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1 **Synthesis and application of hypercrosslinked polymers with weak cation-**  
2 **exchange character for the selective extraction of basic pharmaceuticals**  
3 **from complex environmental water samples**

4

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7

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17 **Keywords:** Hypercrosslinked sorbents, polymer microspheres, mixed-mode  
18 weak cation-exchanger, solid-phase extraction, basic compounds

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## 37 **Abstract**

38

39 The synthesis of high specific surface area sorbents (HXLPP-WCX) in the form  
40 of hypercrosslinked polymer microspheres with narrow particle size  
41 distributions, average particle diameters around 6  $\mu\text{m}$ , and weak cation  
42 exchange (WCX) character, is described. The WCX character arises from  
43 carboxylic acid moieties in the polymers, derived from the comonomer  
44 methacrylic acid. A novel HXLPP-WCX sorbent with an attractive set of  
45 chemical and physical properties was then used in an off-line solid-phase  
46 extraction (SPE) protocol for the selective extraction of a group of basic  
47 compounds from complex environmental samples, a priority being the clean  
48 separation of the basic compounds of interest from acidic compounds and  
49 interferences. The separation power of the new sorbent for basic  
50 pharmaceuticals was compared to two commercially available, mixed-mode  
51 sorbents, namely Oasis WCX and Strata X-CW. Under identical experimental  
52 conditions, HXLPP-WCX was found to deliver both higher capacity and better  
53 selectivity in SPE than either of the two commercially available materials. In an  
54 optimised SPE protocol, the HXLPP-WCX sorbent gave rise to quantitative and  
55 selective extractions of low  $\mu\text{g l}^{-1}$  levels of basic pharmaceuticals present in 500  
56 ml of river water and 250 ml of effluent waste water.

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## 63 **Introduction**

64 Solid-phase extraction (SPE) is a powerful analytical tool used widely for  
65 pre-concentration, fractionation and purification of analytes of interest from  
66 complex environmental [1-4] and biological samples (urine, blood and plasma)  
67 [5,6]. In recent years, a group of analytes of increasing interest is  
68 pharmaceuticals, since they are dispersed continuously into the environment as  
69 a result of human use [5,7], which can give rise to problems including health  
70 concerns for humans, therefore there is a demand for analytical methods which  
71 enable the accurate determination of pharmaceuticals in the environment, even  
72 when the pharmaceuticals are present at low levels. To satisfy this demand,  
73 and meet the appropriate detection limits, there is a requirement for suitable  
74 pre-concentration techniques which can both concentrate and clean-up the  
75 analytes present in the complex environmental matrices. SPE is excellent  
76 choice in this regard since it can provide high enrichment factors of the target  
77 compounds and eliminate interferences from the sample to be analysed.  
78 Polymeric materials are the most important group of sorbent used in SPE, since  
79 they offer attractive advantages such as good retention of analytes and sorbent  
80 stability under a much broader range of analysis conditions than for sorbents of  
81 other types (*i.e.*, silica- and carbon-based sorbents). Several polymeric sorbents  
82 have been developed and applied to the extraction of pharmaceuticals [8-10].

83 In recent years, SPE technology has expanded to offer the use of mixed-  
84 mode, polymeric ion-exchange media, which combines the attributes of  
85 reversed-phase chemistry and ion-exchange interactions into one single  
86 material [11]. Mixed-mode ion-exchange sorbents are designed to interact with

87 ionic species, but they can also retain non-charged species effectively through  
88 hydrophobic or hydrophilic interactions [11,12]. Mixed-mode sorbents are  
89 classified as either strong or weak ion-exchange, depending on the ionic groups  
90 tethered to the sorbent. An important advantage of weak ion-exchange sorbents  
91 is that the ionisation state of the resin may be tuned easily by pH, thus adding  
92 more versatility and power to SPE applications [12]. Amongst the most popular,  
93 commercially available mixed-mode sorbents are Oasis MCX, Oasis MAX,  
94 Oasis WCX, and Oasis WAX (all from Waters), which are classified as strong  
95 (MCX, MAX) or weak (WCX, WAX) cation/anion-exchange resins, respectively.  
96 All four of these interesting sorbents are derived from an Oasis HLB polymeric  
97 skeleton [poly(vinylpyrrolidone-co-divinylbenzene),  $\sim 800 \text{ m}^2 \text{ g}^{-1}$ ] which has been  
98 modified chemically with sulfonic acid and quaternary ammonium groups in the  
99 case of the strong ion-exchangers, and carboxylic acid and piperazine groups in  
100 the case of the weak ion-exchangers. In an analogous fashion, Strata-X (a  
101 Phenomenex sorbent), which is based on a poly(styrene-co-divinylbenzene)  
102 skeleton bearing polar vinylpyrrolidone residues, can be modified chemically to  
103 give related sorbents bearing sulfonic acid groups (Strata-X-C), carboxylic acid  
104 groups (Strata-X-CW) or ethylene diamine groups (Strata-X-AW).

105 All of these commercial sorbents have macroreticular structures which give  
106 rise to weaker reversed-phase interactions with analytes than do  
107 hypercrosslinked polymer resins. Hypercrosslinked polymers are a new  
108 generation of permanently porous, polymeric resins with enhanced analyte  
109 retention characteristics arising from their high micropore contents and  
110 correspondingly high specific surface areas ( $>1000 \text{ m}^2 \text{ g}^{-1}$ ) [13]. Recently, we  
111 disclosed the synthesis of mixed-mode hypercrosslinked sorbents with weak

112 anion exchange (WAX) character, and the application of these novel sorbents to  
113 the SPE of acidic pharmaceuticals from aqueous samples [14].

