

1 **Zeolite/iron oxide composite as sorbent for magnetic solid-phase**
2 **extraction of benzene, toluene, ethylbenzene and xylenes from water**
3 **samples prior to gas chromatography-mass spectrometry**

4
5 Elena Fernández, Lorena Vidal* and Antonio Canals*

6 Departamento de Química Analítica, Nutrición y Bromatología e Instituto Universitario de
7 Materiales, Universidad de Alicante, P.O. Box 99, E-03080 Alicante, Spain.

8 *Corresponding authors: Tel.: +34965909790; fax: +34965903697.

9 E-mail addresses: lorena.vidal@ua.es (L. Vidal), a.canals@ua.es (A. Canals).

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12 **Abstract**

13 This study reports a new composite based on ZSM-5 zeolite decorated with
14 iron oxide magnetic nanoparticles as a valuable sorbent for magnetic solid-
15 phase extraction (MSPE). A proposal is made to determine benzene, toluene,
16 ethylbenzene and xylenes (BTEX) as model analytes in water samples using
17 gas chromatography-mass spectrometry. A two-step multivariate optimization
18 strategy, using Plackett–Burman and circumscribed central composite designs,
19 was employed to optimize experimental parameters affecting MSPE. The
20 method was evaluated under optimized extraction conditions (i.e., amount of
21 sorbent, 138 mg; extraction time, 11 min; sample pH, pH of water (i.e., 5.5-6.5);
22 eluent solvent volume, 0.5 mL; and elution time, 5 min), obtaining a linear
23 response from 1 to 100 $\mu\text{g L}^{-1}$ for benzene; from 10 to 100 $\mu\text{g L}^{-1}$ for toluene,
24 ethylbenzene and o-xylene; and from 10 to 75 $\mu\text{g L}^{-1}$ for m,p-xylene. The
25 repeatability of the proposed method was evaluated at a 40 $\mu\text{g L}^{-1}$ spiking level
26 and coefficients of variation ranged between 8 and 11% (n=5). Limits of

27 detection were found to be $0.3 \mu\text{g L}^{-1}$ for benzene and $3 \mu\text{g L}^{-1}$ for the other
28 analytes. These values satisfy the current normative of the Environmental
29 Protection Agency and European Union for BTEX content in waters for human
30 consumption. Finally, drinking water, wastewater and river water were selected
31 as real water samples to assess the applicability of the method. Relative
32 recoveries varied between 85% and 114% showing negligible matrix effects.

33

34 **Keywords:** ZSM-5 zeolite, magnetic solid-phase extraction, gas
35 chromatography-mass spectrometry, BTEX, water samples.

36

37 **1. Introduction**

38 Zeolites are porous crystalline aluminosilicates formed by SiO_4 and AlO_4
39 tetrahedra interconnected by corner oxygen atoms. Briefly, zeolites can be
40 considered as pure silica frameworks with Si^{4+} substituted by Al^{3+} . The
41 presence of each AlO_4 tetrahedron generates an overall negative charge, which
42 is compensated by extra-framework cations (e.g., Na^+ , K^+ , Ca^{2+}) placed within
43 the pores and channels [1].

44 Zeolites exist as natural minerals (e.g., mordenite, clinoptilolite) and in
45 synthetic forms (e.g., Y, ZSM-5, beta), and both types of zeolites have been
46 used in industrial and commercial applications [2]. Nowadays, almost 200
47 different structures are known and accepted by the Structure Commission of the
48 International Zeolite Association [3]. Both morphology (i.e., pores and channels
49 size and shape) and chemical composition confer different properties to
50 zeolites, which may be considered for specific applications. In general,
51 frameworks with a high Si/Al ratio possess greater thermal stability, higher

52 hydrophobicity and acidity, and lower ion-exchange capacity [1]. The fact that
53 zeolites can be designed with different morphologies, chemical composition and
54 properties has enabled their extensive application to different fields, mainly: (i)
55 catalysis (e.g., cracking processes in petrochemical industry [4]); (ii) adsorption
56 (e.g., purification of gas streams in petrochemical industry [4] or the removal of
57 organic compounds from contaminated waters [5]); and (iii) ion-exchange (e.g.,
58 water softening in detergents or heavy metal removal from contaminated waters
59 [1,5]). Additionally, the ever growing interest in the synthesis of new zeolites has
60 promoted innovative applications such as solar cell technology,
61 microelectronics, medicine and holographic sensors [6,7].

62 Solid-phase extraction (SPE) is one of the most widely used sample
63 preparation techniques providing analyte isolation, preconcentration and sample
64 clean-up [8]. Typically, SPE consists of cartridges packed with sorbent where
65 the analyte is retained when liquid samples flow through it. Then, a proper
66 solvent is employed to elute and recover the analyte for further determination
67 [8]. Several sorbents with different properties have been employed in SPE,
68 including inorganic oxides, porous polymers, molecularly imprinted polymers,
69 biosorbents or nanomaterials [8–10]. In addition, some publications have
70 reported the use of zeolites for the preconcentration of inorganic [11–13] and
71 organic [14,15] analytes. The original SPE has been modified several times to
72 date, mainly related to miniaturization or automation [16]. Zeolites have also
73 been proposed as sorbents in new SPE protocols [17–21]. Recently, magnetic
74 solid-phase extraction (MSPE) has received great interest since it reduces
75 sample preparation time and facilitates sorbent manipulation [16,22]. In MSPE,
76 the magnetic sorbent is dispersed into the aqueous phase and after extraction,

77 it is easily separated from the sample solution by applying an external magnetic
78 field, and thus avoids time-consuming filtration or centrifugation steps for phase
79 separation. Next, target analytes can be desorbed using a proper eluent solvent
80 or temperature for further determination [16,22]. A recent publication has
81 reported the use of zeolite/Fe₃O₄ composite as new sorbent for MSPE for the
82 first time [23]. In this work, phthalates are determined at trace levels in aqueous
83 samples after extraction with clinoptilolite zeolite loaded on Fe₃O₄ nanoparticles
84 [23].

85 Benzene, toluene, ethylbenzene and xylene (BTEX) are compounds of
86 public health concern due to their toxic and carcinogenic properties [24]. BTEX
87 are extensively used in many industrial processes and benzene, toluene and
88 ethylbenzene are considered priority pollutants by the Environmental Protection
89 Agency (EPA) [24]. Therefore, their presence in the environment must be
90 controlled. Numerous methods including SPE techniques have been proposed
91 for BTEX determination in water samples [25–30].

