Hypercrosslinked polymer microspheres decorated with anion- and cation-exchange groups for the simultaneous solid-phase extraction of acidic and basic analytes from environmental waters

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ABSTRACT

Mixed-mode ion-exchange sorbents were introduced to improve the selectivity and retention of solid-phase extraction (SPE) sorbents,. Mixed-mode ion-exchange sorbents integrate reversed-phase chemistry with ion-exchange groups to promote favourable interactions with ionic species. Nevertheless, a need to extract analytes with acidic and basic properties simultaneously within the same SPE cartridge led to the introduction of novel amphoteric/zwitterionic sorbents, which incorporate cation- and anion-exchange moieties within the same functional group attached to the polymeric network.

In the present study, the development, preparation and SPE evaluation of two novel hypercrosslinked zwitterionic polymeric sorbents, functionalised with either strong anion-exchange (SAX) and weak cation-exchange (WCX) or weak anion-exchange (WAX) and strong cation-exchange (SCX) groups (namely HXLPP-SAX/WCX and the HXLPP-WAX/SCX), is presented for the simultaneous retention of acidic and basic compounds. The sorbents were prepared by a precipitation polymerisation route which yielded poly(divinylbenzene-*co*-vinylbenzylchloride) as a precursor polymer; subsequently, the precursor polymer was hypercrosslinked, to increase the specific surface areas and capacities of the sorbents, and then functionalised to impart the zwitterionic character. The HXLPP-SAX/WCX sorbent was decorated with quaternised sarcosine groups and the HXLPP-WAX/SCX sorbent was decorated with taurine moieties. The SPE parameters were optimised to exploit the ionic interactions between compounds and the functional groups. The optimal conditions involve a washing step to remove the compounds retained by hydrophobic interactions, thus increasing the selectivity. The optimised SPE protocol used the quaternised sarcosine-based sorbent followed by liquid chromatography and tandem mass spectrometry, and was applied to determine compounds with acidic and basic properties from environmental samples, such as river water and effluent wastewater samples, with excellent selectivity and matrix effect values below -30% and apparent recovery results ranging from 52% to 105% for most of the compounds. The analytical method was validated for environmental water samples and used in the analysis of samples in which some of the target compounds were found at ng L^{-1} concentration levels.

1. Introduction

Sample pre-treatment is an essential step in the determination of organic contaminants present at low concentrations in complex samples. This is because it is necessary to not only preconcentrate the contaminants prior to analysis, but also to remove the interferences that are present in complex samples which can lead to signal interference during analysis (*e.g.*, ion-suppression in mass spectrometry (MS) analysis). Sorptive extraction techniques, such as solid-phase extraction (SPE), solid-phase microextraction and stir bar sorptive extraction, can achieve high degrees of preconcentration and selectivity (by decreasing matrix effects (ME%) and thereby improve sensitivity and reproducibility. SPE is the pre-eminent technique due to its simplicity, high preconcentration factors and the availability of a range of distinct sorbents [1–4].

Nowadays, the range of SPE sorbents available is very broad, ranging from sorbents that promote capacity enhancements, such as hypercrosslinked polymers, to sorbents that promote selectivity enhancements, such as molecularly imprinted polymers. However, capacity and selectivity enhancements can be offered in a single material by mixed-mode ion-exchange sorbents [5]. Mixed-mode ion-exchange sorbents can be silica-based or polymer-based, with immobilised functional groups conferring the ion-exchange properties onto the sorbents. The sorbents are classified in four main groups: strong anion-exchange (SAX), strong cation-exchange (SCX), weak anion-exchange (WAX) and weak cation-exchange (WCX). Strong ion-exchangers are charged throughout the entire pH range, and are normally based upon sulfonic acids or quaternary amines to give strong cationic and anionic exchangers, respectively. Conversely, the ionisation state of weak ion-exchangers vary depending upon the pH, and the sorbents are normally functionalised with carboxylic acids or tertiary, secondary or primary amines to give weak cation- and anion-exchangers, respectively [5]. Commercial polymeric mixed-mode sorbents, such as Strata-X-A or Oasis WCX, are available and have been used to extract ionisable analytes [6–8]. Alternatively, commercial silica sorbents have been developed, albeit to a lesser extent, including Bond Elut Certify II among others [9].

Non-commercial, silica-based mixed-mode sorbents have also been developed [10–13]. For instance, Wójciak-Kosior *et al.* [12] developed a polyaniline-based sorbent by the *in situ* polymerisation of aniline on silica; the sorbent was used to extract three alkaline plant metabolites (benzophenanthridine, protoberberine and protopine alkaloids) from *Chelidonium majus* extracts. The protonated polyaniline suggested that retention of the analytes was by π-π and ionic interactions. Excellent recoveries (> 96%) were reported for the compounds investigated.

Regarding non-commercial, polymer-based mixed-mode ion-exchange sorbents, most of them are based on hypercrosslinked polymer networks. Mixed-mode hypercrosslinked materials were developed and evaluated in our group as SPE sorbents for the extraction of ionisable compounds in aqueous samples [14–17]. The fact that they are hypercrosslinked means that they typically have high micropore contents and correspondingly high specific surface areas, which enhances the capacity and leads to an effective retention of analytes when loading samples by SPE [18]. For instance, Bratkowska *et al.* [14] prepared two different hypercrosslinked sorbents (HXLPP-SAXa and HXLPP-SAXb) in a microsphere format and applied these materials as SAX sorbents in SPE for the extraction of acidic analytes from environmental samples.

All of the mixed-mode materials described thus far focus on the retention of one type of ionic species, either cationic or anionic. In order to retain both acidic and basic compounds simultaneously, different strategies have been devised, such as multi-layered and tandem approaches. Studies using various mixed-mode ion-exchange sorbents in tandem for sample clean-up have been published recently [19–22]. For example, the combination of Oasis MAX with Oasis MCX was reported to be the optimal SPE configuration, with excellent recoveries ranging from 91% to 99% [19]. Additionally, Salas *et al.* [22] combined pairs of commercially available mixed-mode sorbents (including SCX/SAX, SCX/WAX, WCX/SAX, and WCX/WAX) bearing opposite charges within one SPE cartridge to achieve simultaneous extractions of acidic and basic analytes with results comparable to other studies. A washing step consisting of 15 mL MeOH was used in the SPE protocol, which proved to be sufficient to remove the neutral interferences.

An alternative strategy towards the simultaneous extraction of acidic and basic analytes is to bring cation- and anion-exchange groups together in a single SPE sorbent, as was reported recently by our group [23]. In this study, a sorbent based on hypercrosslinked polymer microspheres bearing WAX and WCX moieties (HXLPP-WAX/WCX) was developed to extract acidic and basic analytes from environmental waters, where the sorbent displays amphoteric character by virtue of immobilised sarcosine (*N*-methyl glycine) residues. In a separate study, an amphoteric methacrylate-based material with sulfoethylbetaine groups was synthesised and applied as SPE sorbent to retain hydrophilic solutes by the water retention capacity of the sorbent surface [24].

The current paper presents the design, preparation and SPE evaluation of two novel hypercrosslinked polymeric sorbents functionalised with amphoteric groups. The immobilised groups are either quaternised sarcosine groups, in which case the sorbent offers SAX and WCX character and is referred to as HXLPP-SAX/WCX, or taurine residues in which case the sorbent offers WAX and SCX character and is referred to as HXLPP-WAX/SCX. An optimisation of the SPE parameters was conducted, including the elution conditions, washing step and sample volume. An SPE method using the best-performing sorbent was then validated and applied to environmental waters, with quantification by LC-MS/MS.

2. Experimental

2.1. Reagents and standards

The polymer microspheres were synthesised using the vinyl monomers 4-vinylbenzyl chloride (VBC) (90% technical grade) and divinylbenzene (DVB) (80% technical grade), both provided by Sigma Aldrich (St. Louis, MO, USA). The monomers were purified by passing them through a short column of neutral alumina (Sigma Aldrich). The solvents used were toluene (99.3% LabReagent), methanol (MeOH) (≥ 99% analytical specification), acetonitrile (ACN) (99.9% HPLC grade), acetone (≥ 99% analytical specification), dimethylsulfoxide (DMSO) (≥ 99% analytical specification), 1,2-dichloroethane (DCE) (99.8% anhydrous), ethanol (EtOH) (≥ 99.8%) and diethyl ether (99.8% ACS reagent), also purchased from Sigma-Aldrich. Iron (III) chloride (96% anhydrous), purchased from BDH LabSupplies, was employed for the hypercrosslinking reactions. 2,2-Azo*bis*isobutyronitrile (AIBN) (97%), purchased from BDH Lab Supplies (Poole, UK), was recrystallized at low temperature from cold acetone and used as initiator. Sarcosine methyl ester hydrochloride and taurine, sourced from Sigma-Aldrich and of high purity (≥99%), tetra-*n*-butylammonium bromide (TBAB) (>98%) purchased from Alfa Aesar, iodomethane (MeI) (≥ 99%) from Sigma-Aldrich, THF (>99.7%) and potassium hydroxide (KOH), both supplied by VWR International, were employed as received from the suppliers. Tetrahydrofuran (THF) and nitric acid (65%), both supplied by Sigma-Aldrich, potassium carbonate (K_2CO_3) and sodium hydrogen carbonate

($NaHCO_3$), both provided by VWR International (Leuven, Belgium), were the reagents used to prepare the washing solutions.

