A modified ZSM-5 zeolite/Fe_2O_3 composite as a sorbent for magnetic dispersive solid-phase extraction for the preconcentration of nonsteroidal anti-inflammatory drugs in water and urine samples†

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Abstract

This study is the first to use a new ZSM-5 zeolite-based composite decorated with iron oxide magnetic nanoparticles and modified with hexadecyltrimethylammonium bromide surfactant (i.e., HDTMA-ZSM-5/Fe_2O_3) as an efficient sorbent for magnetic dispersive solid-phase extraction (MDSPE) of nonsteroidal anti-inflammatory drugs in water and urine samples with subsequent measurement by liquid chromatography diode array detection. Experimental factors affecting MDSPE were optimized using a multivariate optimization strategy. The optimum experimental conditions were: amount of sorbent, 40 mg; sample pH, 2.2; NaCl concentration, 2.5%; extraction time, 2 min; eluent solvent, methanol; eluent solvent volume, 424 µL; and elution time, 2 min. The linearity of the method was studied from 3.3 to 400 µg L^{-1} (N=8) for ketoprofen, from 1.7 to 400 µg L^{-1} (N=8) for felbinac, from 6.6 to 400 µg L^{-1}

(N=7) for diclofenac and from 9.9 to 400 µg L^{-1} (N=6) for ibuprofen. Method repeatability was evaluated at 10 and 200 µg L^{-1} spiking levels, obtaining coefficients of variation between 2 and 5% (n=6). Limits of detection, determined empirically, were 1.0 µg L^{-1}, 0.5 µg L^{-1}, 2.0 µg L^{-1} and 3.0 µg L^{-1} for ketoprofen, felbinac, diclofenac and ibuprofen, respectively. Tap water, reservoir water, wastewater and five urine samples were selected to assess method applicability. Recovery values ranged between 86-107% and 80-112% for water and urine samples, respectively, showing negligible matrix effects. Finally, this method was employed to monitor ibuprofen excretion in real urine samples.

Keywords: zeolite, surfactant, magnetic dispersive solid-phase extraction, nonsteroidal anti-inflammatory drugs, water samples, urine samples.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications worldwide due to their analgesic, antipyretic and anti-inflammatory properties [1]. These drugs are used to treat chronic pain, osteoarthritis, rheumatoid arthritis, gout, dysmenorrhea, dental pain and headache [2]. NSAIDs are readily available over-the-counter without medical prescription. Short-term NSAIDs usage is believed safe; however, acute overdosage or chronic abuse can cause adverse side-effects such as gastrointestinal bleeding, acute kidney injury and cardiovascular risk [3]. To diagnose cases of acute overdosage or chronic abuse or, more importantly, assess differential diagnostic exclusion, an analytical procedure is required to

detect these drugs in human urine prior to their quantitation in plasma. Therefore, determination of drugs in urine is essential to monitor drugs concentration [4]. Moreover, NSAIDs are continually being loaded into waters, mainly indirectly by excreta, through disposal of unused or expired drugs, or directly in discharges from pharmaceutical-manufacturing plants [5]. The release of these NSAIDs residues into environmental aqueous systems is toxic to many animal species and human beings. Several NSAIDs have been detected in wastewaters and rivers [6–9], showing the need for NSAIDs monitoring in aqueous environments. All the above reasons show the importance of developing a simple and fast method to determine NSAIDs in environmental (e.g., water) and biological samples (e.g., urine) in order to prevent negative health effects.

NSAIDs are usually determined by chromatographic techniques such as gas chromatography (GC) [6,10], liquid chromatography (LC) [7–9,11,12] or capillary electrophoresis [13] combined with different detectors. Since GC requires a previous derivatization step [6,10], separation is typically performed by LC. However, direct determination of NSAIDs in environmental and biological samples is problematic due to their low concentration and matrix complexity, making a sample pretreatment step necessary prior to chromatographic analysis. Solid-phase extraction is the most commonly used sample pretreatment procedure to determine NSAIDs [14] using different sorbents such as C_{18} [15], modified polymers [16,17], molecular imprinted polymers (MIPs) [12,18] and carbon nanotubes (CNTs) [19]. However, the original SPE has undergone numerous modifications to date, mainly related to miniaturization or automatization [20]. Different modalities of miniaturized SPE such as solid-

phase microextraction (SPME) [21–24], stir-bar sorptive extraction (SBSE) [25,26] and microextraction by packed sorbent (MEPS) [27,28] have also been used for the determination of NSAIDs. Magnetic dispersive solid-phase extraction (MDSPE), also called magnetic solid phase extraction (MSPE), uses magnetic or magnetically modified sorbents. This method, having first been used for analytical purposes by Šafaříková and Šafeřík in 1999 [29], has recently become popular because it reduces sample preparation time and facilitates sorbent manipulation [20,30]. In MDSPE, the magnetic sorbent is directly added and dispersed in the sample solution. After extraction, the magnetic sorbent is easily separated from the sample by using an external magnetic field (i.e., neodymium magnet) without requiring filtration or centrifugation steps and thus reducing time and energy [30]. This makes the extraction process simpler, faster and portable. Finally, analytes can be eluted using a proper solvent or thermally desorbed for further determination. Some works describe magnetic materials used as sorbent, such as magnetic melamine-formaldehyde resin [31], modified magnetic nanoparticles [32,33], magnetic graphene composite [34,35], magnetic sporopollenin-cyanopropyltriethoxysilane [36], and magnetic metal organic framework [9], for determination of NSAIDs in environmental and biological samples. However, to our knowledge there are no reported methods based on MDSPE using zeolites modified with magnetic nanoparticles as sorbent for preconcentration of NSAIDs [37].

Zeolites are ordered crystalline aluminosilicates constituted by a framework structure composed of TO_4 tetrahedra (T= Si, Al) interconnected through O [38]. The presence of Al atoms into the structure makes the framework negatively

charged due to the difference between the $(AlO_4)^{5-}$ and $(SiO_4)^{4-}$ tetrahedral. This negative charge is compensated by extraframework cations (e.g., alkaline and alkaline earth) [37–40]. These materials possess unique and fascinating properties such as high surface area, high adsorption capacity and molecular selectivity, chemical and thermal stability, ion-exchange capacity, low cost extraction and synthesis. Additionally, ease of modification provides a wide range of zeolite-based materials, which convert zeolites into potential sorbents for extraction procedures. In many cases, zeolites cannot adsorb organic molecules because its pore size is smaller than the dimensions of organic compounds. For this reason, in order to increase organic compounds preconcentration capacity, zeolites have been modified with cationic surfactants. The cationic surfactants commonly used to modify zeolites are long alkyl chains with a quaternary ammonium group at one end of the chain. Since the channel diameter of the zeolite is considered sufficiently large for exchangeable cations, but too small for the cationic surfactant, the sorption of surfactant molecules on zeolite is limited to the external surface sites [41]. Several publications have reported the use of cationic surfactant modified zeolites to determine organic compounds [42–45]. However, to our knowledge there are no published methods with analytical purposes to extract NSAIDs by magnetic zeolites modified with cationic surfactants [37].

Therefore, this work aims to develop a simple MDSPE method, employing a ZSM-5 zeolite-based composite decorated with iron oxide magnetic nanoparticles and modified with hexadecyltrimethylammonium bromide surfactant (i.e., HDTMA-ZSM-5/Fe_2O_3) as a valuable sorbent for the simultaneous separation and preconcentration of four NSAIDs (i.e., ketoprofen,

felbinac, diclofenac and ibuprofen) (Table S1) from both water and urine samples for subsequent separation/quantification by liquid chromatography-diode array detection (LC-DAD). To our knowledge, this is the first report of an analytical method in which MDSPE employing a zeolite is used to determine NSAIDs in water and urine samples. Several of the main factors affecting the MDSPE have been optimized by a two-step multivariate strategy, using Plackett–Burman and circumscribed central composite designs. Finally, the reported method has been validated and successfully applied to analyse real water and real urine samples.

2. Experimental

2.1. Reagents

Ketoprofen (KET; 2-(3-benzoylphenyl)propanoic acid), felbinac (FEL; 4-biphenylylacetic acid), diclofenac sodium salt (DIC; 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetic acid) and ibuprofen (IBU; 2-(4-isobutylphenyl)propanoic acid) were obtained from Sigma Aldrich (St. Louis, MO, USA). Individual stock standard solutions containing 1000 mg L^{-1} of KET, FEL, DIC and IBU and mixed stock solutions containing the four NSAIDs (5 and 100 mg L^{-1}) were prepared in HPLC-grade methanol from Sigma-Aldrich and were stored in the dark at 4 ºC. NSAIDs working solutions (0.5-100 µg L^{-1}) were prepared by proper dilution of mixed stock standard solution with deionized water.

HPLC-grade acetonitrile from Sigma-Aldrich, ultrapure water (resistivity of 18.2 MΩ cm at 25 °C) obtained from a Millipore Direct System Q5^{TM} purification system from Ibérica S.A. (Madrid, Spain), H_3PO_4 (85% purity) from Scharlau

Chemie (Sentmenat, Spain) and KH_2PO_4 pro-analysis from Merck (Darmstadt, Germany) were used to prepare the mobile phase of the LC system.

ZSM-5 zeolite (CBV 3024E, SiO_2/Al_2O_3 mole ratio=30) in the ammonium nominal cation form was obtained from Zeolist International (Conshohocken, PA, USA). $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ reactive grade were obtained from Sigma-Aldrich and NaOH (97% purity, pellets) from Scharlau Chemie. Hexadecyltrimethylammonium bromide (HDTMABr, ≥99% purity) from Sigma-Aldrich was employed for preparing a 0.5% (w/v) solution in deionized water/ethanol mixture (50/50, v/v), being HPLC-grade ethanol absolute from Scharlau Chemie.

H_3PO_4, KH_2PO_4 and K_2HPO_4 pro-analysis from Merck were employed for buffering and sodium chloride (99% purity) from Scharlau Chemie was employed to adjust NaCl concentration of water and urine samples prior analysis. Finally, sodium thiosulphate pentahydrate (99.5% purity) from Scharlau Chemie was added to a tap water sample before analysis to remove chlorine interference.

2.2. Samples

Real water samples used were reservoir water from Murcia (Spain), wastewater from a wastewater treatment plant in Barcelona (Spain) and tap water from Barcelona (Spain). Samples were collected in amber glass containers and stored in the dark at 4 ºC. As real urine samples, on the one hand, five urine samples were provided by healthy human volunteers, who had not been treated with anyone of the drugs studied. On the other hand, several urine samples were obtained from one volunteer who had been orally treated

with ibuprofen (200 mg) to study the applicability of the proposed method. All urine samples were collected in sterile containers and stored at 4 ºC.