92 The purpose of our work is to develop an analytical method in which a
93 composite based on ZSM-5 zeolite and iron oxide magnetic nanoparticles is
94 presented as a valuable new sorbent for MSPE. BTEX determination in water
95 samples has been proposed as a model analytical application using gas
96 chromatography-mass spectrometry (GC-MS) for analysis. To the best of our
97 knowledge, this is the first time that ZSM-5/iron oxide magnetic composite has
98 been employed for BTEX extraction and preconcentration. Various parameters
99 of the proposed MSPE have been optimized by the use of a multivariate
100 optimization strategy. Finally, the applicability of the proposed method to
101 determine BTEX at trace levels in real water samples has been evaluated.

102 **2. Experimental part**

103 **2.1. Reagents and water samples**

104 Benzene, ethylbenzene and xylene isomers of analytical standard grade
105 were obtained from Fluka (Steinheim, Germany), whereas toluene LC grade (\geq
106 99.9%) was bought from Scharlau Chemie (Barcelona, Spain). Mixed stock
107 standard solution of BTEX was prepared at a concentration of $1000 \mu\text{g L}^{-1}$ in
108 acetone LC grade from Sigma-Aldrich (Steinheim, Germany) and stored in the
109 dark at $4 \text{ }^\circ\text{C}$. Working solutions ($1\text{-}100 \mu\text{g L}^{-1}$) were prepared by diluting stock
110 solution with acetone.. Aqueous working solutions were daily prepared in
111 ultrapure water (resistivity of $18.2 \text{ M}\Omega \text{ cm}$ at 25°C) from a Millipore Direct
112 System Q5TM purification system from Ibérica S.A. (Madrid, Spain). H_3PO_4
113 (85%) from Scharlau Chemie and KH_2PO_4 and K_2HPO_4 pro-analysis from
114 Merck (Darmstadt, Germany) were employed for preparing buffer solutions
115 during sample pH optimization. $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ and $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ reactive grade
116 were obtained from Sigma-Aldrich and NaOH (97%, pellets) from Scharlau
117 Chemie.

118 ZSM-5 zeolite (CBV 3024E, $\text{SiO}_2/\text{Al}_2\text{O}_3$ mole ratio = 30) in the ammonium
119 nominal cation form was purchased from Zeolist International (Conshohocken,
120 PA, USA).

121 As real water samples, we used drinking water from a drinking-water
122 treatment plant in Albacete (Spain), industrial wastewater from Zaragoza
123 (Spain) and river water from Murcia (Spain). Samples were collected in amber
124 glass containers and stored in the dark at $4 \text{ }^\circ\text{C}$. Drinking and river waters were
125 used without any further pretreatment but wastewater was filtered with a 0.45
126 μm pore-size nylon filter before use. Water samples were analyzed under

127 optimized conditions of the proposed method, and target analytes were not
128 detected.

129

130 **2.2. Instrumentation**

131 X-ray photoelectron spectroscopy (XPS) was used for the chemical
132 characterization of iron oxide in the ZSM-5/iron oxide composite. XPS
133 measurements were performed with an automatic K-Alpha spectrometer from
134 Thermo-Scientific (Waltham, MA, USA). The spectra were recorded with pass
135 energy of 50 eV, x-ray spot size of 400 μm , and step size of 0.1 eV.

136 Surface area (BET) and pore volume (t-plot method) of ZSM-5 zeolite
137 and ZSM-5/iron oxide composite were measured by nitrogen adsorption at 77 K
138 using an Autosorb-6 analyzer from Quantachrome Instruments (Hook, UK).

139 Two chromatographic systems were employed for MSPE optimization,
140 and method validation and real samples analysis, respectively. For MSPE
141 optimization, chromatographic analysis were performed on a gas
142 chromatograph (model 7890A) from Agilent Technologies (Santa Clara, CA,
143 USA) equipped with a splitless/split automatic injector and a flame ionization
144 detector. A capillary column HP-5 (5% phenyl-95% dimethylpolysiloxane, 30 m
145 \times 0.32 mm I.D., 0.25 μm film thickness) was from J&W Scientific (Folsom, CA,
146 USA). The injector temperature was maintained at 200 $^{\circ}\text{C}$ and the injection
147 volume was 1.0 μL in the split mode (split ratio 1:10). The oven temperature
148 program was initially set at 40 $^{\circ}\text{C}$ and was raised by 6 $^{\circ}\text{C min}^{-1}$ up to 100 $^{\circ}\text{C}$
149 (held 5 min). Helium (99.999%) from Air Liquide (Madrid, Spain) was used as
150 the carrier gas at a constant flow rate of 1 mL min^{-1} . The detector temperature
151 was set at 300 $^{\circ}\text{C}$.

152 Method validation and real samples analysis were carried out using GC-
153 MS. The gas chromatograph (model 6890N) was from Agilent Technologies and
154 the capillary column TRB-624 (6% cyanopropylphenyl-94%
155 dimethylpolysiloxane, 30 m × 0.25 mm I.D., 1.4 μm film thickness) was from
156 Teknokroma (Barcelona, Spain). Samples (2 μL) were injected in the split mode
157 (split ratio 1:5) with the injector maintained at 250 °C. The injection liner (19251-
158 60540 straight split) was from Agilent Technologies. The oven temperature was
159 initially set at 35 °C for 5 min, followed by a ramp of 5 °C min⁻¹ up to 150 °C.
160 Helium (99.999%) was used as the carrier gas at a constant flow rate of 1 mL
161 min⁻¹. The MS detector (model 5973N) was from Agilent Technologies. Electron
162 impact ionization was used with ionization energy of 70 eV. The mass source
163 and quadrupole were set at 250 and 150 °C, respectively. Measurements were
164 taken with a solvent delay of 7 min and in selected ion monitoring (SIM) mode
165 at the following mass/charge ratios: 77 and 78 from minute 7 to 11 for benzene
166 determination; 91 and 92 from minute 11 to 16 for toluene; and 91 and 106 from
167 minute 16 to 28 for ethylbenzene and xylenes determination. Figure 1 shows
168 typical chromatograms after MSPE of a blank and a standard solution spiked at
169 100 μg L⁻¹ with target analytes. It should be mentioned that the
170 chromatographic separation of m-xylene and p-xylene isomers was not
171 performed under the above conditions.

