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Cover letter

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Editorial Office

Journal of Chromatography A

Tarragona, 17th March 2014

Dear Prof. Zou,

Please find attached the manuscript number JCA-13-2216R1 entitled "Evaluation of strong cation-exchange polymers for the determination of drugs by solid-phase extraction-liquid chromatography- tandem mass spectrometry" by Núria Miralles, Norhayati Abdullah, Arlene Davies, Núria Gilart, P.A.G. Cormack and myself**,** to be considered for publication in the special virtual issue of "Secyta2013" of Journal of Chromatography A. The manuscript has been revised and modified according the reviewers comments.

Looking forward to hearing from you

Yours sincerely

Dr. Núria Fontanals

RESPONSE TO REVIEWERS –JCA-13-2216R1

Reviewer #1: *JCA-13-2216R1*

The manuscript deals with the preparation and use of polymeric solid-phase extraction materials for the determination of a series of trace basic drugs and other contaminants in sewage waters. Microparticles of polymeric precursors were obtained via non-aqueous dispersion polymerization or precipitation polymerization. The polymer beads were then subjected to hypercrosslinking and sulfonation. The great success in the use of the SPE material for solving important practical problems, which was convincingly shown in the major part of the manuscript, was entirely due to the combination of crucial properties of the SPE material, namely, its micron-range size of the polymer particles and their hypercrosslinked internal morphology. Unfortunately, authors avoid mentioning any of seminal publications of pioneers who first suggested the above crucial procedures of preparing monosized polymeric microbeads and providing hypercrosslinked structure to polystyrene. Instead, they give a biased reference list where 10 of 29 numbers belong to the first author of present manuscript. Indeed she used already earlier the procedures under discussion, but ignoring the origins of the crucial techniques is not consistent with the publication ethics.

We had already included one reference (ref. 12) from Davankov, even the hypercrosslinking strategy used in the present study is not the same as the one used by Davankov. In any case, as we also agree that Davankov pioneered in the development of hypercrosslinked structures we have added an additional reference (ref. 13). Moreover, we have deleted three of our references and grouped in one single.

As to a less important comment, the Reviewer would suggest to give a note concerning the possibility of reusing the cartridges for subsequent analysis.

We have provided this information in the text.

When suggesting the acceptance of the otherwise high quality manuscript for publication, I would strongly recommend giving due acknowledgement to pioneers of break-through techniques in chemistry and analytics.

Reviewer #2: *Changes had been made accordingly. However, based on scientific background, the reviewer still could not accept the used of English 'expression' in text line 75 (…thanks to the careful…) and 102 (…thanks to the different…).*
â€¦).
We arranged these two expressions in the text.

Reviewer #3: *I have read with interest the revised version of the manuscript.*
Authors have taken into consideration the majority of my remarks and suggestions. I would like to repeat once again that the proper QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) systems is vital for reliability of the results of work.
ADDITIONAL CHAPTER DEALING WITH THIS ASPECT OF THE WORK HAS TO BE ADDED TO THE TEXT- I REPEAT IT WITH CONVICTION.
It would be useful if Authors will add some additional reference to the LIST OF REFERENCES. these reference should deal with description of green sample preparation techniques before

chromatographic determination of residue of pharmaceuticals in suitable extracts by LC-MS/MS techniques.

We have added information regarding to QA/QC in the text.

We have added one sentence and its corresponding reference concerning to the use of green sample preparation techniques in the introduction.

Highlights

- We prepare eight different strong-cation exchange (SCX) materials.
- The new SCX materials are evaluated in SPE/LC-MS/MS.
- The materials are evaluated in terms of recovery and matrix effect.
- A selective method to determine basic drugs in sewage samples is developed.

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EVALUATION OF STRONG CATION-EXCHANGE POLYMERS FOR THE DETERMINATION OF DRUGS BY SOLID-PHASE EXTRACTION-LIQUID CHROMATOGRAPHY- TANDEM MASS SPECTROMETRY

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Keywords: strong cation-exchange / solid-phase extraction / recovery / matrix effect / sewage

ABSTRACT

This paper presents eight distinct strong cation-exchange resins, all of which were derived from precursor resins that had been synthesised using either precipitation polymerisation or non-aqueous dispersion polymerisation. The precursor resins were transformed into the corresponding strong cation-exchange resins by hypercrosslinking followed by polymer analogous reactions, to yield materials with high specific surface areas and strong cation-exchange character.

These novel resins were then evaluated as strong cation-exchange (SCX) sorbents in the solid-phase extraction (SPE) of a group of drugs from aqueous samples. Following preliminary experiments, the two best-performing resins were then evaluated in solid-phase extraction-liquid chromatography-tandem mass spectrometry (SPE/LC-MS/MS) to determine a group of drugs from sewage samples. In general, use of these sorbents led to excellent recovery values (75% - 100%) for most of the target drugs and negligible matrix effects (ME) (< 20% ion suppression/enhancement of the analyte signal), when 50 mL and 25 mL of effluent and influent sewage water samples, respectively, were percolated through the resins. Finally, a validated method based on SPE/LC-MS/MS was used to quantify the target drugs present in different sewage samples.

1. INTRODUCTION

In modern society, the numbers of drugs in widespread use is increasing. These drugs can be released into the environment *via* sewage systems or incorrect disposal methods. Although these waters pass through sewage treatment plants (STP), these drugs are often not removed completely by the treatments processes. As a result, they are often found in surface and wastewaters at ng/L levels [1,2]. In view of this, new analytical techniques should be developed. These analytical techniquesinclude sample preparation followed by liquid chromatography coupled with mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS), to enable the determination of these drugs at the required low concentration levels [3-5].