Before use, both water and urine samples were adjusted to pH 2.2 with a buffer solution of 0.0025 M H_3PO_4/0.0029 M KH_2PO_4 and adjusted to 2.5% (w/v) of NaCl concentration. Only tap water was treated with sodium thiosulphate solution (i.e., 33.3 µL of 0.05 M $Na_2S_2O_3$ was added to 0.1 L of tap water) [46]. Samples were filtered with 0.45 µm pore size nylon filters from Millipore (Madrid, Spain) in order to remove suspended particles before use. Therefore in this case the measurands are the soluble NSAIDs [47]. Water and urine samples were initially analysed under optimized conditions of the proposed method and NSAIDs content was undetectable.

2.3. Materials and instrumentation

A Ni-coated neodymium magnet (S-45-30-N), N45 grade, dimensions 45x30 mm from Supermagnete (Gottmadingen, Germany) was used as an external magnetic field. The sample compartment was a 22 mL glass vial with screw top (solid green Melamine cap and PTFE liner) from Supelco (Bellefonte, PA, USA).

The chromatographic analysis were performed by an Agilent 1260 Infinity LC system (Agilent Technologies, Waldbronn, Germany), constituted by the following modules: vacuum degasser, quaternary pump (G1311C), autosampler (G1329B), thermostated column compartment (G1316A) and diode array detector (G4212B). Instrumental control and data acquisition and processing were carried out using the software OpenLab (Agilent Technologies). A Kinetex® 5 µm EVO C18 100 Å column (150 mm x 4.6 mm i.d.) from

Phenomenex (Torrance, California, USA) was used to separate the analytes. A mixture of 0.01 M phosphate buffer (pH = 4.2) and acetonitrile (50:50, v/v) was employed as mobile phase for the separation at a flow rate of 1 mL min^{-1}. The injection volume was 20 µL. The detection was performed at 225 nm for IBU and DIC and 258 nm for KET and FEL.

Magnetic susceptibility measurements were performed at 300 K in the magnetic field range -50 to 50 kOe using a MPMS XL (SQUID) magnetometer from Quantum Design (San Diego, CA, USA) for composite characterization (i.e., HDTMA-modified ZSM-5/Fe_2O_3).

2.4. Synthesis of ZSM-5/Fe_2O_3 composite

Synthesis of ZSM-5/Fe_2O_3 composite, described in detail in our previous work [48], was performed in a ZSM-5/Fe_2O_3 weight ratio of 3:1. This weight ratio was chosen to avoid a decrease in available surface area and for ease of composite manipulation under a magnetic field [48]. Briefly, the composite was prepared from a suspension of 3 g of ZSM-5 zeolite in 250 mL solution with $FeCl_3 \cdot 6H_2O$ (2.335 g) and $FeSO_4 \cdot 7H_2O$ (1.201 g). Then, 15 mL of 5 M NaOH was added dropwise to precipitate the iron oxide. The mixture was stirred at room temperature for 2 h. The composite was separated from the solution using a Ni-coated neodymium magnet as external magnetic field. The obtained composite was washed with deionized water until washing water became clear at neutral pH. Lastly, the composite was dried at 110 ºC overnight.

2.5. Modification of ZSM-5/Fe_2O_3 composite

The modification of ZSM-5/Fe_2O_3 composite with HDTMABr surfactant was

described in detail in a previous work [49]. Briefly, surfactant modification consisted in firstly, stirring 10 g of ZSM-5/Fe_2O_3 composite with 150 mL of the HDTMABr solution (0.5%, w/v) for 24 h at room temperature. Secondly, HDTMA-modified ZSM-5/Fe_2O_3 composite (i.e., HDTMA-ZSM-5/Fe_2O_3) was filtered in a Büchner funnel connected to a vacuum pump and washed with deionized water several times. And finally, the obtained composite was dried at 120 ºC for 3 h.

The structure of ZSM-5/Fe_2O_3 composite modified with HDTMABr surfactant is schematically shown in Fig. 1. It can be observed that the HDTMABr surfactant forms a bilayer on the external surface of the zeolite. This occurs when the concentration of the surfactant exceeds its CMC. The CMC of HDTMABr is 0.9 mmol L^{-1} at 25 ºC [42] and in our case, surfactant concentration has 13.7 mmol L^{-1}, being the surfactant concentration 15 times higher than its CMC. Therefore, the formation of HDTMA bilayer can be confirmed. Briefly, this modification was carried out by as follows: firstly, the surfactant is sorbed on the zeolite by cation exchange (i.e., the exchange capacity of the mineral surface for surfactant depends on the external cation exchange capacity (ECEC) [52]) forming a monolayer or “hemimicelle”, but if the surfactant concentration in solution exceeds the CMC, as in this case, then the hydrophobic tails of the surfactant molecules associate to form a bilayer or “admicelle” [53]. This bilayer formation results in a charge reversal on the external surface of zeolite from negative to positive and the positively charged outward-pointing head groups of HDTMA bilayers are balanced by bromide counterions [54,55].

252 2.6. Magnetic dispersive solid-phase extraction

253 Firstly, 40 mg of HDTMA-ZSM-5/Fe_2O_3 composite were placed in a 22 mL
254 vial glass. Then, 20 mL of standard solution or sample solution adjusted to pH
255 2.2 and 2.5% of NaCl concentration was added and the mixture was shaken
256 vigorously for 2 min. After extraction, the composite was separated from the
257 solution using a Ni-coated neodymium magnet. A glass pipette was used to
258 remove the aqueous phase. Then, the adsorbed analytes were eluted with 424
259 µL of methanol using an ultrasonic bath for 2 min. Finally, eluate was separated
260 from the composite using again the neodymium magnet, withdrawn with a
261 syringe, filtered with 0.22 µm pore size nylon filters and transferred to a vial for
262 further determination by LC-DAD.

263

264 2.7. Data processing

265 A multivariate approach was used to determine the optimum conditions for
266 MDSPE. Firstly, a Plackett-Burman design was used as a screening study to
267 identify the significant factors. Then, a circumscribed central composite design
268 (CCCD) was employed to optimize the significant ones. The statistical software
269 mainly used to build the experiment matrices and evaluate the experimental
270 results in Plackett-Burman and CCC designs (i.e., response surfaces) was
271 NEMRODW® ("New Efficient Methodology for Research using Optimal Design")
272 (LPRAI, Marseille, France). In addition, the statistical software Statgraphics®
273 Centurion (Statpoint Tecnhologies Warreton, USA) was used as supplementary
274 support to evaluate the significant factors of CCCD using Pareto chart and to
275 compare the optimum value with the one obtained with NEMRODW®. A 20 mL
276 of an aqueous standard with 100 µg L^{-1} of KET, FEL, DIC and IBU was used for
277 the optimization MDSPE experiments. Peak areas of KET, FEL, DIC and IBU

obtained with LC-DAD were individually used as response functions for optimization.

3. Results and discussion

3.1. Characterization of HDTMA-ZSM-5/ Fe_2O_3 composite

In our previous work [49], X-ray photoelectron spectroscopy (XPS) and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) were used to investigate the oxidation state of Fe in ZSM-5/Fe_2O_3 and the loading of HDTMABr surfactant on ZSM-5/Fe_2O_3, respectively. Fig. S1 shows the magnetic susceptibility of the studied HDTMA-ZSM-5/Fe_2O_3 at 300 K in the magnetic field range -50 to 50 kOe. The magnetization curve shows a sigmoid shape that passes approximately through the origin with an extremely thin hysteresis loop. In addition, the values of coercivity field (H_C) and residual magnetization (M_R) are very small, 6 Oe and 0.08 emu g^{-1}, respectively. Therefore, it could be concluded that the synthesized Fe_2O_3 presents a superparamagnetic behaviour at room temperature [56].

3.2. Preliminary experiments

3.2.1. Extraction efficiency of composites

The effect of two composites (i.e., ZSM-5/Fe_2O_3 and HDTMA-ZSM-5/Fe_2O_3) for the extraction of KET, FEL, DIC and IBU was studied preliminarily in aqueous standards (data showed in Fig. S2). Extraction experiments were carried out by shaking 50 mg of each composite in 20 mL of aqueous standard with 500 μg L^{-1} of KET, FEL, DIC and IBU for 5 min. After extraction, the aqueous standard was removed and sorbents were eluted with 500 μL of methanol using an ultrasonic bath for 3 min. Fig. S2 shows that the signal

obtained with HDTMA-ZSM-5/Fe_2O_3 was much higher than the signal obtained with ZSM-5/Fe_2O_3 for all analytes. In the case of ZSM-5/Fe_2O_3, the analytes were retained in the composite pores; however, NSAIDs adsorption was negligible. In the case of HDTMA-ZSM-5/Fe_2O_3, the high adsorption efficiency of NSAIDs was attributed to zeolite surface modification with HDTMABr surfactant, which increased hydrophobicity of the sorbent surface and, therefore, provided a high affinity for organic molecules [57,58]. Consequently, HDTMA-ZSM-5/Fe_2O_3 composite was chosen as sorbent.

Related to the affinity of the HDTMA-ZSM-5/Fe_2O_3 composite towards the NSAIDs, two parameters commonly used are: the binding capacity (B, μg g^{-1}) and distribution coefficients (K_D, L g^{-1}), defined according to the following equations [59]:

$$B = \frac{(C_i - C_f)V}{m} \quad (1)$$

$$K_D = \frac{(C_i - C_f)V}{C_f m} \quad (2)$$

where V represents the volume of the solution (L), C_i is the initial solution concentration (μg L^{-1}), C_f is the solution concentration after extraction (μg L^{-1}) and m is the mass of sorbent (g). These parameters were calculated using a solution spiked at 10 mg L^{-1} of each NSAID and the results are presented in Table S2. It should be noted that these results are correlated with enrichment factors obtained in Table 1 (i.e., KET < FEL ≈ IBU < DIC).

