest concentrations (653–1233 ng/L), while the concentrations of PRO and MTO were lower (29–113 ng/L). These levels were comparable to those quantified previously with similar samples. When Gilart et al. [32] measured ATE, RAN, TRI, MTO and PRO from effluent wastewater treatment plant samples, obtaining concentrations values similar to the ones presented in Table 5. PRO, for example, was found between 50 and 100 ng/L while RAN was found at around 1000 ng/L. Nadal et al. [36], for their part, measured MTO, ATE, PRO and TRI (among others) in effluent wastewater treatment plants and also found similar concentrations to those in Table 5. Moreover, when Iancu et al. [37] quantified ATE and PRO (among others) in effluent wastewater samples from Romania, the concentrations of PRO ranged from 5 to 40 ng/L, which are close to those found in our study (48–82 ng/L). On the other hand, the average concentrations of ATE (94.6 ng/L) was lower than ours (234–282 ng/L).

## 4. Conclusions

In this study, we developed and evaluated four novel mixed-mode zwitterionic-exchange sorbents to determine pharmaceuticals in environmental water samples through SPE followed by LC-MS/MS. It has been shown that the sol-gel approach successfully works for the preparation of a series of mixed-mode zwitterionic silica-based sorbents.

When applying a clean-up step to achieve selective extraction, only basic compounds were retained through ion-exchange interactions, whereas acidic compounds were mainly retained by reversed-phase interactions. A method based on the best-performing sorbent was successfully developed to selectively determine the basic drugs in environmental samples. The application of this method in environmental samples is therefore interesting due to the low matrix effect achieved thanks to the clean-up step.

The results obtained in this study, especially when it comes to the low matrix effect, are encouraging for the determination of basic compounds in complex samples. Moreover, the sorbents used could be interesting for extracting other compounds in the future and applying them to other matrices.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## CRediT authorship contribution statement

**Alberto Moral:** Investigation, Resources, Validation, Writing – original draft. **Francesc Borrull:** Supervision, Funding acquisition. **Kenneth G. Furton:** Supervision, Conceptualization. **Abuzar Kabir:** Project administration, Conceptualization, Methodology. **Núria Fontanals:** Project administration, Conceptualization, Writing – original draft. **Rosa Maria Marcé:** Methodology, Writing – original draft, Writing – review & editing.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.463237.

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