The compounds selected to evaluate the retention properties were: saccharin (SAC), naproxen (NAP), ibuprofen (IBP), fenoprofen (FEN), diclofenac (DICLO), clofibric acid (CLO AC) (a metabolite of clofibrate) and potassium acesulfame (ACE) as acid compounds; and trimethoprim (TRI), ranitidine (RAN), propranolol (PROP), metoprolol tartrate salt (MTP), methadone (MET), mephedrone hydrochloride (MEP) and atenolol (ATE) as basic compounds. They were all purchased as pure standards with > 99% purity from Sigma-Aldrich, except MEP from LGC Standards. The compounds selected and their pK_a values are shown in **Table 1**.

The main stock solutions of the acidic and basic standards were prepared individually in methanol and stored at -20 °C in the dark. The stock concentration level was 1000 mg L^{-1}. Mixed working solutions including all the compounds were prepared weekly by mixing with proper amounts of each stock individual solution followed by dilution with ultrapure water (50/50, v/v), and kept at 4 °C in the dark.

The solvents used to prepare the mobile phase and the solutions for the SPE procedure were as follows: MeOH and ACN HPLC-grade, supplied by J. T. Baker (Deventer, The Netherlands), ammonium hydroxide (NH_4OH) and formic acid (HCOOH) purchased from Scharlab (Barcelona, Spain). Ultrapure water was provided by a Synergy UV water purification system (Merck Millipore, Burlington, USA).

Six water samples, three from Ebre river water and three from effluent wastewater from a treatment plant near Tarragona, were taken to be analysed. Prior to the SPE protocol, the river and effluent wastewater samples were filtered using a 1.2 μm glass-fibre membrane filter and then using a 0.45 μm nylon membrane filter (Fisherbrand, Loughborough, UK).

2.2. Synthesis and characterisation of the polymer microspheres

The hypercrosslinked polymer-based materials decorated with ion-exchange groups were prepared following a three-step procedure: firstly, the synthesis of poly(DVB-*co*-VBC) microspheres (PP) by the precipitation polymerisation of the monomers DVB and VBC; secondly, the hypercrosslinking of the poly(DVB-*co*-VBC) microspheres to give hypercrosslinked polymer microspheres (HXLPP); thirdly polymer-analogous reactions on HXLPP to yield hypercrosslinked polymer microspheres decorated with ion-exchange groups. The polymer-analogous reactions involved either the reaction of HXLPP with sarcosine methyl ester hydrochloride under basic conditions (to give an HXLPP-WAX material) followed by methylation of the amine and ester hydrolysis to yield the HXLPP-SAX/WCX sorbent, or treatment of HXLPP with taurine to give the HXLPP-WAX/SCX sorbent.

2.2.1. Preparation of poly(DVB-*co*-VBC) microspheres

The synthesis of the hypercrosslinked polymer microspheres was based on a published synthetic method [25]. The monomers DVB (5.027 g, 38.4 mmol) and VBC (15.049 g, 98.4 mmol) (25/75, w/w) were used in a precipitation polymerization to give poly(DVB-*co*-VBC) microspheres, employing ACN (500 mL) as solvent and mixing all the reagents in a Nalgene bottle (1 L). The monomer mixture was introduced in an ultrasonic bath for 15 min. at room temperature (r.t.) and then introduced in an ice-bath and sparged with oxygen-free N_2 for 15 min. AIBN (0.542 g, 3.3 mmol, 2 mol% relative to the number of moles of polymerisable double bonds) was added then the Nalgene bottle sealed under nitrogen and placed on a low-profile roller housed inside a temperature-controlled incubator. The temperature of the incubator was raised from ambient to 60 °C for a period of 2 h and kept at 60 °C for 48 h, whereupon a milky suspension of polymer particles formed. The particles were filtered on a 0.45 µm nylon membrane filter by vacuum, isolated and washed with acetone and ACN and dried overnight *in vacuo* (70 °C, 60 mbar), yielding free-flowing, white poly(DVB-*co*-VBC) microspheres (5.217 g, 26%). A replicate synthesis yielded 6.108 g (30%) of product. See Supplementary Information (SI) for the polymer characterisation data on this intermediate product.

2.2.2. Preparation of hypercrosslinked poly(DVB-*co*-VBC) microspheres (HXLPP)

Poly(DVB-*co*-VBC) microspheres (4.605 g, 20.5 mmol of Cl) and DCE (90 mL) were introduced into a 250 mL round-bottomed, three-necked flask equipped with an overhead stirrer and reflux condenser, and immersed in a temperature-controllable oil bath. The mixture was stirred at 100 rpm for 1 h at r.t. and left to swell in the solvent under N_2. The addition to the flask of a second portion of DCE (70 mL) and iron (III) chloride (3.057 g, 18.9 mmol) was conducted and the mixture was heated to 80 °C and left to react for a further 10 min. After that time, the dark purple mixture was cooled to r.t. and filtered by vacuum on a 0.45 µm nylon membrane filter. The filtered product was washed first with MeOH, 2 M aqueous HNO_3, MeOH and acetone, and secondly with acetone by Soxhlet extraction for 24 h. A 0.45 µm nylon membrane filter was used to filter the product by vacuum and washed again with MeOH and diethyl ether. Then, the particles were dried overnight *in vacuo* (70 °C, 60 mbar) to obtain brown-coloured, free-flowing HXLPP particles (4.325 g). A replicate synthesis used 4.577 g of the second batch of the poly(DVB-*co*-VBC) microspheres and yielded 4.242g of HXLPP product. See SI for the polymer characterisation data on this intermediate product.

2.2.3. Preparation of the HXLPP-SAX/WCX sorbent

The HXLPP particles (2.514 g, 6.064 mmol Cl) were subjected to chemical functionalisation with sarcosine methyl ester hydrochloride to yield an HXLPP-WAX material. Sarcosine methyl ester hydrochloride (3.317 g, 23.8 mmol), HXLPP (2.514 g, 6.064 mmol Cl), sodium hydrogen carbonate (3.980 g, 47.4 mmol) and tetra-*n*-butylammonium bromide (1.850 g, 5.740 mmol) were introduced into a round-bottomed, three-necked flask fitted with an overhead stirrer and a reflux condenser. A temperature-controllable oil bath was used to immerse the flask. Toluene (50 mL) was added into the flask and it was heated at 80 °C for 24 h. The product was cooled and isolated by vacuum filtration on a 0.45 µm nylon membrane filter. Soon after, the mixture was washed with water and acetone, before drying for 24 h *in vacuo* (70 °C, 60 mbar) to obtain free-flowing, orange-coloured HXLPP-WAX particles (2.586 g). See ESI for the polymer characterisation data on this intermediate product.

The tertiary amine groups in the polymer-bound sarcosine methyl ester residues were methylated in order to convert the WAX groups into SAX groups and deliver an HXLPP-SAX material. For this, HXLPP-WAX (2.224 g, 2.891 mmol of sarcosine methyl ester groups) and tetrahydrofuran (50 mL) were added to a round-bottomed, three-necked flask fitted with a reflux condenser and an overhead stirrer, and the flask immersed in a temperature-controlled oil bath. The mixture was stirred at 100 rpm for 30 min at r.t. prior to the addition of MeI (1.731 g, 12.195 mmol), after which time the flask contents were refluxed for 48 h. After allowing the contents to cool to room temperature, the product was filtered by vacuum on a 0.45 μm nylon membrane filter and washed with a 0.1 M aqueous NaOH solution, water, and acetone before drying for 24 h *in vacuo* (70 °C, 60 mbar). HXLPP-SAX was isolated as a free-flowing, orange-coloured powder (2.061 g). See SI for the polymer characterisation data on this intermediate product.

