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3 **determination of pharmaceutical compounds by bar adsorptive**  
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6  
7 **Carbon-based sorbent coatings for the determination of**  
8 **pharmaceutical compounds by bar adsorptive microextraction**

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19  
20 **Abstract**

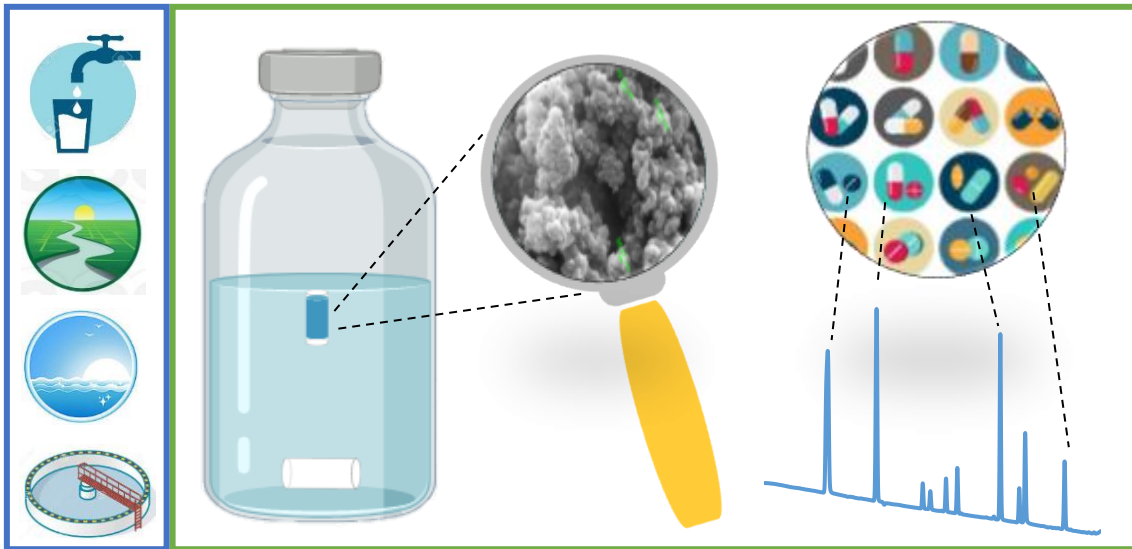
21 Thirteen carbon materials comprising commercial activated carbons and lab-  
22 made materials (hydrochars and activated carbons) were assayed as  
23 enrichment phase in bar adsorptive microextraction to monitor trace levels of ten  
24 common pharmaceutical compounds (PhCs) in environmental water matrices,

25 including surface water, sea water, tap water and wastewater. Polar and non-  
26 polar pharmaceuticals were selected – sulfamethoxazole, triclosan,  
27 carbamazepine, diclofenac, mefenamic acid, 17 $\alpha$ -ethinylestradiol, 17 $\beta$ -estradiol,  
28 estrone, gemfibrozil and clofibrac acid – as model compounds to cover distinct  
29 therapeutic classes. Despite having a less-developed porosity, data showed  
30 that “in-house” prepared nanoporous hydrochars obtained from carbohydrates  
31 at low temperature (e.g., 180 °C) in the presence of an eutectic salt mixture  
32 compete with the best commercial activated carbons for this particular  
33 application. The combination of a micro and mesopore network with a rich  
34 oxygen-based surface chemistry yielding an acidic nature allowed these  
35 hydrochars to present the best overall recoveries (between 20.9 to 82.4 %) for  
36 the simultaneous determination of the ten target PhCs with very distinct  
37 chemical properties, by high performance liquid chromatography-diode array  
38 detection (HPLC-DAD).

39

40

41 **Table to Contents (TOC)**



42

43 **Keywords**

44 Carbon hydrochars; Activated Carbons; Sorbent coating; Bar adsorptive

45 microextraction (BA $\mu$ E); HPLC-DAD; Pharmaceutical compounds;

46 Environmental water matrices.

## 47        **1. Introduction**

48    Pharmaceuticals and personal care products (PPCPs) encompass many  
49    different substances, such as drugs used in human and veterinary medicine,  
50    fragrances, sunscreen agents, and cosmetics ingredients. More than 3000  
51    PPCPs are currently marketed and new molecules enter the market yearly and  
52    they are ubiquitously detected in treated wastewater [1]. Moreover,  
53    pharmaceuticals compounds (PhCs) are generally biologically active  
54    compounds that are often water soluble, therefore can be found in wastewaters  
55    and easily end up in natural waters, including ground water, river water, sea  
56    water and surface waters [2–5], usually at the trace level, ranging from  $\mu\text{g L}^{-1}$  to  
57     $\text{ng L}^{-1}$  [6]. PhCs include multiple medicines classes, namely lipid regulators,  
58    antibacterials/antifungals,  $\beta$ -adrenergic blocking agents, analgesics,  
59    tranquilizers, hormones, and antiseptics, among many others [7]. The concern  
60    for the possible ecotoxicological effects of these compounds is reflected on  
61    decisions of the European Parliament in the field of water policy. In 2013, three  
62    widely used PhCs, including the hormonal preparations  $17\alpha$ -ethinylestradiol and  
63     $17\beta$ -estradiol, as well as the painkiller diclofenac, were listed in the watch list of  
64    substances to be monitored in the European Union (EU) to support the  
65    determination of appropriate measures to address the risk posed by them  
66    (Directive 2013/39/EU) [8]. The first revision of this list in 2015 maintained these  
67    three pharmaceuticals (Directive 2015/495/EU) [9] but in the most recent  
68    revision diclofenac, along with three other substances, was removed from the  
69    watch list since the commission concluded that sufficient high-quality monitoring  
70    data was already available (Directive 2018/840/EU) [10]. For these reasons,  
71    there is a need for innovative analytical approaches that allow for their

72 quantification in environmental water matrices. Furthermore, considering the EU  
73 decision on water policy, the development of a simple methodology that allows  
74 the simultaneous analysis of the most representative emerging PhCs in water  
75 matrices continues to be a hot research topic. As water bodies contain trace  
76 amounts of a large range of PhCs with distinct physicochemical properties, the  
77 challenge relies on the development of quantification techniques comprising  
78 both polar and non-polar compounds.

79 Many analytical approaches have been proposed to analyze trace levels of  
80 PhCs in environmental water matrices; these always include a sample  
81 enrichment step prior to gas or liquid chromatography [1,8-10]. In the last  
82 decades, the development of miniaturized passive sorption-based techniques  
83 for sample enrichment has increased, covering *e.g.*, solid phase microextraction  
84 (SPME), stir bar sorptive extraction (SBSE) [14–16], and bar adsorptive  
85 microextraction (BA $\mu$ E) as most representative for trace analysis of polar to  
86 nonpolar analytes in aqueous media [11-13]. BA $\mu$ E is based on the floating  
87 sampling technology enrichment process, allowing for the possibility of selecting  
88 the most convenient sorbent coating upon the target PhCs involved [11,18,19].  
89 In this regard, and recognizing the relevance of activated carbons as  
90 adsorbents of micropollutants, it is interesting to evaluate the potential of  
91 different types of carbon materials -*e.g.*, carbohydrate-derived hydrochars and  
92 their activated counterparts-, as coating phases for a selective extraction of a  
93 wide range of PhCs. Hydrochars can be obtained by the hydrothermal  
94 carbonization of a carbohydrate precursor under mild synthesis conditions using  
95 water as solvent. As opposed to activated carbons prepared from conventional  
96 activation routes, hydrochars are essentially non-porous solids characterized by

97 displaying a surface chemistry rich in oxygen groups. The addition of eutectic  
98 salt mixtures during the hydrothermal carbonization allows to obtain nanoporous  
99 hydrochars with hierarchical porosity [20] and a rich amphoteric surface  
100 chemistry. The combination of these features will prove to play a crucial role in  
101 their high performance toward the quantification of PhCs with distinct  
102 physicochemical properties.

103 This work aims to evaluate the performance of BA $\mu$ E devices coated with  
104 various carbon materials to simultaneously monitor traces of pharmaceutical  
105 compounds (*i.e.* sulfamethoxazole, triclosan, carbamazepine, diclofenac,  
106 mefenamic acid, 17 $\alpha$ -ethinylestradiol, 17 $\beta$ -estradiol, estrone, gemfibrozil and  
107 clofibrac acid) in deionized water and in environmental water matrices. The  
108 BA $\mu$ E was used as the first step in the quantification and identification of the  
109 PhCs, followed by microliquid desorption ( $\mu$ LD) and then high performance  
110 liquid chromatography with diode array detection (HPLC-DAD). The  
111 performance of the distinct carbon materials covering activated carbons,  
112 nanoporous carbohydrate-based hydrochars and superactivated hydrochars  
113 has been discussed in the light of their porous and chemical features. The  
114 validation and application of the optimized methodology (including the influence  
115 of polarity, back-extraction time and solvent, stirring rate) for real water matrices  
116 is fully discussed, as well as the comparison with other microextraction-based  
117 techniques.