114 The present study describes the synthesis of hypercrosslinked polymer  
115 resins with weak cation-exchange (WCX) character, where the WCX properties  
116 are derived from the presence of carboxylic acid moieties, and the application of  
117 these sorbents to the SPE of basic pharmaceuticals from complex  
118 environmental samples. The new materials have been bench-marked against  
119 Strata-X-CW and Oasis WCX. Although previously porous polymer containing  
120 methacrylic acid (MAA) in monolith format has been applied to in-tube-solid-  
121 phase microextraction (SPME)-liquid chromatography (LC) for the extraction of  
122 drugs from complex sample matrices [15]; as far as we are aware, this is the  
123 first time that a hypercrosslinked sorbent has been used as a weak cation-  
124 exchanger for the selective extraction of basic pharmaceuticals from complex  
125 environmental samples, which also contain acidic and neutral compounds.

126

## 127 **2. Experimental**

### 128 **2.1 Reagents and standards**

129 The reagents used for the polymer syntheses were divinylbenzene (DVB)  
130 (80% grade) supplied by Aldrich (Steinheim, Germany), methacrylic acid (MAA)  
131 (98% grade) and *para*-vinylbenzyl chloride (VBC) (95% grade) supplied by  
132 Fluka (Buchs, Switzerland). DVB and VBC were purified by passing through  
133 short columns packed with neutral alumina. MAA was purified by vacuum  
134 distillation. The 2,2'-azobisisobutyronitrile (AIBN) used as initiator was supplied  
135 by BDH (Poole, UK) and purified by recrystallisation from acetone (Merck,

136 Darmstadt, Germany). Ferric chloride ( $\text{FeCl}_3$ ) and anhydrous 1,2-dichloroethane  
137 (DCE), from Aldrich, were used in the hypercrosslinked reactions.

138 The pharmaceutical analytes selected to evaluate the performance of the  
139 sorbents in SPE were: acetaminophen, caffeine, antipyrine, propranolol,  
140 carbamazepine, naproxen and diclofenac (all obtained from Sigma-Aldrich). The  
141 chemical structures and  $\text{pK}_a$  values of the analytes are presented in Table 1.

142 Standard solutions at  $1000 \text{ mg l}^{-1}$  in methanol were prepared for each  
143 analyte. The mixture of all the analytes was prepared by diluting the standard  
144 solutions with  $\text{MeOH:H}_2\text{O}$  (1:1, v/v).

145 LC-grade acetonitrile and methanol (SDS, Peypin, France) and Milli-Q  
146 water (Millipore, Bedford, MA, USA) were used to prepare the mobile phases.  
147 Hydrochloric acid (Probus, Barcelona, Spain) was used to adjust the pH of the  
148 mobile phase and the sample before SPE. Other reagents used in SPE  
149 procedures were: ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) (Merck), formic acid  
150 ( $\text{HCOOH}$ ) (Probus) and trifluoroacetic acid (TFA) (Fluka).

## 151 **2.2 Resin preparation and characterisation**

152 The micron-sized spherical particles (PP-WCX) used as swellable  
153 precursors in the production of the hypercrosslinked resins (HXLPP-WCX),  
154 were synthesised using an optimised precipitation polymerisation (PP) protocol  
155 [16]. The monomers (10% MAA, 50% VBC and 40% DVB [% w/w]) and AIBN (2  
156 mol% relative to polymerisable double bonds) were dissolved in acetonitrile  
157 (200 ml) in a polypropylene bottle (250 ml) at a total monomer concentration of  
158 2% w/v. The monomer solution was de-oxygenated with  $\text{N}_2$  at  $0 \text{ }^\circ\text{C}$  and the  
159 bottle then placed on a low-profile roller (Stovall, Essex, UK) in a temperature-  
160 controllable incubator (Stuart Scientific, Surrey, UK). The temperature was



161 ramped from ambient to 60 °C over a period of ~ 2 hours and the polymerisation  
162 allowed to proceed at 60 °C for a further 46 hours. The resulting particles were  
163 filtered on a 0.2 µm nylon membrane filter and washed successively with  
164 MeOH, toluene and acetone, before overnight drying *in vacuo* at 40 °C.

165 The hypercrosslinked reactions of the MAA-VBC-DVB precursors were  
166 carried out as described in previous study [16], using a well-established reaction  
167 for the VBC-DVB precursor.

168 The hypercrosslinked resin (HXLPP-WCX) was characterised by  
169 measuring specific surface area using a BET treatment of N<sub>2</sub> sorption isotherm  
170 data generated on a Micromeritics ASAP 2000 porosimeter. The carbon (83.1%  
171 w/w), hydrogen (7.2% w/w), chlorine (2.5% w/w) and oxygen (6.3% w/w,  
172 calculated by difference) contents for the resin were obtained by elemental  
173 microanalysis using a Carlo-Erba EA 1106 instrument. The cation-exchange  
174 capacity was calculated from the microanalytical data using the theoretical  
175 values. The average microsphere diameter and homogeneity in size (particle  
176 size distribution) were calculated using ImageJ software from the image  
177 analysis of 100 individual particles in scanning electron microscopy (SEM)  
178 images, which were acquired using a JEOL 6400 Instrument. SEM image for  
179 the HXLPP-WCX resin is included in Figure 1S of the Supported Information  
180 Section. A schematic representation of the structure of HXLPP-WCX is depicted  
181 in Figure 1. The characterisation data for all the sorbents studied is detailed in  
182 Table 2.

### 183 **2.3 Chromatographic equipment and conditions**

184 The chromatographic experiments were performed with an HP 1090 Liquid  
185 Chromatograph equipped with an injection valve with a 20 µl loop and an

186 Agilent 1200 UV spectrophotometric detector (Agilent, Waldbronn, Germany).  
187 The analytical column was a 250 mm × 4.6 mm i.d. stainless-steel column  
188 packed with Kromasil 100 C<sub>18</sub>, 5 μm (Teknokroma, Barcelona, Spain).