The reutilization of HDTMA-ZSM-5/Fe_2O_3 composite was also studied. Three consecutive extractions were carried out using the same composite and results showed that the sorbent was still extracting NSAIDs but extraction efficiency for all analytes decreased around 15% approximately from first

328 extraction to the second one. This could be due to the removal of HDTMABr
329 surfactant in the elution step. Hence, it can be concluded that HDTMA-ZSM-
330 5/Fe_2O_3 composite cannot be reused. However, this is not an inconvenient
331 since the sorbent (i.e., zeolite) is of low cost and composite synthesis (i.e.,
332 HDTMA-ZSM-5/Fe_2O_3) is very simple.
333
334 3.2.2. Elution solvent nature
335 In this study, based on previous experiences [4,60], the type of eluent
336 solvent was investigated. It should be mentioned that this factor was not include
337 in the screening study because the Plackett-Burman design investigates the
338 factors at two levels and, in this case, four solvents were tested. Acetone,
339 ethanol, acetonitrile and methanol were selected for analyte elution from the
340 sorbent. Extraction experiments were performed by shaking 50 mg of HDTMA-
341 ZSM-5/Fe_2O_3 in 20 mL of aqueous standard with 500 μg L^{-1} of KET, FEL, DIC
342 and IBU for 5 min. After extraction, the aqueous standard was removed and the
343 sorbent was eluted with 500 μL of acetone, ethanol, acetonitrile and methanol
344 using an ultrasonic bath for 3 min. The results are shown in Fig. S3. Acetone,
345 ethanol and acetonitrile obtained a slightly higher peak area than methanol.
346 However, for practicality, methanol was selected as eluent solvent because
347 phase separation performance was better than with the other solvents.
348
349 3.3. Adsorption mechanisms
350 As previously mentioned, the HDTMABr molecules form a bilayer on the
351 external surface of the zeolite (Fig. 1), which results in a reversal charge on the
352 external surface of zeolite providing sites where anions might be retained while

353 neutral species could partition into the hydrophobic cores [61]. Previous works
354 have proposed several adsorption mechanisms for different analytes (i.e., DIC
355 [57,62], IBU [62], tannic acid [63], humic acid [64], bisphenol A [65], among
356 others) by zeolites modified with surfactants. The adsorption mechanisms are
357 described below. First, electrostatic interaction takes places between the
358 positively charged outward-pointing head groups of HDTMA bilayer and the
359 negatively charged NSAIDs molecules [63]. This mechanism is pH dependant,
360 i.e., at pH below the pKa of studied NSAIDs, they are in a neutral form; while
361 when the pH is clearly above the pKa of these analytes, NSAIDs are negatively
362 charged, and there is electrostatic attraction between NSAIDs and positive
363 charge of head groups of HDTMA bilayer. It is important to point out that the
364 optimum pH was 2.2 (section 3.4.2), and the studied NSAIDs have pKa values
365 ranging from 4-5 (Table S1), therefore, the latter mechanism did not take place.
366 The second mechanism that could act in the adsorption of NSAIDs involves π-
367 cation interaction between the aromatic rings of NSAIDs and quaternary
368 ammonium groups of HDTMA bilayer [51]. Thirdly, hydrogen bonding also could
369 play an important role in the adsorption between the nitrogen atoms of HDTMA
370 bilayer and carboxylate groups of NSAIDs molecules since nitrogen atoms may
371 act as hydrogen bonding acceptors and carboxylate groups may act as
372 hydrogen bonding donors [63,64]. Finally, the last mechanism involved
373 hydrophobic interactions between the hydrophobic C chains of HDTMA bilayer
374 and the hydrophobic functional groups of NSAIDs molecules (i.e., aromatic
375 rings) [63]. Therefore, in our case, it is likely the main mechanisms governing
376 the adsorption of NSAIDs onto HDTMA-ZSM-5/Fe_2O composite were: (i) π-
377 cation interaction, (ii) hydrogen bonding and (iii) hydrophobic interactions.

3.4. MDSPE optimization

3.4.1. Screening study

Numerous factors can affect extraction yield in the MDSPE procedure. Therefore, optimization through a multivariate approach is recommended. One particular strategy is the Plackett-Burman design, which is a two-level fractional factorial design to study k=N-1 factors in N runs, where N is a multiple of 4 [66]. This design assumes that the interactions between factors can be ignored; therefore the main effects can be calculated with a reduced number of experiments, saving time and resources. A Plackett-Burman design was used to construct the matrix of experiments, including five factors in eight runs. Based on previous experience of the research group in MDSPE [48,49,67], the factors investigated at two levels in this work were: sample pH, NaCl concentration, extraction time, eluent solvent volume and elution time. The amount of sorbent is another key factor in MDSPE technique, however, this factor was not included in the present study since it was optimized later by fixing the concentration of the analytes at the upper threshold of the working range and fixing the others factors at their optimum values. Table S3 shows the considered experimental factors and levels in the Plackett-Burman design. The eight experiments were randomly carried out using 20 mL of aqueous standard spiked with 100 μg L^{-1} of each NSAID. Peak area of each analyte was individually used as response functions.

The data obtained were evaluated by ANOVA and the results are showed in the Pareto charts in Fig. 2. The length of each bar is proportional to the relative influence of the corresponding factor, and those bars that exceed reference

403 vertical lines (dashed lines) can be considered significant with 95% probability.
404 In addition, positive and negative bars revealed if the responses increase or
405 decrease, respectively, when passing from a lower to upper level of the
406 corresponding factor. Fig. 2 shows all the Pareto charts present a similar
407 response for each factor, except DIC Pareto chart, where NaCl concentration
408 and elution time have a different effect, however none of them is significant.
409 According to Fig. 2, the significant factors were: sample pH and NaCl
410 concentration for KET (Fig. 2(a)) and eluent solvent volume for KET, FEL and
411 IBU (Fig. 2(a), 2(b) and 2(d)). The sample pH presents a negative effect for all
412 analytes and was significant for KET. The pKa values of KET, FEL, DIC and
413 IBU range from 4-5 (Table S1). At sample pH below their pKa, the studied
414 analytes are neutral molecules; therefore adsorption is due to π-cation
415 interaction, hydrogen bonding and hydrophobic interactions. When the sample
416 pH is above their pKa, the NSAIDs molecules are transformed into their anionic
417 forms; therefore in this case adsorption is mainly due to electrostatic
418 interactions between the negatively charged NSAIDs molecules and positively
419 charged head groups of HDTMA bilayer, in addition to the abovementioned
420 mechanisms. In this case, all analytes presented a negative effect for sample
421 pH. Therefore, the decrease in NSAIDs adsorption when passing from the lower
422 (pH=3) to the upper level (pH=9) might be attributed to the fact that each type of
423 interaction did not present the same significance at the two pH levels. The NaCl
424 concentration presents a negative effect for FEL, IBU and KET, being
425 significant for the latter, and a positive effect for DIC. The effect of this factor will
426 be explained in the next section (section 3.4.2). Extraction time presents a
427 negative effect for all analytes. The negative effect could be due to the fact that

428 the sorption of HDTMABr surfactant on ZSM-5/Fe_2O_3 surface is by cation
429 exchange, so longer agitation time could cause surfactant losses and,
430 therefore, the concentration of analyte remaining in the supernatant is higher. In
431 addition, this negative effect revealed a rapid and effective mass transfer in the
432 proposed MDSPE method (i.e., two minutes were enough to reach the
433 adsorption equilibrium). The eluent solvent volume presents a negative effect
434 for all analytes since the lower the volume of eluent solvent the higher the
435 analyte concentration in the eluate. Finally, the elution time presents a negative
436 effect for all NSAIDs, except for DIC, although it was not significant in any case.
437 For KET, FEL and IBU one possible explanation is that at longer elution times
438 analyte concentration in the eluate is smaller due to one possible degradation of
439 NSAIDs by ultrasounds energy [68]. However, in the case of DIC, the elution
440 time presents a positive effect, which could be explained by additional hydrogen
441 bonding DIC of its amino group, presenting more sites to interact with the
442 sorbent and, therefore, a longer elution time was necessary to break these
443 interactions. According to results obtained from the Plackett-Burman design,
444 sample pH, NaCl concentration and eluent solvent volume were chosen as
445 significant factors. Thus, these factors were optimized in the next optimization
446 step. The other factors (i.e., extraction time and elution time) were fixed at the
447 most convenient level. Both extraction time and elution time were fixed at low
448 level (i.e., 2 min).
449
450 3.4.2. Optimization study
451 Different experimental designs are reported in the literature, many of which
452 are based on the so-called response surface designs. Circumscribed central

composite design (CCCD) is one of the most frequently used as response surface designs. This type of design was employed to assess the main effects, interactions effects and quadratic effects of significant factors of a previous screening step (i.e., sample pH, NaCl concentration and eluent solvent volume). It consists of a two-level factorial design (2^k), with a central point which is repeated n times and 2k star points, where k is the number of factors to optimize [66]. The value of star points depends on the desired design properties and number of factors. Star points were fixed at $\alpha = \sqrt[4]{2^k} = 1.68$ in order to ensure the rotatability of the model and the central point was repeated five times to ensure its orthogonality [66]. Low, central and high levels, and the star points of the studied factors are shown in Table S4. The overall matrix of CCCD design involved 19 experiments.

The data obtained were evaluated by ANOVA test and the effects were shown in response surfaces from NEMRODW® (Fig. S4-S6) and Pareto charts from Statgraphics® (Fig. S7). The repeatability of the central point (n=5) was assessed, obtaining coefficients of variation between 2 and 10%. Table S5 shows the optimum MDSPE conditions obtained from the response surface, which were confirmed with optimum value obtained from Statgraphics®. It can be observed that both, the values obtained for sample pH and those obtained for eluent solvent volume, were similar for all analytes investigated. However, optimum values for NaCl concentration were analyte-dependent and hence a compromise value was chosen in order to select the most favourable conditions for the simultaneous MDSPE procedure of the four NSAIDs investigated. As shown in Fig. S4-S6 and Fig.S7, all three variables considered were significant. Firstly, regarding eluent solvent volume, the response surfaces (Fig. S4)

showed higher signals at the lowest eluent solvent volume and Paretos charts confirmed the significant negative effect for all analytes (Fig. S7(a), S7(c) and S7(d)) except for FEL (Fig. S7(b)). According to Table S5, the optimum eluent solvent volume was 424 µL of methanol in all cases. This result can be easily explained since the lower the volume of eluent solvent the higher the analyte concentration in the extract, as previously explained. Secondly, with regard to sample pH, the response surfaces (Fig. S5) showed higher signals at the lowest pH values for all analytes except for DIC (Fig. S5(c)). Same results were obtained with Paretos charts since all analytes except DIC (Fig. S7(c)) presented a significant negative effect for sample pH (Fig. S7(a), S7(b) and S7(d)). According to Table S5, the optimum values for the sample pH were 2.4, 2.0, 2.5 and 2.0 for KET, FEL, DIC and IBU, respectively. They were very similar, and since pH was not significant for DIC, 2.2 was chosen as the optimal value for sample pH (i.e., compromise value between values of KET, FEL and IBU). Finally, regarding NaCl concentration, the response surface of DIC (Fig. S6(c)) showed that the highest signal was around the high level (i.e., NaCl concentration=4%) and the response surface of KET (Fig. S6(a)) showed higher signals at the lowest NaCl concentration. These results were confirmed by Paretos charts (Fig. S7) since NaCl concentration presented a positive effect for FEL, IBU and DIC, being significant only for the latter (Fig. S7(c)), and a negative effect for KET (Fig. S7(a)). For FEL, IBU and DIC one possible explanation of enhanced adsorption in the presence of NaCl in solution was probably due to the salting out effect decreases NSAIDs solubility in the aqueous sample. Similar results were obtained for bisphenol A [65]. However, in the case of KET, the negative effect might be related with the ketone group,

since this analyte is the only one with that functional group. According to Table S5, the optimum NaCl concentration values were 0, 2.7, 3.9 and 3.1 for KET, FEL, DIC and IBU, respectively. A compromise value of 2.5% NaCl was selected as an optimum value since when the NaCl concentration exceeded this value, the KET signal decreased severely.

