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Development of a maleic acid-based material to selectively solid-phase extract basic compounds from environmental samples

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ABSTRACT

This study presents a novel mixed-mode weak cation-exchange (WCX) material. This material was prepared by means of the functionalization of a mesoporous divinylbenzene (DVB) resin with maleic acid (maleic acid-DVB), which yielded a high carboxylic moiety content resulting in WCX interactions as well as suitable specific surface area for reversed-phase interactions. After the optimization of the solid-phase extraction (SPE) protocol to enhance the selectivity of the sorbent, this material was evaluated as a novel WCX sorbent in the SPE of a group of drugs from environmental water samples. The method is based on SPE followed by liquid chromatography (LC) coupled to high resolution mass spectrometry (HRMS) with an Orbitrap analyzer, and was validated and applied for the determination of basic drugs in river, effluent and influent wastewater samples. Maleic acid-DVB sorbent yielded suitable recovery rates (57% to 89%) and an acceptable matrix effect (<32%) thanks to the effective washing step included when these environmental waters were loaded through the novel resin. The method was applied to different environmental water samples and some basic drugs were suitably quantified in these environmental samples.

1. Introduction

The analysis of complex samples to determine target compounds at trace levels is a major challenge in analytical chemistry. To respond to this challenge, analytical methods are being developed based on high-tech techniques such as hyphenated chromatography-mass spectrometry. However, even when these techniques are applied, sample treatment is mandatory to both enrich the compounds and clean up the sample matrix.

Among the different extraction techniques for liquid samples, solid-phase extraction (SPE) remains the most commonly used, mainly because of the availability of sorbents that cover the different interactions with the target compounds. Because of this, several applications have been described in the literature where SPE coupled to chromatography with mass spectrometry (MS) -based detectors are reported to determine different types of compounds from a broad range of samples [1–4].

Numerous SPE materials are currently available. On the one hand, there are materials that exploit capacity, such as silica modified with C_{18} or polymer-based materials, including macroporous crosslinked polymers and hypercrosslinked polymers both hydrophobic and hydrophilic. In addition, there are emerging materials like highly ordered mesoporous silica, carbon nanotubes, or hybrid materials such as metal-organic frameworks or magnetic nanoparticles [1,5,6]. On the other hand, there are materials developed to improve selectivity, such as molecularly imprinted polymers (MIPs) [7].

Furthermore, capacity and selectivity can be combined in a single material, giving rise to mixed-mode ion-exchange materials. These materials combine silica- or polymer-based skeleton (non-specific interactions) functionalized with ionizable moieties (specific ionic interactions with ionic compounds). Depending on the moiety attached, mixed-mode ion-exchange materials can be classified as strong cation-exchange (SCX – sulfonic group), weak cation-exchange (WCX – carboxylic group), strong anion-exchange (SAX – quaternary amine) and weak anion-exchange (WAX – tertiary or secondary amine). Strong ion-exchange materials remain charged regardless of pH, whereas the charge or lack of charge of weak ion-exchange materials is pH dependent. Therefore, ion-exchange interactions enable ionizable compounds or interferences to be selectively extracted by controlling the charge state of the

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compounds/material through the pH in the SPE protocol [5,8]. In addition, these ion-exchange interactions remain bound after a washing step using an organic-based solvent (i.e. methanol - MeOH), which is capable of washing out compounds/interferences bound to the sorbent through non-specific interactions. This effective washing simplifies the extraction process and favors the reduction of the matrix effect (ME).

In view of these advantages, commercially available mixed-mode ion-exchange sorbents are sold by a variety of different manufacturers, including Oasis, Strata, and the Isolute series, among others. Numerous examples can be found in the literature of the application of these commercial mixed-mode ion-exchange sorbents to selectively extract different types of compounds, including drugs [9,10], herbicides [11], and amino acids [12], among other compounds, from complex samples such as biological fluids [12], environmental waters [10,11] and food [9]. For example, cysteine (an amino acid) was effectively extracted (recoveries of 93–97%) from saliva, human serum and pharmaceutical preparations using a Strata-X-C cartridge (30 mg) (SCX material) and applying a washing step based on 0.5 mL of MeOH [12]. Sartan drugs were completely extracted (recoveries of 93–104%) from tap, river, effluent and influent sewage samples using Oasis WAX (150 mg) (WAX material) with a washing step of 5 mL of MeOH/water (50/50, v/v) [10]. In addition, this washing step minimized the ME which, in the case of this study, was at most 24% in influent wastewater (the most complex sample analyzed) [10].

Apart from commercially available materials, some researchers have prepared in-house mixed-mode ion-exchange materials that present outstanding features. For instance, a series of mixed-mode ion-exchange materials developed from hypercrosslinked skeletons (improved capacity) was prepared (i.e. HXLPP-SCX [13], HXLPP-SAX [14], HXLPP-WCX [15] and HXLPP-WAX [16]) and successfully evaluated by our research group to selectively extract different types of compounds from environmental samples [13–16]. Specifically, HXLPP-SCX (hypercrosslinked resin functionalized with lauroyl sulfate that acts as SCX) was successfully applied for the determination of a group of drugs in environmental waters, achieving ME values not higher than 30% as a result of the washing step (5 mL of MeOH) included [13]. Other authors, such as Meischl et al. [17], also functionalized vinylbenzyl chloride-ethyleneglycol dimethacrylate (VBC-EGDMA) with trimethylamine, imidazole, piperidine and pyrrolidone to prepare four mixed-mode ion-exchange materials with anion-exchange capabilities. They were then evaluated for the selective extraction of pharmaceuticals from tap and river water. Other examples include a hybrid silica monolith functionalized with sulfonic moieties that was successfully prepared and evaluated to extract sulfonamides in milk [18], or the functionalization of silica coated magnetic nanoparticles with 3-aminopropyltriethoxysilane moieties to obtain a material that displays SAX interactions [19]; in this last example, magnetic nanoparticles were used to extract herbicides from environmental samples.