189 The mobile phases were Milli-Q water adjusted to pH 3 with HCl (solvent  
190 A) and acetonitrile (solvent B). The flow rate was 1 ml min<sup>-1</sup> and the  
191 temperature of the column oven was set at 65 °C. The gradient profile was from  
192 10% to 15% ACN in 5 min, then to 100% ACN in 25 min (held for 2 min), then  
193 the mobile phase was returned to the initial conditions (10% ACN) in 3 min.

194 The detection wavelength for all the compounds was 210 nm.

#### 195 **2.4 Solid-phase extraction**

196 SPE cartridges (6 ml, polypropylene) were packed with 200 mg of the  
197 HXLPP-WCX sorbent. The sorbent was retained by two frits: a 2 μm pore size  
198 metal frit at the bottom, and a 20 μm pore size polyethylene frit at the top. The  
199 retention capabilities of the novel sorbent was compared to commercial SPE  
200 cartridges from Phenomenex (Strata-X-CW; 200 mg/6 ml) and Waters (Oasis  
201 WCX; 200 mg/6 ml) (which was packed manually). A vacuum manifold  
202 (Teknokroma) was used to manipulate the cartridges in the off-line SPE  
203 process. One single sorbent cartridge of each type was used for the whole  
204 study. The three sorbent structures are presented in Figure 1.

205 Prior to the SPE extractions, the pH of the sample was adjusted to 7 with  
206 HCl or NaOH. The procedure used for all cartridges was identical: the cartridge  
207 was activated with 5 ml of MeOH followed by 2 ml of Milli-Q water, and the  
208 sample then loaded at a flow rate 10 ml min<sup>-1</sup>. After equilibration, the cartridge  
209 was washed with 2 ml 5% NH<sub>4</sub>OH in MeOH. Finally, the compounds were  
210 eluted from the cartridge using 5 ml of 2% TFA in MeOH.

211 Prior to LC analyses, the SPE eluates were evaporated to dryness and  
212 then reconstituted in 1 ml of MeOH:H<sub>2</sub>O (1:1, v/v).

213 To keep the samples under proper conditions, real water (Ebre river water  
214 and effluent waste water from a treatment plant) were adjusted to ~ pH 3 with  
215 HCl and kept at 4 °C before analysis. They were filtered through 0.22 μm nylon  
216 membranes (Supelco, Bellefonte, PA, USA) prior to the preconcentration step to  
217 eliminate the particulate matter which is normally present in real samples.

218

### 219 **3. Results and discussion**

#### 220 **3.1 Preparation of the HXLPP-WCX sorbent**

221 The novel WCX hypercrosslinked sorbent (HXLPP-WCX) was derived from  
222 a swellable copolymer precursor (PP-WCX) prepared by precipitation  
223 polymerisation (PP). PP is a simple, straightforward and reproducible method  
224 for obtaining, in one single preparative step, spherical polymer particles with  
225 average diameters in the low micron size regime which, as has been  
226 demonstrated previously [14,17,18], perform well as novel sorbents in SPE  
227 applications. The aim of the present work was to synthesise a hypercrosslinked  
228 derivative of the terpolymer poly(MAA-co-VBC-co-DVB), and thereby access a  
229 resin which combined both weak cation-exchange character (through the MAA  
230 residues present) and high specific surface area derived from its high micropore  
231 content (from hypercrosslinking reactions which consume the pendent  
232 chloromethyl groups). During the production of the poly(MAA-co-VBC-co-DVB)  
233 precursor polymer, various comonomer ratios were evaluated (data not shown);  
234 the comonomer ratio reported in the present manuscript (*i.e.*, 10% MAA,

235 50%VBC, 40% DVB [w/w]) was found to offer the optimal balance of properties,  
236 *i.e.*, suitable ion-exchange capacity and particle size, and high specific surface  
237 area.

238 The resin characterisation data for HXLPP-WCX is detailed in Table 2. The  
239 specific surface area was  $1125 \text{ m}^2 \text{ g}^{-1}$  and the cation-exchange capacity  $0.72$   
240  $\text{meq g}^{-1}$ . Following on from the development of a convenient synthetic route into  
241 an HXLPP-WCX resin, our aim was to evaluate the potential benefits in SPE of  
242 introducing carboxylic acid moieties into hypercrosslinked polymer  
243 microspheres, and to compare the performance of this new resin to the  
244 commercially available sorbents (more specifically, Oasis-WCX and Strata-X-  
245 CW). The characterisation data for all three sorbents is detailed in Table 2. The  
246 ion-exchange capacity is similar for all three sorbents ( $\sim 0.75 \text{ meq g}^{-1}$ ), however  
247 the particle size of the HXLPP-WCX sorbent ( $\sim 6 \mu\text{m}$ ) is markedly lower than  
248 either of the other two materials. The lower particle size of the sorbent might  
249 provide better contact with the analytes to be extracted, and, thus, benefit in the  
250 SPE process.

251

### 252 **3.2 SPE optimisation**

253 Pharmaceuticals bear a variety of functional groups and they can be  
254 cationic, anionic or zwitterionic depending on the sample pH. Some  
255 pharmaceuticals contain nitrogen-containing functional groups which are basic  
256 and will therefore be readily protonated to give a cation under certain conditions  
257 [19,20]. To evaluate the cation-exchange properties of the HXLPP-WCX  
258 sorbent, and establish the scope of its sorption characteristics, we selected a  
259 group of acidic and basic pharmaceuticals with variable  $\text{pK}_a$  values (Table 1).

260 Besides the selection of analytes with a wide range of acidic/basic  
261 properties, to test the performance of the WCX sorbents in an accurate and  
262 reliable manner it was necessary to optimise the SPE conditions in such a way  
263 as to maximise the retention of the analytes on the sorbents. Optimal retention  
264 conditions are those for which ionic interactions between the MAA residues in  
265 the sorbent and the cationic forms of the analyte are maximised.

