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# Selective monitoring of acidic and basic compounds in environmental water by capsule phase microextraction using sol-gel mixed-mode sorbents followed by ~~LC-MS/MS~~liquid chromatography-mass spectrometry in tandem

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## Highlights

- Development of novel sol-gel mixed-mode MECs.
- Evaluation of mixed-mode ion-exchange MECs by CPME.
- Highly selective extraction of acidic or basic analytes.
- Satisfactory recoveries from optimised CPME parameters.
- Successful application in the extraction of ionisable compounds from environmental samples.


## ABSTRACT

In addition to the diverse extraction techniques available, capsule phase microextraction (CPME), which ~~is performed by means~~uses ~~of~~ a microextraction capsule (MEC), has recently been introduced as a sorptive-based sample preparation technique. In this study, two different MECs (MEC-C18/SAX and MEC-C18/SCX) based on mixed-mode ion-exchange technology were synthesized and evaluated for the selective extraction of a group of ionizable compounds, including acidic and basic analytes. A sulfonic acid was used as the cation-exchange group in MEC-C18/SCX, and a quaternary amine as the anion-exchange group in MEC-C18/SAX. The extraction parameters optimized were sample pH, elution solvent, sample/elution volume and extraction/elution time. The optimized CPME ~~–~~method followed by LC-MS/MS was used to determine the ionizable compounds in environmental water samples, including river water and effluent wastewater, with excellent selectivity and matrix effect values below -30% (except -47% for methadone) and apparent recovery results ranging from 40% to 69%, except for ibuprofen (< 35%) and atenolol (< 25%). The analytical method was validated for environmental water samples, and used in the analysis of several samples in which some of the target compounds were found at ng $L^{-1}$ concentration levels.

## 1. Introduction

Sample treatment and extraction techniques, especially those involving miniaturization like microextraction techniques, have recently become the focus of much attention. Microextraction techniques involve non-exhaustive miniaturized sample preparation techniques which, using high preconcentration factors, employ a small volume of the extracting phase relative to the sample volume, and offer multiple other advantages. They therefore represent an important contribution to the enhancement of sample preparation performance [1,2].

Microextraction techniques are classified depending on the type of extraction media used to extract the analytes: they can be liquid-based if the extraction media is a solvent, such as liquid-phase microextraction (LPME), dispersive liquid-liquid microextraction (DLLME), and hollow fibre liquid-phase microextraction (HF-LPME), among others; or sorptive-based, if the extraction media is a solid or semi-solid material, such as solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), rotating disk sorptive extraction (RDSE), fabric phase sorptive extraction (FPSE) and capillary microextraction (CME), among others [2–5]. Recently, Kabir and Furton introduced a new sample preparation technique known as capsule phase microextraction (CPME) to improve and simplify ongoing sample preparation [6]. CPME is based on the principle of equilibrium extraction and uses a microextraction capsule (MEC) as the extraction medium. The MEC is made up of two fused porous tubular polypropylene membranes, one to accommodate the sorbent through sol-gel technology and the other to encapsulate

a magnetic metal rod. These MECs hold different sorbents, such as the non-polar sol-gel polydimethylsiloxane (PDMS) or sol-gel $C_{18}$ and the polar sol-gel Carbowax 20M. CPME has been used to extract personal care products from environmental water samples [7] and sulfonamides from milk [8].

One of the key considerations in the discussion of sorptive extraction techniques is the range of different materials available. For instance, in SBSE, although PDMS (intended to determine non-polar compounds) is the most frequently used coating and the one that offers the best chemical and mechanical stability, other coatings, such as EG silicone (ethylene glycol-silicone) and PA (acrylate) for polar compounds, have been developed and discretely used to extract analytes from environmental samples [9–11]. However, these polar coatings present some mechanical and thermal stability problems. Aiming to increase the polarity as well as the stability of the coating, several new in-house synthesized materials have been developed [1,12]. Sol-gel technology has become one of the most popular methods for preparing novel coatings for microextraction techniques, and this approach has led to the development of many novel materials with large surface areas and high thermal and solvent stabilities [13–16]. For example, metal organic framework and PDMS coated stir bar were both prepared by means of sol-gel technology [16]. And, it was successfully applied to the extraction of a group of organophosphorus pesticides in environmental water samples. Moreover, other authors have presented strategies to develop polar coatings using the monolithic approach [17,18].

In addition, in the past few years mixed-mode ion-exchange materials using either silica or polymeric-based materials to which ionic moieties are introduced have been progressively applied to different microextraction techniques [19]. Different coatings have been developed using either the sol-gel or monolithic approach [20–23] in order to promote the selective extraction of the ionic compounds (through ionic interactions) while maintaining the reversed-phase interactions between the backbone and the remaining compounds. For instance, Huang et al. [21] designed a novel mixed-mode ion-exchange coating for the determination of quinolones using SBSE. It consisted of a monolithic material synthesized by means of the copolymerization of methacrylic acid-3-sulfopropyl ester potassium salt (MASE) and divinylbenzene (DVB). In the coating, the sulfonic groups promoted ionic interactions with the amino groups of the quinolones and DVB interacted through reversed-phase interactions with the ring alkyl groups and the benzene present in the structure of the quinolones.