3.4.3. Study of sorbent amount

The amount of sorbent is also a factor that affects the MDSPE procedure. This factor was not included in optimization studies because the amount of sorbent depends significantly on the analyte concentration. Therefore, the amount of sorbent was studied fixing the concentration of NSAIDs at the upper threshold of the working range (i.e., 400 μg L^{-1}). Different amounts of HDTMA-ZSM-5/Fe_2O_3 (i.e., 10, 20, 30, 40 and 50 mg) were tested to evaluate the effect of the sorbent quantity on the extraction yield of NSAIDs. Extraction experiments were performed under MDSPE optimized conditions. The results are shown in Fig. 3. Peak area of analytes increased on increasing the amount of sorbent from 10 to 40 mg. Then the adsorption of NSAIDs did not increase by increasing the amount of sorbent to 50 mg. Therefore, the sorbent amount of 40 mg was selected as the optimum value under the studied concentration.

According to the results of the optimization study, the MDSPE optimum conditions selected for simultaneous extraction of KET, FEL, DIC and IBU were: amount of sorbent, 40 mg; sample pH, 2.2; NaCl concentration, 2.5%; extraction time, 2 min; eluent solvent, methanol; elution solvent volume, 424 μL; and elution time, 2 min.

528 3.4. Validation of the method

529 Analytical figures of merit of the proposed method were assessed under
530 MDSPE optimized conditions (Table 1). The working range was from 3.3 to 400
531 μg L^{-1} for KET, from 1.7 to 400 μg L^{-1} for FEL, from 6.6 to 400 μg L^{-1} for DIC
532 and from 9.9 to 400 μg L^{-1} for IBU. The lower concentrations of working ranges
533 were limited by the limit of quantification (LOQ). The resulting calibration curves
534 gave a high level of linearity with correlation coefficients (r) of 0.998 (N=8) for
535 KET, 0.999 (N=8) for FEL, 0.997 (N=7) for DIC and 0.995 (N=6) for IBU.
536 Instrumental measurement sensitivity was estimated by the slope of the
537 calibration curves being 1.74 ± 0.02 mAU min μg^{-1} L, 3.18 ± 0.05 mAU min μg^{-1}
538 L, 1.38 ± 0.02 mAU min μg^{-1} L and 1.16 ± 0.06 mAU min μg^{-1} L for KET, FEL,
539 DIC and IBU, respectively. Method repeatability, expressed as a coefficient of
540 variation (CV), was evaluated by six replicate analyses of aqueous standard at
541 NSAIDs concentrations of 10 and 200 μg L^{-1}. CV values ranged between 2 and
542 5% (Table 1). Enrichment factors (EFs) were calculated as the ratio of the
543 signals obtained at 400 μg L^{-1} with and without MDSPE. As shown in Table 1,
544 EFs were similar for FEL, DIC and IBU (i.e., values ranged between 29.7 ± 0.5
545 and 36.4 ± 1.3). However, KET gave lower extraction performance than the
546 other NSAIDs, with an EF value of 26.1 ± 0.6. The low EF value obtained for
547 KET can be explained by the optimized extraction conditions chosen. Optimum
548 NaCl concentration for KET was 0% (Table S5). However, NaCl concentration
549 of 2.5% was chosen as optimum extraction conditions for the proposed method
550 because NaCl concentration presented a positive effect for FEL, IBU and DIC,
551 being significant for the latter in CCCD Pareto charts (Fig. S7(c)). The limit of
552 detection (LOD) was determined empirically, progressively measuring more

553 diluted concentrations of the NSAIDs [69,70]. The LOD for each NSAID was the
554 lowest concentration whose signal could be clearly distinguished from blank.
555 The LOD values were 1.0 μg L^{-1}, 0.5 μg L^{-1}, 2.0 μg L^{-1} and 3.0 μg L^{-1} for KET,
556 FEL, DIC and IBU, respectively.
557
558 3.5. Analysis of real samples
559 The applicability of the proposed method to determine NSAIDs in real water
560 and urine samples was assessed. Three water samples (namely tap water,
561 reservoir water and wastewater) and five urine samples taken from healthy
562 human volunteers were employed to assess matrix effects using recovery
563 studies. Fig. 4 shows typical chromatograms after MDSPE under optimal
564 conditions of samples non-spiked and spiked at 50 μg L^{-1} of each NSAID.
565 Preliminary analyses with the proposed method revealed that none of the
566 selected water and urine samples had initial detectable NSAIDs concentrations
567 (i.e., it can be seen in the chromatograms (a and c)). Consequently, all
568 investigated samples were spiked at two different levels (i.e., 10 and 200 μg L^{-1})
569 and analysed in triplicate. Results are summarized in Table 2 and Table 3 for
570 water and urine samples, respectively. These tables show the relative
571 recoveries determined as the ratio of the signals found after MDSPE in real
572 samples and deionized water spiked at the same concentration levels.
573 For water samples, results showed relative recoveries varying from 86 and
574 107% and CV values ranged between 1 and 8%. And for urine samples, results
575 showed relative recoveries varying from 80 and 112% and CV values ranged
576 between 1 and 14%. It should be noted that obtained relative recoveries for
577 KET in urine samples were lower than those obtained in water samples. It could

be due to the effect of ionic strength (i.e., urine samples have a content of salts in their composition which could affect extraction). In addition, initial relative recoveries for DIC in tap water sample were 0 and 36% for 10 and 200 μg L^{-1}, respectively. This was corrected by adding sodium thiosulphate to the tap water sample prior analysis to capture free chlorine that might be interacting with DIC [46], obtaining 86 and 106% for DIC at 10 and 200 μg L^{-1}, respectively. Finally, according to results, it can be concluded that matrix effects were not significant for the determination of NSAIDs in the studied water and urine samples.

3.6. Excretion study of IBU in real urine samples

The described MDSPE-LC-DAD method was successfully applied to the analysis of urine samples taken from one human volunteer who was orally treated with IBU (200 mg). Urine samples were collected at 0, 2, 3, 4, 6 and 8 h after drug administration. Fig. S8 shows the concentration-time curve of IBU. As can be seen, the urinary excretion of IBU increases to a maximum and then decreases, reaching maximum urinary excretion of the drug at 3 h post IBU dosing. IBU was detected in human urine between 2 h and 8 h at a concentration ranging from 97 ± 2 μg L^{-1} to 1087.1 ± 1.0 μg L^{-1} (n=3) and the mean IBU concentration in urine during this time period was 447 ± 4 μg L^{-1}.

3.7. Comparison with other methods

For comparative purposes, the characteristics of previously reported MDSPE-based methods using different magnetic sorbents to determine NSAIDs in water and urine samples are summarized in Table 4. As can be seen, the proposed method has the shortest extraction time. In some cases the difference

603 is outstanding, for instance, the methods developed by Li Xu et al. [31,32],
604 require 45 min to extract the analytes whereas in our method 2 min were
605 enough to reach adsorption equilibrium, which indicates the rapid and effective
606 mass transfer of the proposed method. The amount of sorbent in our work is
607 comparable to those in previous works. However, the sorbent used in this work
608 (i.e., HDTMA-ZSM-5/Fe_2O_3) has numerous advantages over the other sorbents.
609 On the one hand, in previous works magnetic nanoparticles were solvothermally
610 synthesized [31,32,71] or heated under N_2 atmosphere [34,51], while in this
611 work they were synthesized at room temperature. On the other hand, in most
612 reported methods sorbent modification is a tedious and time-consuming
613 process including the use toxic organic solvents. The empirical LODs of this
614 work are similar or slightly higher than those obtained in previous publications
615 using statistical methods (i.e., calculated using signal-to-noise ratio or the
616 standard deviation of the blank). It should be pointed that the empirical method
617 provided much more realistic LOD values [69]. Finally, extraction recoveries
618 were comparable to those obtained in previous publications.
619

620 **4. Conclusions**

621 A simple, fast, economical and user-friendly MDSPE-LC-DAD method has
622 been developed to determine NSAIDs in water and urine samples. The
623 proposed sorbent is HDTMA-ZSM-5/Fe_2O_3 composite, based on ZSM-5 zeolite
624 decorated with iron oxide magnetic nanoparticles and modified with HDTMABr
625 surfactant. Due to its magnetic properties, not only is the sorbent easy to handle
626 but also the method is time-saving since filtration and centrifugation steps are
627 unnecessary. The simple modification with a cationic surfactant provides high

extraction capacity and rapid extraction. In addition, the sorbent is economical since zeolites are low cost materials, be they synthetic or of natural origin.

The applicability of the proposed method has been successfully tested to extract NSAIDs from water and urine samples. Finally, the method enabled subsequent analysis of urine samples taken from one human volunteer orally treated with IBU. These results show the proposed method is applicable to urinary monitoring.

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642 **References**

643 [1] I. Pountos, T. Georgouli, H. Bird, P.V. Giannoudis, Nonsteroidal anti-
644 inflammatory drugs: Prostaglandins, indications, and side effects, Int. J.
645 Interf. Cytokine Mediat. Res. 3 (2011) 19-27.
646 https://doi.org/10.2147/IJICMR.S10200.

647 [2] C.K.S. Ong, P. Lirk, C.H. Tan, R.A. Seymour, An evidence-based update
648 on nonsteroidal anti-inflammatory drugs, Clin. Med. Res. 5 (2007) 19–34.
649 https://doi.org/10.3121/cmr.2007.698.