### 266 *3.2.1 Sample loading*

267 Since HXLPP-WCX is a cation-exchange material, the analyte retention  
268 mechanism is based on ionic interactions between carboxylic acid groups in the  
269 polymer and the pharmaceuticals. Thus, the pH of the sample during analyte  
270 extraction by the sorbent is an important parameter to be optimised.

271 To investigate the retention properties of the HXLPP-WCX sorbent, 100 ml  
272 volumes of two separate samples (at pH 3 and pH 7, respectively) were  
273 percolated through SPE cartridges packed with the sorbent. At pH 3 the  
274 carboxylic acid groups of the acidic compounds and the sorbent are primarily in  
275 their non-ionised form, whereas the basic compounds are fully ionised. In  
276 contrast, at pH 7 the carboxylic acid-containing acidic compounds are  
277 deprotonated and are eluted during the SPE washing step, while the carboxylic  
278 acid residues in the polymer are ionised and retain the basic pharmaceuticals  
279 (protonated) by ionic interactions. Thus, cation-exchange phenomena are  
280 expected to be more effective at pH 7 than at pH 3. When preliminary SPE  
281 experiments were performed to confirm these expectations, the recoveries of  
282 the basic compounds were found to be lower at pH 3 and very high at pH 7. For  
283 this reason, samples were adjusted to pH 7 in all the subsequent SPE  
284 experiments.

### 285 3.2.2 *Washing step*

286 The aim of the washing step was to eliminate interferences (including  
287 acidic and neutral compounds) bound to the sorbent through reserved-phase  
288 mechanisms, while retaining on the sorbent the basic compounds bound  
289 through cation-exchange interactions. 1 ml volumes of various neat organic  
290 solvents (such as methanol and acetonitrile) were applied in the washing step,  
291 but in such cases all the analytes were eluted. Thus, we decided to use a  
292 solution of NH<sub>4</sub>OH in organic solvent as the washing solution, to maintain the  
293 desired ionisation state of the analytes and the sorbent. In this regard, the  
294 following solutions were evaluated: 5% NH<sub>4</sub>OH in MeOH; 5% NH<sub>4</sub>OH in ACN;  
295 5% NH<sub>4</sub>OH in MeOH/ACN (1/4). Of these three options, 5% NH<sub>4</sub>OH in MeOH  
296 gave higher recoveries for all analytes than the other two washing solution and  
297 was thus selected as the washing solvent of choice. Thereafter, the next step  
298 was to evaluate the optimum volume of the washing solvent to be used in the  
299 SPE protocol. For these experiments, where the sample matrix was Milli-Q  
300 water, 1 ml of 5% NH<sub>4</sub>OH in MeOH was used initially. Although this volume of  
301 washing solvent was found to be not enough to elute all the acidic compounds  
302 quantitatively, 2 ml of 5% NH<sub>4</sub>OH in MeOH was found to be effective for this  
303 purpose so was established as the optimal volume of washing solvent required  
304 to elute acidic compounds and interferences, whilst still allowing total retention  
305 of the analytes of interest (*i.e.*, basic compounds).

### 306 3.2.3 *Elution of basic compounds*

307 For the elution step, in which the aim was to elute the basic compounds  
308 bound to WCX sorbents through ionic interactions, various acidic solutions were  
309 tested (acidification protonates the carboxylic acid residues on the sorbents,

310 break the cation-exchange interactions and leads to release of the basic  
311 analytes from the sorbents thanks to the elution strength of the organic solvent  
312 also present in the solution). For this purpose, 5 ml aliquots of 2% HCOOH in  
313 MeOH, 2% TFA in MeOH and 2% TFA in MeOH/ACN (1/4) were investigated.  
314 Since 2% TFA in MeOH delivered the best results (higher recoveries than for  
315 2% HCOOH in MeOH), and did not give any significant disturbance in the LC  
316 separation of the analytes, it was selected for use in the elution step. 2% TFA in  
317 MeOH/ACN (1/4) delivered good results also, but required longer evaporation  
318 times. 5 ml of 2% TFA in MeOH was found to be sufficient to elute completely  
319 all of the basic compounds, so was set as the optimal volume of elution solvent.

#### 320 *3.2.4 Volume of sample*

321         Once the SPE protocol had been established, the effect of varying the  
322 volume of sample in the loading step (from 100 to 1000 ml) was investigated as  
323 a manner to predict the extraction capacity of the sorbent. The HXLPP-WCX  
324 sorbent gave rise to good recoveries of analytes even when the sample volume  
325 was 1000 ml (Table 3). Typically, the recoveries of the basic analytes were  
326 close to 100% for the HXLPP-WCX sorbent. Only for antipyrine did the HXLPP-  
327 WCX resin gave rise to a small degree of fractionation; for example, when 1000  
328 ml of sample spiked at  $20 \mu\text{g l}^{-1}$  with the analyte mixture were extracted, the  
329 recovery of antipyrine in the elution step was 79%, with the remainder (15%)  
330 being eluted in washing step. In view of the  $\text{pK}_a$  (13.3) and chemical structure of  
331 antipyrine, this behaviour may be attributable to the stronger retention of  
332 antipyrine through hydrophobic interactions.

333         Therefore, we have demonstrated that the HXLPP-WCX sorbent is highly  
334 effective in extracting basic analytes in a quantitative manner from high volume

335 (1000 ml) aqueous samples, after a washing step with 2 ml of 5% NH<sub>4</sub>OH in  
336 MeOH, a feature which helps greatly in the removal of interferences from the  
337 sample matrix.

### 338 *3.3 Comparison to commercial sorbents*

339 The SPE performance of the HXLPP-WCX sorbent was compared to  
340 Strata-X-CW and Oasis WCX. The former had a specific surface area of 1125  
341 m<sup>2</sup> g<sup>-1</sup> (arising from the high micropore content) whereas the commercially  
342 available sorbents, which are not hypercrosslinked, have lower specific surface  
343 areas (800 m<sup>2</sup> g<sup>-1</sup>). A second notable difference between the HXLPP-WCX  
344 sorbent and the commercially available sorbents is the particle size; the  
345 HXLPP-WCX sorbent is in the form of microspheres with average particle  
346 diameter around 6 µm, whereas the average particle size of Strata X-CW and  
347 Oasis WCX are both significantly larger at around 30 µm.