172

173 **2.3. Synthesis of ZSM-5/iron oxide composite**

174 The composite was synthesized considering a ZSM-5/iron oxide ratio 3/1
175 (w:w), being a compromise value between iron oxide load (proven to decrease
176 the available surface area and micropore volume [31]) and the easy

177 manipulation of the composite under a magnetic field. Thereby, the composite
178 was prepared from a suspension of 1 g of ZSM-5 zeolite in 250 mL of water
179 solution with $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.778 g) and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.400 g). After
180 homogeneous mixing, 5 mL of NaOH 5 M was added drop wise to precipitate
181 the iron oxide. The mixture was then stirred for 2 h at room temperature. The
182 resulting composite was cleaned with ultrapure water until clear washing waters
183 were obtained, using a neodymium magnet to separate magnetic from non-
184 magnetic material. Finally, the composite was dried at 100 °C overnight. Figure
185 S1 (Supplementary material) shows a photograph of the magnetic attraction
186 between the synthesized composite and the magnet. Before MSPE, the
187 composite was kept at 200 °C for 2 h in order to remove the excess of
188 physisorbed water and other residual compounds.

189

190 **2.4. Magnetic solid-phase extraction**

191 Firstly, 138 mg of the magnetic composite were placed in a 22 mL glass
192 vial. Then, 20 mL of aqueous standard or sample solution were added and the
193 mixture was shaken vigorously for 11 min. After extraction, the neodymium
194 magnet was used to attract the composite to the bottom of the vial and, thus,
195 the water phase was easily removed using a glass pipette. Then, elution was
196 carried out using 0.5 mL of acetone and shaking gently for 5 min in order to
197 prevent sorbent sticking in the vial walls. Acetone was chosen as eluent since
198 previous results (data not shown) revealed it performed better than other
199 organic solvents (e.g., acetonitrile, methanol). Finally, the organic phase was
200 separated from the magnetic composite using the neodymium magnet, retrieved
201 with a syringe and filtered with 0.45 μm pore-size nylon filters for injection in

202 GC-FID (for MSPE optimization studies) or GC-MS (for method validation and
203 real sample analysis).

204

205 **2.5. Data processing**

206 A two-step multivariate optimization strategy, using Plackett-Burman and
207 circumscribed central composite designs, was carried out to determine the
208 optimum conditions for MSPE. The statistical software NEMRODW[®] ("New
209 Efficient Methodology for Research using Optimal Design") from LPRAI
210 (Marseille, France) was used to build the experimental design matrices and
211 evaluate the results. Peak areas of benzene, toluene and ethylbenzene
212 obtained with GC-FID were individually used as response functions for
213 optimization. It should be mentioned that xylenes could not be included in
214 optimization studies since the lower sensitivity of the detector used in this step
215 (i.e., GC-FID) did not allow their quantification.

216

217

218 **3. Results and discussion**

219 **3.1. Characterization of ZSM-5/iron oxide composite**

220 Figure 2 shows a high resolution XPS spectrum in the Fe 2p region.
221 Comparing the spectrum obtained with database spectra [32], it can be
222 concluded that iron is in its highest oxidation state (Fe^{3+}) forming Fe_2O_3 . The
223 results suggest that, although Fe^{2+} and Fe^{3+} salts (i.e., $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and
224 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, respectively) were employed to synthesize iron oxide
225 nanoparticles, Fe^{2+} was oxidized to Fe^{3+} during composite preparation and
226 Fe_2O_3 was finally obtained instead of the mixed oxide (i.e., Fe_3O_4) [31].

227 According to bibliography [33], both van der Waals and electrical forces are
228 involved in the interaction between zeolites and Fe_2O_3 particles obtained by
229 chemical precipitation.

230 For pure ZSM-5 zeolite and ZSM-5/iron oxide composite, BET surface
231 areas of $442 \text{ m}^2 \text{ g}^{-1}$ ($V_{\text{micropores}} = 0.161 \text{ cm}^3 \text{ g}^{-1}$) and $292 \text{ m}^2 \text{ g}^{-1}$ ($V_{\text{micropores}} = 0.073$
232 $\text{cm}^3 \text{ g}^{-1}$), were respectively obtained from nitrogen adsorption isotherms (Fig.
233 S2) and BET theory. It is interesting to note the decrease in BET surface area
234 and micropore volume in the magnetic composite, due to the presence of iron
235 oxide nanoparticles [31]. Additionally, Figure S2 shows that adsorption at high
236 pressures was greater for ZSM-5/iron oxide composite which could be related
237 with the creation of mesopores on zeolite surface due to poorly dispersed iron
238 oxide nanoparticles. Despite the decrease in surface area of ZSM-5/iron oxide
239 compared with pure zeolite, the use of the magnetic composite as sorbent
240 provides unique advantages due to its easy manipulation and shorter extraction
241 times. In addition, the proposed method provides limits of detection and
242 quantification low enough to satisfy current normative [34,35] (see Section 3.4.).

243 Finally, it is important to point out that extractions using Fe_2O_3 as
244 extractant phase (without zeolite) were carried out and analytes were not
245 detected in the eluates, showing that Fe_2O_3 did not possess a sorption capacity
246 for target analytes.

247

248 **3.2. Multivariate optimization**

249 **3.2.1. Screening**

250 Screening experiments initially include many factors in order to identify
251 which ones have important effects [36]. Fractional factorial designs are the most

252 widely used in screening experiments. Plackett-Burman is a two-level fractional
253 factorial design for studying up to $k=N-1$ variables in N runs, where N is a
254 multiple of 4 [36]. Plackett-Burman design assumes that interaction between
255 factors can be ignored so the main effects can be calculated with a reduced
256 number of experiments, thereby saving time and resources. A Plackett-Burman
257 design was used to construct the matrix of experiments, including five factors
258 studied in eight runs. The five experimental factors selected at two levels were:
259 amount of sorbent, sample pH, extraction time, eluent solvent volume and
260 elution time. Table 1 shows the experimental factors and levels considered in
261 the Plackett–Burman design whereas Table S1 shows the matrix of
262 experiments. The eight experiments were randomly performed using 20 mL of
263 aqueous standards with 20 mg L^{-1} of benzene, toluene and ethylbenzene due to
264 preliminary investigations (about the extraction) at higher concentrations than
265 20 mg L^{-1} revealed that sorbent capacity was not saturated. The analysis was
266 carried out using GC-FID.

267 The data obtained were evaluated by ANOVA and the results were
268 visualized with the Pareto charts shown in Fig. S3. The length of each bar was
269 proportional to the influence of the corresponding factor while the effects that
270 exceed reference vertical lines can be considered significant with 95% of
271 probability. In addition, negative and positive signs reveal whether the system
272 response decreases or increases, respectively, when passing from the lowest to
273 the highest level of the corresponding factor.