In this study, we propose a material prepared via the suspension polymerization of divinylbenzene (DVB), which gives the sorbent particles and their porous structure great mechanical strength. The material was further modified through a Diels-Alder reaction with maleic anhydride, and subsequent base hydrolysis generated the carboxyl groups on the polymer surface, which are responsible for cation-exchange properties. Therefore, it is possible to use this sorbent in SPE systems as a mixed-mode WCX material applicable, for example, in the determination of basic compounds in environmental waters. The implementation of carboxyl groups in a polymer structure may also result in another specific interaction, like hydrogen bonding. This property of the proposed sorbent was used in the sorption of triazine-based herbicides from aqueous solutions [20–22], suggesting that it would yield satisfactory results in sorption from environmental samples as well [23].

In this paper, we present the preparation of a novel WCX material based on mesoporous poly(DVB) functionalized with maleic acid and its application in SPE. The performance of this material is evaluated as a mixed-mode WCX material to selectively extract a group of drugs from environmental samples such as river, effluent and influent sewage. Then, the developed method was applied to different environmental water samples.

2. Materials and methods

2.1. Materials, reagents and standards

The following chemicals were used for the preparation of the materials: DVB (80%), benzoyl peroxide (BPO), sodium chloride, 88% hydrolyzed polyvinyl alcohol (PVA), toluene, and n-heptane purchased from Sigma-Aldrich (St. Louis, MO, USA). The monomer was purified by distillation before use. Ultrapure water (Merck Millipore, Burlington, MA, USA) was used for the aqueous phase in the polymerization reaction.

For the analytical evaluation, standards of atenolol (ATE), ranitidine (RAN), trimethoprim (TRI), caffeine (CAFF), metoprolol (MET), propranolol (PROP), naproxen (NAP), fenoprofen (FEN) and diclofenac (DICLO) were purchased from Sigma-Aldrich. Standard solutions of each analyte were prepared at 1000 mg/L in MeOH and stored at -20°C. Solution of all analytes was prepared at 100 mg/L concentration in MeOH: H_2O (1/1, v/v) and also stored at -20°C. Working solutions of all analytes were prepared weekly and stored at 4°C. The compound structure and its pK_a are shown in Table S1.

HPLC grade MeOH and acetonitrile (ACN) were supplied by J.T. Baker (Deventer, the Netherlands). The ultrapure water was provided by means of a Synergy UV water purification system (Merck Millipore). Formic acid (HCOOH) and ammonium hydroxide (NH_4OH) purchased from Sigma-Aldrich, and hydrochloric acid (HCl) (Scharlab, Barcelona, Spain) were needed for the regulation of pH in the mobile phase and extraction solutions.

2.2. Preparation and characterization of polymer material

PolyDVB polymer beads were synthesized in radical suspension polymerization. The continuous water phase comprised 2% w/w sodium chloride and 1% w/w PVA (calculated for the organic phase). The dispersed organic phase contained monomer (DVB), initiator (BPO – 0.5% w/w calculated for the organic phase) and the solvents toluene and n-heptane (1:7 w/w). The prepared polymer beads were modified with maleic anhydride in the Diels-Alder reaction as described in [24]. First, maleic anhydrate was dissolved in toluene and then the previously prepared polyDVB beads were added. The modification was carried out at 110°C for 48 hours. After that, base hydrolysis was performed using 3 mol/L sodium hydroxide. The products were then placed in ion-exchange columns and washed with water, HCl and again with water because they contained carboxyl groups in acidic form. A detailed summary and the modification are described in [25].

The material obtained (maleic acid-DVB) was fully characterized by calculating the water regain, carboxylic acid content, and measuring the specific surface area and pore size.

The water regain, W (g/g), of the material was determined using the centrifugation method and was calculated using Equation (1):

$$W = (m_w - m_d)/m_d \quad (1)$$

where m_w (g) is the weight of the wet polymer after centrifugation in a small column with a fritted-glass bottom and m_d (g) is the weight of polymer after drying at 100°C overnight.

The content of the carboxyl groups was determined by reversed HCl titration. First, the polymer beads were presoaked in water for 24 hours. They were subsequently centrifuged for 5 minutes at 3000 rpm, and then the analyzed material (~1.2 g) plus 50 mL of 0.1 mol/L sodium hydroxide were placed in the shaker for 24 hours. Finally, 20 mL of solution was sampled and titrated with 0.1 mol/L HCl using phenolphthalein as an indicator.

Pore size and surface area were obtained by examining nitrogen adsorption at the temperature of liquid nitrogen using a Micromeritics ASAP 2020 analyzer. The resulting data were subjected to a Brunauer-Emmett-Teller (BET) analysis. The total pore volume was estimated from a single point adsorption at relative pressure of 0.988. Scanning electron micrographs (SEM) were taken to obtain more direct insight into the porous polymer structure. The morphology of the obtained polymer beads was assessed using a scanning electron microscope manufactured by FEI (Quanta 250). The topography of the surface was evaluated using a secondary electron detector.