348 The SPE results arising from use of the three different resins are  
349 presented in Table 3. It can be seen that the analyte recoveries were higher for  
350 all compounds with HXLPP-WCX than either Strata-X-CW or Oasis WCX.  
351 When varying sample volumes were percolated through the Strata-X-CW and  
352 Oasis WCX cartridges, most of the compounds were either eluted in the  
353 washing step or fractionated between the washing and the elution steps; the  
354 retention of certain analytes was also low compared to HXLPP-WCX.

355 When 1000 ml of sample was percolated through Strata-X-CW, the  
356 recoveries of the acidic analytes in the washing step were 9% for  
357 acetaminophen, and close to 50% for naproxen and diclofenac. It was also  
358 observed that all of the basic analytes were fractionated (see Table 3).



359           The Oasis WCX sorbent, which has properties similar to Strata-X-CW,  
360 was found to be even less useful than Strata-X-CW for the capture of basic  
361 pharmaceuticals; for Oasis WCX all the compounds retained very poorly and  
362 were eluted primarily during the washing step.

363           Another interesting feature relates to the retention behavior of naproxen  
364 and diclofenac, which have  $pK_a$  values of 4.8 and 4.2, respectively. These  
365 compounds were eluted nearly quantitatively (%R ~100%) during the washing  
366 step when percolated through the HXLPP-WCX sorbent, but they were not  
367 recovered completely by the commercial sorbents, which can be attributed to  
368 losses of these analytes during the loading step. This behaviour for this pair of  
369 analytes may be due to the weaker reversed-phase retention mechanisms  
370 operating for the commercially available sorbents. In any case, it is evident that,  
371 the HXLPP-WCX sorbent gives higher recoveries for all of the target analytes  
372 than the two commercial WCX sorbents, which, due to its not suitable results  
373 were not further tested.

374

### 375 **3.3 Application to real samples**

376           Given the highly promising SPE data obtained with HXLPP-WCX when  
377 the SPE protocol was applied to Milli-Q water, an analogous protocol was  
378 applied to the analysis of Ebre river water and effluent waste water. As is  
379 common practice, for the analysis of real water samples the sample volume  
380 loaded onto the SPE cartridges is normally lower than the sample volume  
381 applied when the analytes are in Milli-Q water due to the presence of  
382 interferences in real samples which compete with the analytes for binding to the  
383 sorbent and thereby reduce the analyte capture efficiency. To establish the

384 utility of the HXLPP-WCX sorbent for the analysis of real water samples, the  
385 initial SPE experiments involved the percolation of 500 ml sample of Ebre river  
386 water spiked at  $1 \mu\text{g l}^{-1}$  through cartridges packed with the sorbent (thereafter,  
387 the remainder of the SPE protocol was as detailed in Section 2). Table 4  
388 summarises the recovery values obtained for the various analytes on the  
389 HXLPP-WCX sorbent. From these results it can be observed that when 500 ml  
390 of a river water sample was loaded onto the SPE cartridge the recovery values  
391 for the analytes were high and similar to those obtained for Milli-Q water, with  
392 the exception of antipyrine which showed a higher level of fractionation than for  
393 the Milli-Q water case.

394 Fig. 2 shows the chromatograms obtained following preconcentration on  
395 HXLPP-WCX of 500 ml of non-spiked (Fig. 2b, 2d) and spiked (at  $1 \mu\text{g l}^{-1}$  for  
396 each analyte; Fig. 2a, 2c) Ebre river water. For the river water samples, a signal  
397 was detected at the retention time corresponding to caffeine (see the non-  
398 spiked Ebre river water chromatogram, Fig. 2d), but further analysis by a  
399 confirmatory technique such as mass spectrometry (MS) may be appropriate  
400 here. Typical chromatograms for the washing step, where all the interferences  
401 and acidic analytes retained on the cartridges through reversed-phase  
402 mechanism are eluted from the sorbents, are shown in Fig. 2a (spiked) and Fig.  
403 2b (non-spiked). Typical chromatograms for the elution step, where the target  
404 analytes retained through weak cation-exchange interactions (*i.e.*, mainly the  
405 basic analytes) are eluted from the sorbents, are shown in Fig. 2c (spiked) and  
406 Fig. 2d (non-spiked). It is important to note the cleanliness of the  
407 chromatograms, an observation which is particularly striking when one  
408 considers the fact that a non-selective detector (UV) was used in these

409 analyses. Both the selectivity and sensitivity of the analyses could be improved  
410 further by using more powerful detector such as mass spectrometer.

411 To demonstrate the selectivity of the HXLPP-WCX sorbent, a further set  
412 of SPE experiments was performed using dirtier sample matrices, including  
413 effluent water from a wastewater treatment plant (WWTP). The recovery values  
414 obtained when 250 ml of effluent WWTP samples, spiked at  $5 \mu\text{g l}^{-1}$ , was  
415 percolated through the HXLPP-WCX sorbent, is shown in Table 4. In general,  
416 the HXLPP-WCX sorbent gave good recoveries for most of the analytes  
417 studied, with the exception of antipyrine and carbamazepine. The recovery of  
418 carbamazepine in the elution step was 70%, the remaining 30% being eluted in  
419 washing step. As regards antipyrine, its elution profile, when loaded onto the  
420 HXLPP-WCX sorbent, was the reverse of that expected, *i.e.*, it was eluted in the  
421 washing step. In fact, antipyrine had already presented retention problems  
422 when present in other aqueous matrices, and these problems may be magnified  
423 when antipyrine is present in more complex samples since natural organic  
424 matter and other compounds present in wastewater matrices give rise to  
425 increased competition for binding to the sorbent.

