Determination of four bisphenols in water and urine samples by magnetic dispersive solid-phase extraction using a modified zeolite/iron oxide composite prior to liquid chromatography diode array detection†

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Abbreviations

BPA	Bisphenol A
BPAF	Bisphenol AF
BPAP	Bisphenol AP
BPP	Bisphenol P
BPs	Bisphenols
CV	Coefficient of variation
dSPE	Dispersive solid-phase extraction
DSPME	Dispersive solid-phase microextraction
EEs	Extraction efficiencies
EFs	Enrichment factors
HDTMABr	Hexadecyltrimethylammonium bromide
$K_{o/w}$	Partition coefficient
LC-DAD	Liquid chromatography-diode array detector
LOD	Limit of detection
LOQ	Limit of quantification
MDSPE	Magnetic dispersive solid-phase extraction
NSAIDs	Nonsteroidal anti-inflammatory drugs
r	Correlation coefficients
SPE	Solid-phase extraction
ZSM-5/Fe_2O_3	Zeolite-based magnetic composite

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Abstract

A novel approach is presented to determine four bisphenols in water and urine samples, employing magnetic dispersive solid-phase extraction combined with liquid chromatography and diode array detection. A modified zeolite-based magnetic composite was used as an efficient sorbent, combining the advantages of magnetic materials with the remarkable properties of zeolites. A multivariate optimization design was employed to optimize some experimental factors affecting magnetic dispersive solid-phase extraction. The method was evaluated under optimized conditions (i.e., amount of sorbent, 50 mg; sample pH, unadjusted; NaCl concentration, 1.25%; extraction and elution time, 2 min; eluent solvent, ethanol; eluent solvent volume, 400 µL), obtaining good linearity with correlation coefficients ranging between 0.995 and 0.999 (N=5) (from 2 to 250 µg L^{-1} for bisphenol A, bisphenol AP and bisphenol P and from 5 to 250 µg L^{-1} for bisphenol AF). Method repeatability was assessed obtaining coefficients of variation between 3 and 11% (n=6). Finally, the method was applied to spiked real samples, obtaining for water samples relative recoveries between 83 and 105% and, for urine samples between 81 and 108% for bisphenol A, bisphenol AP and bisphenol AF, and between 47 and 59% for bisphenol P.

1. Introduction

Bisphenols (BPs) are a series of synthetic organic compounds with two hydroxyphenyl functional groups that include several analogous structures such as bisphenol A (BPA), bisphenol AP (BPAP), bisphenol AF (BPAF), bisphenol P (BPP),

among others. The bisphenol used widely is the BPA. It is mainly used as a monomer to synthesize polymeric materials such as polycarbonates and epoxy resins in the plastics industry for food containers and packaging materials, coatings on metal cans and bottle tops [1]. Human exposure to BPA primarily occurs from oral ingestion and dietary sources and through inhalation and dermal routes [2]. BPA is one of the most documented endocrine disruptors in humans due to its structural similarity to natural hormones such as estradiol and diethylstilbestrol. The estrogenic activity of BPA was first reported by Krishnan et al. in 1993 [3]. Subsequent studies showed that BPA has adverse effects on the immunological system [4], thyroid hormone action [5] and hepatic function [6] and recent studies suggest that BPA is associated with obesity, diabetes and cardiovascular diseases [7]. Given the potentially adverse effects of BPA in humans, there has been growing concern about BPA release into food and environmental matrices worldwide in recent years. Therefore, many analytical methods for BPA determination in environmental, food and biological samples have been developed in the last two decades [2,8,9].