2.3. Solid-phase extraction procedure

200 mg of the in-house prepared sorbent (maleic acid-DVB) was manually packed into a 6 mL cartridge with two polyethylene 10 µm frits. The commercial sorbent, Strata-X-CW 33u Polymeric Weak Cation from Phenomenex (Torrance, CA, USA), was also evaluated for comparison purposes. The cartridges were placed in a vacuum manifold (Teknokroma, Barcelona, Spain) for the off-line SPE. The optimized SPE protocol was the same for both sorbents. The cartridges were preconditioned with 5 mL of MeOH and 5 mL of ultrapure water (pH 7). Then, samples also adjusted to pH 7 were loaded at a flow rate of about 10 mL/min. The cartridges were washed with 5 mL of MeOH and the analytes were eluted with 5 mL of 15% HCOOH in MeOH. The extract was evaporated to dryness using a miVac Duo centrifuge evaporator (Genevac, Ipswich, UK) and reconstituted with 1mL of MeOH: water (1/9, v/v). The extracts were filtered with 0.45 µm PTFE syringe filters (Scharlab) before being injected into the liquid chromatograph.

The SPE protocol was initially evaluated in ultrapure water and then tested using river water from the Ebre River and effluent and influent wastewater from a treatment plant in the region of Tarragona, Spain. All water samples were collected in pre-cleaned bottles and stored at -20°C until analysis. The water samples were filtered through a 1.2 μm glass-fiber membrane filter (only wastewater) and through a 0.45 μm nylon membrane filter (Fisherbrand, Loughborough, UK) prior to analysis.

2.4. Instrumentation and chromatographic conditions

For SPE evaluation, we used an Agilent 1260 system (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump, a solvent degasser, a manual injector and a column heater coupled to a UV detector. To analyze the environmental water samples, we used an Accela 1250 UHPLC system coupled to an Exactive Orbitrap™ mass spectrometer from Thermo Scientific (Bremen, Germany). The UHPLC was equipped with a quaternary pump (1250 bar), a refrigerated automatic injector (10°C) and a column oven (thermostatized at 30°C). The mass spectrometer worked with a heated electrospray ionization (HESI) source and a higher-energy collisional dissociation (HCD) cell to fragment the analytes for confirmation.

The analytical column was a Luna Omega Polar C_{18} (150 mm x 3 mm i.d., 5 μm) from Phenomenex, and the injection volume was 20 μL in all instances. The mobile phase was a mixture of 0.1% HCOOH in H_2O (solvent A) and ACN (solvent B), and was pumped at 0.4 mL/min. The signal was measured at 220 nm for all the compounds.

The optimal gradient profile started with 5% of solvent B, which was maintained for 2 minutes, and then, increased to 50% in 7 minutes, and then increased again to 100% within 2 minutes, which was held for 3 minutes; then, it was returned to initial conditions within 2 minutes, being maintained for 4 minutes. The total analysis time lasted 20 minutes.

In the ion source, ATE, RAN, TRIM, MET, PROP, CAFF and NAP were ionized in positive mode using the following optimal parameters: spray voltage, 4 kV; skimmer voltage, 25 V; capillary voltage, 40 V; and tube lens voltage, 80 V. FEN and DICLO were ionized in negative mode, with the optimal parameters as follows: spray voltage, 3.5 kV; skimmer voltage, -18 V; capillary voltage, -25 V; and tube lens voltage, -75 V. Gas flow rates and temperatures were the same for both ionization modes: sheath gas, 30 AU (adimensional units) and auxiliary gas, 5 AU; and, the heater and capillary temperatures were 350°C in both cases. The probe position settings were side to side 0, vertical C, and micrometer 0.5.

Acquisition was performed for 14 minutes using two time windows. The first one (0–11 minutes) only operated in positive mode, whereas the second one (11–14 minutes) operated in both modes. In both windows, two scan events were used for each ionization mode: a full scan (at 50,000 FWHM with 250 ms of injection time) and a fragmentation scan (at 10,000 FWHM with 50 ms of injection time). Those in the first window comprised two scan events, while four scan events were used in the second window, as it operated in both ionization modes. The optimal voltage in the HCD cell selected in the fragmentation was 25 eV for the positive analytes and 15 eV for the negative ones. For quantification, the protonated or deprotonated ions were measured (with a mass extraction window of 5 ppm) and the selected fragments and ion ratios were considered for confirmation. Table S1 shows the exact mass for the monitored ions of each compound. Figure S1 shows an extracted ion chromatogram of a standard solution.

3. Results and discussion

3.1. Material characterization

To obtain the polymer beads, the polyDVB was synthesized in radical suspension polymerization, resulting in a product in the form of regular microspheres with diameters in the range of 0.4–0.8 mm. The polymer was chemically modified by means of a Diels-Alder reaction in which polyDVB reacts as a diene and maleic anhydride as a dienophile. The efficiency of the modification reaction of polyDVB microspheres with maleic anhydride was 54%. The generation of the carboxyl groups capable of specific interactions was followed by ring opening of the maleic anhydride with base hydrolysis. The cycloaddition reaction scheme and basic hydrolysis data are shown in Fig. 1.

Suspension polymerization of DVB yielded spherical white polymer beads. Further modification with maleic anhydride and sodium hydroxide resulted in slightly beige spheres. Their SEMs are presented in Fig. S2 (supplementary material).