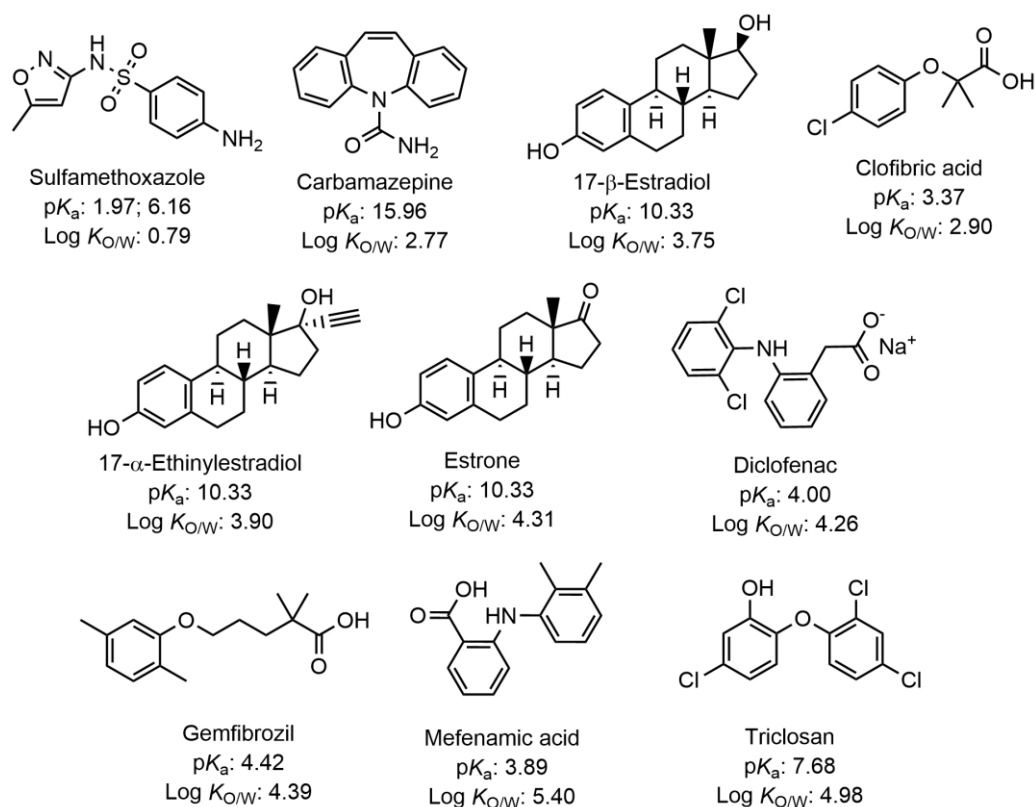
## 118 **2. Experimental**

### 119 **2.1 Reagents**

120 Glucose, G (> 99 %), fructose, F (> 99 %) and sucrose, S (> 99 %) were  
121 obtained from AnalaR NORMAPUR (Leuven, Belgium). Lithium chloride (LiCl,

122 99 %) was acquired from Merck (Darmstadt, Germany), and zinc chloride (ZnCl,  
123 98 %) was acquired from Acros Organics (Geel, Belgium). Carbamazepine  
124 (99.0 %), triclosan (97.0 %), diclofenac sodium salt (98.0 %) 17- $\beta$ -estradiol  
125 (98.0 %), estrone (99.0 %), gemfibrozil (98.5 %), mefenamic acid (98.5 %),  
126 clofibrac acid (98.0 %), sulfamethoxazole (98.0 %) and potassium carbonate  
127 ( $K_2CO_3$ , 99 %) were purchased from Sigma-Aldrich (Steinheim, Germany). 17-  
128  $\alpha$ -ethinylestradiol (98.0 %) was purchased from Riedel-de-Haën (Seelze,  
129 Germany). Figure 1 depicts the chemical structures, as well as the  $pK_a$  and log  
130  $K_{OW}$  values of the PhCs studied. The solvents used were HPLC-grade  
131 methanol (MeOH, 99.8 %), and acetonitrile (ACN, 99.8 %), obtained from Carlo  
132 Erba (Arese, Italy). Sodium chloride (NaCl, 99.5 %) was purchased from Merck  
133 Millipore (Darmstadt, Germany). Sodium hydroxide (NaOH, 98.0 %) pellets  
134 were obtained from AnalaR (BDH chemicals, Lutterworth, U.K.). Hydrochloric  
135 acid (HCl, 37 %), acetic acid (99.5 %) and potassium hydroxide (KOH, 85 %)  
136 were purchased from Panreac (Barcelona, Spain). The acrylic acid (AA,  $\geq 99$  %)  
137 was purchased from Merck (Hohenbrunn, Germany). All chemicals were used  
138 without further purification. Ultra-pure water was obtained from the Milli-Q water  
139 purification systems (Merck Millipore, Darmstadt, Germany).





140

141

Figure 1 – Chemical structures, pK<sub>a</sub> and log K<sub>OW</sub> values of the analysed PhCs [21].

142

## 2.2 Carbon materials synthesis and characterization

143

The hydrochars were prepared by hydrothermal carbonization of

144

carbohydrates: 15 cm<sup>3</sup> of 1.5 mol L<sup>-1</sup> glucose or sucrose solutions introduced in

145

an autoclave and heated at 190 °C during 5 h [22,23]. These samples are

146

labeled as HG or HS according with the carbohydrate precursor (glucose and

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sucrose, respectively). Modified sucrose-derived hydrochars were prepared by

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a similar protocol but adding acrylic acid (e.g., 7, 14 or 27 wt.%) to the starting

149

sucrose aqueous solution [24,25]. The modified hydrochars were labeled as

150

HSAAx, where x stands for the amount of acrylic acid. The sucrose-derived

151

hydrochar was chemically activated as indicated elsewhere [22,23]. Briefly,

152

superactivated carbons HS/H800 and HS/C800 were prepared by activation of

153

1 g of sucrose-derived hydrochar (HS) with 4 g of, respectively, KOH and

154

K<sub>2</sub>CO<sub>3</sub> during 1 h at 800 °C.

155 The synthesis of nanoporous hydrochars was inspired on the studies  
156 reported by Fechler *et al.* for glucose [20]. Briefly, 11.25 g of eutectic LiCl/ZnCl<sub>2</sub>  
157 salt mixture (23 molar % LiCl and the remaining corresponds to ZnCl<sub>2</sub>) were  
158 ground and homogenized in an agate mortar; then 4.5 g of a carbohydrate  
159 precursor (G - glucose, F - fructose or S - sucrose) were added, and the  
160 resulting mixture was grounded until obtaining a homogeneous sample. The  
161 mixture carbohydrate:eutectic salt was introduced in a Teflon-line stainless steel  
162 autoclave (35 cm<sup>3</sup>). After adding 2.3 cm<sup>3</sup> of ultra-pure water, the autoclave was  
163 closed, shaken vigorously, and placed in an oven (Medline Scientific Limited,  
164 model ON-02G) pre-heated to 180 °C. The hydrothermal treatment was  
165 performed during 17 h, after what the autoclave was cooled down to room  
166 temperature, and the porous hydrochar was washed with distilled water up to no  
167 detection of chlorine anions (*i.e.* no precipitation when AgCl is added). After  
168 washing, the samples were dried at atmospheric pressure, ground and sieve to  
169 store particles with dimensions < 0.297 mm. The porous hydrochars were  
170 labelled as npHy, where y stands for the carbohydrate precursor (G, F, or S:  
171 npHG, npHF and npHS, respectively).

172 An activated carbon prepared by steam activation of expanded corkboard  
173 granules at semi-industrial scale following the procedure reported in ref. [26]  
174 was also used (Cork/S800). Commercial powdered activated carbon (PAC)  
175 materials CN1 from Cabot-Norit (Com/CN1, supplied by Salmon & Cia. (Lisbon,  
176 Portugal)) and R from Riedel-de-Haën (Com/R, Seelze, Germany) were tested  
177 for comparison purposes. Further details on the synthesis and characterization  
178 of some of these carbons were previously reported (see cited references), and it  
179 is presented on table 1 for comparison purposes.

181 Table 1 – Summary of the main textural and surface properties of the carbon materials used as  
 182 coatings (apparent surface area –  $A_{\text{BET}}$ , micropore volume –  $V_{\text{micro}}$  (pores with widths < 2 nm),  
 183 mesopore volume –  $V_{\text{meso}}$  (pores with widths between 2 and 50 nm), pH at the point of zero  
 184 charge –  $\text{pH}_{\text{PZC}}$ ).