Due to the extremely low concentration of BPs in real samples and the great complexity of matrices (e.g., biological samples), it is necessary to develop sensitive BP extraction techniques for sample preparation. Solid-phase extraction (SPE) is by far the most common extraction technique to preconcentrate BPA due to easy operation mode and effective purification [9]. However, SPE has several drawbacks (i.e., packing columns, long extraction times due to the time-consuming process of loading large-volume samples, high organic-solvent consumption, among others) and has thus undergone several modifications to date, mainly related to dimension reduction. Recently, magnetic dispersive solid-phase extraction (MDSPE) has received attention due to its advantages over SPE. Typically, MDSPE consists,

firstly, of directly dispersing a magnetic sorbent into the sample; secondly, after extraction, the magnetic sorbent enriched with analytes is separated from the solution by applying an external magnetic field; and subsequently, analytes can be desorbed using a proper eluent solvent or temperature for further determination [10,11]. Compared with conventional SPE, the main advantage of MDSPE concerns increased extraction rates (i.e., rapid mass transfer of analyte) due to improved contact area between sorbent and sample. In addition, the sorbent is easily and quickly separated from the sample by using an external magnetic field [12]. Both advantages significantly cut the extraction procedure duration. Recently, the number of studies reporting BP preconcentration using magnetic or magnetizable materials has increased (i.e., modified magnetic nanoparticles [13], carbon microspheres [14,15], graphene [16,17], molecularly imprinted polymer [18,19] and metal organic framework [20,21], among others). Nowadays, one of the main MDSPE-related research topics involves the search for new sorbents with good selectivity and high adsorption capacity, which are thermally and chemically stability, economical and environmentally friendly. Zeolites, which are microporous crystalline aluminosilicates, are good MDSPE-sorbent candidate due to their simple modification to obtain the desired physical properties (i.e., magnetism). Recently, zeolite-based magnetic composites have been proposed for organic-compound [22–26] and metal [27–30] extraction from environmental, biological and food samples. Furthermore, the external surface of zeolites can easily be modified with functional groups, thus altering zeolite adsorption properties. Accordingly, the main modification to increase preconcentration capacity of organic compounds is cationic surfactant treatment by associating hydrophobic tails of the surfactant molecules in order to form a bilayer on the zeolite surface. Cationic surfactants offer faster extraction and can provide a

wide range of interactions between modified zeolite-based composites and organic compounds: (i) hydrophobic, (ii) hydrogen bonding, (iii) π-cation and (iv) electrostatic. To date, surfactant modified zeolite-based composites have been employed to preconcentrate carbamates [31,32], mycotoxins [33] and nonsteroidal anti-inflammatory drugs (NSAIDs) [26] with analytical purposes. However, to the best of our knowledge, there are no published methods based on MDSPE using zeolite-based composites as magnetic sorbent for preconcentration of BPs in water and biological samples.

The purpose of this study is to present a simple and fast analytical method to determine four bisphenols (i.e., BPA, BPAP, BPAF and BPP) (**Table S1**) in water and urine samples using a zeolite-based magnetic composite modified with the hexadecyltrimethylammonium bromide surfactant (i.e., HDTMA-ZSM-5/Fe_2O_3) as a sorbent for MDSPE and liquid chromatography-diode array detector (LC-DAD) for analysis. The proposed HDTMA-ZSM-5/Fe_2O_3 composite was employed in a previous work [26] to determine NSAIDs in water and urine samples. However, in this study BPs have been determined due to the aforementioned concern about the release of BPs into food and environmental samples, and the need to monitor their presence in biological fluids to prevent or diagnose adverse health effects. The aforementioned method was validated and finally successfully applied to analyse BPs in water and urine samples.

2. Materials and methods

2.1. Reagents

Bisphenol A (BPA), bisphenol AP (BPAP), bisphenol AF (BPAF) and bisphenol P (BPP) were all obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock standard solutions of individual compounds (1000 mg L^{-1}) and mix stock solutions containing

the four BPs (5 and 100 mg L^{-1}) were prepared in LC grade methanol from Sigma-Aldrich and were stored in the dark at 4 ºC.

LC grade acetonitrile from Sigma-Aldrich and ultrapure water (resistivity of 18.2 MΩ cm at 25 °C) obtained from a Millipore Direct System Q5™ purification system from Ibérica S.A. (Madrid, Spain) 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). Reactive grade $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ and hexadecyltrimethylammonium bromide (HDTMABr, ≥99% purity) were obtained from Sigma-Aldrich. NaOH (97% purity, pellets) and LC grade ethanol absolute were obtained from Scharlau Chemie (Sentmenat, Spain).