274 As shown in Fig. S3, the amount of sorbent, extraction time and eluent
275 solvent volume were the most important factors, having the same sign for the
276 three studied analytes and, therefore, showing analogous behaviors during

277 extraction. Although these factors were not significant in all cases, they were
278 shown to have the greatest effects and were selected for the next optimization
279 step. The amount of sorbent and extraction time, both with positive effects, were
280 studied in the circumscribed central composite design (CCCD) whereas finally
281 eluent solvent volume could not be considered. The eluent solvent volume
282 showed a negative effect, which is easily explained considering that when one
283 employs a smaller volume of eluent solvent one obtains a higher analyte
284 concentration in the extract. However, volumes lower than 0.5 mL could not be
285 easily handled after elution and, therefore, a volume of 0.5 mL of acetone was
286 finally fixed for subsequent experiments. Sample pH and elution time showed
287 non-significant effects and they were fixed at the most convenient experimental
288 levels, without adjusting water pH, and with a 5 min elution time.

289 **3.2.2. Optimization**

290 Circumscribed central composite design (CCCD) was employed in this
291 optimization step. This design combines a two-level full factorial design ($2k$) with
292 $2k$ star points, where k is the number of factors being optimized, and one point
293 at the center of the experimental region, which can be run n times. In order to
300
348 $42\sqrt{2}=1.41$ whereas the central point was repeated three times to provide an
349 orthogonal design [36]. CCCD was used to evaluate the main effects,
350 interaction effects and quadratic effects of the amount of sorbent and extraction
351 time. Table 2 shows the low, central and high levels, and the star points of the
352 considered factors. Eleven experiments (Table S2) were randomly carried out
353 using 20 mL of aqueous standards with 20 mg L⁻¹ of benzene, toluene and
354 ethylbenzene. GC-FID was used for the analysis.

355 Data obtained were also evaluated by ANOVA. The quadratic regression
356 model was significant in all cases (data not shown). Coefficients of variation
357 (CV) at central point (n=3) were 6%, 7% and 11% for benzene, toluene and
358 ethylbenzene, respectively. Response surfaces of the CCD are shown in
359 Figure 3. As can be observed, the higher the extraction time, the bigger the
360 signals; an optimum value of 11 min was set for all analytes under study.
361 Nevertheless, the influence of the extraction time was less important in
362 benzene, probably because this analyte is easier to extract due to steric effects
363 and size. Furthermore, an increase amount of sorbent led to an initial increase
364 in signals, followed by a decrease. This could be related with nanoparticle
365 aggregation turning into: (i) lower surface area available for extraction; and/or,
366 (ii) less desorption efficiency in the elution step. Optimum values for the
367 amount of sorbent were 127, 142 and 144 mg for benzene, toluene and
368 ethylbenzene, respectively. A compromise value (i.e., mean value) of 138 mg
369 was adopted to validate the method.

370 Based on the results shown above, the MSPE experimental conditions
371 selected for BTEX extraction were: amount of sorbent, 138 mg; extraction time,
372 11 min; sample pH, water pH without adjustment; eluent solvent volume, 0.5
373 mL; and elution time, 5 min.

374

375 **3.3. Sorbent reutilization study**

376 The possibility of reusing ZSM-5/iron oxide composite was studied
377 considering the environmental importance of saving chemicals and wastes.
378 Twelve consecutive extractions were conducted using the same ZSM-5/iron
379 oxide composite, which was dried at 200 °C for 2 h after each extraction. The

380 experiments were carried out in triplicate, employing 10 mg L⁻¹ of benzene,
381 toluene and ethylbenzene aqueous standard solutions and GC-FID as detector.
382 As mentioned before, xylenes were not included in this study since the lower
383 sensitivity of GC-FID did not allow their quantification. As shown in Figure 4,
384 signals remained practically constant during the twelve experiments with CV
385 values ranged between 2 and 15% and, thus demonstrating the possibility of
386 reusing ZSM-5/iron oxide composite at least twelve times.

387

388 **3.4. Analytical figures of merit**

389 Quality parameters of the proposed method were assessed under MSPE
390 optimized conditions. The concentration range studied was from 1 to 100 µg L⁻¹
391 for all the analytes and the final linear working ranges are shown in Table 3.
392 Other main analytical parameters of the proposed method are also summarized
393 in Table 3. The lower concentrations of linear ranges were limited by the limit of
394 quantification (LOQ). The upper end for m,p-xylene was established at 75 µg L⁻¹
395 since standards of 75 µg L⁻¹ and 100 µg L⁻¹ provided the same response.
396 Correlation coefficients (r) ranged from 0.950 to 0.997 (Table 3). Student's t-test
397 [37] was applied to assess linearity, obtaining the following *t* calculated values:
398 16.3 (r=0.993; N=6) for benzene; 14.5 (r=0.991; N=6) for toluene; 13.3 (r=0.990;
399 N=6) for ethylbenzene; 20.9 (r=0.997; N=5) for m,p-xylene; and 5.84 (r=0.950;
400 N=6) for o-xylene. In all cases, the null hypothesis of no correlation for a 5%
401 significance level and 3 or 4 degrees of freedom ($t_{0.05,3}=3.18$ and $t_{0.05,4}=2.78$,
402 respectively) could be rejected and we concluded that a significant correlation
403 does exist. The sensitivity of the instrumental measurements estimated by the
404 slope of the calibration curves ranged between (24800±1700) au µg⁻¹ L for

405 toluene and (180 ± 30) au μg^{-1} L for o-xylene. The repeatability of the proposed
406 method, expressed as CV, was evaluated by analyzing five aqueous standards
407 at BTEX concentration of $40 \mu\text{g L}^{-1}$. CV values ranged between 8 and 11%
408 (Table 3). Enrichment factors (EFs) were evaluated through the slope ratio of
409 calibration curves with and without MSPE. As can be seen in Table 3, EFs were
410 very similar for benzene, toluene and ethylbenzene. However, xylene isomers
411 gave lower extraction performance, with the EF value below 1 for o-xylene. This
412 means that there was no preconcentration for this analyte, probably due to
413 sterically hindered extraction. These EFs values agree with the efficiency for the
414 complete sample preparation procedure which was 19% for benzene, 17% for
415 toluene, 16% for ethylbenzene, 5% for m,p-xylene and 2% for o-xylene. Limit of
416 detection (LOD) and LOQ were estimated according to $3S_b$ and $10S_b$ criteria,
417 respectively, where S_b is the standard deviation of the blank [37]. The obtained
418 values (Table 3) satisfy current normative according to EPA [34] and the
419 European Union [35] for BTEX content in waters intended for human
420 consumption. EPA establishes maximum contamination levels of $5 \mu\text{g L}^{-1}$ for
421 benzene, 1 mg L^{-1} for toluene, 0.7 mg L^{-1} for ethylbenzene and 10 mg L^{-1} for
422 xylenes [34]. The European Union is more restrictive with levels of benzene in
423 drinking waters due to its carcinogenic properties, and establishes a threshold
424 of $1 \mu\text{g L}^{-1}$. By contrast, toluene, ethylbenzene and xylenes are not included in
425 European legislation [35]. Finally, we want to point out that LODs obtained for
426 benzene and toluene are lower than those previously reported (i.e., $11 \mu\text{g L}^{-1}$
427 ¹ and $13 \mu\text{g L}^{-1}$, respectively) for a method in which ZSM-5 zeolite was
428 employed as sorbent for headspace SPE [21].