Type	Carbon material	Textural properties	Surface properties
Activated carbons	Commercial (Com/CN1 and Com/R)	$A_{\text{BET}} \approx 1000 \text{ m}^2/\text{g}$ Micro+mesopores Similar $V_{\text{micro}}$ but $V_{\text{meso}}$ of Com/CN1 is the double of Com/R	$\text{pH}_{\text{PZC}}(\text{Com/CN1}) = 5.1$ $\text{pH}_{\text{PZC}}(\text{Com/R}) = 6.5$
	Steam activated cork (Cork/S800) <sup>a</sup>	BET area = $750 \text{ m}^2/\text{g}$ Micro+mesopores	$\text{pH}_{\text{PZC}} \geq 11$
Hydrochar	Glucose and sucrose derived hydrochars (HG and HS) <sup>c</sup>	Non-porous	$\text{pH}_{\text{PZC}} = 2$
	Acrylic acid modified sucrose-derived hydrochar (HSAA7%, HSAA14% and HSAA27%) <sup>d</sup>	Non-porous	$\text{pH}_{\text{PZC}} \approx 2$ Carboxylic, lactonic and phenolic groups in higher percentage than in non-modified HS
Activated carbons from hydrochars	Glucose, fructose and sucrose derived nanoporous hydrochars npHG, npHF and npHS)	$A_{\text{BET}}(\text{npHG}) = 529 \text{ m}^2/\text{g}$ $A_{\text{BET}}(\text{npHF}) = 407 \text{ m}^2/\text{g}$ $A_{\text{BET}}(\text{npHS}) = 487 \text{ m}^2/\text{g}$ Micro+mesopore samples with predominance of mesopores	$\text{pH}_{\text{PZC}} = 2.7 - 3.1$
	Chemically activated sucrose-derived hydrochar (HS/C800 and HS/H800) <sup>b</sup>	$A_{\text{BET}}(\text{HS/C800}) = 1350 \text{ m}^2/\text{g}$ $A_{\text{BET}}(\text{HS/H800}) = 2431 \text{ m}^2/\text{g}$ Both microporous but HS/H800 $V_{\text{micro}}$ is almost the double of HS/C800	$\text{pH}_{\text{PZC}} \approx 4$

185 <sup>a</sup> Synthesis and characterization reported in [26]

186 <sup>b</sup> Synthesis and characterization reported in [22,23]

187 <sup>c</sup> Synthesis reported in [23,24] and characterization reported in [24]

188 <sup>d</sup> Synthesis and characterization reported in [24]

189

## 190 **2.3 Characterization of the carbon materials**

191 The textural properties of the porous hydrochars and commercial PACs  
 192 were characterized by  $\text{N}_2$  adsorption isotherms at  $-196 \text{ }^\circ\text{C}$  in an automatic  
 193 volumetric apparatus from Micromeritics (ASAP 2010). Before  $\text{N}_2$  adsorption-

194 desorption the samples (60 – 100 mg) were outgassed at 120 °C overnight (ca.  
195 17 h) under vacuum (pressure < 10<sup>-2</sup> Pa). The apparent surface area,  $A_{\text{BET}}$ , was  
196 estimated from the N<sub>2</sub> adsorption data in the  $p/p^0$  range of the BET plot that  
197 assures: positive interception on the ordinate of the BET plot ( $C > 0$ ) and  $n_{\text{ads}}(1-$   
198  $p/p^0)$  continuously increases with  $p/p^0$  [27,28]. The total pore volume,  $V_{\text{total}}$ , was  
199 quantified with the Gurvich rule [29], the micropore volume,  $V_{\text{micro}}$ , was  
200 calculated with the  $\alpha_s$  method taking as reference the isotherm reported by  
201 Rodríguez-Reinoso *et al.* [30]. ACRESCENTAR ultra e super... The mesopore  
202 volume,  $V_{\text{meso}}$ , corresponds to the difference  $V_{\text{total}} - V_{\text{micro}}$ .

203 The surface chemistry properties of the synthesized porous hydrochars and  
204 commercial PAC were assessed through the determination of the pH at the  
205 point of zero charge,  $\text{pH}_{\text{PZC}}$ , according to the reverse mass titration method [32]  
206 using a Symphony SP70P pH meter. Diffuse reflectance infrared Fourier  
207 transform (DRIFT) spectra of the porous hydrochars were recorded in a Nicolet  
208 Magma-IR560 spectrometer provided with a high sensitivity mercury cadmium  
209 telluride detector (MCT-A) that operates at cryogenic temperature. Each  
210 spectrum was obtained using the powdered samples with no KBr addition and  
211 corresponds to the accumulation of 256 scans, recording with a spectral  
212 resolution of 2 cm<sup>-1</sup> in the mid-IR (4000 – 650 cm<sup>-1</sup>) spectral range. The thermal  
213 analysis (Setaram Labsys) was carried out on 15 mg of sample under a  
214 nitrogen flow rate of 50 cm<sup>3</sup> min<sup>-1</sup> up to a final temperature of 900 °C (heating  
215 rate of 15 °C min<sup>-1</sup>). The ash content of the glucose-derived porous char was  
216 determined in the same equipment after exposure of the sample at 600 °C in air  
217 (50 cm<sup>3</sup> min<sup>-1</sup>) for 3 h (constant mass).

218 The morphology of the porous hydrochars was evaluated by Scanning  
219 Electron Microscopy (SEM) performed at a Zeiss Supra 55 VP apparatus with 5  
220 kV as accelerating voltage and using the powdered samples coated with  
221 iridium.

#### 222 ***2.4 Standard preparation and water samples***

223 Stock solutions of individual analytes ( $1,000.0 \text{ mg L}^{-1}$ ) used for the working  
224 standard mixture were prepared in MeOH and stored at  $4 \text{ }^{\circ}\text{C}$  and renewed  
225 every month. Working standard mixtures of  $1.0 \text{ mg L}^{-1}$  were daily prepared in  
226 MeOH and used for spiking sample assays. For instrumental calibration,  
227 standard mixtures were prepared in MeOH by appropriate dilution from stock  
228 solutions.

229 The water samples were obtained in the metropolitan area of Lisbon  
230 (Portugal). The sea water samples were collected in the coastal area near  
231 Estoril and Costa da Caparica, the lake water from an artificial lake (Campo  
232 Grande) and the tap water samples from the public water supply system of  
233 Lisbon and Almada. The estuarine water samples were collected at Ponta do  
234 Mato, a Tagus river estuary beach. The wastewater treatment plant (WWTP)  
235 samples were obtained from Alcântara WWTP after primary decantation and  
236 filtration. All samples were collected in clean amber glass bottles and filtered  
237 with paper filters (125 mm of diameter, 10-13  $\mu\text{m}$  of pore size, Cat No 1001 125,  
238 Whatman; Amersham, U.K.) and kept refrigerated at  $-20 \text{ }^{\circ}\text{C}$  until being used.

#### 239 ***2.5 BA $\mu$ E- $\mu$ LD methodology***

240 The BA $\mu$ E devices (7.5 mm in length and 3 mm in diameter) were in-house  
241 prepared as indicated in previous works [17,33]. After being produced, the

242 devices were stored at room temperature in closed glass flasks. The BA $\mu$ E  
243 devices were cleaned with MeOH and ultra-pure water before use. Considering  
244 that microextraction bars are relatively inexpensive and easy to prepare, and  
245 aiming to avoid any possible contamination, they were discarded after each  
246 use.

247 The BA $\mu$ E- $\mu$ LD procedure was performed by placing a 25 mL aliquot of the  
248 water samples in a 25 mL sampling glass flask, followed by the introduction of a  
249 BA $\mu$ E device, previously coated with npHS, and a conventional Teflon magnetic  
250 stirring bar. The assays were performed in a multipoint agitation plate  
251 (Variomag H+P Labortechnik Multipoint 15, Oberschleissheim, Germany) at  
252 room temperature using 1,000 rpm for 16 h (pH 2.0). After microextraction, the  
253 devices were removed from the samples with clean tweezers, dried for a few  
254 seconds using a lint-free tissue and placed into glass vial inserts containing 100  
255  $\mu$ L of ACN, ensuring their total immersion prior to ultrasonic treatment (Branson  
256 3510, Zurich, Switzerland) at room temperature for 60 min. After  $\mu$ LD, the  
257 devices were removed, 100  $\mu$ L of ultra-pure water was added, the vials  
258 vortexed for 10 s, and closed, followed by HPLC-DAD analysis. The standard  
259 addition method (SAM) was applied in real samples assays using four  
260 concentration levels ranging from 8.0  $\mu$ g L<sup>-1</sup> to 104.0  $\mu$ g L<sup>-1</sup> for all PhCs under  
261 study. Blank assays were also performed using the same procedure but without  
262 spiking (“zero-point” assays). The assays on real matrices were performed in  
263 triplicate.

## 264 ***2.6 Instrumental set-up***

265 HPLC-DAD analysis were carried out on a benchtop Agilent 1100 series LC  
266 chromatographic system (Agilent Technologies, Waldbronn, Germany)

267 equipped with a vacuum degasser (G1322A), autosampler (G1313A),  
268 thermostated column compartment (G1316A), quaternary pump (G1311A) and  
269 a diode array detector (G1315B). The data acquisition and system control were  
270 performed by the software LC3D ChemStation (version Rev.A.10.02[1757],  
271 Agilent Technologies). Analyses were performed on a Kinetex hexyl phenyl  
272 column, 150.0 × 4.6 mm, 2.6 µm particle size (Phenomenex, Torrance, U.S.A.).  
273 The samples were analysed using a gradient mobile phase consisting of 2.5 %  
274 acetic acid/ultra-pure water (v/v, solvent A) and ACN (solvent B). The employed  
275 elution gradient was as follows: 0 min – 80/20 (solvent A/B); 24 min – 20/80  
276 (solvent A/B); 27 min - 20/80 (solvent A/B); 30 min – 80/20 (solvent A/B); 35 min  
277 – 80/20 (solvent A/B). All solvents were previously filtered (125 mm in diameter,  
278 10-13 µm in pore size, 1001 125, Whatman, U.K.) to remove suspended  
279 particles, if any. The detector was set at 280 nm and the column temperature at  
280 20 °C. The injection volume was 40 µL with a draw speed of 200 µL min<sup>-1</sup> and  
281 the flow rate was set at 0.6 mL min<sup>-1</sup>. For identification purposes, standard  
282 addition was used, by spiking the samples with pure standards, as well as by  
283 comparing the relative retention time and peak purity with the UV/vis spectral  
284 reference data. For quantification purposes, calibration curves using the  
285 external standard methodology were performed. Instrument linearity was  
286 calculated by injecting ten standard solutions having concentration from 10.0 or  
287 30.0 µg L<sup>-1</sup> (depending on the compound) to 5,000.0 µg L<sup>-1</sup>, where it was  
288 possible obtain determination coefficients ( $r^2$ ) higher than 0.99 for the target  
289 compounds. For recovery calculations, peak areas obtained from each assay  
290 were compared with the peak areas of standard controls used for spiking. Peak  
291 areas were obtained by integration of each target compounds corresponding