Other strategies, involving nanomaterials such as metallic and carbon-based nanoparticles (magnetically modified graphene) and polymer-based nanocomposites combined with mixed-mode polymeric resins have been used as novel coatings for SBSE and have generated increasing interest among researchers exploring sample preparation [24,25].

In the present study, for the first time, two novel mixed-mode ion-exchange materials that combine $C_{18}$ with quaternary amine moieties were developed to achieve strong anion exchange (MEC-C18/SAX) and, with sulfonic moieties, strong cation exchange (MEC-C18/SCX). These MECs were applied in CPME followed by liquid chromatography with mass spectrometry in tandem (LC-MS/MS) for the selective determination of a group of acidic or basic analytes in environmental samples. The CPME parameters including sample pH, sample/elution volume, extraction/elution time and washing/elution solvent were thoroughly optimized to promote selective interactions with the target compounds.

## 2. Experimental part

### 2.1 Materials, reagents and standards

For the preparation of the MECs, Accurel® porous capillary membranes were purchased from 3M Inc. (St. Paul, MN, USA). Cylindrical magnets (1/16" x 3/4") were from K&J Magnetics Inc. (Pipersville, PA, USA). Tetramethoxyorthosilicate (TMOS) and methyl trimethoxysilane (MTMOS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Isopropanol, methylene chloride, hydrochloric acid (HCl), ammonium hydroxide ($NH_4OH$), sulphuric acid ($H_2SO_4$) and hydrogen peroxide ($H_2O_2$) were obtained from Fisher Scientific (Milwaukee, WI, USA). N-trimethoxysilylpropyl-N,N,N-trimethyl ammonium chloride and 3-mercaptopropyl trimethoxysilane (3-MPTMS) were purchased from Gelest Inc. (Morrisvile, PA, USA).

A 2510 Branson Ultrasonic Cleaner (Branson Inc., USA) was used to sonicate sol solutions. A Barnstead NANOPure Diamond (Model D11911) deionized water system (Dubuque, IA, USA) was used to collect deionized water for sol-gel synthesis.

The 14 model compounds selected for this study, including pharmaceuticals, illicit drugs and artificial sweeteners, were potassium acesulfame (ACE), atenolol (ATE), clofibric acid (CLO AC) (clofibrate metabolite), diclofenac (DICLO), fenoprofen

(FEN), ibuprofen (IBP), methadone (MET), metoprolol tartrate salt (MTP), naproxen (NAP), propranolol (PROP), saccharin (SAC) and trimethoprim (TRI), and were all purchased as pure standards from Sigma-Aldrich. Mephedrone hydrochloride (MEP) was supplied by LGC Standards (Luckenwalde, Germany). All standards were of a purity exceeding 96%. The selected compounds and the $pK_a$ are shown in Table ~~1S~~S1.

Stock solutions of individual standards at 1000 mg $L^{-1}$ were prepared in MeOH and stored at -20°C. Working solutions of mixtures of all compounds were prepared weekly in an ultrapure water and MeOH solution (50:50) and were stored in the dark at 4°C. The ultrapure water was provided by a Synergy UV water purification system (Merck Millipore, Burlington, MA, United States) and HPLC-grade MeOH and ACN were purchased from J. T. Baker (Deventer, The Netherlands). Formic acid (HCOOH) and $NH_4OH$ from Sigma-Aldrich and HCl from Scharlab (Barcelona, Spain) were used to prepare the mobile phase and the solutions for the CPME.

### 2.2 Preparation of sol-gel mixed-mode sorbents encapsulated in microextraction capsules

Creating sol-gel mixed mode sorbents in MECs involves several steps: (a) preparing the Accurel S6/2 tubular membranes; (b) preparing the sol solution for the sol-gel mixed-mode sorbents; (c) *in situ* creation of monolithic sorbent bed inside the membrane; (d) aging, conditioning and cleaning the microextraction capsules.

#### 2.2.1 Preparing the Accurel S6/2 tubular membranes

The Accurel® polypropylene S6/2 capillary membranes were first cut into 3 cm pieces. The membranes were then rinsed with methylene chloride and subsequently air dried at room temperature for 30 min. The cleaned membranes were stored in an airtight glass bottle. A cylindrical magnet (2.54 cm) was inserted into one porous tubular membrane, and then the two tubular membranes (one empty and the other containing the cylindrical magnet) were impulse heat sealed (Pasco Inc., Rocky Mount, MO, USA) on both sides. The microextraction capsules were then ready for the *in-situ* creation of sol-gel mixed sorbents.

#### 2.2.2 Preparing the sol solution

The sol solutions for sol-gel mixed-mode sorbents (C18/SAX and C18/SCX) were prepared separately. The sol solution for sol-gel C18/SAX was prepared by sequentially adding the following reagents at the proportion in molar ratio in parenthesis: TMOS(1), MTMOS (1), isopropanol (30), ODSTMS (0.2), N-trimethoxysilylpropyl-N,N,N-trimethyl ammonium chloride (0.2), HCl (0.04) and deionised water (8), in an amber glass reaction bottle. The acid hydrolysis process took place at room temperature for 12 h.