Sample preparation must enrich the analytes, and should reduce the matrix effect (ME) on subsequent LC-MS analysis to obtain reliable and repeatable results. For liquid samples, some green solvent-extraction techniques have been developed [6]; regarding to sorptive-extraction techniques, solid-phase extraction (SPE) is usually the technique of choice [7] because of its versatility arising from the ready availability of different sorbent types. In recent years, research into SPE sorbents has focused on improving capacity (enhancing the preconcentration factor) and selectivity (improving the clean-up effectiveness) within a single material, leading to the emergence of what are known as mixed-mode polymeric sorbents. These sorbents combine a polymeric skeleton with ionic groups, with two types of interactions available: reversed-phase (RP) (from the skeleton) and ion-exchange (from the ionic groups). Mixed-mode sorbents are classified depending on whether the ionic group attached to the resin is cationic or anionic, but also whether the ionic group is strong or weak. The most common of these ionic groups are sulfonic acids and carboxylic acids for strong and weak-cation exchange sorbents, respectively; and quaternary amines for strong anion-exchange, and tertiary, secondary and primary amines for weak anion-exchange. A benefit of mixed-mode sorbents is that the ion-exchange interaction between the sorbent and the analytes and/or interferences is turned on and off by the careful control of the pH of the washing and elution solvents, resulting in the selective protonation or deprotonation of the analytes or interferences, and even the sorbent (in the case of weak ion-exchange sorbents). Thus, the interferences and analytes can be eluted separately during the washing and elution steps, respectively, ~~thanks~~ as a consequence of ~~to~~ the careful, rational selection of pH and the solvent in each SPE step [8].

At present, mixed-mode sorbents are one of the main focuses of research for manufacturers and companies. One of the reasons for this is, generally, the need for cleaner extracts from SPE and, in particular, avoidance of ion-suppression/enhancement when these extracts are

injected into LC-MS or LC-MS/MS systems [4,9-11]. Therefore, despite being relatively new, they have been applied in various fields to extract different types of analytes in a selective manner from the matrix interference usually present in complex samples, such as those of biological, foodstuff and environmental origin [8,9]; thus, it is a constant evolving field.

In recent years, several SPE sorbent companies have launched strong or weak cation-exchange (SCX or WCX, respectively), and strong or weak anion-exchange (SAX or WAX, respectively) variants of such well-known sorbent precursors, including Oasis (Waters), Strata (Phenomenex), Bond Elut Plexa (Agilent Technologies), and Evolute (Biotage), among others. These commercially available mixed-mode sorbents are characterised by their macroreticular structures, specific surface areas from 600 – 800 m^2/g, mean particle diameters from 50 -100 µm and, in some cases, a degree of hydrophilicity [8].

To improve the features of the mixed-mode sorbents available in the market, our research group has been working on improving the morphological properties (by exploiting hypercrosslinked polymer microspheres ~~[12]~~pioneered by Davankov in the early 90s' [12,13]) and the introduction of ionic moieties, so that they can exhibit higher levels of RP and ionic interactions with the analytes. So far, we have prepared and evaluated, *via* SPE, hypercrosslinked polymer microspheres modified with 1,2-diethylamine and piperazine moieties ~~[14]~~, dimethylbutylamine ~~[15]~~ and carboxylic acid ~~[16]~~ moieties to impart WAX, SAX and WCX character onto the sorbents. The results obtained with these resins were promising and better than those from commercial available sorbents ~~–~~[8,~~14-16~~]. These suitable results were attributed to the enhanced structural properties of these mixed-mode sorbents.

In view of this, we present eight different in-house prepared SCX ~~materials,~~ materials. These materials ~~which~~ differ in terms of their morphological properties and ion-exchange capacity, which are ~~thanks~~attributed to the different synthetic approaches used during their preparation. We have evaluated these different SCX materials in the following terms: capacity to enrich target analytes and effectiveness in cleaning-up the interferences in the matrix samples; ability to reduce the ME encountered when analytes are determined by LC-MS/MS from complex environmental samples.

2. EXPERIMENTAL PART

2.1. MATERIALS

The reagents used for the polymer synthesis were *para*-vinylbenzyl chloride (VBC) (95% grade), divinylbenzene (DVB) (80 % grade), styrene (St) (99% grade), ethylene glycol dimethacrylate (EGDMA) (98% grade), poly(*N*-vinylpyrrolidone) (PVP) 55 (M_W ~55,000) and Triton X-305, all supplied by Sigma-Aldrich (Steinheim, Germany). The monomers were purified by passing them through a short column of neutral alumina. 2,2'-Azo*bis*(isobutyronitrile) (AIBN), supplied by BDH (Poole, U.K.) was recrystallised from acetone at low temperature. Anhydrous 1,2-dichloroethane (DCE), iron (III) chloride, chlorosulfonic acid, lauric acid and tetraethyl ammonium bromide (TEABr) (or sodium chloride – NaCl) were supplied by Sigma-Aldrich; all were of high purity as supplied and not purified further prior to use.

The additional reagents used in the preparation of the HXLNAD-SCX sorbents were 1,2-dichloroethane (DCE) (99.8% grade) and concentrated sulfuric acid (95-97%), both supplied by Sigma-Aldrich, and distilled water. All reagents were used as received.

We selected some therapeutic drugs with basic and acidic groups to evaluate the performance of the different sorbents. They included: trimethoprim, caffeine, antipyrine, atenolol, ranitidine, metoprolol, propranolol, carbamazepine, clofibric acid, salicylic acid, ibuprofen and diclofenac. They were obtained from Sigma-Aldrich. Standard stock solutions of each analyte were prepared at 1000 mg/L in methanol (MeOH). We also selected the following illicit drugs: morphine, cocaine, methadone and codeine, and their metabolites, 6-acetylmorphine, benzoylecgonine, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and dihydrocodeine, respectively. They were purchased from Cerilliant (Round Rock, TX, USA) as solutions in MeOH at a concentration of 1000 mg/L. A mixed solution of all analytes in MeOH at 50 mg/L was prepared weekly. All standard solutions were stored at -20 ºC. Working solutions were prepared daily by an appropriate dilution of the mixed solution with ultrapure water, which was obtained from a water purification system (Veolia, Sant Cugat del Vallès, Spain). Table 1 presents the pK_a values of these analytes.