292 peak using the mentioned software. The sensitivity of the instrumental system  
293 was checked through the limits of detection (LODs) and quantification (LOQs)  
294 calculated with signal-to-noise (S/N) of 3/1 and 10/1, where it ranged from 1.0  
295  $\mu\text{g L}^{-1}$  to 10.0  $\mu\text{g L}^{-1}$  and between 5.0  $\mu\text{g L}^{-1}$  to 27.0  $\mu\text{g L}^{-1}$  for all the PhCs under  
296 study, respectively. The instrumental precision was evaluated by consecutively  
297 injecting a standard mixture ( $n = 6$ , 1.0  $\text{mg L}^{-1}$ ), resulting in relative standard  
298 deviations (RSD) lower than 2.9 %.

299

### 300 **3. Results and discussion**

#### 301 **3.1 Carbon material selectivity as sorbent coating**

302 To maximize the microextraction efficiency by the BA $\mu$ E- $\mu$ LD process,  
303 thirteen carbon materials were tested as enrichment phase for the recovery of  
304 PhCs using carbamazepine, 17 $\alpha$ -ethinylestradiol, 17 $\beta$ -estradiol, diclofenac and  
305 triclosan as model compounds. These carbon materials were selected to cover  
306 a large range of textural features (from non-porous to highly porous samples  
307 with  $A_{\text{BET}}$  up to 2400  $\text{m}^2/\text{g}$ ) and surface properties (acidic, neutral or basic, with  
308  $\text{pH}_{\text{PZC}}$  ranging from 2 to 11), as it can be observed in tables 1 and 2. Additional  
309 information on the physicochemical properties of the studied carbons is  
310 summarized in the Supporting Information (topic S1). The evaluation assays  
311 were made in triplicate using ultra-pure water samples spiked at the 16.0  $\mu\text{g L}^{-1}$   
312 level.

313 As presented in figure 2a, the recovery efficiency of the carbon materials  
314 for the five selected PhCs is strongly dependent on both the type of material  
315 and the target pharmaceutical compound.



316

317 Table 2 – Textural properties of the porous hydrochars and commercial activated carbons, and  
 318  $\text{pH}_{\text{PZC}}$ .

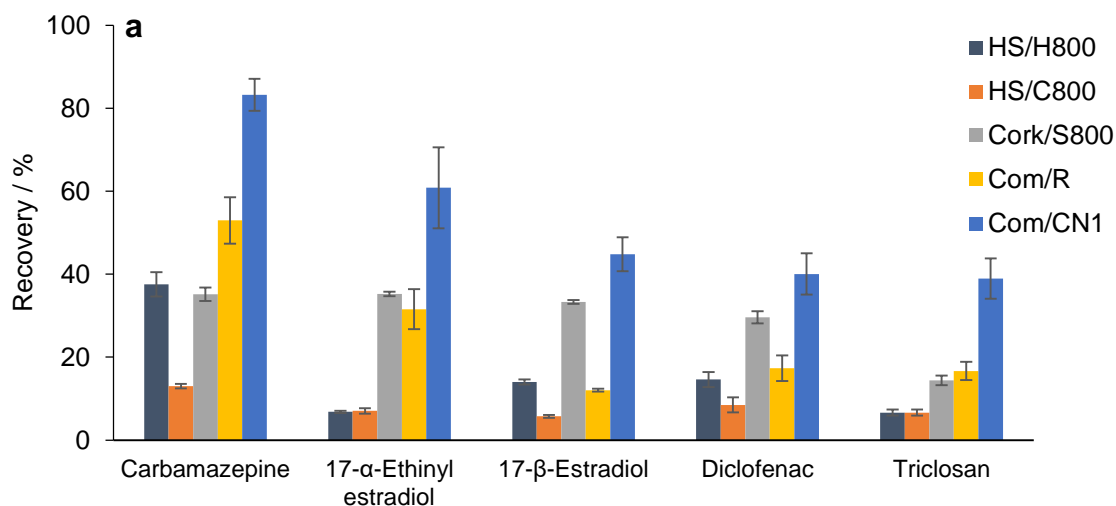
Sample	$A_{\text{BET}}$ ( $\text{m}^2 \text{g}^{-1}$ )	$V_{\text{total}}^{\text{a}}$ ( $\text{cm}^3 \text{g}^{-1}$ )	$V_{\text{meso}}^{\text{b}}$ ( $\text{cm}^3 \text{g}^{-1}$ )	$\alpha_s$ Method			$\text{pH}_{\text{PZC}}$
				$V_{\alpha \text{ total}}$ ( $\text{cm}^3 \text{g}^{-1}$ )	$V_{\alpha \text{ ultra}}$ ( $\text{cm}^3 \text{g}^{-1}$ )	$V_{\alpha \text{ super}}$ ( $\text{cm}^3 \text{g}^{-1}$ )	
<b>npHG</b>	529	0.58	0.47	0.11	0.00	0.11	3.0
<b>npHF</b>	407	0.28	0.16	0.12	0.00	0.12	2.7
<b>npHS</b>	487	0.47	0.35	0.12	0.00	0.12	3.1
<b>Com/CN1</b>	1179	0.98	0.68	0.30	0.00	0.30	5.1
<b>Com/R</b>	964	0.65	0.37	0.28	0.10	0.18	6.5

319 <sup>a</sup> Evaluated at  $p/p^0 = 0.975$  in the  $\text{N}_2$  adsorption isotherms at  $-196^\circ\text{C}$

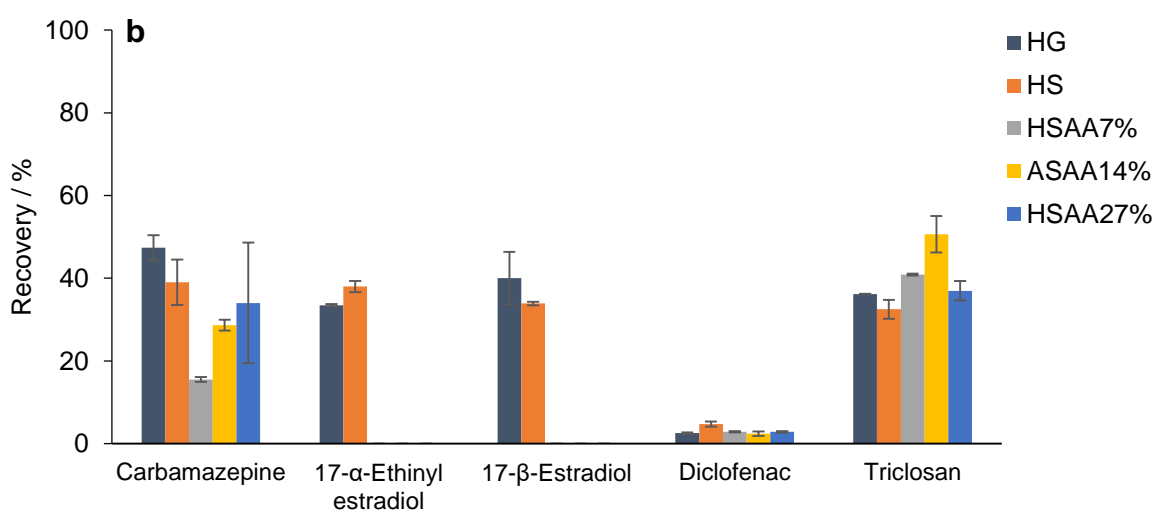
320 <sup>b</sup> Difference between  $V_{\text{total}}$  and  $V_{\alpha \text{ total}}$