Reactive grade NaCl (99% purity) was obtained from Scharlau Chemie to adjust NaCl concentration of water and urine samples before analysed. Finally, H_3PO_4 (85% purity) from Scharlau Chemie, KH_2PO_4 and K_2HPO_4 pro-analysis from Merck (Darmstadt, Germany) were employed to prepare buffer solutions during sample pH optimization.

For comparative purpose, QuEChERS (6 g $MgSO_4$ and 1.5 g CH_3COONa) and dSPE (25 mg C18 and 150 mg $MgSO_4$), both supplied by Agilent Technologies (Lake Forest, CA, USA), were employed.

2.2. Samples

Samples of drinking water from Valencia (Spain), river water from Alicante (Spain) and wastewater from a wastewater treatment plant in Barcelona (Spain) were employed as real water samples. Water samples were collected in amber glass containers, and urine samples from healthy human volunteers were collected in

sterilized containers. Both samples were stored in the dark at 4 ºC. Before use, 1.25% (w/v) of NaCl was added to water and urine samples, bearing in mind that urine samples present an initial salt content. Samples were filtered with 0.45 µm pore-size nylon filters from Millipore, therefore, the soluble BPs in samples were the measurand.

2.3. Materials and instrumentation

The sample compartment was a 22 mL glass vial with a screw top (solid green Melamine cap and PTFE liner) from Supelco (Bellefonte, PA, USA). A disc-shaped neodymium (Nd) magnet with a nickel-plated (Ni-Cu-Ni) coating (ref. S-45-30-N, N45 grade, 45 mm diameter, 30 mm height) from Supermagnete (Gottmadingen, Germany) was used to handle the magnetic sorbent during synthesis and phase separation after MDSPE.

The chromatographic analyses were performed with an Agilent 1260 Infinity LC system (Agilent Tecnhologies, 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 Tecnhologies). A Synergy™ Fusion-RP 80 Å C18 column (250 mm x 4.6 mm I.D., 4 µm particle size) from Phenomenex (Torrance, CA, USA) was employed for analyte separation. Analytes were eluted in gradient mode using LC grade acetonitrile (mobile phase A) and ultrapure water (mobile phase B) with a flow rate of 1 mL min^{-1}. Elution program was as follows: firstly, 50% mobile phase A and 50% mobile phase B was kept for 15 min; then, mobile phase A was increased to 80% from 15 min to 16 min and this condition was kept constant up to 21 min; finally from 21 min to 22 min, mobile phase A was returned to the initial percentage

(50%) and this condition was held constant until the end of the chromatographic analysis (25 min). The volume injection was 20 µL. The detection was set at 210 nm. **Fig. 1** shows a chromatogram of a urine sample spiked at 1 mg L^{-1} and a chromatogram of extract of urine sample spiked at 250 µg L^{-1} after MDSPE under optimized conditions.

2.4. Synthesis of zeolite/iron oxide magnetic composite

The synthesis of the ZSM-5/Fe_2O_3 composite was carried out following the procedure described in our previous works [26,28] (see the ESI† for further details).

2.5. Modification of zeolite/iron oxide magnetic composite

The modification of the ZSM-5/Fe_2O_3 composite with HDTMABr surfactant to obtain the HDTMA-ZSM-5/Fe_2O_3 composite was carried out following the procedure described in our previous work [26] (see the ESI† for further details). According to a previous publication [26], the HDTMABr surfactant forms a bilayer (i.e., surfactant concentration is higher than the critical micelar concentration) on the external surface of the zeolite, resulting in a charge reversal on the external surface of the zeolite from negative to positive. The positive charge, which is balanced by bromide counterions of surfactant, is given by outward-pointing head groups of HDTMA bilayers [34].