429

430 **3.5. Analysis of real water samples**

431 The applicability of the proposed method to determine BTEX in real water
432 samples was evaluated. Three water samples (namely drinking water,
433 wastewater and river water) were employed to assess matrix effects using
434 recovery studies. It should be noted that in previous analyses with the proposed
435 method, none of the selected water samples had initial detectable BTEX
436 concentrations. Three replicated analyses of each water sample were carried
437 out at 40 $\mu\text{g L}^{-1}$ spiking level. Table 4 shows the relative recoveries determined
438 as the ratio of the signals found after MSPE in real and ultrapure water samples
439 spiked at the same concentration level. Results showed relative recoveries
440 varying from 85 and 94% for drinking water, between 93 and 113% for
441 wastewater, and between 111 and 114% for river water. In all cases, CV values
442 were below 11%. According to these results, we can conclude that matrix
443 effects were not significant for the determination of BTEX in the three selected
444 water samples.

445

446 **4. Conclusions**

447 A new MSPE-GC-MS procedure using ZSM-5/iron oxide composite as
448 sorbent has been proposed in this work. The magnetic composite presents
449 remarkable advantages such as low cost, rapid and simple synthesis, easy
450 manipulation under a magnetic field and reuse options. To our knowledge, this
451 is the first time that ZSM-5/iron oxide composite has been employed for BTEX
452 extraction and preconcentration from water samples. Good extraction
453 efficiencies were obtained for benzene, toluene and ethylbenzene. However,
454 EFs were lower for xylenes, and preconcentration for o-xylene isomer was not

455 obtained, probably due to steric effects. Nevertheless, the results showed LODs
456 that satisfy the current EPA and European Union normative for BTEX content in
457 waters for human consumption, especially for benzene, which presents the
458 most restrictive levels (i.e., 5 and 1 $\mu\text{g L}^{-1}$, respectively). In addition, LODs were
459 lower than those previously reported for benzene and toluene in a method using
460 ZSM-5 zeolite as sorbent for headspace SPE. Finally, our study demonstrates
461 the ability of the proposed method to determine BTEX at trace levels in real
462 water samples.

463 Zeolites possess unique sorbent properties, such as ordered crystalline
464 structure with well-defined pore size and shape. Therefore, zeolites can act as
465 molecular sieves, which are very useful for selective extractions. This work can
466 be considered as a starting point for the use of ZSM-5/iron oxide composite in
467 MSPE. In future works, the application of ZSM-5/iron oxide composite can be
468 extended to different target analytes (i.e., organic and inorganic) and samples
469 (e.g., environmental, food and biological).

470

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477

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608

609 **Figure captions**

610 **Fig. 1** Total ion chromatograms obtained in the SIM mode after MSPE of a
611 blank (green line) and a standard solution spiked at $100 \mu\text{g L}^{-1}$ with target
612 analytes (black line).

613 **Fig. 2** High resolution XPS spectrum in the Fe 2p region for ZSM-5/iron oxide
614 composite.

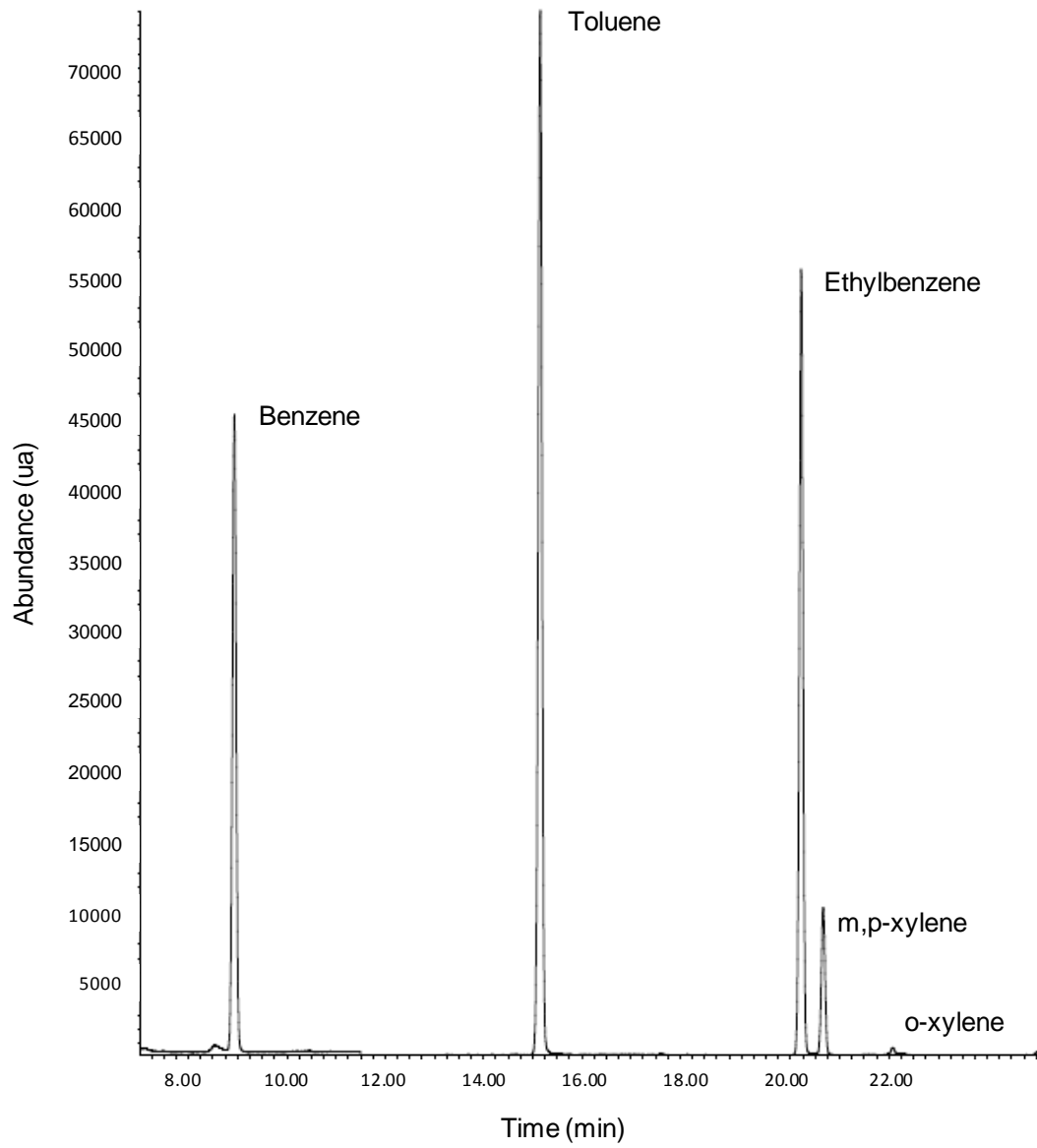
615 **Fig. 3** Response surfaces of CCD obtained by plotting the amount of sorbent
616 vs. extraction time for: (a) benzene; (b) toluene; and (c) ethylbenzene.