The sol solution for sol-gel C18/SCX was prepared by sequentially adding TMOS (1), MTMOS (1), isopropanol (30), ODSTMS (0.2), 3-MPTMS (0.5), HCl (0.04) and deionized water (8), in an amber glass reaction bottle. The acid hydrolysis process took place at room temperature for 12 h.

### 2.2.3 In situ creation of the monolithic sorbent bed inside the membrane

Next, the hydrolyzed C18/SAX sol solution was transferred to a wide-mouth glass container and $NH_4OH$ was added to this solution in droplets with continuous stirring at a TMOS:$NH_4OH$ molar ratio of 1:0.18. Twenty pieces of MECs were immediately added to the sol solution and the solution was sonicated for 5 min. The sol solution formed a solid gel in 30 min.

The hydrolyzed sol solution for C18/SCX was transferred into another wide-mouth glass container and condensation was initiated by slowly adding $NH_4OH$ at a TMOS:$NH_4OH$ molar ratio of 1:0.18. The sol solution was sonicated in an ultrasound water bath for 5 min. The sol solution formed a transparent gel in 1 h.

### 2.2.4 Conditioning and cleaning the microextraction capsules

Subsequent to the gelation of the sol-gel sorbents, the gels were aged and conditioned at 50ºC for 24 h. The MECs were then cleaned, and the gels adhered the microextraction capsules outside were removed by gently rubbing them against one another. The MECs were then cleaned using a mixture of MeOH:methylene chloride (50:50 v/v) under sonication for 30 min. Next, the MECs were then dried at 50ºC in a neutral environment under continuous helium gas flow. Finally, the MECs with encapsulated sorbents were ready for use.

## 2.3 Capsule phase microextraction conditions

Two different protocols were used: one for MEC-C18/SAX, which retained the acidic analytes and another for MEC-C18/SCX, which retained the basic analytes. Each MEC was first placed in a 50 mL glass vial and conditioned with 10 mL of MeOH for 10 min followed by 10 mL of ultrapure water adjusted to the same pH as the sample.

For MEC-C18/SAX, 25 mL of sample adjusted to pH 7 was extracted by stirring at 600 rpm for 180 min. Then, as the washing step, the MEC-C18/SAX was added to another glass vial containing 3 mL of ultrapure water, and placed in an ultrasonic bath for 1 min. After that, liquid desorption (LD) was performed in an ultrasonic bath using 3 mL MeOH containing 5% HCOOH for 5 min.

For MEC-C18/SCX, 25 mL of sample adjusted to pH 5 was extracted by stirring at 600 rpm. After that, the same washing procedure was applied with 3 mL ultrapure water. LD of MEC-C18/SCX was performed using 3 mL MeOH containing 5% $NH_4OH$ and placed in an ultrasonic bath for 5 min. The elution solutions of each MEC were placed separately in a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) to evaporate the extract until dry, and later it was reconstituted with 1 mL of mobile phase (ultrapure water adjusted to pH 2.8 with HCOOH/ACN, 90/10, v/v). All fractions were filtered with 0.45 µm PTFE syringe filters (Scharlab) before being injected into the chromatographic system. After each use, the MECs were cleaned twice with the corresponding elution solution and two more times with MeOH in the ultrasonic bath for 10 min each, dried and stored in a small glass vial until the next experiment. These MECs can be reused more than 30 times when analyzing environmental samples.

The methods developed were tested using two different types of water: river water from the Ebre River and effluent wastewater from a treatment plant near Tarragona, Spain. Both water samples were filtered through a 1.2-µm glass-fibre membrane filter and through a 0.45 µm nylon membrane filter (Fisherbrand, Loughborough, UK) prior to analysis.

### 2.4 Liquid chromatography-mass spectrometry analysis

An Agilent model 1200 series LC coupled with a 6460 QqQ mass spectrometer (MS/MS) detector with an electrospray ionization (ESI) interface was used. The LC system was equipped with an autosampler, a degasser, an oven and a quaternary pump (Waldbronn, Germany). The optimal mobile phase was a mixture of ultrapure water at pH 2.8 with HCOOH (solvent A) and ACN (solvent B). Two different gradient profiles

were used depending on the compounds analyzed as one MEC selectively extracted the acidic compounds and the other selectively extracted the basic ones. For the acidic compounds, the gradient profile started with 15% of B, which was raised to 85% within 9 min and then to 100% within 4 min. It was subsequently held at 100% for 3 min before returning to the initial conditions after 2 min. The initial conditions were then maintained for another 2 min. For the basic compounds, the gradient profile started with 15% of B, which was raised to 30% within 6 min and then to 100% within 4 min. It was then was held at 100% for 3 min, and returned to the initial conditions after 2 min, which were maintained for another 2 min. The chromatographic column used in both separations was the Tracer Excel 120 $C_8$ (150 mm × 4.6 mm i.d., 5 µm particle size) supplied by Teknokroma (Sant Cugat del Vallès, Spain). The column was maintained at 30°C and the mobile phase flow rate was 600 µL $min^{-1}$. The injection volume was 20 µL.