426 Fig. 3 shows the elution chromatograms obtained after percolation of 250  
427 ml of an effluent WWTP sample through the HXLPP-WCX sorbent without (Fig.  
428 3b) and with (Fig. 3a) the addition of the mixture of analytes at the  $5 \mu\text{g l}^{-1}$  level.  
429 To emphasise the importance and effectiveness of the washing step for this  
430 complex sample matrix, we performed this particular analysis without a washing  
431 step; after the loading of 250 ml of effluent wastewater spiked at the  $5 \mu\text{g l}^{-1}$   
432 level with the mixture of analytes, all the analytes were eluted directly with 5 ml  
433 of 2% TFA in MeOH without any prior washing step being used. The effect of

434 re-introducing the methanol-based washing step was then examined in an effort  
435 to remove interferences. Fig. 4 shows the washing (Fig.4a, 4b) and elution  
436 (Fig.4c, 4d) chromatograms obtained after the percolation of a 250 ml effluent  
437 WWTP sample through the HXLPP-WCX sorbent without (Fig. 4b, 4d) and with  
438 (Fig. 4a, 4c) the addition of the mixture of analytes at the  $5 \mu\text{g l}^{-1}$  level. For the  
439 effluent WWTP sample, peaks were observed at the retention times  
440 corresponding to antipyrine, naproxen and diclofenac (Fig. 4b) and caffeine  
441 (Fig. 4d), but these results should be affirmed by a more powerful detector.

442 In addition to the marked improvements in the quality of the  
443 chromatograms, the new sorbent allows a more accurate quantification of  
444 analytes at lower concentration levels in complex matrices without the analytes  
445 being masked by interferences. The main point of all is the fact that the  
446 recovery of all the basic analytes of interest is complete in these complex  
447 environmental samples, on account of the WCX interactions which lead to high  
448 analyte recoveries (and clean chromatograms). In addition, the cleanliness of  
449 the extracts obtained after SPE with the HXLPP-WCX sorbent is an added  
450 advantage in respect of the potential to reduce or avoid ion-suppression effects  
451 in the case of determination by LC-MS with electrospray ionisation.

452 In validation studies using 500 ml of river water and 250 ml of effluent  
453 WWTP, all the basic analytes exhibited good linearity. In river water all the  
454 analytes exhibited a linear range from  $0.5\text{-}50 \mu\text{g l}^{-1}$ , with determination  
455 coefficients ( $r^2$ ) greater than 0.992. The limits of detection (LODs), calculated on  
456 the basis of a signal to noise ratio  $\geq 3$ , were  $0.1 \mu\text{g l}^{-1}$  for all the basic analytes.  
457 The repeatability and reproducibility of the method, expressed as the relative  
458 standard deviation (RSD) of three analyses of 500 ml of Ebre river water spiked

459 at  $1 \mu\text{g l}^{-1}$ , were less than 14% for all the basic analytes. For effluent waste  
460 water, all the analytes exhibited a good linear range ( $1\text{-}50 \mu\text{g l}^{-1}$ ) with  $r^2$  greater  
461 than 0.984. The LODs were  $0.5 \mu\text{g l}^{-1}$  for most of compounds, with the  
462 exception of caffeine where the LOD was  $1 \mu\text{g l}^{-1}$ . Although the LODs are not as  
463 low as those reported for some environmental water samples [21,22], they  
464 could be decreased markedly by the introduction of a more sensitive detection  
465 system, such as tandem mass spectrometry. Moreover, the cleanliness of the  
466 chromatograms will tend to reduce or prevent ion-enhancement/suppression  
467 effects when LC-MS is used.

468

#### 469 **4. Conclusions**

470 In this study the synthesis of hypercrosslinked polymer resin with weak cation-  
471 exchange is described, and a detailed investigation carried out with respect to  
472 the application of this sorbent to the SPE of basic pharmaceuticals from  
473 complex environmental samples. The resin was produced *via* the  
474 hypercrosslinking of swellable polymers precursors which were synthesised *via*  
475 precipitation polymerisation. The WCX properties are derived from the presence  
476 of carboxylic acid moieties in the polymer.

477 This is the first time that a hypercrosslinked polymer resin has been exploited  
478 as weak cation-exchanger for the SPE of basic pharmaceuticals. Following  
479 optimisation of the SPE protocol, it was found that the novel HXLPP-WCX  
480 sorbent enabled essentially quantitative recovery, and adequate selectivity, of  
481 most of the analytes tested, and performed well as a weak cation-exchanger. In  
482 contrast, the commercially available sorbents Strata-X-CW and Oasis WCX  
483 were unable to completely retain basic analytes *via* an ion-exchange

484 mechanism and remove acidic analytes during the washing step. The highest  
485 extraction efficiency was achieved with the HXLPP-WCX sorbent. Overall, the  
486 HXLPP-WCX sorbent proved to be highly effective for the preconcentration of  
487 basic analytes present in complex environmental water samples.

488

#### 489 **Acknowledgements**

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493

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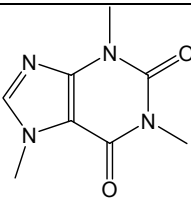
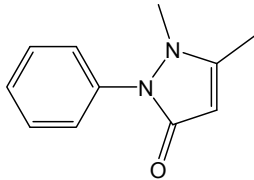
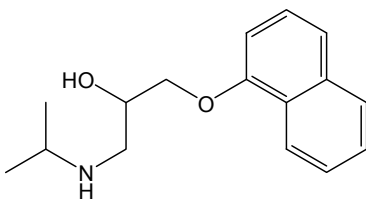
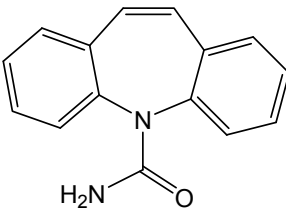
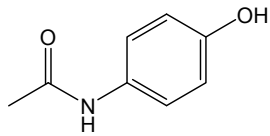
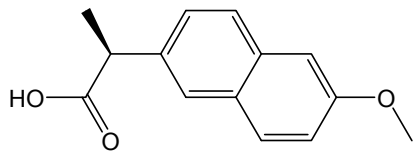
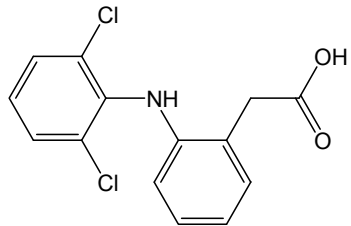
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**Table 1.** Chemical structures and pK<sub>a</sub> values of the selected analytes.