Sorption capacities are highly dependent on the porous structure of the material used. In the case of polymeric materials, their porous structures are designed by modifying the solvent mixtures used during the polymerization step. The appropriate solvents used in the polymerization process were selected based on the value called the solubility parameter (δ). Solvents having a δ similar to the δ of the monomer resulted in smaller pores and a higher surface area (sol solvents). The use of non-sol solvents (the difference in solubility parameters is greater than 1 Hildebrand unit) resulted in worse solvation of monomer and, consequently, increased pore size, which in turn led to a decreased surface area. The solvents responsible for the creation of the porous structure in the material obtained were toluene and *n*-heptane (1:7 w/w). The solubility

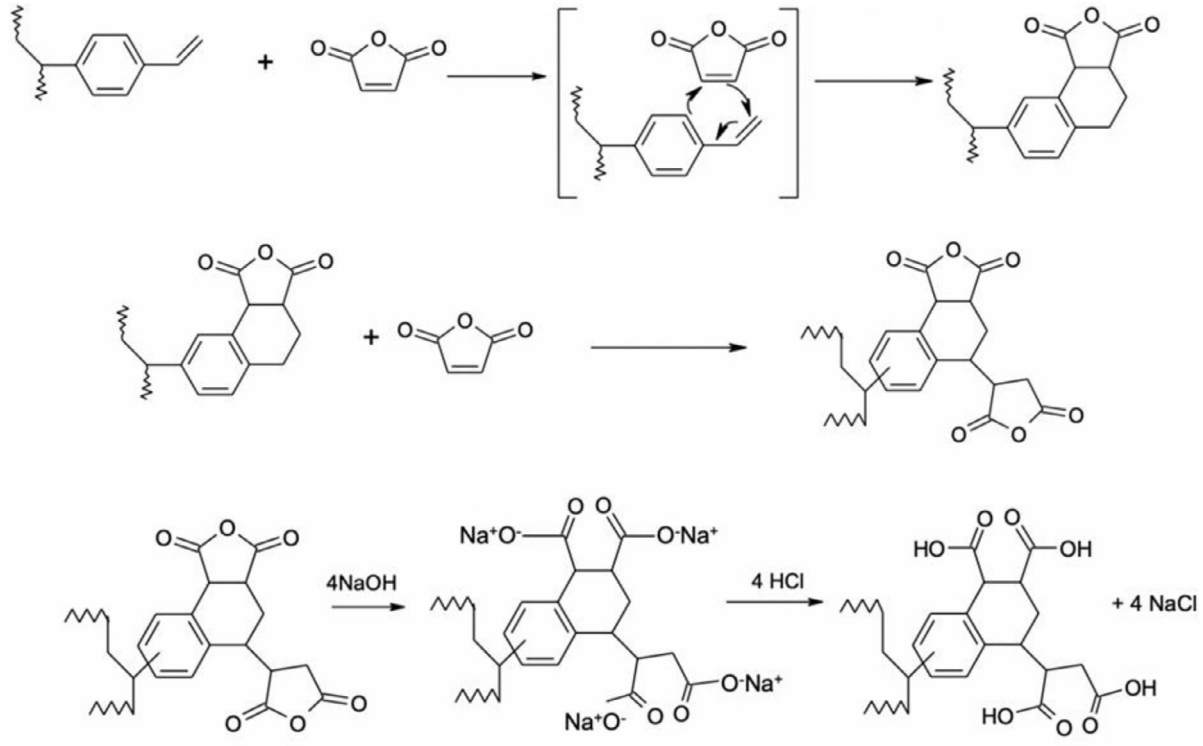

Fig. 1. Scheme of the modification of polyDVB with maleic anhydride to obtain maleic acid-DVB.

parameter values for toluene and n-heptane are 18.2 $MPa^{1/2}$ and 15.1 $MPa^{1/2}$, respectively, whereas the δ for DVB is 18.2 $MPa^{1/2}$. The pore structure of the synthesized adsorbent is noticeable in the scanning electron microscopy images, which are presented in Fig. S3 (supplementary material).

The parameters of the porous structure, including pore size (6.62 nm), pore volume (0.96 cm^3/g) and surface area (578 m^2/g), were calculated on the basis of nitrogen sorption measurements at liquid nitrogen temperature. The study shows that the tested sorbent has a mesoporous structure and a well-developed surface area (578 m^2/g). The water regain (2.54 g/g) of the tested polymer is characteristic for porous materials and is the result of the retention of water in the pores of the polymer.

The synthesized material has an acidic group content of 3.58 mmol/g, which indicates the presence of carboxyl groups in the sorbent structure that have been introduced through the modification of the free vinyl groups in the polyDVB. These groups can increase the sorption capacity by creating specific interactions between the sorbent and sorbate. More importantly from an analytical point of view, the presence of carboxyl groups in the polymer structure also adds ion-exchange properties to the resulting sorbent. Moreover, the ion-exchange capacity (3.58 mmol/g) is larger than other already reported ion-exchange sorbents. This is attributed to the four carboxylic acid groups per pendent moiety, whereas most of the already developed ion-exchange sorbents developed only own one ionic group per pendent moiety.

It is important to note that the presence of this high carboxylic acid content (3.58 mmol/g) may induce an improved ion-exchange capacity compared to other sorbents also modified with carboxylic acid moieties but with lower acidic group contents. For instance, the commercially available sorbent Oasis WCX, functionalized with carboxylic acid obtained via the oxidation of the intermediate chlorinated resin, contains about 0.75 mmol/g of acidic groups [26]. A similar content (0.72 mmol/g) was found in the in-house prepared sorbent based on terpolymer: methacrylic acid (MAA – containing the carboxylic acid moieties), vinylbenzyl chloride (VBC) and DVB [15]. Therefore, our next aim is to further exploit the WCX features of maleic acid-DVB sorbent, whose features envisaged an outstanding selectivity during its application in analytical fields.