Acetonitrile (ACN) and MeOH were of HPLC grade and from Prolabo (Llinars del Vallès, Spain). Nitrogen (N_2) was supplied by Carburos Metálicos (Tarragona, Spain). Hydrochloric acid (HCl) (37%), formic acid (HCOOH) (≥95%), ammonium hydroxide solution (NH_4OH) (28%) and sodium hydroxide (NaOH) (≥98%) were purchased from Sigma-Aldrich.

2.2. RESIN SYNTHESIS

Two type of polymerisations, namely non-aqueous dispersion polymerisation (NAD) and precipitation polymerisation (PP) were adopted to prepare the precursor particles [14] ~~[17]~~.

For NAD polymerisations, PVP-55 (6% w/w), Triton X-305 (2% w/w), AIBN (2-6% w/w), styrene (50-90% w/w) (all the percentages are relative to the total mass of monomer in the monomer feed), half of the VBC and 47.5 mL of ethanol were added into a 500 mL five-necked, round-bottomed flask fitted with an overhead stirrer, condenser and nitrogen inlet. Once a homogenous solution had formed at room temperature, the solution was bubbled with nitrogen gas at room temperature for 30 minutes. The flask was then placed into an oil bath set at 70 °C, and stirred mechanically using a four-bladed PTFE stirrer at 160 rpm. EGDMA (1% w/w relative to total mass of monomer in the feed) and the second half of the VBC were dissolved in a second portion of ethanol (47.5 mL) at 70 °C under nitrogen. One hour after the start of the polymerisation, the hot solution containing EGDMA and VBC was added dropwise into the reaction vessel. The reaction was continued for a further 24 hours under constant agitation. The particles that were obtained were centrifuged for 10 minutes at 3,000 rpm and then washed 2 times in ethanol and 2 times in methanol (the particles were suspended in the appropriate wash solvent and centrifuged between each washing step).The particles were filtered using vacuum filtration on a 0.2 µm nylon membrane filter and dried overnight in *vacuo* at 40 °C.

For the PP polymerisations, the comonomers (75% (w/w) VBC and 25% (w/w) DVB) (2% w/v total monomer in feed relative to solvent) and AIBN (2 mol% relative to polymerisable double bonds) were added to ACN (200 mL) in a polypropylene bottle (250 mL). The monomer solution was deoxygenated with N_2 at 0 ^{o}C and then the bottle placed on a low-profile roller (Stovall) in a temperature-controllable incubator (Stuart Scientific, Surrey, UK). The temperature was ramped from ambient to 60 ^{o}C over a period of ~2 hours and the polymerisation allowed to proceed at 60 ^{o}C for a further 46 hours. The resulting particles were separated from the reaction medium by filtration on a 0.2 µm nylon membrane filter, washed successively with MeOH, toluene and acetone, before drying *in vacuo* at 40 ^{o}C overnight.

For the hypercrosslinking reactions the NAD or PP precursor particles (~ 1.2 g) were added to 1,2-dichloroethane (DCE) (40 mL) in a round-bottomed flask (100 mL) and were left to swell fully under a stream of N_2 at room temperature for 1 hour. $FeCl_3$ (1:1 mole ratio with respect to the CH_2Cl content of particles) in DCE (40 mL) was added and the mixture heated at 80 ^{o}C for 18 hours. The hypercrosslinked particles (HXLPP and HXLNAD for PP and NAD precursors, respectively) were recovered as described above and washed with MeOH and several times with aqueous HNO_3 (pH 1). The particles were then extracted overnight with acetone in a

Soxhlet extractor and were washed again with MeOH and diethyl ether before drying *in vacuo* overnight at 40 °C.

For the sulfonation procedure, either HXLPP or HXLNAD particles were charged to a three-necked, round-bottomed flask fitted with an overhead mechanical stirrer and a reflux condenser, under an N_2 atmosphere. Anhydrous DCE (30 mL) was added. This was left for 1 hour to wet the beads. For sulfonation of the HXLPP particles, the lauroyl sulfate solution (obtained by reaction of lauric acid and chlorosulfonic acid in cyclohexane (3 mL) under an N_2 atmosphere and stirred at room temperature for 1 hour [~~18~~15]) was added to the HXLPP beads *via* syringe and the reaction was heated to 50 °C, with stirring, for 24 hours. After 24 hours, the product was recovered by filtration on a 0.2 μm nylon filter membrane and washed with petroleum ether (b.p. 60-80 °C), and further extracted overnight in a Soxhlet apparatus with the same solvent. The product, in the form of a brown powder, was then washed with diethyl ether (b.p. 30-40 °C) and oven-dried *in vacuo* at 40 °C for 24 hours. For sulfonation of the HXLNAD particles, sulfuric acid was added to the HXLNAD beads *via* syringe and the mixture was then heated rapidly to 60 °C under nitrogen, with continuous mechanical stirring during the reaction. The sulfonated hypercrosslinked particles were then allowed cool on an ice-water bath to quench the reaction, washed with an excess of distilled water until the pH of the filtrate was neutral, and then filtered using vacuum filtration on a 0.2 µm nylon membrane filter. They were then dried overnight in *vacuo* at 40 °C.

Variable amounts of the sulfonation reagent were added so that we obtained resins with different loadings of sulfonic acid groups. Table 2 details all the resin compositions.

The HXLPP-SCX and HXLNAD-SCX resins were characterised by measuring their specific surface areas using N_2 sorption isotherm data generated on a Micromeritics ASAP 2000 porosimeter. Microsphere diameters and particle size distributions were calculated using ImageJ software from the image analysis of 100 individual particles in scanning electron microscopy (SEM) images, which were acquired using a Cambridge Instruments Stereoscan 90 instrument. The ion-exchange capacity (IEC) was calculated by the titration method using NaCl or TEABr. The carbon, hydrogen, sulfur, and nitrogen contents of the polymers were obtained by elemental microanalysis using a Perkin Elmer 2400 Series II analyser.The characterisation data obtained from the resin evaluation is detailed in Table 2.

2.3. INSTRUMENTATION

For the SPE evaluation, an Agilent Technologies 1100 series HPLC system, equipped with a quaternary pump, UV detector, a solvent degasser unit, a 20 µL loop manual injector and a column heater was used.