Compound	Compound type	Chemical Structure	pK <sub>a</sub> <sup>a</sup>
Caffeine	CNS <sup>b</sup> stimulant		13.4
Antipyrine	Analgesic		13.3
Propranolol	β-blocker		9.5
Carbamazepine	Anti-epileptic		13.7
Acetaminophen	Analgesic		9.7
Naproxen	NSAID <sup>c</sup>		4.8
Diclofenac	NSAID <sup>c</sup>		4.2

<sup>a</sup> pK<sub>a</sub> values calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (© 1994-2009 ACD/Labs)

<sup>b</sup> Central nervous system

<sup>c</sup> Non-steroidal anti-inflammatory drug

**Table 2.** Characterisation data for the sorbents tested in SPE.

	<b>HXLPP-WCX<sup>a</sup></b>	<b>Oasis WCX<sup>b</sup></b>	<b>Strata-X-CW<sup>b</sup></b>
	Laboratory synthesised	Waters	Phenomenex
Yield (%) <sup>c</sup>	85	n.d.	n.d.
I.E.C. <sup>d</sup> (meq g <sup>-1</sup> )	0.72	0.75	0.74
Specific surface area (m <sup>2</sup> g <sup>-1</sup> )	1125	800	800
Average particle diameter (μm)	6.1±1.6	30	33

n.d. no data

<sup>a</sup> Data measured experimentally

<sup>b</sup> Data provided by the supplier

<sup>c</sup> Relative to the mass of the corresponding (non-hypercrosslinked) precursor particles

<sup>d</sup> Ion-exchange capacity

**Table 3.** Recovery values (%) obtained when the HXLPP-WCX, Strata-X-CW and Oasis WCX sorbents were applied in SPE for the preconcentration of 1000 ml of a Milli-Q sample spiked at 20 µg l<sup>-1</sup> with the analyte mixture.

Analytes	Type	HXLPP-WCX		Strata-X-CW		Oasis WCX	
		Wash	Elution	Wash	Elution	Wash	Elution
Caffeine		5	93	47	17	60	5
Antipyrine	Basic	15	79	55	11	91	0
Propranolol		0	93	48	40	73	13
Carbamazepine		4	107	31	46	90	15
Acetaminophen		87	0	9	0	17	0
Naproxen	Acidic	99	6	47	17	74	3
Diclofenac		94	13	45	22	77	11

For the experimental conditions, see text.

% Relative standard deviations (RSDs) (n=3) were lower than 12% for %R >10%.

**Table 4.** Recovery values (%) obtained when the HXLPP-WCX sorbent was applied in SPE for the preconcentration for different real samples spiked with the analyte mixture.

Analytes	Type	Ebre River (1 $\mu\text{g l}^{-1}$ )		Effluent WWTP (5 $\mu\text{g l}^{-1}$ )	
		500 ml		250 ml	
		Wash	Elution	Wash	Elution
Caffeine		20	90	26	82
Antipyrine	Basic	50	54	76	0
Propranolol		0	90	0	92
Carbamazepine		11	90	30	70
Acetaminophen		113	0	100	0
Naproxen	Acidic	94	0	91	0
Diclofenac		98	0	93	0

For the experimental conditions, see text.

% Relative standard deviations (RSDs) (n=3) were lower than 14% for %R >11%.

## Figure captions

Fig. 1 Chemical structures of the sorbents tested: HXLPP-WCX, Strata-X-CW and Oasis WCX

Fig. 2 Chromatograms obtained after off-line trace enrichment with HXLPP-WCX of 500 ml of Ebre river water sample with (a,c) and without (b,d) addition of a  $1 \mu\text{g l}^{-1}$  level of analyte mixture: washing step (a, b) and elution step (c, d).

Fig. 3 Chromatograms obtained after off-line trace enrichment with the HXLPP-WCX sorbent of 250 ml of effluent WWTP sample with (a) and without (b) the addition of a  $5 \mu\text{g l}^{-1}$  level of an analyte mixture (without a washing step).

Fig. 4 Chromatograms obtained after off-line trace enrichment with HXLPP-WCX of 250 ml of effluent WWTP sample with (a,c) and without (b,d) the addition of a  $5 \mu\text{g l}^{-1}$  level of analyte mixture: washing step (a,b) and elution step (c,d).