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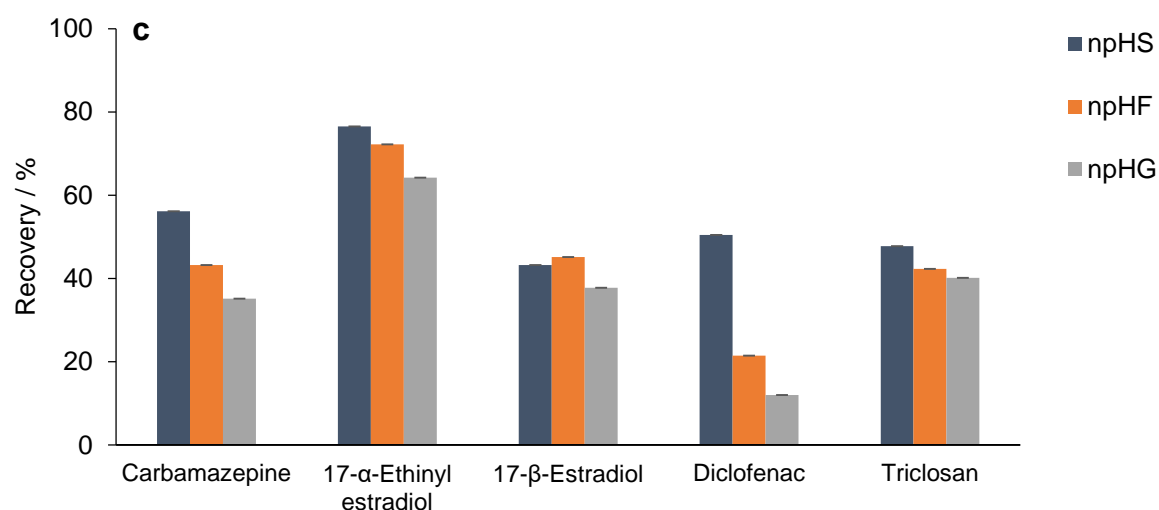
322 From the set of activated carbons tested (samples Com/CN1, Com/R,  
 323 Cork/S800, HS/C800 and HS/H800), the commercial sample Com/CN1 with a  
 324 BET area higher than  $1000 \text{ m}^2/\text{g}$ , a micro+mesopore structure and slightly  
 325 acidic character (*i.e.*,  $\text{pH}_{\text{PZC}}$  of 5.1) outperformed the steam activated cork  
 326 carbon (sample Cork/S800) with lower surface area and basic character, as well  
 327 as the activated sucrose-derived hydrochars (samples HS/H800 and HS/C800)  
 328 with a better-developed micropore structure and acidic nature. The non-porous  
 329 hydrochars (figure 2b) failed to recover diclofenac and, while the samples HG  
 330 and HS were able to recover ca. 40 % of the remaining PhCs, the acrylic acid  
 331 modified hydrochars (HSAAx%) failed the recovery of  $17\alpha$ -ethinylestradiol and  
 332  $17\beta$ -estradiol. On the contrary, the acidic nanoporous hydrochars (figure 2c)  
 333 displaying micro-/mesopore networks and moderate surface areas (Tables 1  
 334 and 2) allowed to recover the five target PhCs. Furthermore, the bar prepared  
 335 using sample npHS as coating allowed to obtain recoveries comparable to the  
 336 best commercial activated carbon for all the PhCs (ranging between 42-78%),  
 337 with the exception of carbamazepine.



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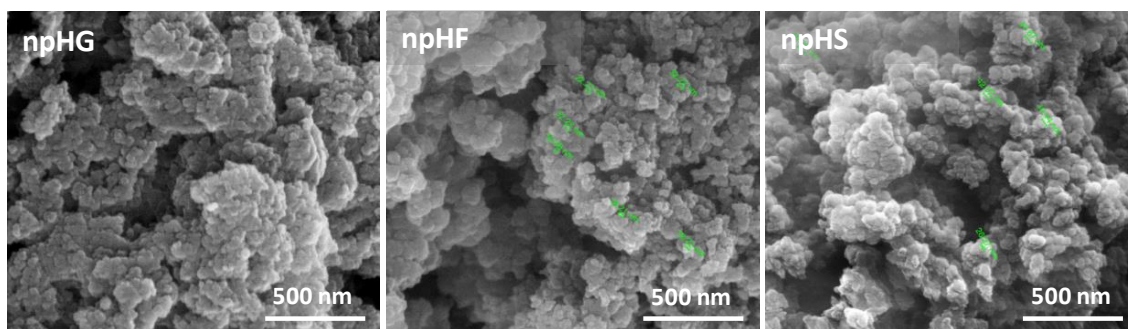
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Figure 2 – Effect of sorbent selectivity on the recovery yields of the five mentioned PhCs using commercial and lab-made activated carbons (a), lab-made hydrochars (b) and lab-made nanoporous hydrochars (c) as sorbent coating obtained by BAμE-μLD/HPLC-DAD. BAμE coating: 1.0-3.0 mg; Spiking level: 16.0 μg L<sup>-1</sup>; Equilibrium: 16 h (1000 rpm) pH 5.5; μLD: ACN (100 μL) 60 min.

347  
348 To gather a deeper understanding of the contribution of the adsorption  
349 and desorption processes in the overall recovery of the lowest performing  
350 materials, three of them were selected for a complementary assay. Samples  
351 HS/H800, HG and HSAA27% were tested for the recovery of the five PhCs  
352 using higher initial concentrations, thereby facilitating the quantification after the  
353 concentration step (figure S3, SI). In the case of sample HS/H800  
354 (superactivated carbon), the low recoveries can be attributed to the irreversible  
355 adsorption of the targets inside the well-developed microporous structure; on  
356 the contrary, for the non-porous hydrochars, most of the molecules remain in  
357 the water matrix, thus the less efficient recovery of polar PhCs seems to be  
358 related with their low affinity towards the carbon coating phase. Under these  
359 experimental conditions, recoveries of 100% were obtained for triclosan -the  
360 most nonpolar target- with these non-porous hydrochars, whereas the recovery  
361 of carbamazepine -the most polar compound of the set- would be hindered by  
362 the irreversible retention on the acidic surface of such carbon coatings.  
363 Interestingly, the relatively high recoveries of herein prepared nanoporous  
364 hydrochars characterized by lower surface areas than commercial activated  
365 carbons (tables 1 and 2) points out to the paramount importance of combining  
366 micro-mesoporosity and appropriate surface chemistry. The porous hydrochars  
367 are composed by agglomerates of interconnected units in the nanometric scale  
368 (20 – 50 nm) that originate a pore system in the mesoscale (figure 3). This  
369 could be important to assure an efficient and fast adsorption and desorption of  
370 the target compounds during the enrichment step.



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Figure 3 – SEM images of the nanoporous hydrochars.

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Figure 4 illustrates the influence of the properties of the above mentioned thirteen carbon materials on their performance as enrichment phases. The four graphics present the correlation between selected carbon properties ( $\text{pH}_{\text{PZC}}$  vs  $A_{\text{BET}}$ ,  $V_{\text{total}}$ , %  $V_{\text{micro}}$  and %  $V_{\text{meso}}$ ) and the percentage of removal attained for the set of five PhCs (carbamazepine,  $17\alpha$ -ethinylestradiol,  $17\beta$ -estradiol, diclofenac and triclosan). The number of occurrences corresponding to removals in the first quartile is systematically higher at low  $\text{pH}_{\text{PZC}}$  values associated with percentages of micropores between 15 % and 50 % and consequently to the presence of a micro+mesopore network.

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Summarising, these set of data points out that the selection of an adequate carbon coating for BA $\mu$ E- $\mu$ LD/HPLC-DAD to assure high recoveries should consider sorbents combining a well-developed pore structure in the full micro to mesopore range, along with acidic surface groups (*i.e.* phenol and carboxylic acid, see DRIFT spectra discussion in Supporting Information).

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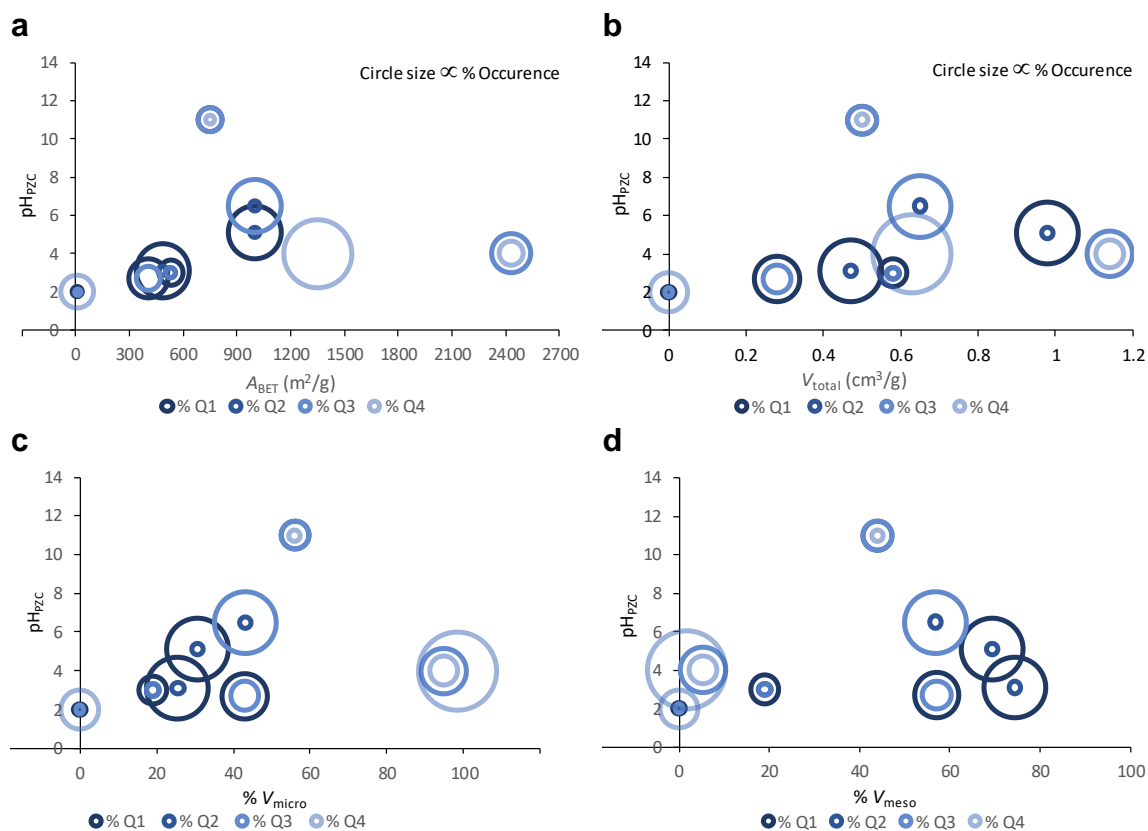
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In light of these preliminary results for the nanoporous hydrochars with the set of five targets, these carbon materials were further tested as enrichment phase for the recovery of the 10 PhCs and benchmarked vs two commercial PACs (figure 5). As seen, under the experimental conditions tested (*ca.* pH 5.5) none of the carbon materials were able to recover clofibric acid, although

392 recoveries up to 50 % were obtained for the other two acidic compounds  
 393 (diclofenac and mefenamic acid).