650 [3] J.M. Misurac, C.A. Knoderer, J.D. Leiser, C. Nailescu, A.C. Wilson, S.P.
651 Andreoli, Nonsteroidal anti-inflammatory drugs are an important cause of
652 acute kidney injury in children, J. Pediatr. 162 (2013) 1153–1159.
653 https://doi.org/10.1016/j.jpeds.2012.11.069.

654 [4] S. Magiera, Ş. Gülmez, A. Michalik, I. Baranowska, Application of
655 statistical experimental design to the optimisation of microextraction by
656 packed sorbent for the analysis of nonsteroidal anti-inflammatory drugs in
657 human urine by ultra-high pressure liquid chromatography, J.
658 Chromatogr. A 1304 (2013) 1–9.
659 https://doi.org/10.1016/j.chroma.2013.06.047.

660 [5] M. Petrović, M.D. Hernando, M.S. Díaz-Cruz, D. Barceló, Liquid
661 chromatography-tandem mass spectrometry for the analysis of
662 pharmaceutical residues in environmental samples: A review, J.
663 Chromatogr. A 1067 (2005) 1–14.
664 https://doi.org/10.1016/j.chroma.2004.10.110.

665 [6] I. Rodríguez, J.B. Quintana, R. Cela, R.A. Lorenzo, A.M. Carro, J.
666 Carpinteiro, Solid-phase microextraction with on-fiber derivatization for

667 the analysis of anti-inflammatory drugs in water samples, J. Chromatogr.
668 A 1024 (2004) 1–8. https://doi.org/10.1016/j.chroma.2003.10.049.
669 [7] J. Debska, A. Kot-Wasik, J. Namiesnik, Determination of nonsteroidal
670 antiinflammatory drugs in water samples using liquid chromatography
671 coupled with diode-array detector and mass spectrometry, J. Sep. Sci. 28
672 (2005) 2419–2426. https://doi.org/10.1002/jssc.200400055.
673 [8] S.S. Zunngu, L.M. Madikizela, L. Chimuka, P.S. Mdluli, Synthesis and
674 application of a molecularly imprinted polymer in the solid-phase
675 extraction of ketoprofen from wastewater, Comptes Rendus Chim. 20
676 (2017) 585–591. https://doi.org/10.1016/j.crci.2016.09.006.
677 [9] T. Wang, S. Liu, G. Gao, P. Zhao, N. Lu, X. Lun, X. Hou, Magnetic solid
678 phase extraction of non-steroidal anti-inflammatory drugs from water
679 samples using a metal organic framework of type Fe_3O_4/MIL-101(Cr), and
680 their quantitation by UPLC-MS/MS, Microchim. Acta 184 (2017) 2981–
681 2990. https://doi.org/10.1007/s00604-017-2319-8.
682 [10] G.G. Noche, M.E.F. Laespada, J.L.P. Pavón, B.M. Cordero, S.M.
683 Lorenzo, Microextraction by packed sorbent for the analysis of
684 pharmaceutical residues in environmental water samples by in situ
685 derivatization-programmed temperature vaporizer-gas chromatography-
686 mass spectrometry, J. Chromatogr. A 1218 (2011) 9390–9396.
687 https://doi.org/10.1016/j.chroma.2011.10.094.
688 [11] H. Alinezhad, A. Amiri, M. Tarahomi, B. Maleki, Magnetic solid-phase
689 extraction of non-steroidal anti-inflammatory drugs from environmental
690 water samples using polyamidoamine dendrimer functionalized with

magnetite nanoparticles as a sorbent, Talanta 183 (2018) 149–157. https://doi.org/10.1016/j.talanta.2018.02.069.

[12] T. Martinez-Sena, S. Armenta, M. de la Guardia, F.A. Esteve-Turrillas, Determination of non-steroidal anti-inflammatory drugs in water and urine using selective molecular imprinted polymer extraction and liquid chromatography, J. Pharm. Biomed. Anal. 131 (2016) 48–53. https://doi.org/10.1016/j.jpba.2016.08.006.

[13] A. Gentili, Determination of non-steroidal anti-inflammatory drugs in environmental samples by chromatographic and electrophoretic techniques, Anal. Bioanal. Chem. 387 (2007) 1185–1202. https://doi.org/10.1007/s00216-006-0821-7.

[14] A.I. Olives, V. Gonzalez-Ruiz, M.A. Martin, Isolation and quantitative methods for analysis of non-steroidal anti-inflammatory drugs, Antiinflamm. Antiallergy. Agents Med. Chem. 11 (2012) 65–95. https://doi.org/10.2174/187152312803476273.

[15] A. Kretschmer, M. Giera, M. Wijtmans, L. de Vries, H. Lingeman, H. Irth, W.M.A. Niessen, Derivatization of carboxylic acids with 4-APEBA for detection by positive-ion LC-ESI-MS(/MS) applied for the analysis of prostanoids and NSAID in urine, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 879 (2011) 1393–1401. https://doi.org/10.1016/j.jchromb.2010.11.028.

[16] T. Kosjek, E. Heath, A. Krbavčič, Determination of non-steroidal anti-inflammatory drug (NSAIDs) residues in water samples, Environ. Int. 31 (2005) 679–685. https://doi.org/10.1016/j.envint.2004.12.001.

715 [17] N.H. Hashim, S.J. Khan, Enantioselective analysis of ibuprofen,
716 ketoprofen and naproxen in wastewater and environmental water
717 samples, J. Chromatogr. A 1218 (2011) 4746–4754.
718 https://doi.org/10.1016/j.chroma.2011.05.046.
719 [18] L.M. Madikizela, L. Chimuka, Determination of ibuprofen, naproxen and
720 diclofenac in aqueous samples using a multi-template molecularly
721 imprinted polymer as selective adsorbent for solid-phase extraction, J.
722 Pharm. Biomed. Anal. 128 (2016) 210–215.
723 https://doi.org/10.1016/j.jpba.2016.05.037.
724 [19] B. Suárez, B.M. Simonet, S. Cárdenas, M. Valcárcel, Determination of
725 non-steroidal anti-inflammatory drugs in urine by combining an
726 immobilized carboxylated carbon nanotubes minicolumn for solid-phase
727 extraction with capillary electrophoresis-mass spectrometry, J.
728 Chromatogr. A 1159 (2007) 203–207.
729 https://doi.org/10.1016/j.chroma.2007.01.092.
730 [20] J. Płotka-Wasylka, N. Szczepańska, M. de la Guardia, J. Namieśnik,
731 Miniaturized solid-phase extraction techniques, Trends Anal. Chem. 73
732 (2015) 19–38. https://doi.org/10.1016/j.trac.2015.04.026.
733 [21] M.E. Torres Padrón, Z. Sosa Ferrera, J.J. Santana Rodríguez, Coupling
734 of solid-phase microextraction with micellar desorption and high
735 performance liquid chromatography for the determination of
736 pharmaceutical residues in environmental liquid samples, Biomed.
737 Chromatogr. 23 (2009) 1175–1185. https://doi.org/10.1002/bmc.1240.
738 [22] R. Mirzajani, F. Kardani, Z. Ramezani, Preparation and characterization
739 of magnetic metal–organic framework nanocomposite as solid-phase

microextraction fibers coupled with high-performance liquid chromatography for determination of non-steroidal anti-inflammatory drugs in biological fluids and tablet formulation samples, Microchem. J. 144 (2019) 270–284. https://doi.org/10.1016/j.microc.2018.09.014.

[23] A. Sarafraz-Yazdi, A. Amiri, G. Rounaghi, H. Eshtiagh-Hosseini, Determination of non-steroidal anti-inflammatory drugs in water samples by solid-phase microextraction based sol-gel technique using poly(ethylene glycol) grafted multi-walled carbon nanotubes coated fiber, Anal. Chim. Acta 720 (2012) 134–141. https://doi.org/10.1016/j.aca.2012.01.021.

[24] A. Sarafraz-Yazdi, A. Amiri, G. Rounaghi, H. Eshtiagh-Hosseini, Determination of non-steroidal anti-inflammatory drugs in urine by hollow-fiber liquid membrane-protected solid-phase microextraction based on sol-gel fiber coating, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 908 (2012) 67–75. https://doi.org/10.1016/j.jchromb.2012.09.040.

[25] Y.B. Luo, H.B. Zheng, J.X. Wang, Q. Gao, Q.W. Yu, Y.Q. Feng, An anionic exchange stir rod sorptive extraction based on monolithic material for the extraction of non-steroidal anti-inflammatory drugs in environmental aqueous samples, Talanta 86 (2011) 103–108. https://doi.org/10.1016/j.talanta.2011.08.020.

[26] W. Fan, X. Mao, M. He, B. Chen, B. Hu, Development of novel sol-gel coatings by chemically bonded ionic liquids for stir bar sorptive extraction - application for the determination of NSAIDS in real samples, Anal. Bioanal. Chem. 406 (2014) 7261–7273. https://doi.org/10.1007/s00216-014-8141-9.