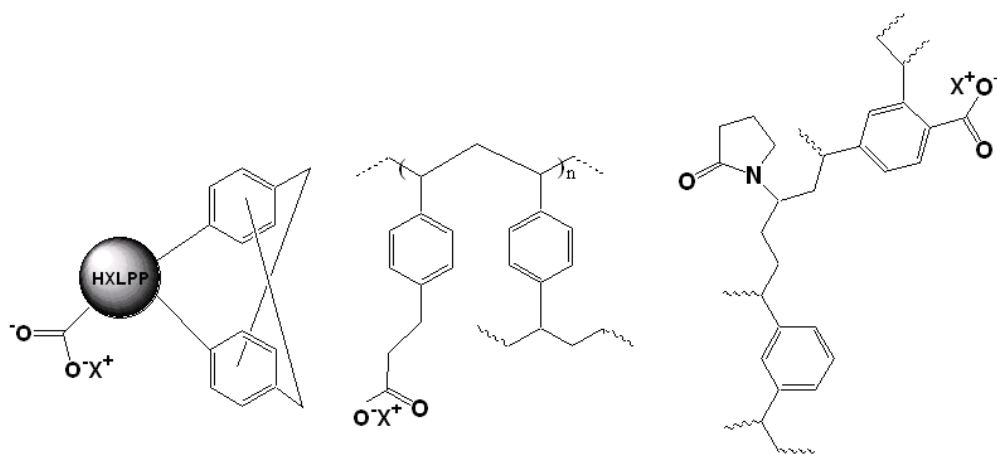
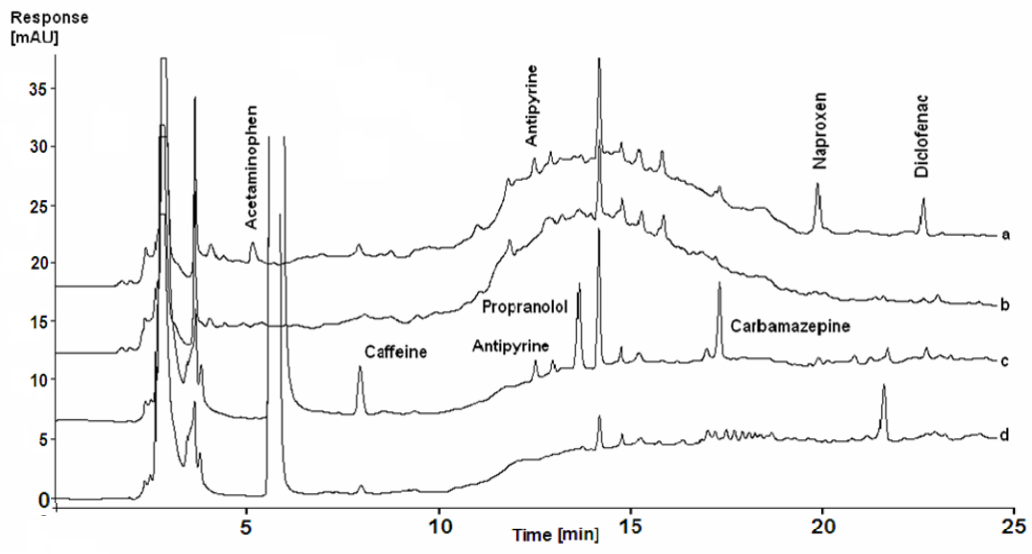
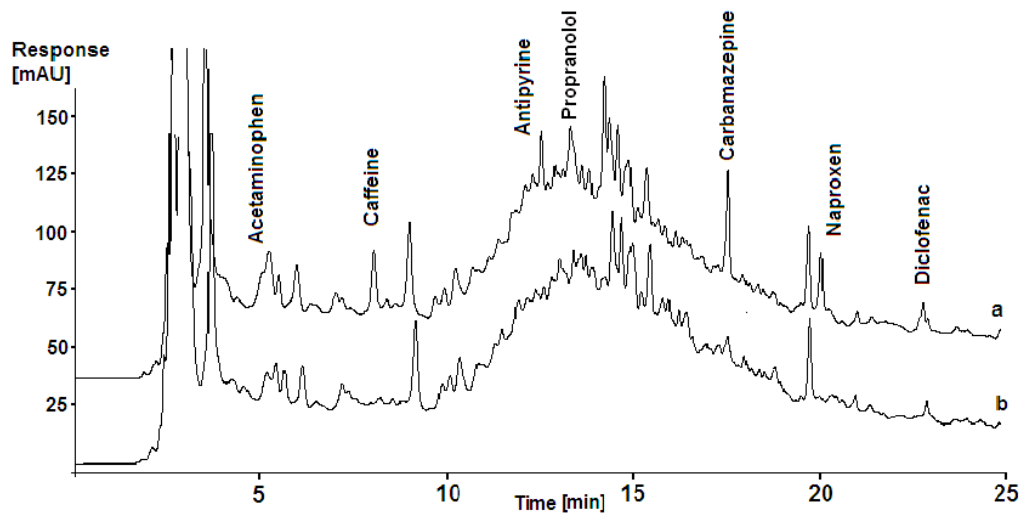


Figure 1

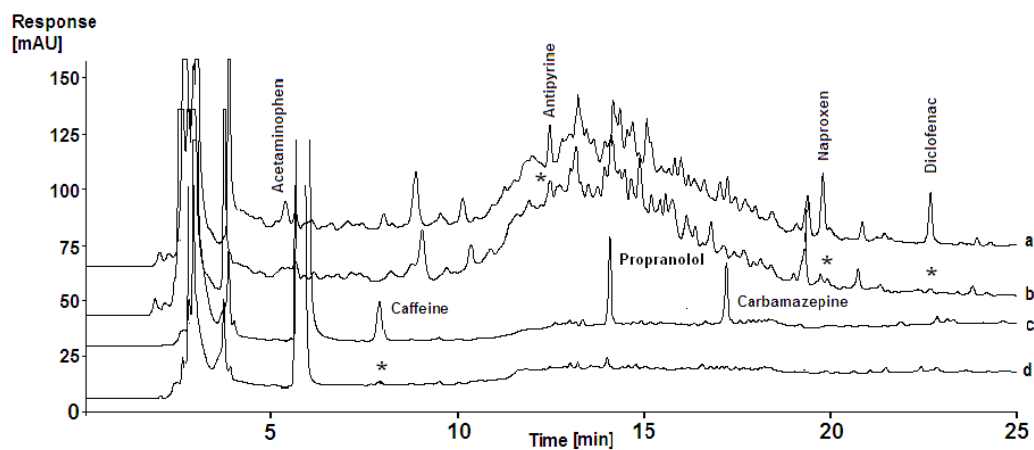


**Figure 2**



**Figure 3**





**Figure 4**