617 **Fig. 4** Study of sorbent reutilization using the same ZSM-5/iron oxide composite
618 in twelve consecutive extractions.

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620

Fig. 1



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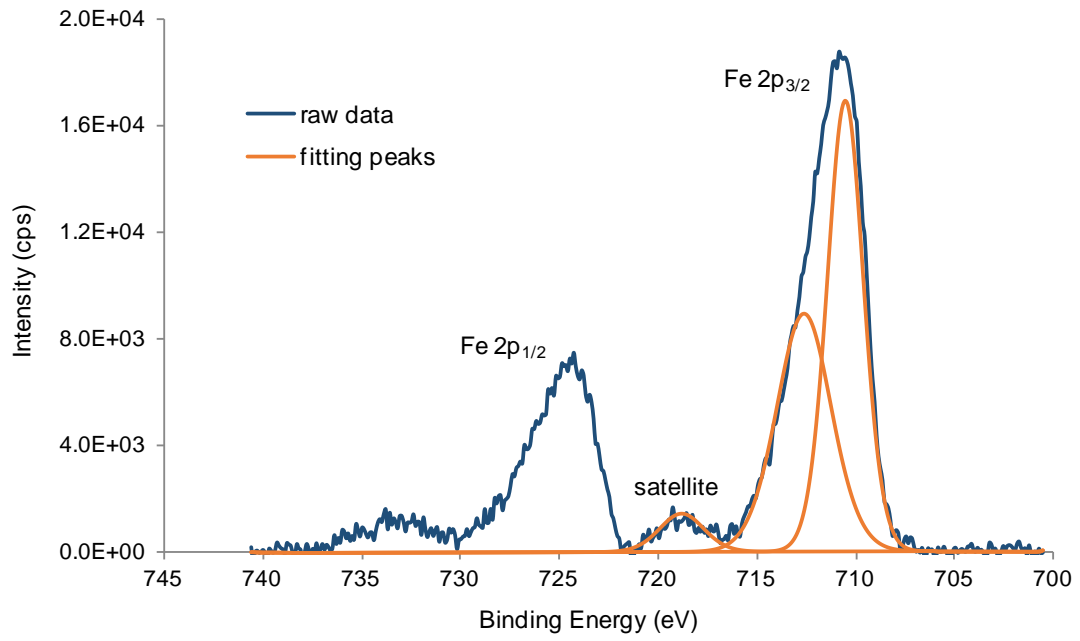
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Fig. 2

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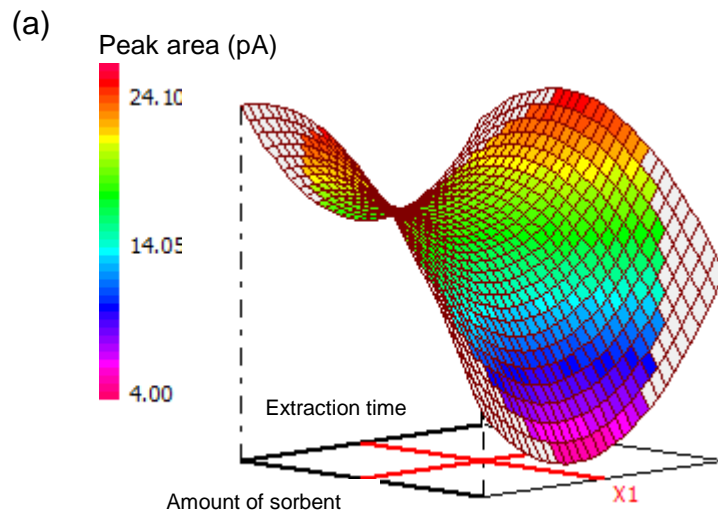
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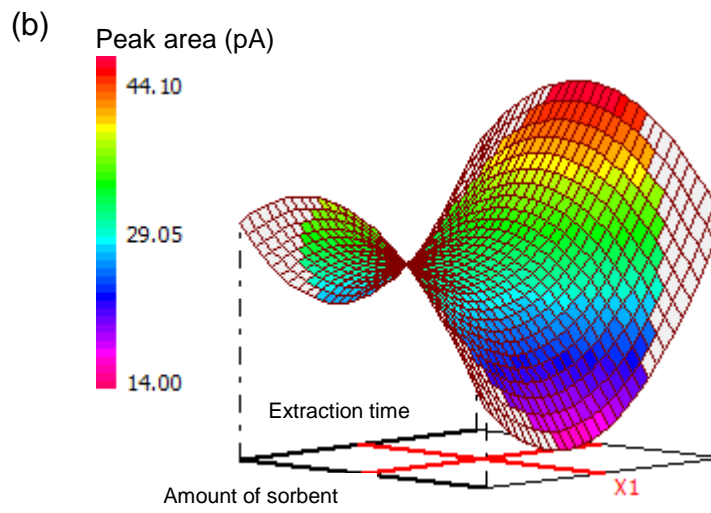
Fig. 3