To obtain the HXLPP-SAX/WCX particles, the methyl ester groups of the HXLPP-SAX product were hydrolysed. For this, HXLPP-SAX (1.969 g, 1.2 mmol of quaternised sarcosine methyl ester groups) was placed into a round-bottomed, three-necked flask equipped with a reflux condenser and overhead stirrer, and immersed in a temperature-controllable oil bath. To this, a solution of KOH (4.276 g, 76.4 mmol) in MeOH (100 mL) was added and the suspension was left stirring at r.t. for 24 h at an agitation speed of 100 rpm. The resulting product was filtered using a 0.45 μm nylon membrane filter by vacuum and subsequently washed with large volumes of MeOH and dried overnight *in vacuo* (70 °C, 60 mbar), yielding orange-coloured HXLPP-SAX/WCX particles in their potassium carboxylate form (1.902 g). A reaction scheme outlining the synthetic procedure used to prepare the HXLPP-SAX/WCX sorbent is presented in **Figure 1a**.

Figure 1SA shows the FT-IR spectrum of HXLPP-SAX/WCX with the following bands ($\bar{\nu}$/cm^{-1}): 3012 (aromatic C-H stretch), 2911 (aliphatic C-H stretch), 2850-2700 (broad, quaternary ammonium C-H stretch), 1750-1650 (broad, carboxylate C-O stretch), 819 (1,3-disubstituted and 1,2,4-trisubstituted aromatic out-of-plane C-H bend), 709 (aromatic ring bend). Elemental microanalysis found for HXLPP-SAX/WCX: C, 84.6 %; H, 7.2 %; N, 0.6 %; Sarcosine loading level = 0.4 mmol/g. SEM microscopy: mean particle diameter = 4.31 μm; C_v = 18.79 %. N_2 sorption analysis: Langmuir specific surface area = 860 m^2/g; specific pore volume = 0.35 cm^3/g; mean pore width = 2.7 nm.

2.2.4. Preparation of the HXLPP-WAX/SCX sorbent

The second batch of poly(DVB-*co*-VBC) microspheres was used for the preparation of the HXLPP-WAX/SCX sorbent. For this, hypercrosslinked poly(DVB-*co*-VBC) microspheres (2.001 g, 2.201 mmol Cl) were introduced together with DMSO (60 mL) into a round-bottomed, three-necked flask fitted with a reflux condenser and overhead stirrer, and the mixture stirred under an N_2 atmosphere for 30 min. Taurine (0.603 g, 4.818

mmol) and K_2CO_3 (0.653 g,4.725 mmol) were dissolved in DMSO (40 mL), the solution added into the round-bottomed, three-necked flask and the mixture stirred and heated at 60 °C for 18 h. After this period, the particles were filtered on a 0.45 µm nylon membrane filter and then washed successively with ACN, MeOH, acetone, THF and acetone before being dried overnight *in vacuo* (70 °C, 60 mbar). HXLPP-WAX/SCX was isolated as a free-flowing, orange-coloured powder (2.304 g). A reaction scheme outlining the synthetic procedure used to prepare the HXLPP-WAX/SCX sorbent is presented in **Figure 1b**.

Figure 1SB shows the FT-IR spectrum of HXLPP-WAX/SCX with the following bands ($\bar{\nu}$/cm^{-1}): 3350-3310 (amine N-H stretch), 3017 (aromatic C-H stretch), 2919 (aliphatic C-H stretch), 1600 (aromatic C=C stretch), 1364 (O=S=O asymmetric stretch), 1260 (CH_2-Cl wag), 1260 (amine C-N stretch), 1183 (O=S=O symmetric stretch), 822 (1,3-disubstituted and 1,2,4-trisubstituted aromatic out-of-plane C-H bend), 707 (aromatic ring bend). Elemental microanalysis found for HXLPP-WAX/SCX: C, 82.4 %; H, 7.2 %; N, 0.7 % S, 0.3%; N loading level = 0.5 mmol/g; S loading level = 0.1 mmol/g. SEM microscopy: mean particle diameter = 6.1 µm; C_v = 5.9 %. N_2 sorption analysis: Langmuir specific surface area = 1310 m^2/g; specific pore volume = 0.51 cm^3/g; mean pore width = 2.8 nm.

2.3. Solid-phase extraction procedure

200 mg of sorbent (either HXLPP-SAX/WCX or HXLPP-WAX/SCX, as appropriate) was packed into a 6 mL SPE tube (Symta, Madrid, Spain) using an upper 10 µm polyethylene frit (Symta) and a lower 10 µm polyethylene frit followed by a 2 µm stainless steel frit (Sigma-Aldrich) to prevent sorbent loss. The cartridge was mounted on the vacuum manifold (Teknokroma, Barcelona, Spain) and conditioned with 10 mL of MeOH followed by 10 mL of ultrapure water adjusted to pH 6 for both sorbents. The samples were adjusted to pH 6 with HCl. A washing step, consisting of 1 mL of MeOH, was introduced to remove interferents from the matrix. The nature of the elution step depended upon which sorbent was in use. For the HXLPP-SAX/WCX sorbent, the elution step used 5 mL of 5% NH_4OH in MeOH . For the HXLPP-WAX/SCX sorbent, the elution step used 7 mL of 5% NH_4OH in ACN . The HXLPP-SAX/WCX sorbent was evaluated

for environmental water samples, and for this work 250 mL of river water and 100 mL of effluent wastewater were loaded thorough the cartridge.

The elution fractions were evaporated to dryness using a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) prior to reconstitution with 1 mL of mobile phase (H_2O/ACN, 90/10, v/v). and filtered through 0.45 µm PTFE syringe filters (Scharlab) for UHPLC analysis.

2.4. Instrumentation and chromatographic conditions

For the evaluation of both sorbents and optimisation of the SPE protocol, an Agilent 1200 UHPLC instrument equipped with autosampler, oven, binary pump, automatic injector and a DAD detector was used (Agilent Technologies, Waldbronn, Germany). Ultrapure water (adjusted to pH 2.8 with HCl) (solvent A) and ACN (solvent B) were used as the mobile phases. A Tracer Excel 120 C_8 (150 mm × 4.6 mm i.d., 5 µm particle size) provided by Teknokroma (Sant Cugat del Vallès, Spain), set at 30 °C, was used as the chromatographic column. The flow rate was 600 µL min^{-1} and the injection volume was 20 µL. The gradient starting conditions were ultrapure water (pH 2.8)/ ACN (90/10, v/v). Then, 10% ACN was ramped to 40% at 12 min and then to 100% ACN at 16 min, kept for 3 min and returned to the initial conditions in 3 min. The signal was measured at 210 nm for all the compounds.

For the method validation, which was performed with the HXLPP-SAX/WCX sorbent only, an Agilent 1200 series LC coupled with a 6460 QqQ mass spectrometer (MS/MS) detector with electrospray ionisation (ESI) interface was used (Agilent Technologies). The LC system was equipped with an autosampler, a degasser, an oven and a quaternary pump (Agilent Technologies, Waldbronn, Germany). The chromatographic conditions in LC-MS/MS were the same as used for LC-DAD.

Regarding the MS/MS detection, the basic compounds (ATE, TRI, MEP, PROP, MET, MTP) were analysed under ESI positive mode, whereas the acidic compounds (ACE, SAC, CLO AC, FEN, DICLO, IBP, NAP) were analysed under ESI negative mode, since better ionisation is achieved in each case. The optimum source conditions for basic compounds were: capillary voltage of 2500 V; nebuliser pressure of 60 psi; nitrogen flow rate of 13 L min^{-1}; source gas temperature of 350 ºC. For the acidic compounds they were:

capillary voltage of 3000 V; nebulizer pressure of 25 psi; nitrogen flow rate of 12 L min^{-1}; source gas temperature of 350 ºC for. The optimal MS/MS parameters are detailed in **Table 1.** The optimum fragmentor voltage was selected for each compound, which was 100 V for all basic compounds and 75 V for the acidic compounds. The collision energy was between 8 and 22 eV (basic compounds) and between 2 and 24 eV (acidic compounds). A precursor ion and two product ions were selected for each analyte, except for IBU for which only one transition was acquired. The most abundant transitions were measured for quantification, and the other transitions and the corresponding ion ratios were used for confirmation purposes in multiple reaction mode (MRM) mode (**Table 1**).

All compounds presented good linearity (r^2=0.998) in LC-MS/MS in the following ranges: 0.01 to 50 µg L^{-1} for ACE, MEP, MTP, PROP and TRI; from 0.05 to 50 µg L^{-1} for ATE, NAP, CLO ACID and IBP; 1 to 50 µg L-1 for SAC and FEN; and, 0.05 to 10 µg L^{-1} for RAN and DICLO.