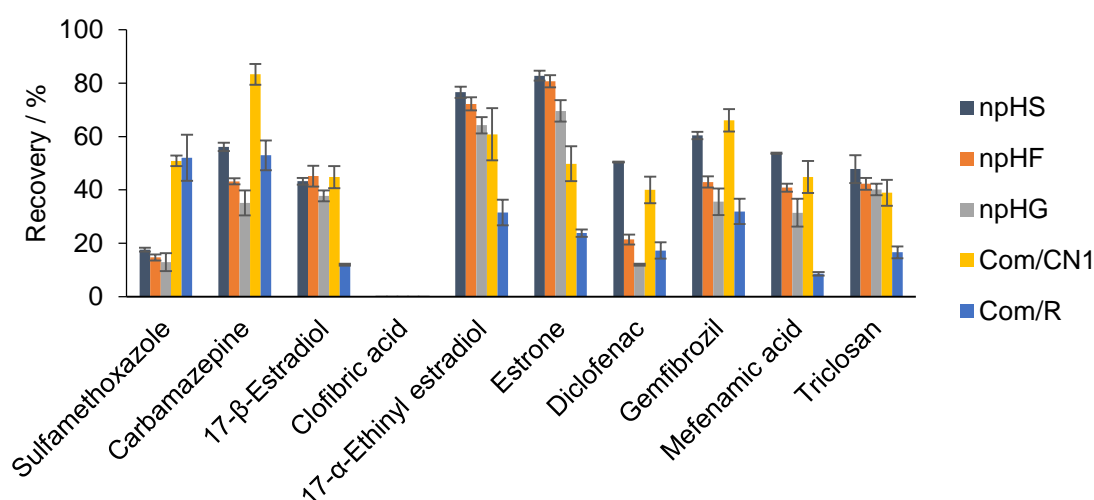
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395 Figure 4 – Influence of carbon materials properties in the removal percentages of five target  
 396 PhCs: pH at the point of zero charge ( $pH_{PZC}$ ) versus BET area (a), total pore volume (b),  
 397 percentage of micropore volume (c), and percentage of mesopore volume (d). Circles represent  
 398 the percentage of removal (in quartiles) adjusted for the removal range of each PhC, Q1  
 399 corresponding to top 25 % removal and Q4 to bottom 25 % removal, and circle sizes are directly  
 400 proportional to the percentage of occurrence.

401

402 Com/R presented the lowest performance for the majority of PhCs, with  
 403 the exceptions were sulfamethoxazole and carbamazepine. Such low  
 404 recoveries may be partially since this carbon was the only material displaying a  
 405 slightly positively charged surface under our experimental conditions (i.e., pH  
 406 solution <  $pH_{PZC}$  6.5). Despite a less developed pore network, the nanoporous  
 407 hydrochars compare favourably with the commercial activated carbons, except  
 408 in the case of sulfamethoxazole and carbamazepine. This might be attributed to  
 409 their negatively charged surface charge (i.e., pH solution >  $pH_{PZC}$  2.7 – 3.1). For

410 the other pharmaceuticals, the recoveries ranged between 40 % (17- $\beta$ -estradiol)  
 411 and 80 % (17- $\alpha$ -ethinyl estradiol and estrone), with the nanoporous  
 412 hydrocarbons prepared from sucrose and fructose competing closely with the  
 413 best performing commercial carbon (sample Com/CN1) for the majority of the  
 414 target compounds. Considering all this, further optimization assays were carried  
 415 out on microextraction bars prepared using samples npHS, npHF and  
 416 Com/CN1 as carbon coatings.



417

418 Figure 5 – Effect of sorbent selectivity on the PhCs recovery yields with different carbon based  
 419 materials as sorbent coating obtained by BA $\mu$ E- $\mu$ LD/HPLC-DAD. BA $\mu$ E coating: 1.0-3.0 mg;  
 420 Spiking level: 16.0  $\mu$ g L<sup>-1</sup>; Equilibrium: 16 h (1000 rpm) pH 5.5;  $\mu$ LD: ACN (100  $\mu$ L) 60 min.  
 421

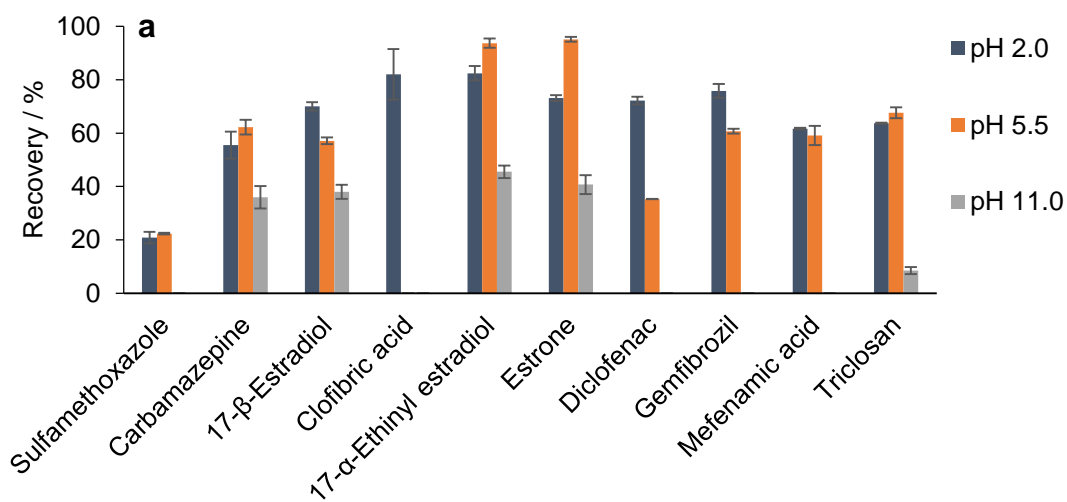
422 After choosing the best performing carbon materials through BA $\mu$ E for the  
 423 determination of the target PhCs from aqueous media, the  $\mu$ LD parameters, as  
 424 well as the equilibrium time, stirring rate, ionic strength, matrix polarity, and  
 425 sample pH were also evaluated. All the results the optimization, with exception  
 426 of the latter which will be discussed in the following, can be consulted in SI.

427 The chemical characteristics of the water matrix were also assessed, in  
 428 particular pH, ionic strength and polarity. Figure 6 shows the recovery yields

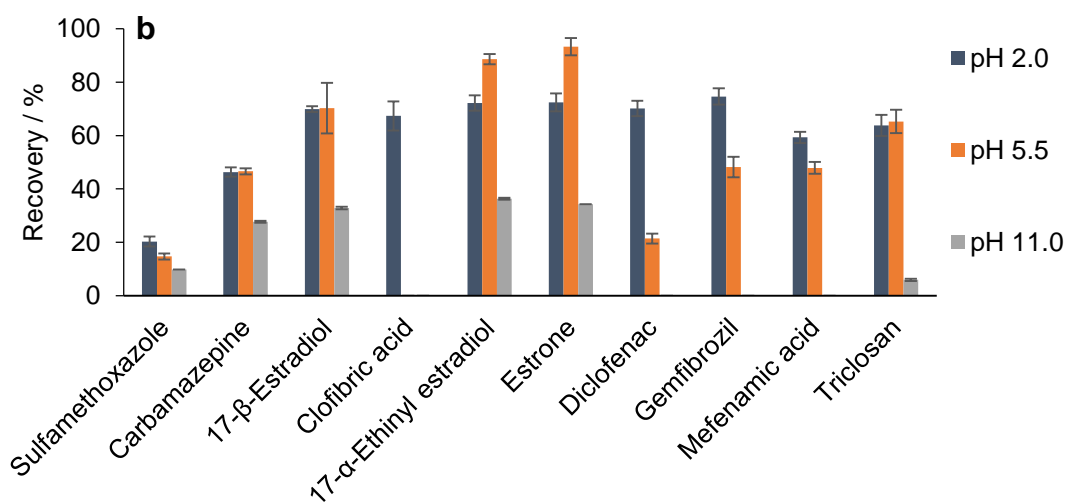
429 obtained for the BA $\mu$ E devices at different solution pH between 2 and 10 for the  
430 three selected carbon coatings. With the exception of carbamazepine recovery  
431 with sample CN1, the solution pH strongly influenced the recovery of all the  
432 target analytes. This was somewhat expected since it is well known that this  
433 parameter affects the ionic or neutral forms of the PhCs in solution [11], as well  
434 as the surface charge of the carbon materials. Under strong acidic pH, clofibric  
435 acid -analyte with the lowest  $pK_a$  value- was recovered with yields between 70  
436 to 90 % for all tested sorbent phases. This points out that the determination of  
437 clofibric acid by BA $\mu$ E using carbon coatings as enrichment phases, is only  
438 possible when this semipolar acidic compound is in the protonated form. Our  
439 previous results using cork-derived activated carbons as enrichment materials  
440 also identified the critical role of pH in the quantification of clofibric acid, with the  
441 best results attained at acidic pH [34].