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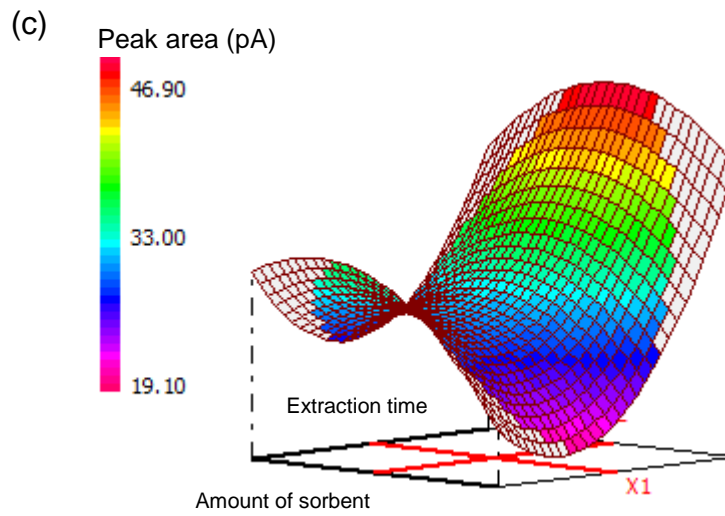
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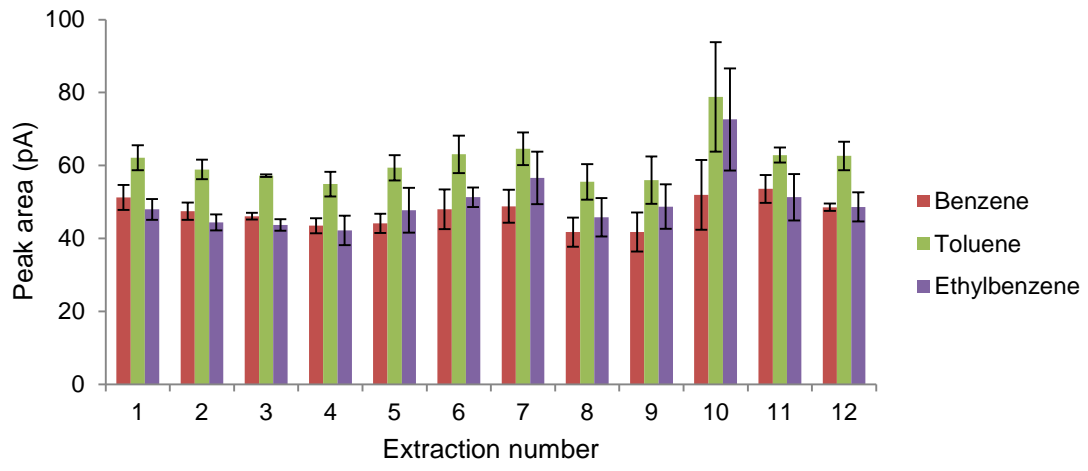
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Fig. 4

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641

642 **Table 1.** Experimental factors and levels of the Plackett–Burman design.

643

Factors	Level	
	Low (-1)	High (+1)
Amount of sorbent (mg)	50	100
Sample pH	3	9
Extraction time (min)	3	6
Eluent solvent volume (mL)	0.5	1
Elution time (min)	5	10

644 **Table 2.** Factors, low, central and high levels, and star points used in CCD design.

Factors	Level			Star points ($\alpha=1.41$)	
	Low (-1)	Central (0)	High (+1)	- α	+ α
Amount of sorbent (mg)	100	150	200	79	221
Extraction time (min)	4	7	10	3	11

645

646

647 **Table 3.** Main analytical parameters of the proposed method.

Analyte	Working range ($\mu\text{g L}^{-1}$)	r^a	CV ^b (%)	LOD ^c ($\mu\text{g L}^{-1}$)	LOQ ^d ($\mu\text{g L}^{-1}$)	EF ^e
Benzene	1-100	0.993 (6)	9	0.3	1	7.6
Toluene	10-100	0.991 (6)	8	3	10	7.0
Ethylbenzene	10-100	0.990 (6)	9	3	10	6.5
m,p-xylene	10-75	0.997 (5)	9	3	10	1.7
o-xylene	10-100	0.950 (6)	11	3	10	0.6

648 ^a Correlation coefficient: number of calibration points in parentheses.

649 ^b Coefficient of variation: mean value for five replicate analyses of a $40 \mu\text{g L}^{-1}$ spiked solution.

650 ^c Limit of detection: calculated using blank signal plus three times its standard deviation.

651 ^d Limit of quantification: calculated using blank signal plus ten times its standard deviation.

652 ^e Enrichment factor: calculated as slope ratio between calibration curves with and without MSPE.

653

654 **Table 4.** Relative recoveries and CV values (in parentheses) obtained for the target analytes in
655 the three studied water samples.

656

Analyte	Relative recoveries ^a		
	Drinking water	Wastewater	River water
Benzene	94 (4)	112 (8)	112 (11)
Toluene	92 (6)	112 (11)	113 (2)
Ethylbenzene	85 (3)	113 (8)	111 (3)
m,p-xylene	93 (9)	98 (11)	114 (4)
o-xylene	87 (8)	93 (2)	112 (8)

657 ^aThree replicate analysis at 40 µg L⁻¹ spiking level.

658

659

660 Electronic Supplementary Material

661

662 **Zeolite/iron oxide composite as sorbent for magnetic solid-phase**
663 **extraction of benzene, toluene, ethylbenzene and xylenes from water**
664 **samples prior to gas chromatography-mass spectrometry**

665

666 Elena Fernández, Lorena Vidal* and Antonio Canals*

667 Departamento de Química Analítica, Nutrición y Bromatología e Instituto Universitario de
668 Materiales, Universidad de Alicante, P.O. Box 99, E-03080 Alicante, Spain.

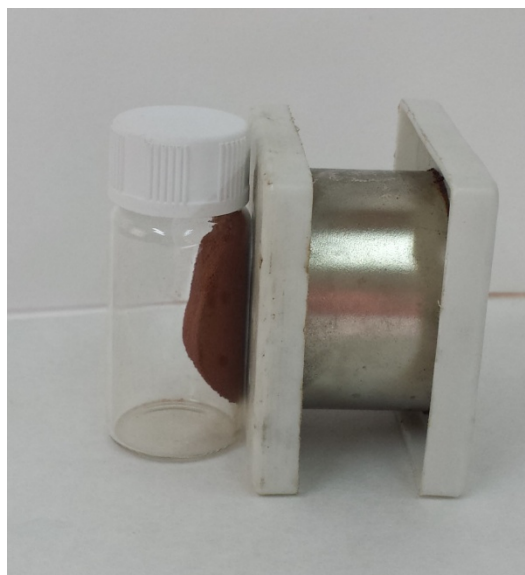
669 *Corresponding authors: Tel.: +34965909790; fax: +34965903697.