For the evaluation of MEs and analysis of sewage samples, an LC-MS/MS system was used. The instrument consisted of an Agilent 1200 series LC, equipped with an automatic injector (volume injected was 50 µL), a degasser, a binary pump and a column oven; and a 6410 series triple quadrupole mass spectrometer using an ESI interface from Agilent Technologies (Waldbronn, Germany).

The analytical column was an Ascentis Express C_{18} (100 mm x 4.6 mm i.d.) with 2.7 µm particle size from Sigma-Aldrich.

2.4. CHROMATOGRAPHIC CONDITIONS

A binary mobile phase was a mixture of two solvents: ultrapure water with 1% HCOOH (pH 3) (solvent A) and ACN (solvent B). The flow rate was 0.6 mL/min. The column temperature was kept at 30 ºC. The gradient was as follows: 7% B to 28% B in 9 min, increased to 100% B in 5 min, constant for 1 min and then decreased to initial conditions in 1 min.

The wavelength used to detect all the compounds during the entire analysis was 210 nm.

The MS/MS parameters were optimised under flow injection of standard solutions of each compound. Both positive and negative ionisation modes were applied to enable a simultaneous determination of the studied analytes. The optimum source conditions were as follows: nebuliser pressure of 45 psi, drying gas (N_2), flow rate of 12 L/min, source temperature of 350 ºC and a capillary potential of 4,000 V. To obtain two multiple reaction monitoring (MRM) transitions for each compounds, cone voltage and collision energies were optimised. Table 1 collects the optimum conditions for each compound.

2.5. SPE PROTOCOL

All the in-house prepared hypercrosslinked SCX sorbents were laboratory packed (60 mg) into 6 mL polyethylene cartridges with two frits (a metal frit of 2 µm pore size at the bottom of the sorbent bed and a polyethylene frit of 10 µm pore size at the top of the sorbent bed). Each cartridge was used throughout all the study. The cartridges were placed in an SPE manifold (Teknokroma, Barcelona, Spain) connected to a vacuum pump. The SPE procedure was the same for all the sorbents. First of all, the cartridges were preconditioned with 5 mL of MeOH

and 5 mL of ultrapure water adjusted at pH 3 with HCl. Subsequently, the samples, also adjusted to pH 3 (HCl), were passed through the cartridges at a flow rate of 5 mL/min. The cartridges were then washed with 5 mL of MeOH. Finally, the analytes were eluted from the cartridges using 5 mL of 5% NH_4OH in MeOH. Prior to the LC injection, the extracts were evaporated to dryness under a gentle stream of N_2 and redissolved in 1 mL of ultrapure water adjusted to pH 3 with HCOOH.

Sewage (influent and effluent) water samples from a treatment plant in the Tarragona surrounding area were collected in a pre-cleaned amber glass bottles from a wastewater treatment plant (WWTP). Subsequently, the samples were filtered using a 0.22 µm nylon membrane (Supelco, Bellefonte, PA, USA) to eliminate the particulate matter prior to the preconcentration step, acidified to pH 3 (HCl) and stored at 4 ºC until analysis.

The quality assurance and quality control procedures included analysis of solvent blanks and duplicates of the samples. One procedural blank was run every five sewage samples to assess potential sample contamination [~~19~~16].

3. RESULTS AND DISCUSSION

Eight distinct SCX materials were prepared with variations in the ion-exchange capacity and specific surface area. Table 2 details the characterisation data for all the materials studied. In-exchange capacities are in the range 0.6 -2.8 mmol g^{-1}. Specific surface areas from ~ 300 – 1,400 m^2 g^{-1}, and the mean particle diameter were in the low micro range (2 – 6 µm is typical).

3.1. EVALUATION OF THE SPE PERFORMANCE

The SPE protocol used was adapted from the method recommended by different manufactures [8], and also based on our previous experiences with the handling and use of ion-exchange resins. The sample was adjusted to pH 3, as all of the basic analytes exist in their protonated form at this pH. During the loading step, the basic analytes are retained on the sorbent by both reversed-phase (RP) and ionic interactions. Any acidic compounds in the sample will also be retained by RP mechanisms; however, there will be no additional ionic interactions with the acidic compounds. In the washing step, MeOH breaks the RP interactions which bind the analytes to the SCX materials. It was not necessary to use any more than a 5 mL volume of MeOH since, even with 5 mL of methanol, trimethoprim (a basic compound) started to elute in the washing step already. With specific regards to the elution step, this was performed using a solution of NH_4OH in MeOH. The NH_4OH serves to break any ionic interactions between the analytes and the sorbent, while the MeOH prevents any new RP interactions from developing. We evaluated two different proportions of basic additive: 5%