3.2. Chromatographic evaluation

Ascentis Express C_{18} (100 mm x 4.6 mm i.d, 2.7 μm) and Luna Omega Polar C_{18} (150 mm x 3 mm i.d., 5 μm) columns were compared for the separation of the compounds. Both columns provided successful separation of the compounds using the optimized gradient (section 2.5) with a similar total elution time (13 minutes at most); however, the Luna Omega Polar C_{18} provided a shift of up to 3 minutes in the retention time of the first eluting compounds. As this shift might be beneficial when injecting complex samples (expected reduction of the ME), the Luna Omega Polar C_{18} was selected for this study.

Solutions of a mixture of all the compounds were infused under the chromatographic mobile phase composition and flow conditions in order to optimize the parameters that affect ionization and transfer to the Orbitrap analyzer. The basic and neutral compounds were tested under positive ionization mode, whereas the acidic compounds were tested in both ionization modes. All analytes exhibited better performance in the positive ionization mode, except for FEN and DICLO, which were ionized in negative mode. In all instances, either the protonated $[M+H]^+$ or deprotonated $[M-H]^-$ ions were selected as diagnostic ions. Next, ranges of gas flow rates, voltages and temperatures and different ionization probe positions were tested to optimize the HRMS conditions. The optimum parameters are those detailed in section 2.5. Fragmentation conditions in the Exactive Orbitrap mass analyzer were optimized for each diagnostic ion separately to achieve the highest abundance of the two fragment ions (Table S1). Collision energies were tested from 0 to 60 eV. A compromise energy of 25 eV was established for the analytes (basic) eluting in the first window, and of 15 eV for those in the second window.

Table S1 details the accurate mass and the chemical formula of the diagnostic ions and fragments. The selected ions agree with those tested in previous studies in which HRMS analyzers such as Orbitrap [27] and Q-Orbitrap [28] were used.

All selected compounds presented good linearity (r^2=0.998) in LC-HRMS, and the linear ranges were between 0.5 and 1000 µg/L for ATE, PROP and DICLO, and between 1 and 1000 µg/L for the rest of the compounds, except RAN which only achieved up to 100 µg/L. Instrumental limits of detection (ILDs) were established as the concentrations whose signal-to-noise ratio (S/N) was greater than 3, and which had one fragment with a signal intensity higher than 1×10^3 in the Orbitrap analyzer. The IDLs ranged from 0.05 to 0.1 µg/L. The instrumental limits of quantification (IQLs) adopted were the lowest concentration in the calibration curve, which also accomplished a S/N of more than 10; they were set at 0.5 and 1 µg/L.

3.3. SPE optimization

To check the WCX features of the maleic acid-DVB sorbent, we selected a group of basic, neutral and acidic pharmaceuticals (pK_a values listed in Table S1) to assess its ionic and reversed-phase interactions. In an SPE protocol that exploits the WCX features of a sorbent, the sample is loaded at basic or neutral pH so that the sorbent is in its anionic form and is able to ionically interact with the basic analytes (protonated), while the neutral and acidic analytes (in neutral form) are only retained through reversed-phase interactions. These neutral and acidic analytes (or interferences) are eluted during the washing step, while the basic analytes are selectively eluted during the elution step, which includes an acidic additive to protonate the sorbent and release the ionic interactions.

The initial experiments for the SPE optimization were performed by loading 100 mL of ultrapure water at pH 7 spiked with the analyte mixture at 0.5 mg/L and eluting the analytes with 5 mL of 5% HCOOH in MeOH. None of the extracts obtained during the SPE optimization were evaporated, instead they were diluted with aqueous solution so that the injection solution was compatible with the initial mobile phase. Furthermore, to keep it simpler, all these extracts were injected into LC-UV.

First, the elution conditions were established by testing different percentages (5%, 10% and 15%) of HCOOH and different volumes (2, 5 and 10 mL) of MeOH. Both 10 mL of 10% HCOOH in MeOH and 5 mL of 15% HCOOH in MeOH provided complete recoveries for all the analytes. 5 mL of 15% HCOOH in MeOH was selected in order to maintain the elution volume as low as possible, because that makes the analysis time shorter if evaporating the extract to dryness. Percentages of up to 10% HCOOH in MeOH are usually selected in the elution solution when WCX sorbents are used [27,29,30]. However, in other cases, a higher percentage of HCOOH (i.e. 40%) [9] or stronger acids (i.e. trifluoroacetic acid) [15] have been selected. In fact, a greater amount of HCOOH required in the elution step is compatible with the high carboxylic acid content (3.58 mmol/g) in the resin. Thus, 15% HCOOH is completely feasible in the elution solution.

Next, a washing step with 2 mL MeOH was used to check the selectivity of the sorbent. As expected, when the washing step was included, the acidic and neutral compounds were washed out, as MeOH broke the reversed-phase interactions, while the basic analytes remained bound through ionic interactions until the elution step. Therefore, the selectivity of the sorbent was successfully demonstrated. The next step was to optimize the type and volume of washing solvent. Different volumes (2, 5 and 10 mL) of ACN and MeOH, and mixtures of the two, were assayed. The elimination of acidic and neutral compounds (also interferences when move to complex environmental samples) was more relevant when using MeOH (up to 100%) than with the other solvents tested (70–90%). As for the volume, when 2 and 5 mL of MeOH were used in the washing step, none of the basic analytes were lost in this step; however, when 10 mL was tested, 22% of RAN and 30% of PROP were washed out, while the rest of the basic compounds remained. Thus, the optimized washing step consisted of 5 mL MeOH. It should be mentioned that in the SPE protocol of other WCX sorbents, a lower volume of methanolic solution [15,31] or even a solely aqueous-based washing solution [9,30] has been applied. In fact, with this volume of pure organic solvent in the washing step, the elimination of a large quantity of interferences present in complex environmental samples is likely, and thus the reduction of the ME.