670 E-mail addresses: lorena.vidal@ua.es (L. Vidal), a.canals@ua.es (A. Canals).

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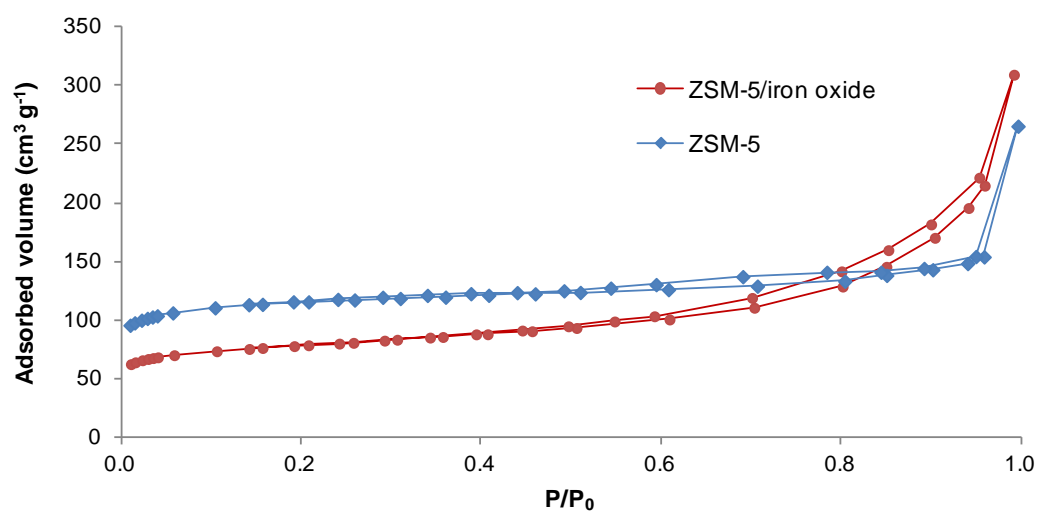
673 **Fig. S1** Photograph showing the magnetic attraction between ZSM-5/iron oxide
674 composite and neodymium magnet.



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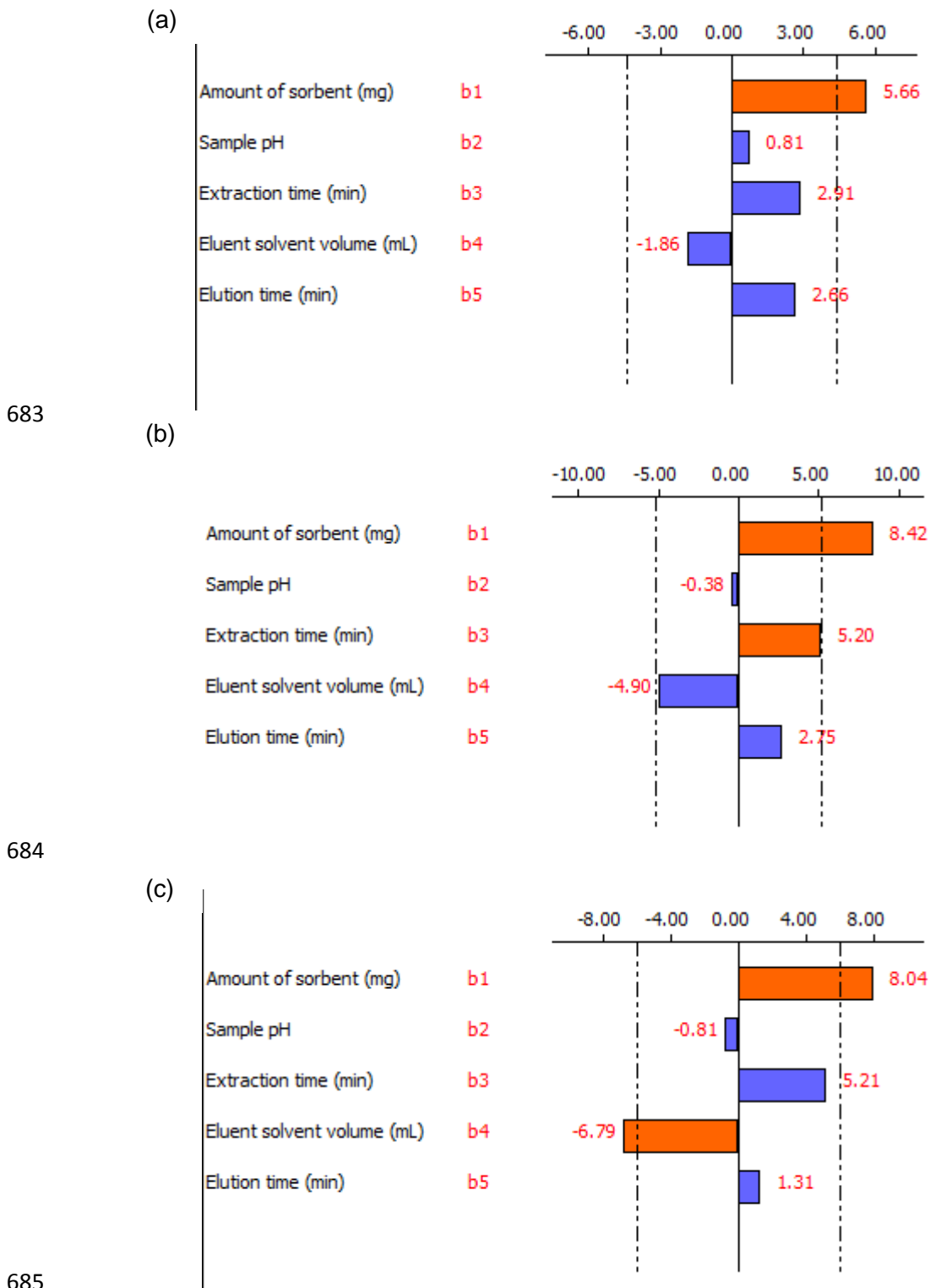
677 **Fig. S2** Nitrogen adsorption–desorption isotherms for pure ZSM-5 zeolite and
678 ZSM-5/iron oxide composite.



679

680

681 **Fig. S3** Pareto charts of the Plackett-Burman design obtained for: (a) benzene;
 682 (b) toluene; and (c) ethylbenzene.



687 **Table S1.** Matrix of experiments of Plackett-Burman design.

Run	Amount of sorbent (mg)	Sample pH	Extraction time (min)	Eluent solvent volume (mL)	Elution time (min)
1	100	9	6	0.5	10
2	50	9	6	1	5
3	50	3	6	1	10
4	100	3	3	1	10
5	50	9	3	0.5	10
6	100	3	6	0.5	5
7	100	9	3	1	5
8	50	3	3	0.5	5

688

689

690 **Table S2.** Matrix of experiments of CCCD.

Run	Amount of sorbent (mg)	Extraction time (min)
1	100	4
2	200	4
3	100	10
4	200	10
5	79	7
6	221	7
7	150	3
8	150	11
9	150	7
10	150	7
11	150	7

691

692