276 and 10% NH_4OH in MeOH. However, an increase in the proportion of NH_4OH from 5% to 10%
277 had no significant effect on the recovery of the basic compounds, leading to the conclusion
278 that 5% NH_4OH in MeOH was a suitable composition for the elution solvent.
279 We then evaluated, as part of a preliminary study, each of the eight materials by loading with
280 50 mL sample of the drug mixture at a level of 0.5 ppm, washing with 5 mL volumes of MeOH
281 and finally eluting with 3x5 mL of a 5 % NH_4OH in MeOH.
282 The compounds eluted *via* the washing step were all acidic, as expected, with the exception of
283 carbamazepine. Carbamazepine is basic and should therefore, in principle, be retained by ion-
284 exchange interactions; however, its aromatic ring structure enhances its retention with the
285 resins through RP interactions rather than SCX interactions. The remainder of the basic
286 analytes tested eluted as expected during the elution step. Figure 1 shows the recovery values
287 obtained with the eight different SCX resins for a representative group of basic compounds.
288 Among the five HXLNAD-SCX resins tested, HXLNAD-SCXa and HXLNAD-SCXb gave the highest
289 recoveries for all the analytes tested; only trimethoprim and morphine experienced a decrease
290 in its recovery (for instance, trimethoprim recoveries were 65% and 55% for HXLNAD-SCXa and
291 HXLNAD-SCXb, respectively). In any case, trimethoprim recoveries were even lower (values not
292 higher than 15%) for the rest of HXLNAD-SCX resins tested. These low recoveries for
293 trimethoprim were, as mentioned already, due to its fractionation between the washing and
294 elution step, which might be attributed to the weak ion-exchange interactions with the
295 sulfonic groups in the resins. The poorer recoveries of the analytes in the HXLNAD-SCXc,
296 HXLNAD-SCXd and HXLNAD-SCXe resins were due to either their lack of retention during the
297 loading step or the fact that they were washed out during the washing step. This behaviour is
298 in agreement with their specific surface areas which are lower than the other two resins (*i.e.*,
299 HXLNAD-SCXa and HXLNADb). Therefore, these three resins were ruled out as candidates for
300 further evaluation. In addition, although the HXLNAD-SCXa resin provided successful results, it
301 was discarded as a candidate since it was necessary to elute the analytes retained on the
302 sorbent with up to 15 mL of elution solution. However, in the rest of HXLNAD-SCX sorbents
303 tested, the elution was complete with the first 5 mL of elution solution. The higher the elution
304 volume the more diluted the analytes and the lower the sensitivity or, in the case of
305 evaporating the eluate to dryness, the longer the analysis time. Thus, the HXLNAD-SCXa resin
306 was also ruled out as a candidate.
307 With regards to the three HXLPP-SCX resins, they, in general, provided recoveries ranging from
308 70% to 100 % for all the analytes tested, with the exception of caffeine where the recoveries
309 dropped to 60% for HXLPP-SCXb and to 40% for HXLPP-SCXa. HXLPP-SCXc gave rise to the best
310 recoveries for all the analytes tested (as shown in Figure 1). These results are in accordance

311 with the morphological and chemical properties of the resin since it combines the largest
312 specific surface area (1370 m^2/g) and the highest ion-exchange content (2.8 mmol/g).
313 After this preliminary study, two highly promising resins were selected for further
314 experiments: HXLNAD-SCXb and HXLPP-SCXc.
315 For experiments involving ultrapure water samples, the breakthrough volume was studied for
316 the analytes percolated through the HXLNAD-SCXb and HXLPP-SCXc resins. As a compromise
317 for all the basic analytes tested, a sample volume of up to 250 mL (which is consistent with the
318 quantity of SPE resins packed -60 mg) can be percolated through both resins without losses of
319 the analytes (recovery values ranging from 60% to 100% in all cases). So far, in the comparison
320 of the performance of HXLPP-SCX with HXLNAD-SCX, no differentiation in terms of %recovery
321 was found.
322

323 **3.2. RECOVERIES AND MATRIX EFFECT IN SEWAGE WATERS**

324 When dealing with complex samples, such as sewage waters, apart from the recovery results
325 *per se*, the ME issue should be considered, and several experiments were conducted in this
326 sense to cover both issues. Firstly, a blank sample was analysed in order to subtract the
327 possible signal of existing analytes that appeared in all instances at low concentration levels.
328 Then, the ME was evaluated, which was calculated by comparing the signal obtained for the
329 analytes (S2) when spiked at 50 µg/L to the blank extract of the SPE of either 50 mL of effluent
330 or 25 mL of influent sewage samples to the signal obtained for these analytes at the same
331 concentration in the injection solution (S1), as described elsewhere [9](%ME = (100 – (S2/S1)) x
332 100).
333 The ME values for both type of samples analysed (*i.e.*, effluent and influent sewage) are
334 detailed in Table 3, where it can be seen that most of the analytes showed ion-suppression
335 (positive values of % ME). In general, the ME is not higher than 10% for most of the analytes in
336 both type of samples, and for both resins tested. Just as was the case for morphine, with
337 atenolol, trimethoprim and caffeine the ME rises to 20% at most when using HXLPP-SCXc.
338 When using HXLNAD-SCXb, the ME values of trimethoprim, caffeine and morphine are about
339 30% (ion-suppression) in influent wastewater samples (and morphine for both type of sample).
340 These values are satisfactory and lower than the usual values encountered when these types
341 of environmental samples are analysed by SPE/LC-MS/MS [4,~~20~~17-~~22~~19]. For instance, Caban
342 *et al.* [4] reported values of ion-suppression from 18 to 41% when a group of β-blockers
343 (including atenolol, metoprolol and propranolol) were extracted from composite (effluent and
344 influent) sewage samples. The low ME values encountered in the present study are due to the

clean-up step performed (with 5 mL of MeOH), which simplifies the complexity of the matrix. In fact, in order to check how the washing step helps to diminish the ME, a set of experiments was conducted in which the washing step was not included. In general, the MEs values increased, especially for the first eluting analytes. For instance, morphine presented values of ion-suppression from 52% to 57% in each of the four cases, and for dihydrocodeine on HXLNAD-SCXb the ion-suppression effect increased dramatically to 65% for effluent wastewater and to 60% for influent water. Similar results were reported [10,~~23~~20,~~24~~21] when different mixed-mode resins were used as SPE sorbents to extract a similar group of drugs and the SPE protocol included a washing step involving an organic solvent. As an example, morphine presented a near 50% level of ion-enhancement when the clean-up was not included using Oasis WCX as sorbent, whereas it presented a ion-suppression of 10% when a clean-up that included MeOH was included [~~23~~20].

Once it was confirmed that the ME was negligible, the recovery values (Table 3) for each resin and type of sample were calculated when 50 mL of effluent and 25 mL of influent spiked at 2 and 4 μg L^{-1}, with the analyte mixture, respectively, were loaded onto the cartridges. All the values were, in general, from 75% to 100% for both types of resins and, as expected, slightly lower for influent wastewater samples (due to the matrix complexity). We should just stress the drop in the recoveries for morphine (near 50%), which comes about because this compound is one of the most-affected by the ME but also because its polarity might hinder its proper retention in the sorbent, but also for methadone in the influent samples with values of 24% and 45% for HXLNAD-SCXb and HXLPP-SCXc, respectively. In addition, and as was reported for the ultrapure water samples, trimethoprim was just partially retained and displayed recoveries not higher than 40%.