Once the SPE protocol had been established, the next step was to evaluate the loading volumes through the maleic acid-DVB cartridge. As shown in Table 1, the recoveries obtained when 100 mL or 250 mL were percolated were very similar to values ranging from 74% to 106% for all basic compounds, with the analytes RAN and PROP (% recovery values of 74–78%) presenting the lowest values. Nonetheless, the values obtained (% recoveries 51–77%) when 500 mL of sample was loaded decreased compared to those when 250 mL was percolated. Thus, the sample volume was fixed at 250 mL. This volume of sample loading was consistent with the quantity of sorbent packed (200 mg). If we were interested in raising the capacity of the sorbent, a larger amount of sorbent would have been packed (i.e. 500 mg). Another interesting feature to observe in Table 1 is the selectivity of the sorbent, since the neutral and acidic compounds are completely eluted in the washing step.

To further demonstrate the WCX features of the maleic acid-DVB sorbent, its performance was compared to the commercially available sorbent Strata-X-CW. This is also a macroporous resin (800 m^2/g) with COOH functionalization (0.74 mmol/g); but Strata-X-CW presents hydrophilic moieties in its structure (DVB functionalized with pyrrolidone moieties), whereas maleic acid-DVB sorbent is completely hydrophobic since it is based solely on DVB. In any case, the results obtained (Table 1) from the Strata-X-CW are very similar to those of the in-house sorbent. Both sorbents present similar mesoporous structure resulting in similar specific surface area. What is more noticeable, as stated in section 3.1, is the difference in carboxylic acid content (3.58 mmol/g of acidic groups in the in-house sorbent versus 0.74 mmol/g in the commercially available product), which might result in a greater number of ion-exchange interactions that enhance the selectivity of the sorbent in comparison to the commercially available ones. Other studies have also evaluated other commercially available sorbents, such as Oasis WCX, for the extraction of a similar group of compounds [15,27]. For instance, a multi-layered approach that consisted of the combination of Oasis WAX and Oasis WCX packed in a single 100 mg cartridge was tested for the same basic compounds. In this case, the recoveries when 50 mL of ultrapure water adjusted to pH 5 were loaded using a protocol that included a washing step with 2 × 1 mL MeOH were between 91% and 56% [27].

3.4. Application to environmental samples

The optimized SPE protocol was then tested in river and wastewater samples to examine the performance of the in-house sorbent when dealing with complex samples. At this stage, the acidic and neutral analytes were not evaluated as it had already been proven that they would be lost during the washing step. The in-house sorbent using the optimum protocol was applied to analyze 250 mL of river water, 100 mL of effluent wastewater and 50 mL of influent wastewater; volumes that were selected based on the ultrapure water results and previous experience. ME (%ME) and apparent recovery ($\%R_{app}$) percentages were assessed and are shown in Table 2. The %ME was calculated from the concentration obtained ($C_{post\text{-}spiked}$) when the sample extract was spiked before

Table 1
Recovery values (%) when the maleic acid-DVB and Strata-X-CW were applied to the preconcentration of ultrapure water samples.

		% RECOVERY							
		Maleic acid-DVB						Strata-X-CW	
		100 mL		250 mL		500 mL		100 mL	
		wash	el	wash	el	wash	el	wash	el
ATE	**BASIC**		106 ± 7		95 ± 7		64 ± 6		112 ± 12
RAN			78 ± 11		76 ± 7		51 ± 10		97 ± 7
TRIM			90± 5		87 ± 4		77 ± 5		95 ± 8
MET			87± 4		84 ± 6		74 ± 7		96 ± 6
PROP			74± 8		76 ± 8		63 ± 6		88 ± 6
CAFF*	**NEUTRAL**	88 ± 5		89 ± 7		87 ± 4		93 ± 6	
NAP*	**ACIDIC**	91 ± 6		98 ± 3		94 ± 5		93 ± 3	
FEN*		96 ± 3		95 ± 5		98 ± 7		98 ± 8	
DICLO*		97± 3		98 ± 7		95 ± 5		97 ± 2	

* recovery values in the washing step

Table 2
Matrix effect (%ME) and apparent recoveries ($\%R_{app}$) percentages for the basic pharmaceuticals when 250 mL of river water spiked at 200 ng/L, 100 mL of effluent wastewater spiked at 500 ng/L and 50 mL of influent wastewater spiked at 1000 ng/L were percolated through maleic acid-DVB sorbent.

	River		Effluent		Influent	
	%ME	$\%R_{app}$	%ME	$\%R_{app}$	%ME	$\%R_{app}$
ATE	-18	74 ± 6	-27	62 ± 5	-28	67 ± 11
RAN	-27	66 ± 8	-32	67 ± 10	-30	57 ± 12
TRIM	+7	89 ± 10	-18	72 ± 11	-24	73 ± 8
MET	-7	83 ± 11	-12	80 ± 16	-18	74 ± 8
PROP	-20	63 ± 6	-15	66 ± 7	-28	58 ± 10

injection into the LC-HRMS compared to the concentration when a standard ($C_{standard}$) was injected into the LC-HRMS as described elsewhere as: $\%ME = 100-[(C_{post\text{-}spiked}/C_{standard})x100]$. The $\%R_{app}$ represents the recovery of the whole method (SPE/LC-HRMS) and it was calculated from the concentration obtained ($C_{pre\text{-}spiked}$) after the entire procedure when the sample was spiked with the analyte mixture before the SPE in comparison to the concentration when a standard ($C_{standard}$) was injected into the LC-HRMS as described elsewhere as: $\%R_{app} = (C_{pre\text{-}spiked}/C_{standard})x100$. In all instances, a non-spiked sample was analyzed and the signal of the natural occurring analytes were subtracted. In fact, initially, the $\%R_{app}$ and %ME tests were performed at two concentration levels (20 ng/L and 200 ng/L for river; 50 ng/L and 500 ng/L for effluent; 100 ng/L and 1000 ng/L for influent); however, due to the presence of almost all analytes in the non-spiked wastewater samples, the results are only presented when the samples were spiked at high concentration levels.