2.6. Magnetic dispersive solid-phase extraction

The overall procedure of MDSPE is graphically described in **Fig. 2**. Firstly, 50 mg of HDTMA-ZSM-5/Fe_2O_3 was placed in a 22 mL glass vial. Next, 20 mL of sample or a standard solution with 1.25% (w/v) of added NaCl was transferred to a vial and the mixture was shaken for 2 min. After extraction, the disc-shaped Nd magnet was used to separate the enriched sorbent from the solution, and the aqueous phase was removed. Then, 400 µL of ethanol was added to elute adsorbed

analytes using an ultrasonic bath for 2 min. Thereafter, the eluate was separated from the composite employing the disc-shaped Nd magnet, withdrawn with a syringe, filtered with 0.22 μm pore size nylon filters and finally transferred to a vial for further determination by LC-DAD.

2.7. Data processing

A multivariate optimization strategy (i.e., Plackett-Burman design) was carried out to determine the optimum conditions for MDSPE. The statistical software NEMRODW® ("New Efficient Methodology for Research using Optimal Design") from LPRAI (Marseille, France) was used to build the experimental design matrix and evaluate the results.

3. Results and discussion

3.1. Synthesis reproducibility of magnetic composite

The synthesis reproducibility of HDTMA-ZSM-5/Fe_2O_3 composite was investigated. To this end, the extraction performance was compared of three HDTMA-ZSM-5/Fe_2O_3 composites synthetized in parallel. Extractions from aqueous standard solutions containing 500 μg L^{-1} of BPA, BPAP, BPAF and BPP were carried out with each sorbent; results are shown in **Fig. S1**. As can be observed, the extractions with the three HDTMA-ZSM-5/Fe_2O_3 composites presented a similar response, thus demonstrating the reproducibility of the synthesis and modification procedures for the intended application.

3.2. Magnetic dispersive solid-phase extraction optimization

First, it should be mentioned that the amount of sorbent is a factor which depends significantly on analyte concentration and, as the screening-study concentration is high, this could lead to no general value. Therefore, this factor was not included in the following multivariate optimization study (see section 3.2.2). Thus,

based on the optimum value obtained for the amount of sorbent in a previous work [26], in which HDTMA-ZSM-5/Fe_2O_3 composite was also employed, and preliminary experiments with BPs (data not shown) revealed that 50 mg was the adequate for the concentration commonly found of model analytes in the investigated samples. Consequently, this value was used for all experiments carried out in this work.

3.2.1. Elution solvent type

The type of eluent solvent was also studied separately and not included in the next multivariate optimization because the Plackett-Burman design investigates the factors at two levels, and four solvents were tested. In this study, extractions from aqueous standard solutions with 500 μg L^{-1} of the four BPs were carried out with each eluent solvent and results are shown in **Fig. S2**. As can be observed, the extractions with methanol showed the lowest signals whereas the extractions with acetone and ethanol provided the highest signals, however the error bars (i.e., standard deviations of three replicates) for acetone were the highest ones, presenting coefficients of variation between 9 and 19%. Signals obtained with acetonitrile and ethanol were similar for BPA and BPAF and, for BPP the signal obtained with acetonitrile was lower than that with ethanol. In addition, BPAP could not be eluted by acetonitrile. For all the reasons mentioned above, ethanol was selected as eluent solvent.

3.2.2. Screening study

Plackett-Burman is a two-level fractional factorial design [35] that was employed to identify those factors that have important effects in MDSPE .Based on the literature and the previous experience of the research group [26], the five considered factors were: sample pH, extraction time, elution time, eluent solvent volume and NaCl concentration. **Table S2** shows the experimental factors and levels

considered and **Table S3** shows the matrix of experiments of the Plackett-Burman design, including five factors studied in eight runs. The eight experiments were carried out randomly using 20 mL of aqueous standards containing 500 μg L^{-1} of each BP. The peak area of each BP was employed as response function in order to evaluate the effect of different factors over each individual compound. It should be noted that, the sum of the peak areas of each BP provided a Pareto chart (data not shown) displaying the same results.

The data obtained were evaluated by ANOVA and the results were visualized with the Pareto charts shown in **Fig. S3**. As can be seen, BPAF (**Fig. S3(c)**) and BPAP (**Fig. S3(d)**) presented similar results to BPA (**Fig. S3(a)**). Those effects exceeding the reference vertical line can be considered significant with 95% of probability. Negative and positive signs revealed whether the system responses decreased or increased, respectively, when passing from the lower to the upper level of the corresponding factor.