In spite of these examples of low recoveries, the extraction performance of both resins were good, and comparable to the results reported already where similar illicit drugs were extracted using Oasis MCX [~~24~~21,~~25~~22] (recovery values on average range from 60 to 130%), or better when therapeutic drugs were extracted using Oasis HLB [~~21~~18,~~26~~23] (recovery values on average range from 20 to 85%). It should be mentioned that in the reported studies cited here higher sample volumes (up to 250 mL) of wastewater were percolated through cartridges packed with 150 mg of sorbent; this should be equivalent in terms of capacity in the present case when 50 mL of sample were loaded through 60 mg of packed resin. Thus, the morphology of the tested resins (*i.e.*, low particle size and hypercrosslinked structure) also helps in the performance of the extraction. This fact is also supported in a previous study [~~27~~24] where two non-hypercrosslinked SCX resins prepared by ~~–~~an alternative polymerization protocol which

gave irregular particles presented similar results in %ME values and %R under the same SPE conditions as used in the present study, but with 200 mg of polymer packed in the cartridge. When comparing HXLPP-SCXb and HXLNAD-SCXc in terms of % recovery and % ME, we cannot draw any significant conclusion since the results are quite similar, although the strong cation-exchange capacity for HXLNAD-SCXb is lower than HXLPP-SCXc. In any case the ion-exchange capacity must be enough for the accessibility of the analytes and their proper retention. Resin HXLNAD-SCXb may potentially provide slightly better results, thus we selected it for further experiments.

3.3. METHOD PERFORMANCE AND ANALYSIS OF SAMPLES

The overall analytical method was evaluated for effluent sewage (Table 3) and considered linearity, limits of quantification (LOQs), limits of detection (LODs), repeatability and reproducibility.

Six-point calibration curves with matrix were constructed with a linearity ranging from 25 to 5000 ng/L for all compounds studied with the exception of morphine, ranitidine, dihydrocodeine and EDDP, with calibration curve range from 50 to 5000 ng/L, and antipyrine (100 to 5000 ng/L). The calibration curves are linear with determination coefficients (r^2) exceeding 0.999on average.

The LOQs for each compound were taken as the lowest concentration level of the calibration curve. The LODs, calculated as the signal-to-noise ratio (S/N) of 3, or when the compounds were present in real samples the LODs were estimated from instrumental parameters and by taking into account the recovery and ME for each compound. The LODs were 5 ng/L, for most of the compounds studied, but 2 ng/L for BE, cocaine and propranolol; 10 ng/L for morphine, ranitidine and EDDP; and, 100 ng/L for trimethoprim (which might be attributed to the low recoveries achieved during the extraction). These LOQs are similar to those reported in the literature [~~24~~21,~~28~~25,~~29~~26] where higher volumes of sample were percolated but the signals were more affected by ME which, in turn, negates the gain in sensitivity.

The repeatability and reproducibility, expressed as a % of relative standard deviation (%RSD), were determined by spiking five replicates at two different levels (LOQs and 100 ng/L) for each type of sample; the results obtained were less than 12% and 19%, respectively.

Finally, the developed SPE/LC-MS/MS method was applied for the determination of drugs in influent and effluent samples from an urban STP with samples taken on different days. The presence of the analytes found was confirmed according to the Commission Decision 2002/657/EC [~~30~~27]. All the analytes studied were found in the analysed samples, but some of

them at levels below the LOQs. Table 4 presents the range of concentration for each analyte for both effluent and influent sewage samples. As an example, Figure 2 shows representative MRM chromatograms from the analysis of one of the influent sewage samples. These values are in line with those reported at similar STPs [~~31~~28-~~34~~31]. We should note that the low concentration of trimethoprim found in all the samples analysed might be attributed to the low recoveries achieved with the present method. In addition, we should mention that some drugs are present at a higher concentration in effluent than in influent water. This might be due to the fact that the sampling of the influent and effluent sewage was not performed in the same time period, but also due to a possible conversion of their conjugated metabolite to the original substance after the treatment processes.

4. CONCLUSIONS

Eight different resins were prepared using different synthetic approaches; these resins boasted differences in their ion-exchange capacities and morphological properties. The benefits arising from a combination of properties became apparent when the resins were exploited as SCX sorbents. The resins that presented the best SCX performance were those that had high ion-exchange capacity and high specific surface area.

The SCX resin properties were attractive and enabled the retention of a group of therapeutic and illicit drugs from sewage samples. Simultaneously, they prevented the ME encountered when complex samples are analysed by LC-MS/MS.

A novel SPE method using HXLNAD-SCXb followed by LC-MS/MS was validated with excellent linearity, limits of quantification (LOQs), limits of detection (LODs), repeatability and reproducibility being observed. Thereafter, the SPE method was extended successfully to the analysis of different types of sewage samples with the target drugs present.

These novel SCX sorbents presented are an alternative to further exploit in the determination of contaminants from complex samples.

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FIGURE CAPTIONS.

Figure 1. Preliminary evaluation of the eight SCX resins based on the % recovery values for four selected basic analytes.

Figure 2. MRM chromatograms of an influent sewage sample. For experimental conditions, see the text.

Figure 1S. SPE protocol used for the evalutation of the SCX sorbents.