The %ME (Table 2) observed was low, in general rising at most to -32% and in the form of ion suppression in all cases, except for TRIM in river water, which presented little ion enhancement (+7%). As expected, the ME values encountered in the river water samples were lower (from -7% to -27%) than in effluent wastewater (from -12% to -32%) or influent wastewater (from -18% to -30%), which is attributed to the decreasing sample volume loaded in agreement with the increasing complexity (content of organic matter). The low ME encountered might be due to high content of carboxylic acid that is able to establish specific interactions and the presence of the washing step, which simplified the complexity of the matrix. As for Strata-X-CW slightly higher %ME were obtained with values ranging from -43% (ATE) to + 23% (PROP) in effluent wastewater samples or from -35% (RAN) to -31% (MET) in influent wastewater. Similar ME values were found when an in-house mixed-mode SCX resin was used with a protocol that involved 5 mL of MeOH in the determination of similar basic compounds from effluent and influent wastewater samples [13]. Nonetheless, in another study where a cartridge combining SCX/SAX was used, the ME values obtained when 100 mL of effluent wastewater were analyzed were similar (from -15% to -28%) with the exception of ATE (-49%) and TRIM (-48%), with values higher than expected considering the volume used for washing (15 mL MeOH) [27]. Other studies have presented methods that involve an SPE protocol without a washing step and they report higher ME values [28, 32, 33]. For instance, Strata X was used for the determination of a group of pharmaceuticals, and presented a ME in effluent wastewater of up to -57% for ATE [28]. In another study, Oasis MCX and Strata X were assembled in series to extract as many compounds as possible (including a similar group of pharmaceuticals); however, this strategy also increased the retention of matrix components, which raised the ME to values between -77% and -84% [32]. Differences in %ME arose when comparing the protocols with and without washing step. In this sense, values higher than 100 % in form of ion enhancement were encountered for cocaine and its metabolite when the extraction with Oasis WCX did not include the washing step. Nonetheless, when the washing step based on MeOH was included, these %ME values decreased to +20% (cocaine) and -18% (its metabolite) [33].

The $\%R_{app}$ (Table 2) obtained were very similar for all three matrices, with values ranging from 57 to 89%, which might be attributed to the sample volume loaded and the complexity of the samples. It should be noted that as these $\%R_{app}$ values are practically not affected by the ME, the $\%R_{app}$ values are better than those reported for similar compounds [13,27,32].

The SPE/LC-HRMS method using maleic acid-DVB as a sorbent was validated for river water and wastewater by assessing the linearity, method detection limits (MDLs) and method quantification limits (MQLs), and repeatability and reproducibility. Table S2 details the validation values obtained with the three type of environmental samples. In the case of river water, the matrix-matched calibration curve was experimentally prepared by spiking the river water at eight concentration levels. Linearity was good ($R^2 \geq 0.996$) between the 2.5 ng/L (for ATE and PROP) or 5 ng/L (for TRIM and MET) and 1000 ng/L, except for RAN (10-500 ng/L). The MDLs were the spiked concentrations (from 0.1 to 1 ng/L) that showed a signal for the most abundant fragment of around 10^3 and they were from 0.25 ng/L to 1 ng/L, while the MQLs (2.5 to 10 ng/L) were the first concentration point of the matrix-matched calibration curve. In the case of wastewater, as all the compounds (with the exception of RAN) were present in the non-spiked samples, the external calibration curves considering $\%R_{app}$ were used for quantification. Similarly, MQLs and MDLs were estimated from LOQs and LODs also considering $\%R_{app}$. MDLs were between 1 and 2.5 ng/L for effluent and between 2 and 5 ng/L for influent; while MQLs ranged from 10 to 25 ng/L for effluent and from 20 and 50 ng/L for in-