As observed in **Fig. S3**, factors showing a significant effect were eluent solvent volume and NaCl concentration, the latter only being significant for BPP (**Fig. S3(b)**). The negative effect of eluent solvent volume was because the lower the volume of eluent solvent, the higher the analyte concentration in the eluate. Although both factors were significant, only NaCl concentration factor was selected for the next optimization step, the effect for which will be explained in depth in the next section (section 3.2.3.). Eluent solvent volume was not considered further because volumes lower than 400 μL (lower level of the Plackett-Burman design) could not be handled easily after elution and, consequently, a volume of 400 μL ethanol was set for subsequent experiments. The other three factors (i.e., sample pH, extraction and elution time) considered in the screening step did not show significant effects.

Regarding sample pH factor, the pka values of BPA, BPAP, BPAF and BPP ranged from 8.7-10.3 (**Table S1**). Based on a previous work [26], the suggested mechanisms governing the adsorption of BPs onto HDTMA-ZSM-5/Fe_2O composite could be: (i) electrostatic interaction between the positive head groups of bilayer and the anionic groups of BPs depending on pH solution; (ii) π-cation interaction between the aromatic rings of BPs and quaternary ammonium groups of bilayer; (iii) hydrogen bonding between the nitrogen atoms of bilayer and phenol groups of BPs; and (iv) hydrophobic interactions between the hydrophobic C chains of bilayer and the aromatic rings of BP. At sample pH below their pKa (i.e., pH sample=3), the studied analytes are neutral molecules whereas above pKa (i.e., pH sample=11), the BPs molecules are in their anionic forms. The sample pH presented a positive effect for all analytes **Fig. S3**. In this case, this behaviour suggested that anionic BPs have higher affinity towards HDTMA bilayer than the neutral ones [36]. Regarding to extraction time, a negative effect was obtained, which is in accordance with a previous publication [26]. It is generally expected that analyte adsorption reaches equilibrium (i.e., signal remains constant) after a certain period of time. However, the negative effect could be related to a longer agitation time, which could cause surfactant losses. In any case, a rapid and effective mass transfer took place in the proposed MDSPE method (i.e., two minutes were sufficient to reach adsorption equilibrium). Finally, the elution time presented positive effect except for BPP (**Fig. S3(b)**). No explanation has been found for this anomalous result. As aforementioned, sample pH, and elution and extraction times showed non-significant effects, therefore they were fixed at the most practical levels. Sample pH was not adjusted, and both elution and extraction times were fixed at low level (i.e., 2 min), thus speeding up the extraction process.

3.2.3. NaCl concentration optimization

The effect of NaCl concentration was investigated keeping the rest of factors at the optimum values obtained in the screening study. Different amounts of NaCl (i.e., 0, 0.5, 1.25 and 2.0 (%, (w/v)) were tested to evaluate the effect of NaCl upon the extraction yield of BPs. To this end, extractions from aqueous standard solutions with 500 μg L^{-1} of the four BPs were carried out. As can be observed in **Fig. S4**, peak area of analytes increased on increasing the amount of NaCl from 0 up to 1.25%, and from this value the signals decreased. The optimum value of NaCl is in accordance with a previous publication [36]. The increased adsorption in the presence of NaCl was probably due to the salting out effect, which decreased the solubility of BPs [36]. However, decreased extraction efficiency at high NaCl values could be related to competitiveness between Cl^- and BPs for the positively charged outward-pointing head groups of HDTMA bilayer.