3. Results and discussion

3.1. Preparation of sorbents

For the synthesis of the sorbents, precipitation polymerisation (PP) was the preferred method of polymerisation since this method delivers high quality polymer microspheres in the low micron size range. Accordingly, DVB and VBC were copolymerised by free radical polymerisation under PP conditions to give poly(DVB-*co*-VBC) microspheres that were lightly crosslinked, had diameters in the low micron size range and which contained reactive chloromethyl groups. Hypercrosslinking of the polymer microspheres was then conducted when the polymer microspheres were in a swollen state, to impart porosity into the polymer microspheres. The hypercrosslinking chemistry is extremely efficient and rapid, thus the hypercrosslinking reaction time was restricted to 10 minutes to leave a portion of the polymer-bound chloromethyl groups unreacted, as disclosed in previous studies [18], and available for subsequent polymer-analogous reactions with sarcosine and taurine.

The HXLPP-SAX/WCX sorbent was prepared by immobilising sarcosine residues on the hypercrosslinked polymer microspheres followed by *N*-alkylation. This sorbent was prepared in good yield and had a convenient beaded format thanks to the method of polymerisation (mean particle diameter = 4.3 µm); **Figure 2a** shows an SEM image of the HXLPP-SAX/WCX microspheres. Well-developed porous morphology is an important feature of high-performance, high capacity sorbents since specific surface area is related to capacity, so it is noteworthy that the HXLPP-SAX/WCX sorbent had a high specific surface area (Langmuir specific surface area = 860 m^2/g). The sarcosine loading level was 0.4 mmol/g.

The HXLPP-WAX/SCX sorbent was prepared by immobilising taurine residues on the hypercrosslinked polymer microspheres. This sorbent also had a beaded format (mean particle diameter = 6 μm) and was prepared in good yield; **Figure 2b** shows an SEM image of the HXLPP-WAX/SCX microspheres. The HXLPP-WAX/SCX sorbent had an even higher specific surface area than the HXLPP-SAX/WCX sorbent (Langmuir SSA = 1311 m^2/g). From the elemental microanalytical data, the functional group loading level (taurine content) of this sorbent was estimated to be 0.1 mmol/g.

3.2. Optimisation of the SPE protocol

For the evaluation of the HXLPP-SAX/WCX and HXLPP-WAX/SCX sorbents, model compounds with acidic and basic properties were selected. The SPE conditions should be carefully optimised to ensure the establishment of ionic interactions between the ionic groups of the sorbent and the ionisable groups in the compounds. The loading pH was selected based on the pK_a values of the model compounds **(Table 1)** and the sorbents, in order to promote ionic interactions between the model compounds and sorbents.

In a previous study where an HXLPP-WAX/WCX sorbent was evaluated [23], we concluded that pH 6 was a suitable pH for sample loading since this allowed ionisation of functional groups on the model compounds and sorbent. In the present study, the HXLPP-SAX/WCX sorbent has a similar WCX group to the HXLPP-WAX/WCX sorbent reported previously but has an SAX group instead of a WAX group (thus the SAX character will be turned on throughout the pH range). Hence, pH 6 should be a good loading pH for the HXLPP-SAX/WCX sorbent as well.

As for the HXLPP-WAX/SCX sorbent, it is expected to function as an anion- *and* cation-exchanger at low pH, since both the amine groups and the sulfonic acid groups will be ionised under these conditions. At pH values above the pK_a of the most weakly acidic model compound (IBP; pK_a = 4.9) and below the pK_a of the amine moieties on the

HXLPP-WAX/SCX sorbent, the analytes and the sorbent are expected to be ionised, thus pH 6 was selected as the loading pH.

The SPE conditions under which the sorbents were evaluated initially were: 200 mg of sorbent (based on previous experience [15,17,26,27]) loaded with 100 mL of ultrapure water adjusted at pH 6 and spiked at 150 µg L^{-1} with the analyte mixture. A washing step comprising of 1 mL MeOH was included to remove those compounds bound to the sorbents by reversed-phase interactions only and to improve the selectivity of the SPE. The elution conditions used were distinct for each sorbent. Based on previous studies [15,26], two different eluents were used in series for the HXLPP-SAX/WCX sorbent: an acidic eluent (5 mL 5% HCOOH in MeOH) followed by a basic eluent (5 mL 5% NH_4OH in MeOH). The elution recoveries were taken as the sum of the recoveries of the acidic and basic elution steps. For the HXLPP-WAX/SCX sorbent, a basic elution solvent (5 mL 5% NH_4OH in MeOH) was employed.

Upon applying the initial SPE conditions to the HXLPP-SAX/WCX sorbent, all of the compounds were extracted successfully with recoveries between 83 and 107%, except for RAN (64%) (**Table 2**). The basic compounds and the weakly acidic compounds were eluted from the sorbent in the acidic elution step, whereas the strongly acidic ACE ($pK_a = -0.3$) and SAC ($pK_a = -1.6$) were eluted in the basic elution step. Upon applying the initial SPE conditions to the HXLPP-WAX/SCX sorbent, the basic compounds were extracted with high recoveries from 70% to 112%. However, MET and PROP were highly retained and a higher elution volume (2 more fractions of 5 mL 5% NH_4OH in MeOH) was required to elute them from the sorbent. Regarding the acidic analytes, some weakly acidic analytes were partially lost in the washing step and so relatively poorly retained (recoveries from 20% to 61%), except CLO AC and FEN for which the recoveries were 94% and 78%, respectively. ACE and SAC were lost during the loading step because of repulsion of these highly polar acids by the sulfonate groups. The recoveries of analytes obtained in the initial SPE evaluation of both sorbents are presented in **Table 2.** Similar recovery levels were achieved for the basic compounds across both sorbents, whereas better recoveries were found for the acidic compounds when the HXLPP-SAX/WCX sorbent was used. The strongly acidic ACE and SAC were only retained by the HXLPP-SAX/WCX sorbent and efficiently eluted using a basic elution.

Moving forward from this initial evaluation, different parameters were optimised to improve the selectivity and capacity of the SPE method for both sorbents. The parameters investigated were the elution conditions, the washing volume and the sample volume.

3.2.1. Elution conditions

Firstly, the elution conditions were evaluated for both sorbents. To compare the results with the elution conditions used initially, for the HXLPP-SAX/WCX sorbent the order of eluents was switched, such that the basic elution (5 mL of 5% NH_4OH in MeOH) was performed before the acidic elution (5 mL of 5% HCOOH in MeOH). For the HXLPP-WAX/SCX sorbent, an acidic elution step comprising 5 mL of 5% HCOOH in MeOH was introduced before the basic elution step.

For the HXLPP-SAX/WCX sorbent, all analytes were eluted efficiently from the sorbent during the basic elution step (5 mL 5% NH_4OH in MeOH) with %R >75%, even the strongly acidic analytes ACE and SAC. Given these results, going forward the optimised elution step for the HXLPP-SAX/WCX sorbent comprised 5 mL of 5% NH_4OH in MeOH. For the HXLPP-WAX/SCX sorbent, the introduction of the acidic elution step before the basic elution step did not lead to any improvements in recovery (%R < 20%). Consequently, a basic elution step comprising 5 mL of 5% NH_4OH in MeOH was used to elute the analytes. However, it was necessary to use more 5 mL aliquots of basic MeOH to elute MET and PROP completely. For this reason, 5% NH_4OH in ACN was investigated as an alternative eluent in an effort to reduce the volume of eluent required; under these modified conditions, MET and PROP were eluted completely by the first two 5 mL aliquots of ACN containing 5% NH_4OH, indicating that the elution strength is increased when MeOH is replaced by ACN.

Then, the elution volume had to be optimised and fixed with the goal being to use the minimum volume necessary to elute all the analytes completely from the sorbent. It was found that 7 mL of 5% NH_4OH in ACN was sufficient to elute MET and PROP completely. Moving forward, for all subsequent experiments with the HXLPP-WAX/SCX sorbent, 7 ml of 5% NH_4OH in ACN containing was the eluent used.