442 The recovery yields increased at pH 2.0 for all compounds and carbon  
443 coatings used, since all the carbon materials display a slightly positively  
444 charged surface under these conditions (solution pH <  $pH_{PZC}$ ). These results  
445 can be rationalized considering the log D values (that corrects the log  $K_{o/w}$  for  
446 the  $pK_a$  of each compound by quantifying the amount of both the ionized and  
447 non-ionized forms in octanol and water, Scheme S1, SI) of the PhCs at the pH  
448 values under study.

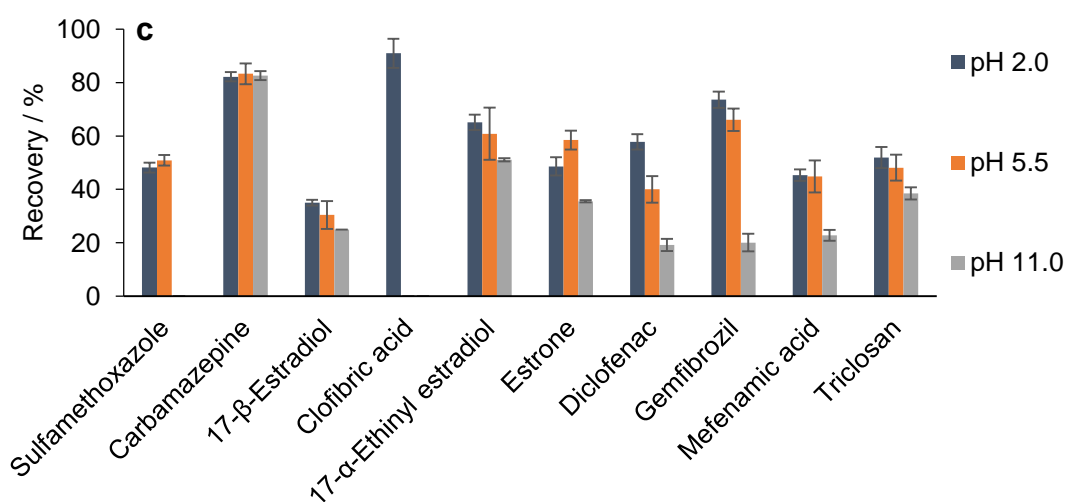
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453 Figure 6 – Effect of matrix pH on the PhCs recovery yields using npHS (a), npHF (b) and  
 454 Com/CN1 (c) as sorbent coating obtained by BA $\mu$ E- $\mu$ LD/HPLC-DAD. BA $\mu$ E coating: 1.0 – 3.0  
 455 mg; Spiking level: 8.0  $\mu$ g L<sup>-1</sup>; Equilibrium: 16 h (1000 rpm);  $\mu$ LD: ACN (100  $\mu$ L) 60 min.



456 Table S1 (SI) showed that regardless the solution pH there is an overall  
457 increase in the recovery yields with the increase in the log D values. In general,  
458 at solution pH 2.0, the selected PhCs present higher octanol solubility (favoring  
459 both adsorption and desorption), thus contributing to higher recovery yields.  
460 When the solution pH is higher than or similar to the  $pK_a$  of a given PhCs, its  
461 recovery gets close to zero, justifying the greater reduction in the recovery  
462 yields in compounds with lower  $pK_a$  values. It can so be concluded that the  
463 lower recoveries are related with the deprotonation of the target compounds,  
464 thus with the higher stability in the water matrix and consequently lower affinity  
465 towards the porous solids.

466

### 467 **3.2 Validation of the BA $\mu$ E(HC-S)- $\mu$ LD/HPLC-DAD methodology**

468 By using the optimized experimental conditions for each carbon-based  
469 sorbents (Com/CN1, npHF and npHS), it was possible to attain average  
470 recoveries between 20.3 and 90.9 % for all target compounds in ultra-pure  
471 water spiked at the 16.0  $\mu\text{g L}^{-1}$  level. Comparatively, better results were  
472 obtained using npHS as carbon coating with most of the PhCs, thus the  
473 validation assays of the BA $\mu$ E(npHS)- $\mu$ LD/HPLC-DAD methodology were  
474 carried out using this sorbent phase (table S1, SI).

475 The sensitivity of the methodology was checked through the LODs (0.5 –  
476 1.5  $\mu\text{g L}^{-1}$ ) and LOQs (1.5 – 5.0  $\mu\text{g L}^{-1}$ ), calculated with signal to noise ratios  
477 S/N of 3/1 and 10/1, respectively. The methodology was also evaluated through  
478 intraday and interday repeatability assays, calculating the corresponding  
479 relative standard deviations (RSD) [35]. Interday repeatability assays were

480 carried out as six replicates a day in three consecutive days and intraday  
481 repeatability assays consisted in six replicates performed in the same day  
482 (using three spiking levels). Good precisions were achieved for the intraday  
483 repeatability assays, with  $RSD \leq 8.4 \%$  (diclofenac), and for interday  
484 repeatability, was  $\leq 12.3 \%$  (mefenamic acid), under optimized experimental  
485 conditions. Assays were also performed in ultra-pure water having  
486 concentrations ranging from 5.0 to 104.0  $\mu\text{g L}^{-1}$ , where convenient linearity was  
487 obtained, with  $r^2$  higher than 0.99 for all the target PhCs under study. Table S2  
488 (SI) summarizes the LODs, LOQs, as well as intraday and interday precision  
489 levels achieved by the proposed methodology, under optimized experimental  
490 conditions.

491

### 492 **3.3 Performance comparison with other microextraction techniques**

493 A comparison of the performance of the BA $\mu$ E(npHS)- $\mu$ LD/HPLC-DAD  
494 methodology and other established static-based microextraction techniques is  
495 shown in table 3 [36–44]. As it can be observed, the developed methodology  
496 using BA $\mu$ E devices coated with sucrose-based hydrochar presents similar or  
497 better recovery yields than other miniaturized enrichment techniques, e.g.,  
498 SBSE coated with PDMS, PA or EG [38] or SBME coated with C8 polymer [39]  
499 for the extraction of carbamazepine; TFME coated with PDMS [44] for the  
500 extraction of 17- $\beta$ -estradiol; SBSE coated with PDMS [42] for the extraction of  
501 clofibric acid; SBSE coated with PU [45] for the extraction of diclofenac,  
502 gemfibrozil or mefenamic acid. Additionally, the BA $\mu$ E(npHS)- $\mu$ LD/HPLC-DAD  
503 methodology sensitivity is similar when compared to analogue instrumental  
504 systems [37,39,45] and/or some mass spectrometry or tandem mass

505 spectrometry instrumental systems [40,44]. On the other hand, instrumental  
506 apparatus that employ mass spectrometry or tandem mass spectrometry  
507 expectedly presented lower LODs than the proposed technique [38,41,44,46].

508

509 Table 3 – Comparison of the LODs, average recovery yields in this work and other static-based microextraction techniques for the determination of the studied  
 510 PhCs in aqueous samples.