In the MS/MS, the ESI negative mode was used for the acidic compounds (ACE, SAC, CLO AC, FEN, DICLO, IBP, NAP) and the positive mode for basic compounds (ATE, TRI, MEP, PROP, MET, MTP), as this configuration yielded better ionization in both cases. The optimal parameters for the acquisition of the acidic compounds were a fragmentor voltage of 75 V, a collision energy between 5 and 28 eV, a source gas temperature of 350ºC, a nitrogen flow rate of 12 L $min^{-1}$, a nebulizer pressure of 25 psi and a capillary voltage of 3000 V. For the acquisition of the basic compounds, the optimal conditions were a fragmentor voltage of 100 V, a collision energy between 8 and 22 eV, a source gas temperature of 350ºC, a nitrogen flow rate of 13 L $min^{-1}$, a nebulizer pressure of 60 psi and a capillary voltage of 2500 V. A precursor ion and two product ions were selected for each analyte. The precursor ions were measured for quantification and the product ions and the corresponding ion ratios were used for confirmation purposes in MRM mode (Table ~~1S~~S1). All selected compounds presented good linearity ($r^2$=0.997) in LC-MS/MS and the linear ranges were between 0.05 and 50 µg $L^{-1}$, except for ATE, MTP and PROP, which were between 0.01 and 50 µg $L^{-1}$, and SAC and ~~IBU~~IBP, which were between 1 and 50 µg $L^{-1}$.

## 3. Results and discussion

### 3.1. Development of mixed-mode microextraction capsules

CPME offers unique advantages in separation science by (a) protecting the sorbents from easy fouling by encapsulating them inside a microporous (0.2 µm pore size) polypropylene membrane, and (b) introducing a built-in bar magnet as an integral part of the device. Figure S1 shows a picture of microextraction tubes that were compared to a US penny (a), and SEM images showing the surface morphology of polypropylene tube at 100x (b) and 10,000x (c) magnifications. The sol-gel sorbents are located inside the tube. Due to the inert nature of the ~~PP~~polypropylene (PP) tube, sol solution does not interact with PP surface. Moreover, ~~Due to~~because of the encapsulation of the sorbent inside a microporous tubular membrane, the device can be directly introduced into the unmodified complex sample. The integration of the magnet into the device design also eliminates potential sample contamination via an external magnet. In addition, MECs can be used and reused many times, which substantially reduces overall sample preparation cost.

The synthesis of sol-gel C18/SCX sorbent involves three distinct steps: (a) the hydrolysis of sol-gel precursors in acidic medium, which is done in the absence of the MECs; (b) the condensation of hydrolysed sol-gel precursors in basic medium in the presence of the MECs, during which the hydrolysed sol-gel precursors rapidly condense to form a 3D network of sol-gel sorbent matrix; and (c) ~~–~~the oxidation of the MECs (after conditioning, cleaning and drying) by means of treatment with 30% $H_2O_2$ for 24 h and 0.05M $H_2SO_4$ for 2 h to oxidise the propyl mercapto ligand into the propyl sulfonic ligand. The sol-gel reaction steps are given in Fig. 1A.

The synthesis of sol-gel C18/SAX sorbent involves two steps: the hydrolysis of sol-gel precursors under acid catalyst and the subsequent polycondensation under basic catalyst. The reactions involved in the creation of sol-gel C18/SAX are provided in Fig. 1B.

### 3.2. Optimization of CPME procedures

To explore the mixed-mode ion-exchange properties of the developed MECs, we needed to carefully optimize the different parameters involved in the CPME procedure so that the MECs could establish both ion-exchange (specific) as well as reversed phase (non-specific) interactions with the ionized and neutral entities simultaneously, and

these interactions could be turned on and off depending on the analytical focus during extraction.

The three different steps of the CPME procedure (i.e. the extraction, washing, and liquid desorption) were optimized in order to achieve the selective retention of the model compounds used. Both coatings, MEC-C18/SAX and MEC-C18/SCX, present strong ionic groups, a quaternary amine and a sulfonic group, respectively, which are charged across the range of the working pH. The model compounds have acidic and basic properties that should establish different interactions with the coating. For this reason, careful attention was paid to the elucidation of the retention mechanisms present during the extraction for each analyte, taking into account the $pK_a$ values of the analytes selected, shown in Table ~~1S~~S1. Monitoring the pH when loading the sample is crucial so that the compounds and the functional groups of the coating are in an ionic state. They can then interact by means of ionic interactions with the ionic groups attached to the coating, in addition to the reversed-phase interactions of the coating. In contrast, in the elution step, the functional groups of the analytes must be in their neutral state using organic solutions at suitable pH conditions to disrupt retention and favour the elution of the analytes, since the coating has strong ion exchangers.