3.2.2. Volume of washing solvent

The next parameter optimised was the volume of MeOH used in the washing step. Since none of the analytes was lost during a washing step involving 1 mL of MeOH, the volume was increased to 3 x 2 mL fractions of MeOH in order to remove compounds bound to the sorbent by non-selective, hydrophobic interactions. Regarding the HXLPP-SAX/WCX sorbent, the acidic compounds were lost, either partially or completely, by the three-fold washing step, whereas the basic compounds were retained strongly and eluted subsequently with R(%) between 91% and 113%, except for RAN (48%). This is likely to be because the basic compounds establish stronger ionic interactions with the anionic moieties of the sorbent than the acidic compounds establish with the cationic moieties. Given these observations, and to minimise losses during the washing step, the optimised washing volume of MeOH used in the washing step for all following experiments was 1 mL. It should be noted that when using volumes of washing solution greater than 1 mL, the sorbent still retains selectivity for the extraction of basic compounds (basic compounds remain bound to the sorbent even after 6 mL of MeOH is applied). Similar results were obtained for the HXLPP-WAX/SCX sorbent; when increasing the volume of MeOH used in the washing step the acidic compounds were lost.

In other studies, a washing step with MeOH has also been applied. For instance, in Prosen's study [28] different washing volumes (from 2-10 mL MeOH) were tested when extracting drugs from environmental waters by SPE/LC-HRMS with Oasis MCX cartridges (500 mg), aiming to get efficient removal of matrix interferences without compromising the recovery of the analytes. Use of 5 mL MeOH ensured the removal of the interferents with minimal losses of analytes, thereby enabling satisfactory recoveries (%R>75%) in ultrapure water. Higher volumes of MeOH were used as washing solvent since the amount of sorbent in the SPE cartridge was 500 mg.

In another study, Deeb *et al.* [19] used Oasis MAX and Oasis MCX sorbents in tandem to extract micropollutants and their transformation products from water and wastewater samples. They introduced two different washing solutions, one for each sorbent: 2 mL 5% NH_4OH in water to remove basic interferents from the Oasis MAX sorbent, and 2 mL 2% HCOOH in water to remove acidic interferents from the Oasis MCX sorbent. Satisfactory recoveries (≥90%) were achieved for all analytes in different water samples, and the matrix interferents were thoroughly removed. However, they only include an aqueous washing whose elution strength for effectively removing those compounds bound by reversed-phase interactions might be limited as compared to a washing step based on pure organic solvent.

3.2.3. Sample volume

The next parameter to optimise in order to improve the sensitivity of the method was the sample volume. The sample volume was increased from 100 mL to 250 mL and then to 500 mL. The data is presented in **Table 3**. For the HXLPP-SAX/WCX sorbent, the recoveries achieved when the sample volume was 250 mL were very similar to the recoveries obtained when the sample volume was 100 mL (successful retention of all compounds). When the sample volume was increased from 250 mL to 500 mL, the recoveries of the basic and strongly acidic compounds were maintained, whereas the recoveries of the weakly acidic compounds slightly decreased. Consequently, sample volumes greater than 500 mL were not evaluated. Going forward, the optimised sample volume used for the HXLPP-SAX/WCX sorbent was 500 mL.

As can be seen in **Table 3**, for the HXLPP-WAX/SCX sorbent the recoveries of analytes from a 250 mL sample were similar to those from a 100 mL sample. However, when the sample volume was increased to 500 mL, the recoveries of some compounds decreased slightly although the recoveries of others were maintained. For instance, the recoveries of DICLO decreased from 85% to 64%. The basic compounds showed excellent retention at all sample volumes tested, however ACE and SAC were always lost in the loading and washing steps. In view of these results, 250 mL was used as the sample volume in subsequent experiments.

As a comparison between sorbents, the HXLPP-SAX/WCX sorbent gave slightly better results for some of the basic compounds. Recoveries of 102% and 94% were achieved for MEP and MET with the HXLPP-SAX/WCX sorbent, respectively, whereas the analogous recoveries were 67% and 82% with the HXLPP-WAX/SCX sorbent. As for the weakly acidic compounds, HXLPP-SAX/WCX gave better recoveries except for FEN and DICLO (R (%) = 74% and 61%, respectively for HXLPP-SAX/WCX and 93% and 85%, respectively, for WAX/SCX sorbent). However, ACE and SAC were not retained by the HXLPP-WAX/SCX sorbent whereas they were retained essentially quantitatively by the HXLPP-SAX/WCX sorbent (%R>100%).

This new SPE data is in broad agreement with data from our previous study on an amphoteric sorbent, in which an HXLPP-WAX/WCX sorbent [23] was evaluated (recoveries were from 80% to 95% when the sample volume was 100 mL). However, a significant new finding relates to the ability of the HXLPP-SAX/WCX sorbent to retain strongly acidic analytes (neither the HXLPP-WAX/WCX sorbent nor the newly reported HXLPP-WAX/SCX sorbent are able to retain ACE and SAC in the loading step). We ascribe this to the fact that the HXLPP-SAX/WCX sorbent is the only material with strong anion-exchange character (the other two resins have weak anion-exchange character). Highly polar strong acids, such as the sweeteners ACE and SAC, are normally poorly or excessively retained in sorptive extraction techniques [29], thus it is very pleasing to see that the HXLPP-SAX/WCX sorbent enables the efficient extraction of both of the polar strong acids (with the basic eluent, the recoveries were 111% and 112%, respectively, when the sample volume was 500 mL).

For all other compounds investigated, results comparable to Segura's study [30] were obtained. In Segura's work, a group of organic contaminants was extracted from 250 mL of surface water by SPE using a number of different commercial mixed-mode cartridges: Strata-X-AW, Strata-X-CW and Strata-X (reversed phase materials with polar groups). Similarly to the recoveries reported in our study, extraction recoveries around 100% were obtained for DICLO, NAP, IBP and MET for the three cartridges tested, except for Strata-X-AW when the recovery of IBP was around 50%. It should be emphasised that in Segura's study only one type of ion-exchange group (either cationic or anionic) was present on any given sorbent, whereas in our study we have two types of ion-exchange group co-exsiting on the same sorbent. In addition, the washing step used by Segura was aqueous-based only which may be less efficient at removing compounds (or interferences) bound by reversed-phase interactions.

Once the SPE procedure had been optimised, the optimal conditions for the SPE protocol were fixed as follows: 200 mg of sorbent; 500 mL of ultrapure water adjusted to pH 6 for the HXLPP-SAX/WCX sorbent and 250 mL of ultrapure water adjusted to pH 6 for the HXLPP-WAX/SCX sorbent; 1 mL MeOH as washing solution; elution with 5 mL of 5% NH_4OH in MeOH for the HXLPP-SAX/WCX sorbent and 7 mL of 5% NH_4OH in ACN for the HXLPP-WAX/SCX sorbent. In order to enhance the sensitivity of the method, the elution extracts were evaporated to dryness and reconstituted in 1 mL of mobile phase (no losses of analytes were observed during the evaporation step). Whilst both sorbents performed well, the best results overall were returned by the HXLPP-SAX/WCX sorbent, thus it was this sorbent which was used in the method validation work as applied to the SPE of environmental water samples.

3.3. Method validation

The SPE method for the HXLPP-SAX/WCX sorbent was validated using river and effluent wastewater samples using the optimised extraction parameters. The method validation was performed by LC-MS/MS to achieve lower detection and quantification limits and increase sensitivity. 0.1 µg L^{-1} and 0.25 µg L^{-1}were the concentration levels selected in river water and effluent wastewater samples, respectively, to calculate the apparent recovery (R_{app} (%)) and the ME (%) for 250 mL river water samples and 100 mL effluent wastewater samples. Lower concentration levels were not considered due to the presence of some of the analytes in non-spiked samples. The method detection limits (MDLs) and method quantification limit (MQLs), repeatability and reproducibility between days were also evaluated for both samples. The R_{app} (%), ME (%), MDLs and MQLs for river and effluent samples are shown in **Table 4**. Non-spiked samples of river water and effluent wastewater were analysed, and the signals obtained subtracted from the signal of the spiked sample in order to calculate the R_{app} (%). R_{app} (%) is the recovery of the whole method and considers the extraction efficiency and the ME (%). To calculate the ME (%), the formula ME (%) = (C_{exp}/C_{theo} x 100%) – 100% was used, where C_{theo} is the concentration of the standard solution and the C_{exp} is the concentration of the analytes when the sample was spiked with the analyte mixture just after the SPE procedure. Depending on the ME (%) obtained, it can increase or decrease the signal depending upon whether there is ion-enhancement or ion-suppression, respectively.

For river water, the R_{app} (%) values ranged from 52% to 93% for all compounds, except MEP and MET (where R_{app} (%) = 18% and 39%, respectively), as seen in Table 4. For effluent wastewater compared to river water, the R_{app} (%) values were similar for all compounds, except for NAP and RAN where the R_{app} (%) values were found to decrease to 41% and 25%, respectively.