Figure1

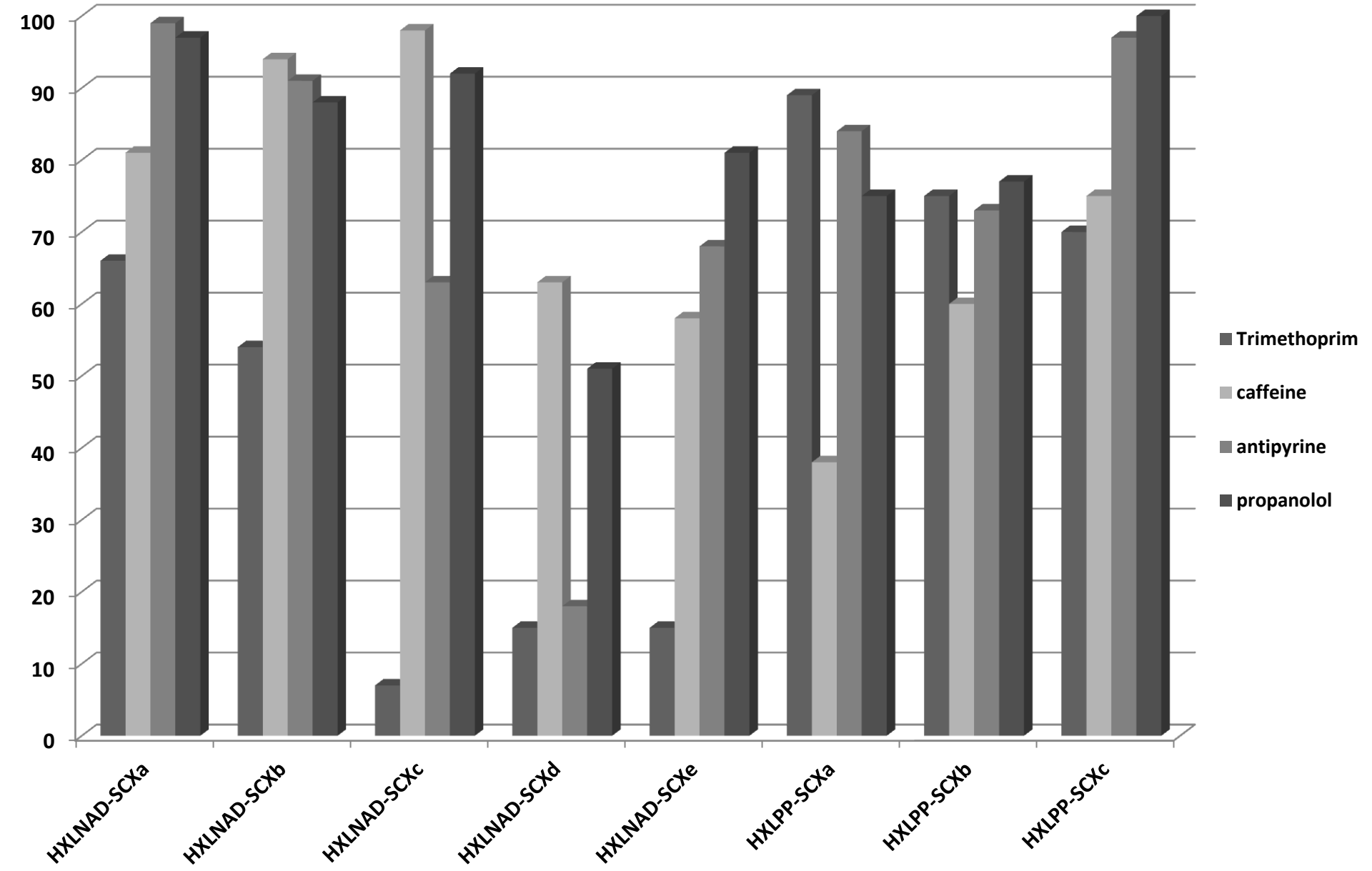

Figure 1

Figure2

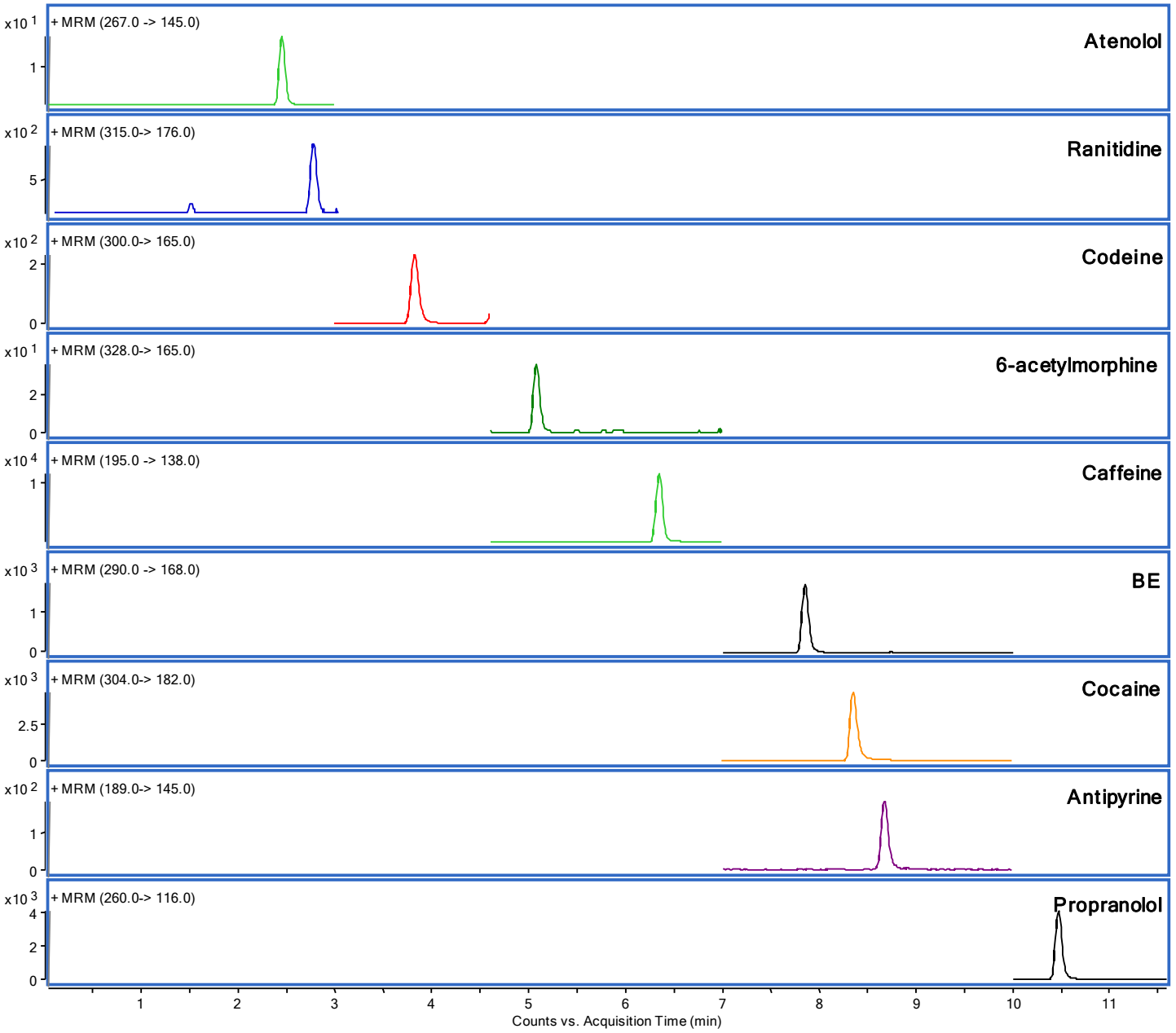

Figure 2

Tables

Table 1. Analyte information (pK_a and retention times) and MS/MS parameters employed for the MRM acquisition for their determination.

Analyte	pK_a[a]	t_R(min)	Ionis. mode	Cone volt. (V)	Parent ion (m/z)	Prod. Ion (m/z)	Collision energy (V)
morphine	8.3	1.8	+	125	286	152	50
						165	50
atenolol	9.4	2.4	+	125	267	145	25
						190	10
ranitidine	8.4	2.7	+	100	315	176	5
						130	15
dihydrocodeine	8.4	4.2	+	150	302	199	25
						128	50
codeine	8.3	4.8	+	150	300	165	50
						153	50
6-acetylmorphine	8.3	5.1	+	150	328	165	50
						211	25
trimethoprim	7.0	6.2	+	125	291	230	15
						123	25
caffeine	14.0	6.4	+	125	195	138	15
						110	25
BE	10.8 3.2	7.9	+	125	290	168	15
						105	25
metoprolol	9.4	8.1	+	125	268	116	15
						159	15

cocaine	8.0	8.4	+	125	304	182 82	15 25
antipyrine	9.86	8.7	+	100	189	145 115	30 30
propranolol	9.5	10.5	+	125	260	116 183	15 15
salicylic acid	3.1	12.3	-	75	137	93 65	15 30
EDDP	7.7	12.6	+	150	278	234 249	25 15
methadone	9.1	13.0	+	100	310	265 105	5 25
carbamazepine	13.94	13.6	+	150	237	179 193	35 35
clofibric acid	3.2	14.7	-	75	213	127 85	10 5
diclofenac	4.2	15.4	-	75	294	250 214	5 15
ibuprofen	4.4	15.6	-	75	205	161	5

Table 2. Properties of the strong cation-exchange resins evaluated.

Polymer type	Material Sample code	IEC[a] (mmol g^{-1})	SSA[b] (m^2 g^{-1})	Mean particle diameter (µm)
HXLNAD	HXLNAD-SCXa	1.3	~~897~~900	3-4
	HXLNAD-SCXb	0.6	~~1021~~1020	2-3
	HXLNAD-SCXc	2.3	~~643~~640	2-3
	HXLNAD-SCXd	1.9	~~772~~770	2-3
	HXLNAD-SCXe	2.1	~~332~~330	2-3
HXLPP	HXLPP-SCXa	1.7	1070	4-6