PPCP	Static based microextraction techniques	Instrumental system	Recovery (%)	LOD ( $\mu\text{g L}^{-1}$ )	Refs.
Sulfamethoxazole	SPME(CW/TPR)	LC-(ESI)MS/MS	59.2	14.0	[36]
	MEPS(C8)	HPLC-DAD	n.a.	LOQ: 5.0	[37]
	BA $\mu$ E(npHS)		20.9	1.5	This work
Carbamazepine	SBSE(PDMS, PA or EG)	LC-(ESI)MS/MS	< 1.0	n/a	[38]
	TFME(PDMS)	GC-MS	67.1	0.72	[44]
	SBME(C8)	HPLC-UV	35.0-42.0	0.7	[39]
	BA $\mu$ E(npHS)	HPLC-DAD	55.5	0.5	This work
17- $\beta$ -Estradiol	SBSE(PDMS)	LDTD/APCI-MS/MS	~ 40.0	2.8	[40]
	TFME(PDMS)	GC-MS	85.0	0.41	[44]
	SPME(PA)		92.0-101.0	0.15	[41]
	SBSE(PDMS)		7.0	0.853	[46]
	BA $\mu$ E(npHS)	HPLC-DAD	70.0	2.0	This work
Clofibric acid	SPME(PA)	GC-MS	95.0-98.0	0.75	[41]
	SBSE(PDMS)		18.5	0.222	[46]

	BA $\mu$ E(AC)	HPLC-DAD	79.7-96.1	0.21-0.28	[34]
	BA $\mu$ E(npHS)		82.0	2.5	This work
17- $\alpha$ - Ethinylestradiol	SBSE(PDMS)	LDTD/APCI-MS/MS	~ 30.0	2.6	[40]
	SPME(PA)	GC-MS	93.0-105.0	0.15	[41]
	SBSE(PDMS)		9.0	0.508	[46]
	BA $\mu$ E(npHS)		HPLC-DAD	82.4	2.0
Estrone	SBSE(PDMS)	LDTD/APCI-MS/MS	~ 50.0	2.2	[40]
	SPME(PA)	GC-MS	92.0-99.0	0.15	[41]
	SBSE(PDMS)		12.7	0.789	[46]
	BA $\mu$ E(npHS)		HPLC-DAD	73.1	2.0
Diclofenac	SBSE(PDMS, PA or EG)	LC-(ESI)MS/MS	< 1.0	n/a	[38]
	SBSE(PDMS)	GC-MS	21.0	0.037	[46]
	SBSE(PDMS)	HPLC-DAD	34.6	1.6	[45]
	SBSE(PU)		77.7	0.7	
	SBME(C8)		47.0-52.0	0.9	[39]
	BA $\mu$ E(npHS)		72.2	0.5	This work
Gemfibrozil	SBSE(PDMS)	GC-MS	15.5	0.013	[46]
	SBSE(PDMS)	HPLC-DAD	73.4	1.7	[45]
	SBSE(PU)		84.0	0.7	

	BA $\mu$ E(HC-S)		75.8	1.0	This work
Mefenamic acid	SBSE(PDMS)	GC-MS)	29.0	0.001	[46]
	SBSE(PDMS)		71.3	1.5	[45]
	SBSE(PU)	HPLC-DAD	48.4	1.3	
	BA $\mu$ E(npHS)		61.7	0.5	This work
Triclosan	SBSE(PDMS, PA or EG)	LC-(ESI)MS/MS	40.0 - 80.0	0.005 – 0.010	[38]
	SPME(PA)		76.0-88.0	0.15	[41]
	SBSE(PDMS)	GC-MS	23.8	0.0002	[46]
	BA $\mu$ E(npHS)	HPLC-DAD	63.7	1.0	This work

511 CW/TPR: Carbowax-templated resin; LC-(ESI)MS/MS: Liquid chromatography coupled to tandem mass spectrometry with electrospray ionization;  
512 LDTD/APCI-MS/MS: Laser diode thermal desorption atmospheric pressure chemical ionization tandem mass spectrometry; MEPS: Microextraction by packed  
513 sorbent; PA: Polyacrylate; PDMS: Polydimethylsiloxane; PEG: Polyethylene glycol; PU: Polyurethane; SBME: Solid bar microextraction; TFME: Thin-film  
514 microextraction;

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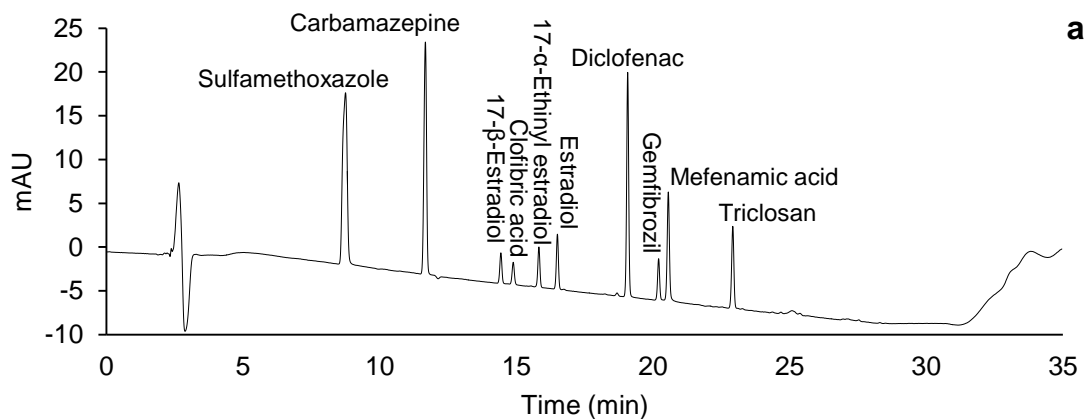
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517 **3.4 Application to environmental water matrices**

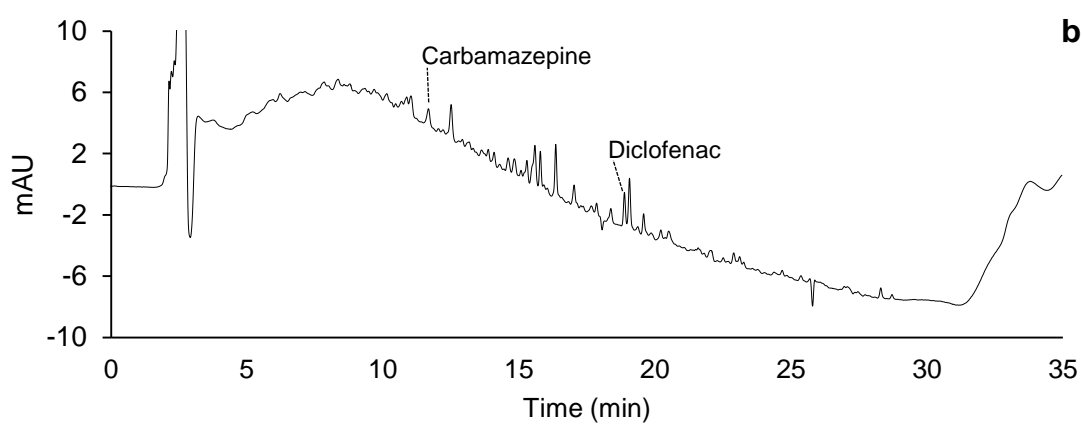
518 To evaluate the applicability of BA $\mu$ E(npHS)- $\mu$ LD/HPLC-DAD methodology  
519 to real matrices, assays were performed in sea, lake, estuarine, tap and WWTP  
520 water samples, through the SAM. This approach is usually the best strategy for  
521 quantification purposes to determine the levels of analytes under study and to  
522 reduce possible matrix interferences in real samples [11,12,47]. In a first step,  
523 the water matrices were spiked with four working standards to produce the  
524 corresponding spiking levels (8.0  $\mu\text{g L}^{-1}$  to 104.0  $\mu\text{g L}^{-1}$ ) for the ten PhCs under  
525 study. "Zero-point" assays were also performed without spiking to ensure  
526 maximum control of the analytical methodology. Good linear correlations were  
527 achieved, with  $r^2$  higher than 0.99. The chromatograms of neat standard mix at  
528 the 8.0  $\mu\text{g L}^{-1}$  level for the ten PhCs (figure 7a) and WWTP sample without  
529 spiking obtained by BA $\mu$ E(npHS)- $\mu$ LD/HPLC-DAD, under optimized  
530 experimental conditions (figure 7b), exemplify the results obtained proving high  
531 sensitivity at the trace level. The proposed methodology allowed to quantify two  
532 of the analytes under study in the WWTP sample (carbamazepine:  $4.01 \pm 0.46$   
533  $\mu\text{g L}^{-1}$ ; diclofenac:  $1.99 \pm 0.21 \mu\text{g L}^{-1}$ ).

534

535



536



537

538 Figure 7 – Chromatograms obtained from assays performed on an ultrapure water sample  
 539 spiked at the  $16.0 \mu\text{g L}^{-1}$  level (a) and a non-spiked WWTP sample (b), performed and by  
 540 BA $\mu$ E(npHS)- $\mu$ LD/HPLC-DAD, under optimized experimental conditions.

541

#### 542 4. Concluding remarks

543 Thirteen carbon sorbents prepared from different precursors and  
 544 methodologies – commercial activated carbons, hydrochars and nanoporous  
 545 hydrochars, and superactivated carbons – were tested as sorbent coatings for  
 546 bar adsorptive microextraction followed by microliquid desorption and high  
 547 performance liquid chromatography-diode array detection (BA $\mu$ E(npHS)-  
 548  $\mu$ LD/HPLC-DAD methodology) for the simultaneous determination of traces of



549 ten PhCs from distinct therapeutic classes in synthetic and environmental water  
550 matrices.

551 The lab-made nanoporous hydrochars proved to compete with commercial  
552 activated carbon adsorbents for the enrichment of the PhCs, due to a  
553 hierarchical pore structure in the full micro-mesopore range combined with a  
554 rich surface chemistry composed of acid O-groups that favour both the  
555 adsorption and desorption of the target compounds, thus contributing to a high  
556 performance at the trace level. The method also demonstrated to be an  
557 environmentally friendly approach, easy to implement, sensitive, robust and  
558 requiring low sample volume. In short, the proposed analytical methodology  
559 seems to be a very effective microextraction-based alternative to fulfill the EU  
560 Decision 2015/495, as well as the USEPA guidelines for PhCs monitoring,  
561 particularly if combined with tandem mass spectrometry systems, which may  
562 provide even better detection limits.

563

#### 564 **Conflicts of Interest**

565 The authors declare no conflict of interest.

566

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576

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