The ME (%) values obtained were similar for both sample types and support ion-suppression for most of the compounds. Low ME (%) values were obtained due to the selectivity of the sorbent and the inclusion of a washing step (1 mL MeOH) to remove matrix interferences. MET and TRI were the compounds with the higher levels of ion-suppression, with ME (%) values of -30% and -20%, respectively, for river samples.

The MDLs and MQLs were determined for the river water samples and the effluent wastewater samples. As some compounds were present in the non-spiked samples, the MDLs and MQLs of the river and effluent samples were estimated from the limits of detection and quantification (LODs and LOQs), considering the R_{app} (%) results and the preconcentration factor, expressed in ng L^{-1}. They ranged from 0.03 ng L^{-1} to 3 ng L^{-1} for the MDLs, and from 0.05 ng L^{-1} to 15 ng L^{-1} for the MQLs. Detailed information appears in **Table 4**.

The method reproducibility between days and repeatability on the same day, expressed as the relative standard deviation (RSD, %) of five replicates of river and effluent samples spiked at a concentration level of 0.1 µg L^{-1} and 0.25 µg L^{-1}, respectively, were lower than 19% and 11%, respectively, and are shown in Table 4.

3.4. Analysis of real samples

The optimised SPE method was applied to river water and effluent wastewater samples to determine the acidic and basic compounds. The concentration levels obtained for the analytes present in three samples of river water and three samples of effluent wastewater are shown in **Table 5**. As a summary, the analytes were found in concentrations between 4-290 ng L^{-1} in river water samples and between <MQL-545 ng L^{-1} in effluent wastewater samples. These concentrations were calculated by taking into account the %R_{app} using an external calibration curve. In both sample types, all analytes were detected. Figure 3 shows the chromatograms obtained when a non-spiked sample of river (A) and effluent wastewater (B) were analysed by SPE/LC-MS/MS. The concentration levels found in river water were lower than the levels found in effluent wastewater samples, as was expected. NAP was found at similar concentration levels in effluent wastewater, but was below the MQL. The concentration levels of the majority of the compounds reported in this study are similar to those reported in other studies [22,31–36]. For instance, CLO AC, MET and ATE were reported in this study to be at concentration levels between 4 and 31 ng L^{-1}, which is the same as the concentration levels reported previously for these compounds present in Ebre River samples (1-50 ng L^{-1}) [22]. Regarding effluent wastewater, MET and ATE were found at concentration levels (17-370 ng L^{-1}) similar to

other reports concerning effluent wastewater from the same treatment plant near Tarragona as used in this study [22]. IBP, MTP and PROP were also found at very similar concentrations to values reported for effluent wastewaters from different treatment plants [33,34,36]. ACE and SAC were found at slightly lower concentration levels than other studies on river water and effluent wastewater [31,32].

4. Conclusions

Two mixed-mode ion-exchange polymeric sorbents have been prepared by decorating hypercrosslinked polymer microspheres with two complementary ion-exchange motifs (one cation-exchange group and one anion-exchange group). More specifically, we have combined SAX character with WCX character in one of the novel sorbents (HXLPP-SAX/WCX) and WAX character with SCX character in the second sorbent (HXLPP-WAX/SCX). The polymers were prepared by polymer-analogous reactions on hypercrosslinked polymer microspheres, where the swellable precursors to the hypercrosslinked materials were prepared by precipitation polymerisation. The sorbents were produced in a convenient beaded format, had high specific surface areas, and were decorated with either sarcosine or taurine residues. It is the sarcosine and taurine motifs that confer the ion-exchange character onto the polymers.

The polymers were exploited as SPE sorbents for the retention of model compounds present in aqueous environments. Several SPE parameters were optimised (including the elution conditions, the washing volume and the sample volume), and the optimised methods enabled many acidic and basic analytes to be extracted efficiently from standard solutions. Both sorbents performed well, however the performance of the HXLPP-SAX/WCX sorbent was particularly impressive (highest recoveries and capacities) so this material was taken forward to method validation and the SPE of analytes from river water samples and effluent wastewater samples. Low %ME values were obtained for all compounds thanks to the inclusion of a washing step in the SPE protocol, and all analytes were quantified successfully at ng L^{-1} levels. The ability to capture anions and cations simultaneously using one single SPE sorbent has been demonstrated clearly in this work.

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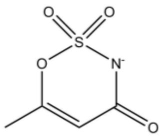
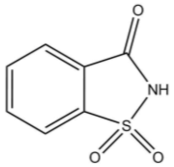
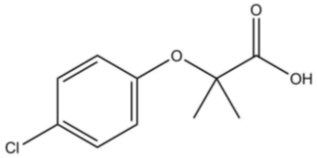
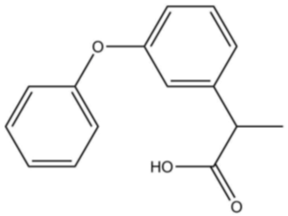
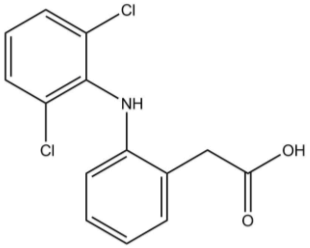
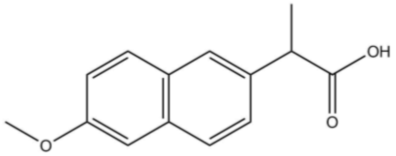
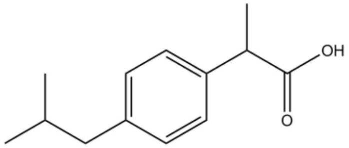
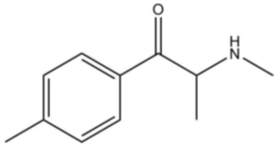
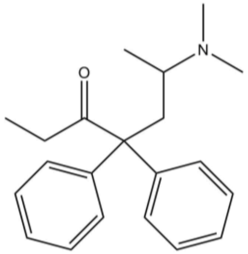
Table 1. Analyte structure, pK_a, MRM transitions and MS/MS parameters for the model compounds.

		Analyte	Formula	Structure	pK_a	Ion. mode	Precu. ion (m/z)	Frag. (V)	Prod. ion (m/z)	CE (eV)	Prod. ion (m/z)	CE (eV)
ACIDIC	STRONG	ACE	$C_4H_4NO_4S$		-0.3	NEG	162	75	82	11	78	15
		SAC	$C_7H_5NO_3S$		1.6	NEG	182	75	105	22	62	24
	WEAK	CLO AC	$C_{10}H_{11}ClO_3$		3.37	NEG	213	75	127	8	85	5
		FEN	$C_{15}H_{14}O_3$		3.96	NEG	241	75	197	2	93	5

		DICLO	$C_{14}H_{11}Cl_2NO_2$		4.00	NEG	294	75	250	5	214	8
		NAP	$C_{14}H_{14}O_3$		4.19	NEG	229	75	185	2	169	4
		IBP	$C_{13}H_{18}O_2$		4.85	NEG	205.1	75	161	2		
BASIC		MEP	$C_{11}H_{15}NO$		8.0	POS	178.1	100	160	8	145	12
		MET	$C_{21}H_{27}NO$		9.1	POS	310.2	100	265	12	223	16

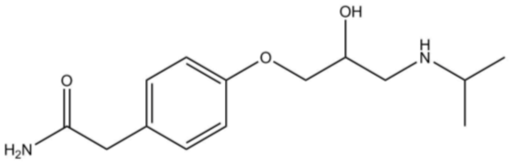
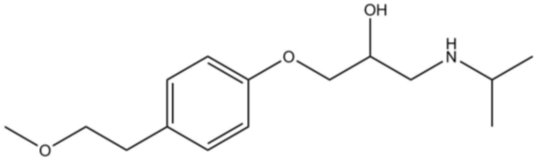
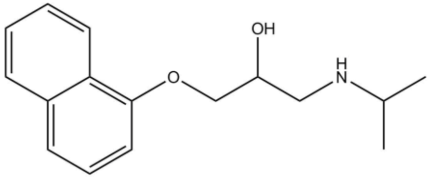
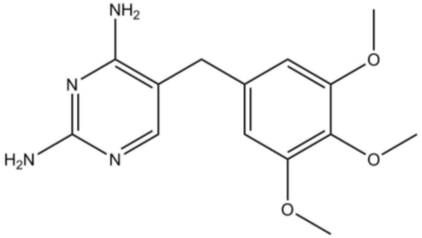
	ATE	$C_{14}H_{22}N_2O_3$		9.67	POS	267.2	100	145	22	74.2	22
	MTP	$C_{15}H_{25}NO_3$		9.67	POS	268.2	100	116	22	74	22
	PROP	$C_{16}H_{21}NO_2$		9.7	POS	260.1	100	183	22	116	22
	TRI	$C_{14}H_{18}N_4O_3$		10.8	POS	291.1	100	261	22	230	22

Table 2. Recovery (R, (%) obtained for both sorbents in initial experiments using the conditions indicated in the text.