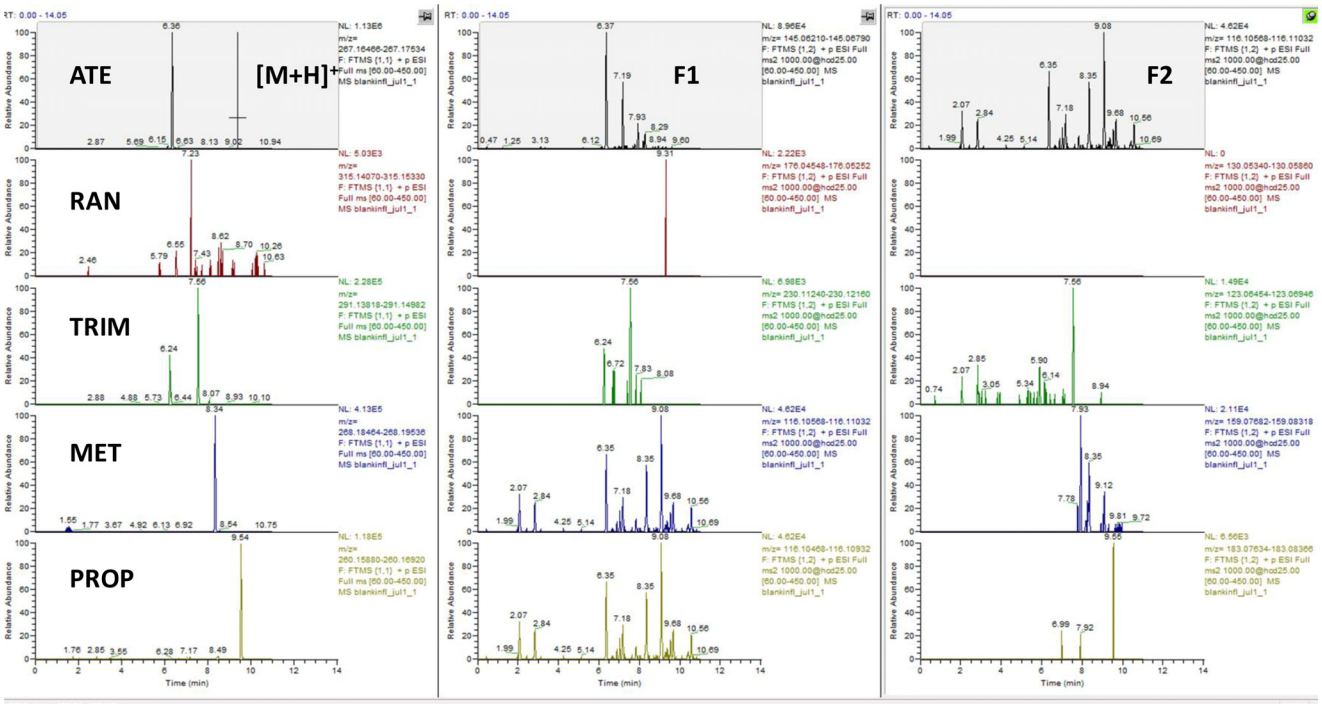

Fig. 2. Extracted ion chromatogram of the diagnostic ions ([M+H]$^+$) and the two fragments (F1, F2) of a non-spiked influent wastewater sample when analyzed by SPE using maleic acid-DVB sorbent and LC-HRMS under the optimum conditions.

Table 3
Concentrations of the analytes found in river, effluent wastewater and influent wastewater when samples were analysed by SPE using maleic acid-DVB followed by LC-HRMS.

	Conc. (ng/L) (N=5)		
	River	**Effluent**	**Influent**
ATE	<MQL – 55.64	67.40 – 539.12	454.25 – 1332.41
RAN	n.d.	n.d.	n.d. - < MQL
TRIM	n.d. -16.61	21.27 – 658.18	8.03 – 183.26
MET	n.d. -15.84	5.09 – 81.75	69.55 – 117.40
PROP	n.d. -2.63	7.31 – 26.57	n.d. – 14.83

fluent. Intra-day repeatability and inter-day repeatability (n=5) of the method expressed as a percentage of relative standard deviation (%RSD) were evaluated for all three matrices, with values from 3% to 22%. It should be mentioned that the figures of merit are in line with those reported in the literature [27,28,34].

The method developed was used to analyze different water samples from the Ebre River and effluent and influent wastewater samples from sewage treatment plants in the Tarragona area (north-eastern Spain). Table 3 summarizes the range of concentrations found in each type of sample. The presence of compounds was confirmed based on an exact mass (error < 5 ppm) of their diagnostic and fragment ions, the ion ratio between the fragment and the diagnostic ion and their retention time (± 0.1 min), ensuring at least four identification points depending on the compound. All the analytes were present in all types of samples, with the exception of RAN which was only present in one influent wastewater sample below MQL. As an example, Figure 2 shows the extract ion chromatogram from the analysis of one influent sewage sample. However, in the river water samples analyzed, as expected, the analytes were either present at low concentration levels or not detected. The concentration values found in effluent wastewater samples were lower or similar to those found in the influent samples; however, in some instances (in one sample for TRIM and in two for PROP) higher concentrations than those in the influent samples were quantified. This might be attributed to the fact that the sampling of the effluent and influent samples was not performed in the same period. This trend was also found in other studies where samples from the same wastewater treatment plants were analyzed [27,35], and in which, in fact, the concentration ranges reported for these pharmaceuticals were also similar. Moreover, the levels reported in influent wastewater samples from other treatment plants were similar, with the exception of ATE for which lower levels (250-400 ng/L [36] or 50-150 ng/L [28]) were reported.

Although the performance of the maleic acid-DVB sorbent has been tested for these analytes in these types of samples, it is possible to test it for a broader range of basic analytes in other complex matrices.

3.5. Conclusions

This work reports the preparation of a novel WCX based on the modification of polyDVB particles with maleic acid. One of the main features of this novel resin is the notable carboxylic acid content compared to that of commercially available WCX sorbents, which enhance the selective ion-exchange interactions with the target compounds.

The WCX features of the maleic acid-DVB resin were successfully exploited in the SPE, where under the optimized protocol involving a washing step with 5 mL MeOH it is capable of selectively extracting a group of basic compounds. This washing step also aided in the reduction of the ME (-32% at most) when different complex samples such as effluent and influent wastewater samples were analyzed by means of SPE/LC-HRMS. The limits of the method were at a low ng/L level, which enables the quantification of most of the compounds present in the environmental samples analyzed.

The developed resin could also be applied in the selective extraction of other basic compounds from different types of complex samples.

Declaration of Competing Interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2021.462165.

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