	HXLPP-SCXb	2.0	1160	2-3
	HXLPP-SCXc	2.8	1370	4-6

[a] Ion-exchange capacity; [b] Specific surface area.

Table 3. Matrix effect and recovery values for target compounds in effluent and influent wastewaters samples using both HXLNAD-SCXb and HXLPP-SCXc as sorbents in SPE/LC-MS/MS.

	HXLNAD-SCXb				HXLPP-SCXc			
	Effluent		Influent		Effluent		Influent	
	% R	% ME	% R	% ME	% R	% ME	% R	% ME
Morphine	52	33	49	30	48	16	43	12
Atenolol	117	12	73	16	94	17	74	21
Ranitidine	92	13	98	17	117	8	95	-4
Dihydrocodeine	115	3	99	-6	97	5	85	-7
Codeine	86	6	80	8	74	9	70	7
6-Acetylmorphine	93	2	109	-5	83	8	95	7
Trimethoprim	41	10	32	30	22	17	31	10
Caffeine	73	24	107	39	64	18	70	14
Metoprolol	120	6	104	6	101	5	86	10
BE	99	5	101	-3	98	4	93	3
Cocaine	80	11	75	6	69	5	62	5
Antipyrine	92	3	69	6	95	4	67	3
Propanolol	97	10	82	7	96	7	95	9
EDDP	75	-15	62	-10	85	-8	63	-8
Methadone	75	7	42	-4	67	7	45	6

Table 4. Concentrations of analytes found in influent and effluent wastewater samples (n=5) when the samples were analysed by SPE/LC-MS/MS using the HXLNAD-SCXb sorbent.

	Concentration (ng L^{-1})	
	Effluent	**Influent**
Morphine	<LOQ – 157	<LOQ
Atenolol	869 – 1323	217 – 563
Ranitidine	<LOQ	<LOQ - 136
Dihydrocodeine	<LOQ	<LOQ
Codeine	<LOQ – 85	<LOQ – 292
6-Acetylmorphine	<LOQ – 87	<LOQ – 124
Trimethoprim	<LOQ	<LOQ
Caffeine	124 – 437	982 – 4577
Metoprolol	<LOQ – 215	<LOQ – 62
BE	331 – 3805	248 – 1311
Cocaine	108 – 344	70 – 602
Antipyrine	35 - 370	<LOQ – 73
Propanolol	75 – 273	<LOQ – 336
EDDP	<LOQ – 84	<LOQ – 213
Methadone	<LOQ – 180	<LOQ – 74

Electronic Supplementary Material (online publication only)

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