765 [27] A.A. D'Archivio, M.A. Maggi, F. Ruggieri, M. Carlucci, V. Ferrone, G.
766 Carlucci, Optimisation by response surface methodology of
767 microextraction by packed sorbent of non steroidal anti-inflammatory
768 drugs and ultra-high performance liquid chromatography analysis of
769 dialyzed samples, J. Pharm. Biomed. Anal. 125 (2016) 114–121.
770 https://doi.org/10.1016/j.jpba.2016.03.045.
771 [28] V. D'Angelo, F. Tessari, G. Bellagamba, E. de Luca, R. Cifelli, C. Celia, R.
772 Primavera, M. di Francesco, D. Paolino, L. di Marzio, M. Locatelli,
773 Microextraction by packed sorbent and HPLC–PDA quantification of
774 multiple anti-inflammatory drugs and fluoroquinolones in human plasma
775 and urine, J. Enzyme Inhib. Med. Chem. 31 (2016) 110–116.
776 https://doi.org/10.1080/14756366.2016.1209496.
777 [29] M. Šafaříková, I. Šafařík, Magnetic solid-phase extraction, J. Magn.
778 Magn. Mater. 194 (1999) 108–112. https://doi.org/10.1016/S0304-
779 8853(98)00566-6.
780 [30] M. Wierucka, M. Biziuk, Application of magnetic nanoparticles for
781 magnetic solid-phase extraction in preparing biological, environmental
782 and food samples, Trends Anal. Chem. 59 (2014) 50–58.
783 https://doi.org/10.1016/j.trac.2014.04.007.
784 [31] S.W. Xue, J. Li, L. Xu, Preparation of magnetic melamine-formaldehyde
785 resin and its application to extract nonsteroidal anti-inflammatory drugs,
786 Anal. Bioanal. Chem. 409 (2017) 3103–3113.
787 https://doi.org/10.1007/s00216-017-0251-8.
788 [32] L.Y. Ma, Q. Li, J. Li, L. Xu, Preparation of highly hydrophilic magnetic
789 nanoparticles with anion-exchange ability and their application for the

790 extraction of non-steroidal anti-inflammatory drugs in environmental
791 samples, J. Sep. Sci. 41 (2018) 678–688.
792 https://doi.org/10.1002/jssc.201700881.
793 [33] M. Amiri, Y. Yamini, M. Safari, H. Asiabi, Magnetite nanoparticles coated
794 with covalently immobilized ionic liquids as a sorbent for extraction of non-
795 steroidal anti-inflammatory drugs from biological fluids, Microchim. Acta
796 183 (2016) 2297–2305. https://doi.org/10.1007/s00604-016-1869-5.
797 [34] A.A. Asgharinezhad, H. Ebrahimzadeh, Poly(2-aminobenzothiazole)-
798 coated graphene oxide/magnetite nanoparticles composite as an efficient
799 sorbent for determination of non-steroidal anti-inflammatory drugs in urine
800 sample, J. Chromatogr. A 1435 (2016) 18–29.
801 https://doi.org/10.1016/j.chroma.2016.01.027.
802 [35] V. Ferrone, M. Carlucci, V. Ettorre, R. Cotellese, P. Palumbo, A. Fontana,
803 G. Siani, G. Carlucci, Dispersive magnetic solid phase extraction
804 exploiting magnetic graphene nanocomposite coupled with UHPLC-PDA
805 for simultaneous determination of NSAIDs in human plasma and urine, J.
806 Pharm. Biomed. Anal. 161 (2018) 280–288.
807 https://doi.org/10.1016/j.jpba.2018.08.005.
808 [36] S.M. Abd Wahib, W.A. Wan Ibrahim, M.M. Sanagi, M.A. Kamboh, A.S.
809 Abdul Keyon, Magnetic sporopollenin-cyanopropyltriethoxysilane-
810 dispersive micro-solid phase extraction coupled with high performance
811 liquid chromatography for the determination of selected non-steroidal anti-
812 inflammatory drugs in water samples, J. Chromatogr. A 1532 (2017) 50–
813 57. https://doi.org/10.1016/j.chroma.2017.11.059.

814 [37] P. Baile, E. Fernández, L. Vidal, A. Canals, Zeolites and zeolite-based
815 materials in extraction and microextraction techniques, Analyst 144
816 (2019) 366–387. https://doi.org/10.1039/c8an01194j.
817 [38] S.M. Auerbach, K.A. Carrado, P.K. Dutta, Handbook of zeolite science
818 and technology, first ed., Marcel Dekker, New York, 2003.
819 [39] W.J. Roth, D. Kubička, J. Čejka, Zeolites in the 21st Century, in: B.
820 Trewyn (Ed.), Heterogeneous catalysis for today's challenges: Synthesis,
821 characterization and applications, Royal Society of Chemistry Publishing,
822 Cambridge, 2015, pp. 77-99.
823 [40] M.G. Valdés, A.I. Pérez-Cordoves, M.E. Díaz-García, Zeolites and
824 zeolite-based materials in analytical chemistry, Trends Anal. Chem. 25
825 (2006) 24–30. https://doi.org/10.1016/j.trac.2005.04.016.
826 [41] P. Chutia, S. Kato, T. Kojima, S. Satokawa, Adsorption of As(V) on
827 surfactant-modified natural zeolites, J. Hazard. Mater. 162 (2009) 204–
828 211. https://doi.org/10.1016/j.jhazmat.2008.05.024.
829 [42] P. Arnnok, N. Patdhanagul, R. Burakham, An on-line admicellar SPE-
830 HPLC system using CTAB-modified zeolite NaY as sorbent for
831 determination of carbamate pesticides in water, Chromatographia 78
832 (2015) 1327–1337. https://doi.org/10.1007/s10337-015-2965-0.
833 [43] T. Pasinli, O. Henden, Solid phase extraction of zearalenone (ZEN) from
834 beer samples using natural zeolite clinoptilolite and organo-zeolites prior
835 to HPLC determination, Ekoloji 22 (2015) 57–66.
836 https://doi.org/10.5053/ekoloji.2013.897.

837 [44] P. Arnnok, R. Burakham, Retention of carbamate pesticides by different
838 surfactant-modified sorbents: A comparative study, J. Braz. Chem. Soc.
839 25 (2014) 1720–1729. https://doi.org/10.5935/0103-5053.20140167.
840 [45] P. Salisaeng, P. Arnnok, N. Patdhanagul, R. Burakham, Vortex-assisted
841 dispersive micro-solid phase extraction using CTAB-modified zeolite NaY
842 sorbent coupled with HPLC for the determination of carbamate
843 insecticides, J. Agric. Food Chem. 64 (2016) 2145–2152.
844 https://doi.org/10.1021/acs.jafc.5b05437.
845 [46] M. Terasaki, M. Makino, Determination of chlorinated by-products of
846 parabens in swimming pool water, Int. J. Environ. Anal. Chem. 88 (2008)
847 911–922. https://doi.org/10.1080/03067310802272663.
848 [47] A. Rodrigues, A. Brito, P. Janknecht, M.F. Proena, R. Nogueira,
849 Quantification of humic acids in surface water: Effects of divalent cations,
850 pH, and filtration, J. Environ. Monit. 11 (2009) 377–382.
851 https://doi.org/10.1039/b811942b.
852 [48] E. Fernández, L. Vidal, A. Canals, Zeolite/iron oxide composite as sorbent
853 for magnetic solid-phase extraction of benzene, toluene, ethylbenzene
854 and xylenes from water samples prior to gas chromatography-mass
855 spectrometry, J. Chromatogr. A 1458 (2016) 18–24.
856 https://doi.org/10.1016/j.chroma.2016.06.049.
857 [49] P. Baile, L. Vidal, M.A. Aguirre, A. Canals, A modified ZSM-5
858 zeolite/Fe_2O_3 composite as a sorbent for magnetic dispersive solid-phase
859 microextraction of cadmium, mercury and lead from urine samples prior to
860 inductively coupled plasma optical emission spectrometry, J. Anal. At.
861 Spectrom. 33 (2018) 856–866. https://doi.org/10.1039/c7ja00366h.

[50] M. Anari-Anaraki, A. Nezamzadeh-Ejhieh, Modification of an Iranian clinoptilolite nano-particles by hexadecyltrimethyl ammonium cationic surfactant and dithizone for removal of Pb(II) from aqueous solution, J. Colloid Interface Sci. 440 (2015) 272–281. https://doi.org/10.1016/j.jcis.2014.11.017.

[51] A.A. Asgharinezhad, N. Mollazadeh, H. Ebrahimzadeh, F. Mirbabaei, N. Shekari, Magnetic nanoparticles based dispersive micro-solid-phase extraction as a novel technique for coextraction of acidic and basic drugs from biological fluids and waste water, J. Chromatogr. A 1338 (2014) 1–8. https://doi.org/10.1016/j.chroma.2014.02.027.

[52] E.J. Sullivan, J.W. Carey, R.S. Bowman, Thermodynamics of cationic surfactant sorption onto natural clinoptilolite, J. Colloid Interface Sci. 206 (1998) 369–380. https://doi.org/10.1006/jcis.1998.5764.

[53] S. Wang, Y. Peng, Natural zeolites as effective adsorbents in water and wastewater treatment, Chem. Eng. J. 156 (2010) 11–24. https://doi.org/10.1016/j.cej.2009.10.029.

[54] Y. Zhan, J. Lin, Z. Zhu, Removal of nitrate from aqueous solution using cetylpyridinium bromide (CPB) modified zeolite as adsorbent, J. Hazard. Mater. 186 (2011) 1972–1978. https://doi.org/10.1016/j.jhazmat.2010.12.090.

[55] Z. Li, R.S. Bowman, Counterion effects on the sorption of cationic surfactant and chromate on natural clinoptilolite, Environ. Sci. Technol. 31 (1997) 2407–2412. https://doi.org/10.1021/es9610693.

[56] B.K. Sodipo, A.A. Aziz, Recent advances in synthesis and surface modification of superparamagnetic iron oxide nanoparticles with silica, J.

887 Magn. Magn. Mater. 416 (2016) 275–291.
888 https://doi.org/10.1016/j.jmmm.2016.05.019.
889 [57] K. Sun, Y. Shi, X. Wang, Z. Li, Sorption and retention of diclofenac on
890 zeolite in the presence of cationic surfactant, J. Hazard. Mater. 323
891 (2017) 584–592. https://doi.org/10.1016/j.jhazmat.2016.08.026.
892 [58] M. Marković, A. Daković, D. Krajišnik, M. Kragović, J. Milić, A. Langella,
893 B. de Gennaro, P. Cappelletti, M. Mercurio, Evaluation of the
894 surfactant/phillipsite composites as carriers for diclofenac sodium, J. Mol.
895 Liq. 222 (2016) 711–716. https://doi.org/10.1016/j.molliq.2016.07.127.
896 [59] P. Luliński, J. Giebułtowicz, P. Wroczyński, D. Maciejewska, A highly
897 selective molecularly imprinted sorbent for extraction of 2-
898 aminothiazoline-4-carboxylic acid – synthesis, characterization
899 andapplication in post-mortem whole blood analysis, J. Chromatogr. A
900 1420 (2015) 16–25. doi:10.1016/j.chroma.2015.09.083.
901 [60] S. Kamaruzaman, M.M. Sanagi, S. Endud, W.A. Wan Ibrahim, N. Yahaya,
902 MCM-41 solid phase membrane tip extraction combined with liquid
903 chromatography for the determination of non-steroidal anti-inflammatory
904 drugs in human urine, J. Chromatogr. B Anal. Technol. Biomed. Life Sci.
905 940 (2013) 59–65. https://doi.org/10.1016/j.jchromb.2013.09.017.
906 [61] Z. Li, T. Burt, R.S. Bowman, Sorption of ionizable organic solutes by
907 surfactant-modified zeolite, Environ. Sci. Technol. 34 (2000) 3756–3760.
908 https://doi.org/10.1021/es990743o.
909 [62] R. Pasquino, M. di Domenico, F. Izzo, D. Gaudino, V. Vanzanella, N.
910 Grizzuti, B. de Gennaro, Rheology-sensitive response of zeolite-
911 supported anti-inflammatory drug systems, Colloids Surfaces B