				Recovery (%)	
				HXLPP-SAX/WCX[a]	**HXLPP-WAX/SCX**
			pK_a	**Elution**	**Elution**
Acidic	Strong	ACE	-0.3	96	-
		SAC	1.6	90	4
	Weak	CLO AC	3.4	95	94
		FEN	4.0	91	78
		DICLO	4.0	92	61
		NAP	4.2	83	20
		IBP	4.9	87	49
Basic		MEP	8.0	84	112
		RAN	8.2	64	70
		MET	9.1	97	99
		ATE	9.7	107	112
		MTP	9.7	97	102
		PROP	9.7	95	89
		TRI	10.8	98	95

[a] For HXLPP-SAX/WCX the elution recoveries were taken as the sum of the recoveries of the acidic and basic elution steps.

RSD (%) (n=5) < 9% when R (%) > 20%.

Table 3. Recovery (%) obtained for both sorbents when different volumes of ultrapure water adjusted to pH 6 and spiked at 150 µg L^{-1} with the analyte mixture were percolated. See the text for the rest of SPE conditions.

			Recovery (%)					
			HXLPP-SAX/WCX			**HXLPP-WAX/SCX**		
			100 mL	**250 mL**	**500 mL**	**100 mL**	**250 mL**	**500 mL**
Acidic	Strong	ACE	106	103	111	3	1	-
		SAC	108	110	112	12	4	6
	Weak	CLO AC	94	96	87	97	94	91
		FEN	90	91	74	95	93	85
		DICLO	80	74	61	91	85	64
		NAP	99	98	98	25	19	30
		IBP	78	89	69	59	53	46
Basic		MEP	107	90	102	85	67	78
		RAN	75	74	74	69	73	67
		MET	104	100	94	95	82	90
		ATE	105	112	111	105	100	89
		MTP	100	105	105	102	99	100
		PROP	93	96	95	93	91	91
		TRI	95	96	97	95	93	98

RSD (%) (n=5) < 10% when R (%) > 25%.

Table 4. R_{app} (%),ME (%),when 250 mL of river water and 100 mL of effluent wastewater spiked at 0.1 μg L^{-1} and 0.25 μg L^{-1}, respectively, were extracted using the HXLPP-SAX/WCX sorbent and analysed by LC-MS/MS. MDLs and MQLs values obtained using this method.

	River water						Effluent wastewater					
Analyte	**R_{app} (%)**	**Repe. (n=5)**	**Repr. (n=5)**	**ME(%)**	**MDLs (ng/L)**	**MQLs (ng/L)**	**R_{app} (%)**	**Repe. (n=5)**	**Repr. (n=5)**	**ME (%)**	**MDLs (ng/L)**	**MQLs (ng/L)**
ACE	89	11	3	-5	0.03	0.1	103	5	3	-10	0.05	0.3
SAC	93	12	5	8	1.0	4.0	101	3	2	-12	2.0	10
CLO AC	80	7	2	3	0.1	0.3	71	5	4	-6	0.3	0.8
FEN	70	18	10	-10	1.0	6.0	69	2	4	-5	3.0	15
DICLO	81	6	5	17	0.05	0.3	61	7	5	-11	0.2	1.0
NAP	60	19	8	-9	0.2	0.4	41	10	10	-5	1.5	4.0
IBP	52	16	12	-9	0.4	0.8	59	11	6	13	0.8	2.0
MEP	18	17	15	-9	0.2	0.4	24	10	10	-12	0.30	1.0
RAN	69	10	5	5	0.1	0.3	25	8	10	-2	0.08	2.0
MET	39	18	11	-30	0.04	0.1	52	11	4	-5	0.05	0.3
ATE	76	6	4	2	0.1	0.5	105	6	8	-9	0.2	1.0
MTP	80	3	4	-4	0.04	0.1	75	9	6	4	0.1	0.3
PROP	60	19	10	-7	0.05	0.1	68	2	2	18	0.1	0.3
TRI	57	5	8	-20	0.05	0.1	92	2	4	-8	0.1	0.2

Table 5. Range of concentrations (in ng L^{-1}) when three different river water samples and three different effluent wastewater samples were analysed using the optimised SPE method with the HXLPP-SAX/WCX sorbent.

	Concentration (ng L^{-1})	
Analyte	**River water**	**Effluent wastewater**
ACE	10-199	332 - 487
SAC	12-112	33-343
CLO AC	4-16	1-10
FEN	7-30	<MQL-37
DICLO	12-70	40-177
NAP	4-27	<MQL-38
IBP	12-29	98-206
MEP	20-39	10-21
RAN	112-225	<MQL-361
MET	8-23	17-56
ATE	6-31	160-370
MTP	8-21	14-174
PROP	10-28	18-47
TRI	62-290	42-545

Figure captions

Figure 1. Reaction schemes showing the synthetic methods used to prepare the HXLPP-SAX/WCX sorbent (a) and the HXLPP-WAX/SCX sorbent (b).

Figure 2. SEM micrographs of the HXLPP-SAX/WCX sorbent (a) and the HXLPP-WAX/SCX sorbent (b). The applied acceleration voltage of the incident electron beam was 25 kV.

Figure 3. Chromatograms obtained when a non-spiked sample of river water (A) and effluent wastewater (B) was analysed by SPE/LC-MS/MS.