The starting conditions were 25 mL of ultrapure water spiked at 0.5 mg $L^{-1}$ with the mixture of analytes and adjusted to pH 3 for the MEC-C18/SCX and at pH 7 for the MEC-C18/SAX, stirred at 600 rpm for 60 min. Based on previous studies [7,8,26,27], elution was performed with 5 mL of MeOH containing 5% $NH_4OH$ for MEC-C18/SCX and 5 mL MeOH containing 5% HCOOH for MEC-C18/SAX, with stirring for 10 min. During optimization, the elution solutions were not evaporated to dryness. Instead, the MeOH elution solution containing 5% HCOOH was diluted to 10 mL with ultrapure water and the MeOH solution containing 5% $NH_4OH$ was neutralized with HCOOH and diluted to 10 mL with ultrapure water to inject into the chromatographic system. These extracts from the optimization of the CPME parameters were injected into a LC-DAD using a gradient that separates all the compounds in the same analysis.

First, we evaluated the selectivity of all the analytes in both MECs. Under the initial conditions, MEC-C18/SAX showed selectivity towards the acidic analytes with recoveries of between 36% and 97%, while the basic analytes were lost in the loading step and recoveries were below 17%. MEC-C18/SCX, on the other hand, was selective towards the basic analytes with recoveries of 28-56%, and the acidic analytes were

completely lost in the loading step. Therefore, MEC-C18/SAX was selected to extract the acidic analytes and MEC-C18/SCX was selected to extract the basic analytes. The acidic analytes established strong anionic interactions with the quaternary amine in MEC-C18/SAX, whereas the basic analytes established strong cationic interactions with the sulfonic acid in MEC-C18/SCX.

### 3.2.1. Sample pH

Once the compounds for each MEC had been established, the first parameter we investigated was sample pH. MEC-C18/SCX, which contains sulfonic moieties, is negatively charged in the entire pH range, as it is a strong cationic exchanger. At pH values below 6, the basic compounds should be in an ionic state according to their $pK_a$ values (Table ~~1S~~S1). Therefore, pH 3 and 5 were tested for MEC-C18/SCX. For all the (basic) compounds, recovery values of 28-56% were found when loading the sample at pH 3 and 42-81% at pH 5, except for MEP, the recovery values of which remained constant at both pH values. In view of these results, pH 5 was established as the optimal pH for loading samples in MEC-C18/SCX for the subsequent analyses.

For MEC-C18/SAX, which contains quaternary amine moieties and is positively charged in the entire pH range, pH 5 and 7 were tested because acidic compounds should be in an ionic state ($pK_a$ in Table ~~1S~~S1) at pH values above 5. The extraction recoveries of all the (acidic) compounds increased from pH 5 to pH 7. For instance, CLO AC, FEN and DICLO attained extraction recoveries of 64%, 75%, and 82% at pH 5, while the recoveries at pH 7 were 70%, 85%, 97%, respectively. Therefore, pH 7 was designated the optimal pH for loading samples using MEC-C18/SAX for the subsequent analyses.

### 3.2.2. Liquid desorption conditions

Desorption parameters such as type and volume of desorption solvent and desorption time were evaluated to select those most effective for the desorption of the selected compounds. We tested different amounts (5% and 10%) of $NH_4OH$ in MeOH for MEC-C18/SCX and HCOOH in MeOH for MEC-C18/SAX and found that the extraction recoveries of all the compounds remained constant, suggesting that the difference between 5% and 10% of $NH_4OH$ and HCOOH was not significant. Thus, MeOH containing 5% HCOOH for MEC-C18/SCX and MeOH containing 5% $NH_4OH$ for MEC-C18/SAX were selected as desorption solutions.

We also tested an increase in desorption time to 15 min. No improvement was observed in either of the MECs when performing the desorption in the ultrasonic bath for 15 min compared to 10 min. Therefore, 10 min was established as the extraction time.

In addition to the starting volume of 5 mL, 3 mL was also tested as a desorption volume. The recoveries of the basic compounds in MEC-C18/SCX and the acidic compounds in MEC-C18/SAX were maintained when decreasing the desorption volume from 5 mL to 3 mL. Therefore, 3 mL was chosen as the optimal desorption volume for both coatings since the posterior evaporation time to dryness decreases as the desorption volume decreases.

### 3.2.3. Sample volume

The next parameter optimized was the extraction sample volume. We tested 50 mL of sample to explore extracting a higher volume. Using 50 mL as the loading volume, the extraction recoveries of the basic analytes in MEC-C18/SCX decreased from 40-65% to 25-53%. With MEC-C18/SAX, the acidic analytes showed a decrease in recoveries from 40-58% when using 25 mL of sample volume to 29-51% when using 50 mL. Consequently, 25 mL was selected as the loading volume for further experiments with both MECs.

### 3.2.4. Washing conditions

To remove interferents from the matrix and therefore increase selectivity, we included a washing step consisting of 3 mL of a washing solution. MeOH, ACN and ultrapure water were tested as solvents for the washing solution for both MECs. During the washing step, each MEC was placed in an ultrasonic bath for 1 min. As shown in Fig. 2. similar results were achieved for both MECs for all of the compounds when using ultrapure water as a washing solution and when no washing solution was used. For MEC-C18/SCX, the basic compounds attained similar results with ACN and ultrapure water. However, with MEC-C18/SAX, the acidic compounds were partially lost during the washing step with ACN. In addition, with both MECs, all the analytes were partially lost during the washing step when MeOH was used as a washing solvent, resulting in a decrease in extraction recoveries. Consequently, ACN and MeOH ~~was~~ were ruled out as possible washing solvents. It should be mentioned that lower volumes of solvent were not tested because 3 mL is the minimum volume that ensures that the MEC is covered. Thus, in view of the results, 3 mL of ultrapure water was selected as the washing

solution; nonetheless, the effectiveness of a water-based washing step was further studied when working with complex environmental samples (section 3.3).