912 Biointerfaces 146 (2016) 938–944.
913 https://doi.org/10.1016/j.colsurfb.2016.07.039.
914 [63] J. Lin, Y. Zhan, Z. Zhu, Y. Xing, Adsorption of tannic acid from aqueous
915 solution onto surfactant-modified zeolite, J. Hazard. Mater. 193 (2011)
916 102–111. https://doi.org/10.1016/j.jhazmat.2011.07.035.
917 [64] Y. Zhan, J. Lin, Y. Qiu, N. Gao, Z. Zhu, Adsorption of humic acid from
918 aqueous solution on bilayer hexadecyltrimethyl ammonium bromide-
919 modified zeolite, Front. Environ. Sci. Eng. China. 5 (2011) 65–75.
920 https://doi.org/10.1007/s11783-010-0277-z.
921 [65] Y. Dong, D. Wu, X. Chen, Y. Lin, Adsorption of bisphenol A from water by
922 surfactant-modified zeolite, J. Colloid Interface Sci. 348 (2010) 585–590.
923 https://doi.org/10.1016/j.jcis.2010.04.074.
924 [66] D.C. Montgomery, Design and analysis of experiments, seventh ed.,
925 Wiley-VCH, New Jersey (USA), 2009.
926 [67] L. Costa dos Reis, L. Vidal, A. Canals, Graphene oxide/Fe_3O_4 as sorbent
927 for magnetic solid-phase extraction coupled with liquid chromatography to
928 determine 2,4,6-trinitrotoluene in water samples, Anal. Bioanal. Chem.
929 409 (2017) 2665–2674. https://doi.org/10.1007/s00216-017-0211-3.
930 [68] M. Stucchi, G. Cerrato, C.L. Bianchi, Ultrasound to improve both
931 synthesis and pollutants degradation based on metal nanoparticles
932 supported on TiO_2, Ultrason. Sonochem. 51 (2019) 462–468.
933 https://doi.org/10.1016/j.ultsonch.2018.07.011.
934 [69] D.A. Armbruster, M.D. Tillman, L.M. Hubbs, Limit of detection (LOD)/limit
935 of quantitation (LOQ): Comparison of the empirical and the statistical

methods exemplified with GC-MS assays of abused drugs, Clin. Chem. 40 (1994) 1233–1238.

[70] Eurachem Guide, The fitness for purpose of analytical methods: A laboratory guide to method validation and related topics, https://www.eurachem.org/index.php/publications/guides/mv, 2014 (accessed 13 February 2019).

[71] Y. Deng, J. Shen, J. Liu, Y. Wei, C. Wang, A magnetic adsorbent grafted with pendant naphthyl polymer brush for enrichment of the nonsteroidal anti-inflammatory drugs indomethacin and diclofenac, Microchim. Acta 185 (2018). https://doi.org/10.1007/s00604-018-2913-4.

Figure captions

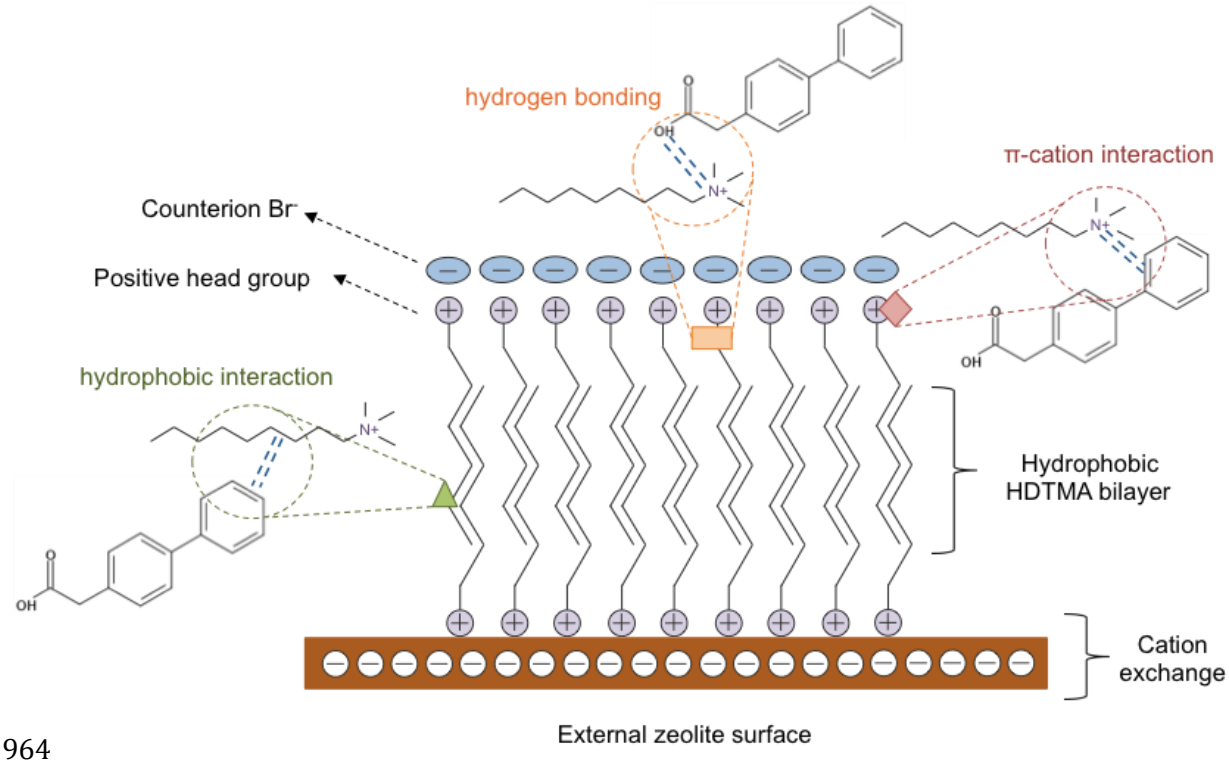
Fig. 1. Scheme of zeolite surface modified by HDTMABr surfactant adapted from [50] and the probable interactions between the analytes and HDTMA-ZSM-5/Fe_2O_3 composite adapted from [51].

Fig. 2. Pareto charts of Plackett-Burman design for: (a) KET; (b) FEL; (c) DIC; and (d) IBU.

Fig. 3. Effect of amount of sorbent. Extraction conditions: concentration of analytes, 400 µg L^{-1}; sample pH, 2.2; NaCl concentration, 2.5%; extraction time, 2 min; eluent solvent, methanol; eluent solvent volume, 424 µL; and elution time, 2 min. The error bars are the standard deviation of three replicates.

Fig. 4. Typical chromatograms after MDSPE under optimal conditions of samples non-spiked and spiked at 50 µg L^{-1} of each NSAID: (a) tap water, (b) spiked tap water, (c) urine, and (d) spiked urine. LC-DAD conditions: mobile phase, 0.01 M phosphate buffer (pH = 4.2) and acetonitrile (50:50, v/v); flow rate, 1 mL min^{-1}; injection volume, 20 µL; and wavelength, 225 nm.

963 **Fig. 1.**



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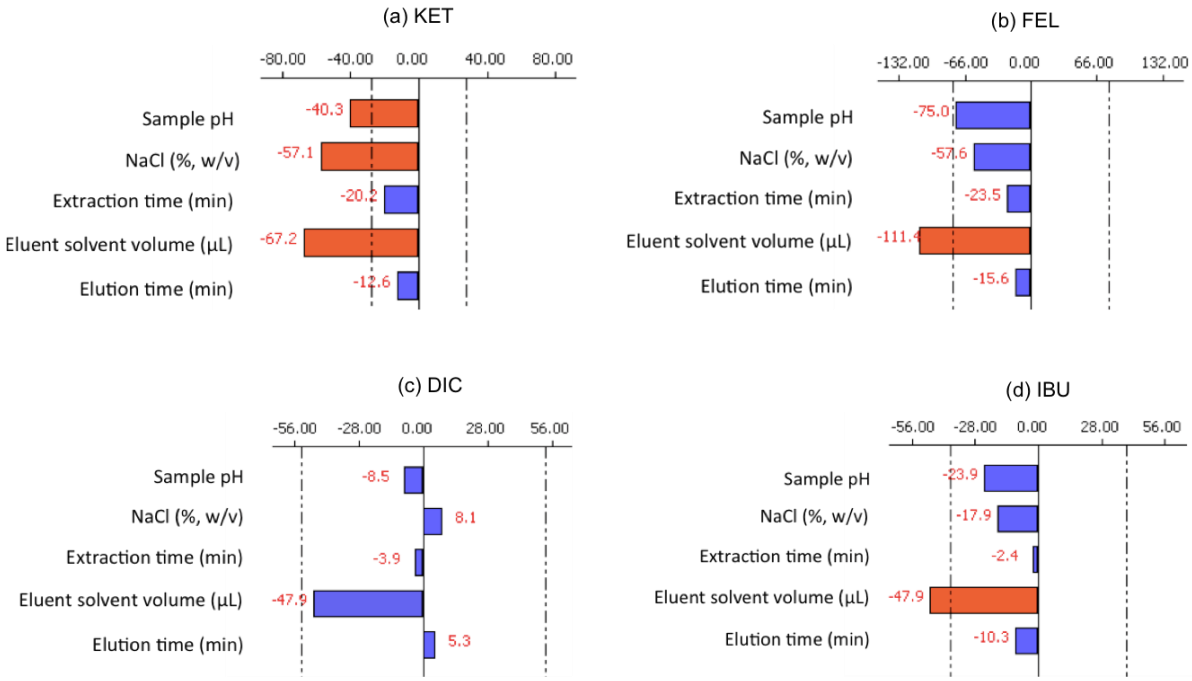
966 **Fig. 2.**