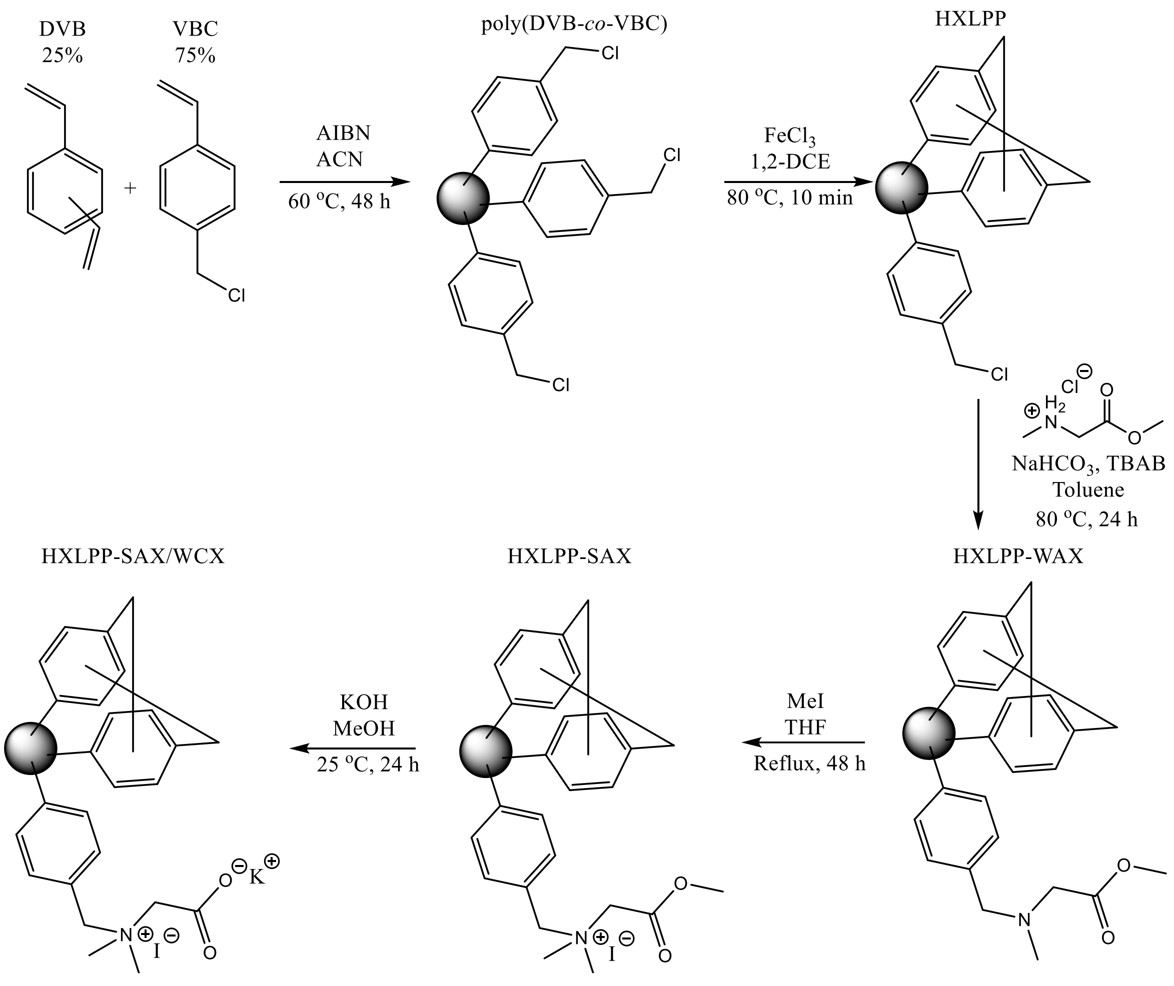


Figure 1a

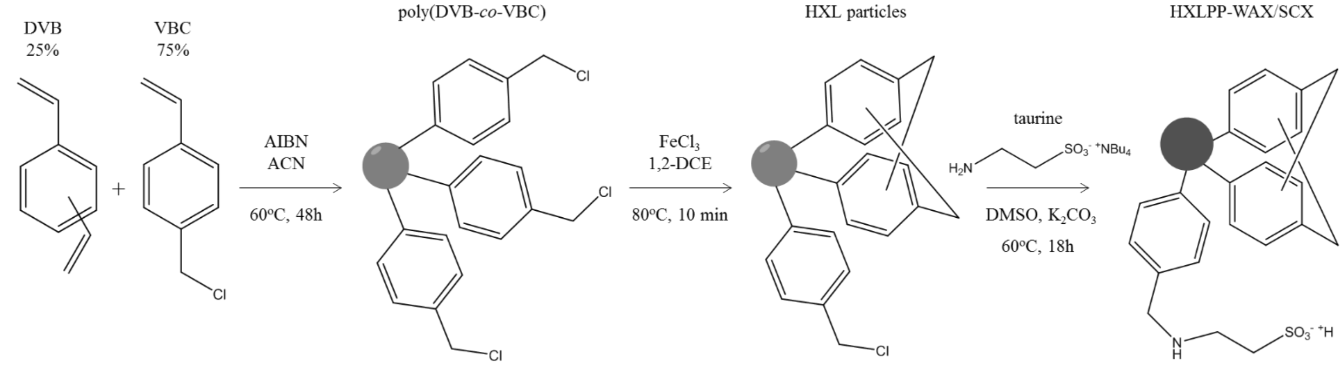


Figure 1b

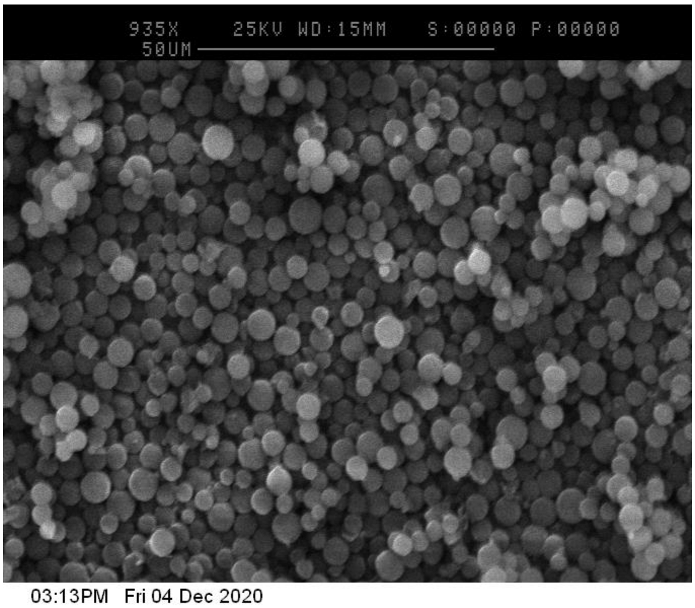


Figure 2a

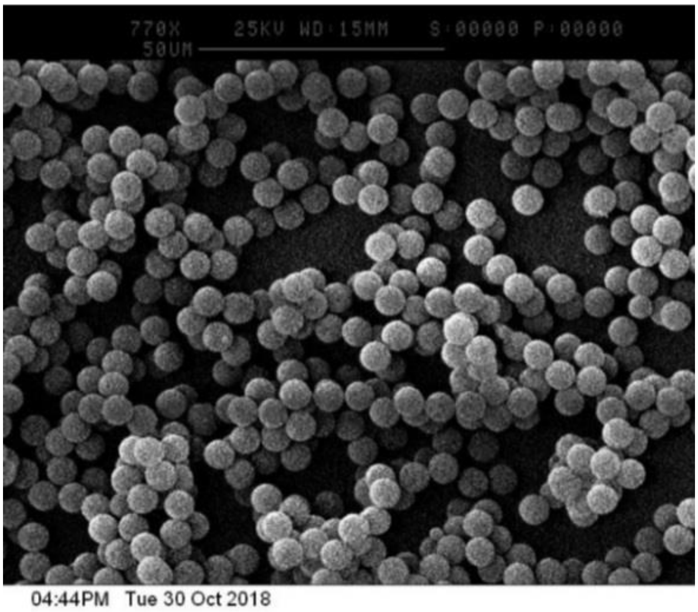


Figure 2b

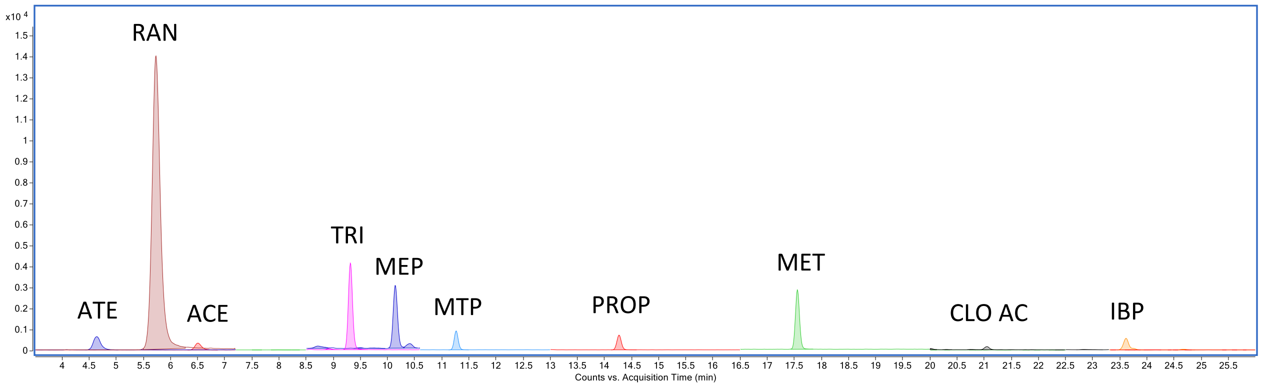


Figure 3A

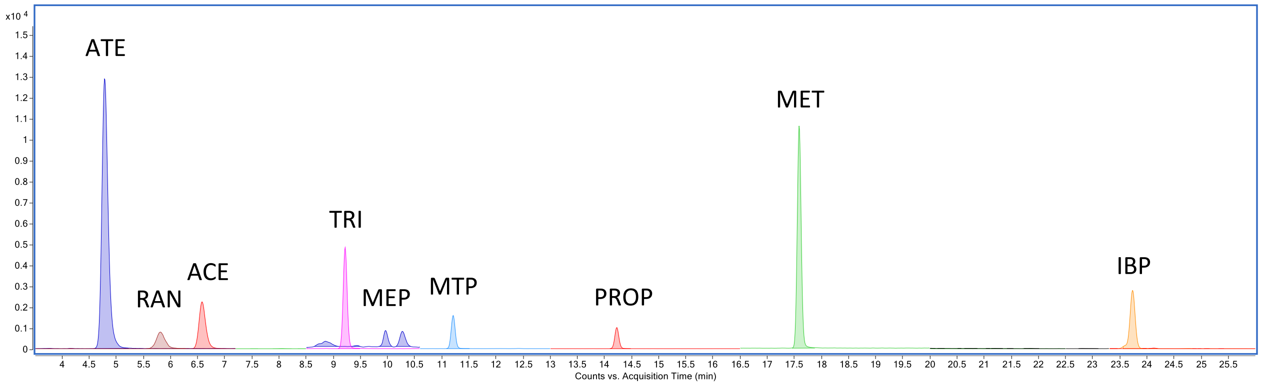


Figure 3B