### 3.2.5. Extraction time

We next studied the parameter of extraction time, examining 30, 60, 120, 180 and 240 min extraction times. The %R obtained in some of the selected compounds when increasing the extraction time from 30 to 180 min are ~~listed~~ shown in Fig. 3, and a similar trend was observed for the remaining compounds. The figure shows the increase of the recovery of the selected compounds ~~analytes present~~ when the extraction time increased~~, resulting in increased recovery in all of the compounds~~.

In both MECs, no change in the recoveries of the analytes was detected when the extraction time was increased from 180 min to 240 min, except for ACE and SAC, which improved their recoveries at 240 min from 46% to 58% for ACE and 50% to 66% for SAC. As a compromise, 180 min was selected as the optimal extraction time for the subsequent analyses.

The CPME recoveries of all the selected compounds in both MECs are summarized in Table 1. The acidic compounds achieved recoveries from 60 to 80%, except the strong acidic compounds, ACE and SAC, which achieved recoveries of 46% and 50%, respectively. Among the basic analytes, MET, MTP and PROP attained good recoveries (77%, 59% and 74%, respectively), although lower recoveries were achieved for ATE and TRI (27% and 45%, respectively). In any case, these recoveries are satisfactory considering that CPME is an equilibrium technique, and not an exhaustive technique. Other equilibrium techniques such as SBSE and dynamic fabric phase sorptive extraction (DFPSE) have also been used to determine these compounds [28,29]. For instance, Aparicio et al. [28] developed a method based on SBSE to determine pollutants of environmental concern, including 14 pharmaceuticals. They tested two stir bar coatings: the classic PDMS coating and the novel EG-silicone coating. The novel EG-silicone coating yielded better results for pharmaceuticals, with recoveries of 55% for DICLO, 45% for CLO AC, 43% for PROP, and 15% for TRI. These recovery rates are lower than those achieved in our study, even though their extraction time was 24 h compared to the 180 min used in our study. In the study by Lakade et al. [29], ionizable compounds such as PROP and DICLO were extracted from environmental water samples by means of DFPSE using sol-gel Carbowax 20M (polar) as a coating. In that

study, the recoveries obtained for PROP were around 30% and for DICLO approximately 85%, whereas in our study recoveries of 74% and 79% were achieved for these compounds, respectively.

### 3.3. Application to environmental water samples and method validation

After the ~~optimised~~optimization, CPME methods were applied ~~with~~ to environmental water samples consisting of river and effluent wastewater samples. At this point, it is important to mention that in order to enhance the sensitivity of the method, the extracts from the LD were evaporated to dryness and reconstituted with 1 mL of mobile phase. No loss of analytes was observed during the evaporation step. In addition, two of the most polar compounds, namely ACE and SAC, were not included in the subsequent application to environmental samples because their recovery rate was low and they were present at high levels in most of the environmental samples analysed.

In a first step, as mentioned in section 3.2.4, the effectiveness of including a washing step using 3 mL of ultrapure water was assayed in effluent wastewater samples. The matrix effect (%ME) was compared after both procedures (including the washing step or not) in effluent wastewater. A blank sample of effluent wastewater was analyzed, and the signal obtained was subtracted from the signal of the spiked sample in order to calculate the %ME. The %ME was calculated using the formula $\%ME = (C_{exp}/C_{theo} \times 100\%) - 100\%$, where $C_{exp}$ is the concentration of the analytes when the extract was spiked with the analyte mixture after the CPME procedure and $C_{theo}$ is the concentration of the standard solution. The %ME obtained can be in form of enhancement if it constitutes an increase in the signal or suppression if it is a decrease in the signal.

The %ME values when the washing step was included or not in the extraction of effluent wastewater samples are listed in Table 2. As expected, the %ME decreased when a washing step was applied. For instance, a significant decrease ranging from -43% and -69% to -14% and -26% in the %ME was observed for the acidic compounds with MEC-C18/~~SCX~~ SAX and 3 mL of ultrapure water as washing solution. The same happened with MEC-C18/~~SAX~~SCX, where the %ME of the basic compounds slightly decreased when a washing step was applied. For instance, %ME results of -34% for MET decreased to -23%, -39% for ATE decreased to -18%, and -38% for PROP

decreased to -18%. Therefore, a washing step consisting of 3 mL of ultrapure water was included in the process.