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969 **Fig. 3.**

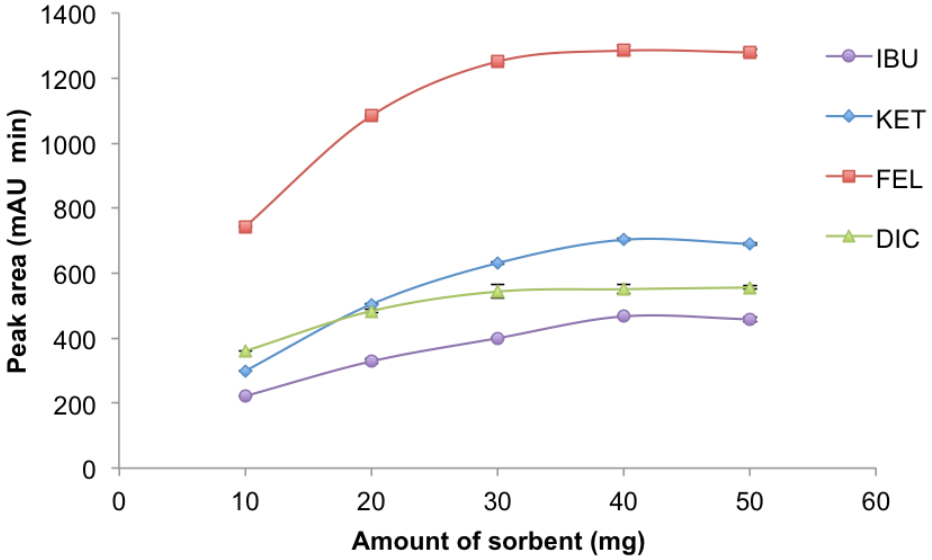

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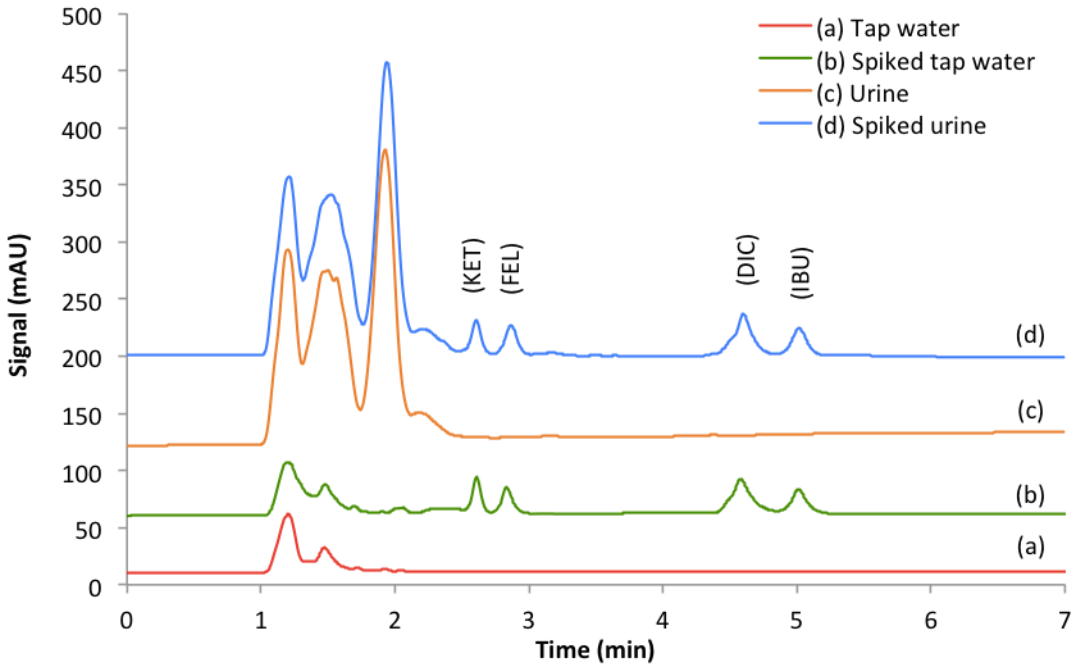
Fig. 4.


(a) Tap water
(b) Spiked tap water
(c) Urine
(d) Spiked urine
Signal (mAU)
Time (min)
(KET)
(FEL)
(DIC)
(IBU)
(a)
(b)
(c)
(d)

TABLES

Table 1. Analytical figures of merit of the proposed method (MDSPE-LC-DAD).

Analyte	Working range ($\mu g\ L^{-1}$)	r[a]	Sensitivity[b] (mAU min μg^{-1} L)	CV[c] (%)		LOD[d] ($\mu g\ L^{-1}$)	LOQ[e] ($\mu g\ L^{-1}$)	EF[f]
				10 $\mu g\ L^{-1}$	200 $\mu g\ L^{-1}$			
KET	3.3-400	0.998 (8)	1.74 ± 0.02	5	2	1.0	3.3	26.1 ± 0.6
FEL	1.7-400	0.999 (8)	3.18 ± 0.05	2	2	0.5	1.7	32.9 ± 0.9
DIC	6.6-400	0.997 (7)	1.38 ± 0.02	4	3	2.0	6.6	36.4 ± 1.3
IBU	9.9-400	0.995 (6)	1.16 ± 0.06	3	2	3.0	9.9	29.7 ± 0.5

[a] Correlation coefficient (r): number of calibration standards in parenthesis.

[b] Slope ± standard deviation.

[c] Coefficient of variation (CV): mean value for 6 replicate analyses of 10 $\mu g\ L^{-1}$ and 200 $\mu g\ L^{-1}$ spiked solutions.

[d] Limit of detection (LOD): determined by the empirical approach. The LODs were the lowest concentration whose signal could be clearly distinguished from blank [69,70].

[e] Limit of quantification (LOQ): calculated as 3.3 times the LOD.

[f] Enrichment factor (EF). EF: calculated as the ratio of the signals obtained at 400 $\mu g\ L^{-1}$ with and without MDSPE.

985 **Table 2.** Relative recoveries and CV values (in parentheses) obtained for the analytes in the three studied real water samples.

Analyte	Relative recoveries (%) and CV values in parentheses (%)[a]					
	Tap water		Reservoir water		Wastewater	
	10 µg L^{-1}	200 µg L^{-1}	10 µg L^{-1}	200 µg L^{-1}	10 µg L^{-1}	200 µg L^{-1}
KET	88 (5)	107 (4)	98 (1)	100 (1)	101 (2)	97 (1)
FEL	90 (3)	106 (2)	92 (2)	102 (1)	94 (1)	99 (1)
DIC	86 (4)	105 (4)	101 (1)	96 (1)	100 (1)	94 (2)
IBU	101 (8)	104 (2)	97 (1)	93 (1)	103 (4)	90 (2)

986 [a]Three replicate analyses at indicated spiking levels.

987

988 **Table 3.** Relative recoveries and CV values (in parentheses) obtained for the analytes in the five studied urine samples.

Analyte	Relative recoveries (%) and CV values in parentheses (%)[a]									
	Urine 1		Urine 2		Urine 3		Urine 4		Urine 5	
	10 µg L^{-1}	200 µg L^{-1}	10 µg L^{-1}	200 µg L^{-1}	10 µg L^{-1}	200 µg L^{-1}	10 µg L^{-1}	200 µg L^{-1}	10 µg L^{-1}	200 µg L^{-1}
KET	80 (8)	84 (1)	85 (9)	88 (10)	87 (3)	87 (3)	85 (14)	95 (3)	85 (7)	94 (2)
FEL	92 (1)	91 (1)	86 (4)	81 (1)	99 (12)	91 (5)	90 (6)	97 (1)	98 (11)	87 (4)
DIC	99 (3)	100 (3)	99 (6)	86 (3)	102 (3)	96 (8)	101 (12)	106 (2)	95 (11)	103 (4)
IBU	112 (4)	102 (11)	107 (4)	88 (14)	111 (6)	87 (8)	107 (6)	107 (1)	90 (13)	98 (6)

989 [a]Three replicate analyses at indicated spiking levels.

990

991 **Table 4.** Comparison of methods based on MDSPE for NSAIDs determination in urine and water samples.

Sorbent	Synthesis MNPs	Sample	Amount of sorbent (mg)	Extraction time (min)	Detection technique	LOD (μg L^{-1})	ER (%)[a]	Ref.
MMFR	Solvothermal method	Urine Milk	20	45	HPLC-UV	0.3 (KET)[b] 0.3 (KET)[b]	78	[31]
Fe_3O_4@SiO_2-MPTMS-DDA	Solvothermal method	Water	20	45	HPLC-UV	1.5-3.0 (KET)[b]	64	[32]
PNA grafted MNPs	Solvothermal method	Water	10	5	HPLC-UV	0.64 DIC[b]	-	[71]
GO/Fe_3O_4@PABT	Heating under N_2 atmosphere	Urine	16	-	HPLC-DAD	0.2 (DIC)[b] 0.3 (IBU)[b]	86 90	[34]
Fe_3O_4@decanoic acid nanoparticles	Heating under N_2 atmosphere	Water Plasma Urine	10	5	HPLC-UV	1.5 (DIC)[b] 3.5 (DIC)[b] 4.5 (DIC)[b]	77 64 67	[51]
MS-CNPrTEOS	Room temperature	Water	40	10	HPLC-UV	0.29-0.45 (KET)[c] 0.24-0.29 (DIC)[c] 0.51-0.34 (IBU)[c]	83-92 72-73 75-87	[36]
HDTMA-ZSM-5/Fe_2O_3	Room temperature	Water Urine	40	2	LC-DAD	1.0 (KET)[d] 0.5 (FEL)[d] 2.0 (DIC)[d] 3.0 (IBU)[d]	55 70 77 64	This work

992 [a]Extraction recovery (ER(%)): it was calculated by the following equation, $ER(\%) = EF \cdot \frac{volume_{final}}{volume_{initial}} \cdot 100$

993 [b]Calculated as a signal-to-noise ratio equal to 3.

994 [c]Calculated using $3s_{blank}/m$, where s_{blank} is the standard deviation of blank and *m* is a slope of the calibration curve.

995 [d]Determined by the empirical approach. The LODs were the lowest concentration whose signal could be clearly distinguished from
996 blank [69,70].

997 MNPs, magnetic nanoparticles; MMFR, magnetic melamine-formaldehyde resin; HPLC-UV, high performance liquid chromatography-ultraviolet; KET,
998 ketoprofen; Fe_3O_4@SiO_2-MPTMS-DDA, diallyldimethylammonium chloride modified magnetite nanoparticles; PNA grafted MNPs, Poly(2-naphthyl acrylate)
999 grafted magnetic nanoparticles; DIC, diclofenac; GO/Fe_3O_4@PABT, Poly(2-aminobenzothiazole)-coated graphene oxide/magnetite nanoparticles; HPLC-DAD,
1000 high performance liquid chromatography-diode array detector; IBU, ibuprofen; MS-CNPrTEOS, magnetic sporopollenin-cyanopropyltriethoxysilane.