We then calculated apparent recovery (%$R_{app}$) at two different concentration levels (0.2 µg $L^{-1}$ and 2 µg $L^{-1}$) and %ME in 25 mL of river water and effluent wastewater samples. The %$R_{app}$ is the recovery of the whole method, calculated as a ratio of ~~from t~~the concentration obtained from a ~~of a~~ sample spiked before the extraction procedure~~,~~ ~~which~~ and the concentration of the ~~pure~~ standard solution. ~~All of t~~The experimental concentrations ~~mentioned~~ were calculated using a calibration curve prepared ~~in pure standard~~ with standard solutions~~was obtained from a calibration curve made of pure standard.~~ . Table 2 shows the %$R_{app}$ and %ME obtained at the lowest concentration level. The %$R_{app}$ of the acidic compounds in river water ranged from 46% to 59%, except for IBP (%$R_{app}$, 35%). The basic compounds in the river sample achieved recoveries between 44 and 68%, except for ATE (25%). In effluent wastewater, the %$R_{app}$ in all the compounds slightly decreased from those obtained in river water, except for DICLO. In addition, the %$R_{app}$ obtained in both samples slightly decreased from the values obtained in ultrapure water due to the %ME present in the samples analyzed.

In both samples, the %ME was acceptable and in the form of ion suppression in the majority of the compounds. The %ME values in MEC-C18/~~SCX~~ SCX were similar in the river and effluent samples. However, slightly higher ion suppression was observed in river water in the %ME of MEC-C18/~~SCX~~ SAX spiked at a lower concentration (Table 2) compared to when it was spiked at a higher concentration (ranging from -7% to -17%). With MEC-C18/~~SAX~~SCX, the %ME at both concentrations showed higher ion suppression in the effluent sample than in the river sample, as expected. MTP and TRI presented ion enhancement at low and high concentration levels in the river sample, as did PROP at high concentration levels in the river sample. Similar %ME results were obtained in previous studies which determined these analytes in complex matrices using other techniques, such as SPE or DFPSE [29,30]. In a study [30] in which SPE with mixed-mode ion-exchange sorbents was used as an extraction technique, %MEs of -21% for CLO AC and -32% for DICLO were reported in river water, which are similar to the values obtained in our study (-18% and -27% for CLO AC and DICLO, respectively). Another study [29] using DFPSE with Carbowax 20M found %MEs of -7% for PROP in river water and -15% in effluent wastewater, comparable values to

512 those observed in our study, which documented %MEs of -13% for this compound in
513 river samples and -18% in effluent samples.

514 We also assessed the method limits (MDLs and MQLs), repeatability, and
515 reproducibility between days in both samples. The MDLs and MQLs of the river and
516 effluent samples were estimated from the instrumental limits (LODs and LOQs),
517 considering the $\%R_{app}$ results and the preconcentration factor, expressed in ng $L^{-1}$. In the
518 river sample, the MDLs ranged between 0.3 ng $L^{-1}$ and 2.2 ng $L^{-1}$ and the MQLs ranged
519 from 0.7 ng $L^{-1}$ to 4.6 ng $L^{-1}$. The exception was ~~IBU~~IBP, whose MDL and MQL were
520 57 and 115 ng $L^{-1}$, respectively, attributed to the high instrumental LODs and LOQs, but
521 also to the low recoveries achieved. In effluent wastewater, MDLs ranged from 0.4 ng
522 $L^{-1}$ to 4.8 ng $L^{-1}$, and MQLs ranged from 0.7 ng $L^{-1}$ to 9.5 ng $L^{-1}$ (except for ~~IBU~~IBP,
523 which were 133 ng $L^{-1}$ and 267 ng $L^{-1}$). The repeatability of the method on the same day
524 and reproducibility between days, expressed as relative standard deviation (%RSD) of
525 five replicates of river and effluent samples spiked at a concentration level of 0.2 and 2
526 µg $L^{-1}$, were less than 21% and 17%, respectively.

527

528 **3.4. Analysis of real samples**

529 The optimized CPME method was employed to determine the model compounds in
530 river and effluent water samples. Three different samples of river water and three of
531 effluent wastewater were analyzed in triplicate. The concentration of the analytes
532 present in the samples were calculated using the external calibration curve and taking
533 into account the $\%R_{app}$ and the concentration factor. Table 3 shows the concentration
534 levels of the selected model compounds found in both samples. All the targeted
535 compounds were detected in both types of sample, except NAP and IBP, due to its high
536 MDLs. As expected, the concentration levels found in the river water samples were
537 lower than those found in the effluent wastewater samples. For instance, MET and TRI,
538 the concentrations of which were lower than the MQLs in the river samples, were
539 quantified in effluent wastewater. However, MEP was present at below the MQLs in
540 both types of sample, and for FEN it was even found at a slightly higher concentration
541 in one of the river samples than in the effluent wastewater samples analysed.

542 The detection of the majority of the selected compounds reported in this work are
543 consistent with those reported in other studies. In a previous study [30] conducted in our

research group in which a similar group of compounds were determined in effluent water from the same sewage treatment plant as in this study, most of the compounds were found at slightly higher concentrations in effluent wastewater than in the present study. Similarly, TRI was found at similar concentration levels and FEN was found below the MQL. In other studies, where other river and wastewater samples were analyzed, NAP was found in quantities below the MQLs [31]. However, in our study NAP was not detected in either the river or the effluent samples. In addition, MTP and PROP were found at similar concentration levels, in both river water and effluent wastewater samples, while DICLO was found at slightly higher concentration levels in other studies [32–35].

## 4. Conclusions

In this study, we successfully pioneered the preparation of mixed-mode ion-exchange MECs using sol-gel technology to accommodate the coating in a porous polypropylene membrane.

The CPME parameters greatly influenced the extraction recoveries of the selected model compounds and a washing step was successfully introduced to the CPME method to reduce the presence of interferents in the matrix and consequently decrease the %ME. MEC-C18/SAX and MEC-C18/SCX were evaluated to selectively retain acidic or basic compounds, respectively, and provided satisfactory extraction efficiency for ionisable compounds.

The method presented here is simple and selective, with promising application for trace analyses in environmental samples such as river water and effluent wastewater samples. The proposed CPME technique using MEC-C18/SAX and MEC-C18/SCX could be expanded to extract other compounds in different samples in the future.

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730

731 Table 1. %R obtained with the MEC-C18/~~SCX~~ SAX and MEC-C18/~~SAX~~ SCX for all
732 the compounds when using the optimized CPME conditions in ultrapure water.

733

| | Analyte | $pK_a$ | %R |
|---|---|---|---|
| MEC-C18/~~SCX~~SAX | ACE | -0.3 | 46 |
| | SAC | 1.6 | 50 |
| | CLO AC | 3.37 | 69 |
| | FEN | 3.96 | 80 |
| | DICLO | 4.00 | 79 |
| | NAP | 4.19 | 78 |
| | IBP | 4.85 | 60 |
| MEC-C18/~~SAX~~SCX | MEP | 8.0 | 56 |
| | MET | 9.1 | 77 |
| | ATE | 9.67 | 27 |
| | MTP | 9.67 | 59 |
| | PROP | 9.7 | 74 |
| | TRI | 10.8 | 45 |

734 % RSD (n=5) <17%

735

Table 2. $\%R_{app}$ and %ME obtained when 25 mL of in river and effluent water samples spiked at 0.2 µg $L^{-1}$ was extracted using MEC-C18/~~SCX~~ SAX or MEC-C18/~~SAX~~ SCX by CPME.

| | | | River | | Effluent wastewater | | |
|---|---|---|---|---|---|---|---|
| | Analyte | ~~pK$_a$~~ | $\%R_{app}$ | %ME | $\%R_{app}$ | %ME | %ME[a] |
| | | | | With washing | | | Without wash |
| MEC-C18/~~SCX~~ SAX | CLO AC | ~~3.37~~ | 59 | -18 | 46 | -21 | -47 |
| | FEN | ~~3.96~~ | 61 | -19 | 47 | -23 | -50 |
| | DICLO | ~~4.00~~ | 60 | -27 | 69 | -26 | -69 |
| | NAP | ~~4.19~~ | 46 | -24 | 21 | -14 | -43 |
| | IBP[b] | ~~4.85~~ | 35 | -12 | 15 | -15 | -50 |
| MEC-C18/~~SAX~~ SCX | MEP | ~~8.0~~ | 44 | -10 | 24 | -33 | -36 |
| | MET | ~~9.1~~ | 53 | -26 | 40 | -23 | -34 |
| | ATE | ~~9.67~~ | 25 | -21 | 22 | -18 | -39 |
| | MTP | ~~9.67~~ | 51 | 8 | 42 | -18 | -21 |
| | PROP | ~~9.7~~ | 68 | -13 | 55 | -18 | -38 |
| | TRI | ~~10.8~~ | 51 | 2 | 43 | -29 | -32 |

[a] Without applying the washing step based on 3 mL of ultrapure water
[b] Spiked at 2 µg $L^{-1}$
% RSD (n=5) <21%

752

753 Table 3. Range of concentration in ng $L^{-1}$ when different river and effluent wastewater
754 samples were analyzed using the CPME/LC-MS/MS developed method.

| | | Concentration (ng $L^{-1}$) | |
|---|---|---|---|
| | Analyte | River water | Effluent wastewater |
| MEC-C18/~~SCX~~SAX | CLO AC | 7-15 | 10-16 |
| | FEN | <MQL-17 | <MQL |
| | DICLO | 23-30 | 7-199 |
| | NAP | n.d | n.d |
| | IBP | n.d | n.d |
| MEC-C18/~~SAX~~SCX | MEP | <MQL | <MQL |
| | MET | <MQL-24 | 44-56 |
| | ATE | 8-45 | 52-847 |
| | MTP | 3-7 | 15-77 |
| | PROP | 3-9 | 59-63 |
| | TRI | <MQL | 321-536 |

755

756

**Figure captions**

**Figure 1.** Reactions involved in different steps in synthesizing sol-gel C18/SCX (A) and sol-gel C18/SAX (B) within the MECs.

**Figure 2**. %R values obtained in the elution step for representative analytes when ultrapure water, ACN, MeOH were used in the washing step and no washing solution were applied.

**Figure 3**. Effect of the extraction time on the recovery of some compounds in the MEC-C18/SCX and MEC-C18/SAX.