Given that this analytical method is devoted to determining BPs in water and urine samples, it is important to point out that optimal conditions for urine were extrapolated from those obtained for water samples, although urine samples contain an amount of different salts [37]. Preliminary studies (data not shown) with urine showed that while, on the one hand, adding the optimum NaCl value obtained (i.e., 1.25 % (w/v)) did not decrease analyte signals, and on the other hand, the NaCl addition is necessary because sorbent manipulation is easier after extraction. This effect can be observed in **Fig. S5**. The figure shows four vials after extraction (**Fig. S5(a)**) and after magnetic separation (**Fig. S5(b)**), respectively. Extraction vials contain the sorbent and: (i) water without NaCl addition; (ii) water with 1.25 % of NaCl; (iii) urine without NaCl addition; and, (iv) urine with 1.25 % of NaCl. In vials without NaCl addition, part of the sorbent is retained in a foam formed on the top of

the liquid phase (vials (i) and (iii) from **Fig. S5(b)**). However, when an amount of NaCl is added this foam is completely free of sorbent (vials (ii) and (iv) from **Fig. S5(b)**). This could be related with the variation of the ionic strength when salt is added.

Finally, based on the whole optimization study, the MDSPE conditions selected for BPs extraction were: amount of sorbent, 50 mg; sample pH, without adjustment; NaCl concentration, 1.25 % (w/v); extraction time, 2 min; eluent solvent, ethanol; eluent solvent volume, 400 µL and elution time, 2 min.

3.3 Validation of the method

The main analytical figures of merit of the proposed method are summarized in **Table 1**. The working range (from 2 to 250 µg L^{-1} for BPA, BPAP and BPP, and from 5 to 250 µg L^{-1} for BPAF) showed good linearity with correlation coefficients (r) of 0.999 (N=5) for BPA, BPAP and BAF and of 0.995 (N=5) for BPP. It should be mentioned that the lower concentrations of working ranges were restricted by the limit of quantification (LOQ). The sensitivity of the instrumental measurements ranged between 1.34 ± 0.02 mAU min μg^{-1} L for BPAF and 2.83 ± 0.04 mAU min μg^{-1} L for BPAP. The repeatability of the method, expressed as coefficient of variation (CV), was evaluated by analysing six aqueous standards spiked at 20 and 100 µg L^{-1}. The obtained CVs varied between 3% and 11% (**Table 1**). Enrichment factors (EFs) were evaluated through the ratio of the signals obtained at 250 µg L^{-1} with and without MDSPE. The limit of detection (LOD) was determined empirically, measuring progressively more diluted concentrations of the BPs [38]. It should be noted that the empirical method provided much more realistic LOD values [38]. The LOD for each BP was the lowest concentration whose signal could be clearly distinguished from blank. The LOD values were 0.6 µg L^{-1} for BPA, BPAP and BPP and 1.5 µg L^{-1} for

BPAF. Finally, EFs were similar for BPA, BPAP and BAF (i.e., values ranged between 32.2 ± 2.3 and 35.8 ± 4.0). However, BPP gave the lowest extraction performance of the studied BPs, with an EF value of 20.1 ± 1.8. The extraction efficiencies (EEs (%)) for the complete sample preparation procedure were 71.6% for BPA, 65.0% BPAP, 64.4% BPAF and 40.2% BPP.

3.4. Analysis of real samples

The applicability of the proposed method to determine BPs in real water and urine samples was evaluated studying matrix effects using recovery studies. Investigated samples were initially analysed and their BP concentration resulted under the LOD of the proposed method. Thus, all investigated samples were spiked at two different levels (i.e., 20 and 100 μg L^{-1}). **Table 2** (i.e., water samples) and **Table 3** (i.e., urine samples) show the relative recoveries determined as the ratio of the signals found after MDSPE in real samples and deionized water spiked at the same concentration levels. For water samples, results showed relative recoveries varying from 83 and 105% (CVs between 1 and 10%). For urine samples, results showed relative recoveries varying from 81 and 108% for BPA, BPAP and BPAF (CVs between 1 and 13%). For BPP in urine samples, relative recoveries ranged from 47 to 59% (CVs between 1 and 13%). Recoveries obtained for BPP in real water samples showed lower values than for the other analytes. In the urine samples this negative matrix effect was accentuated. These values are correlated with EFs obtained in Table 1 since BPP showed the lowest EF. Probably, low recoveries might be related to steric effects or electrostatic interactions impeding BPP extraction. It should be noted that these initial values could be corrected on the one hand, by multiplying them by a factor (i.e., 2) and thus obtaining relative recoveries between 94 and 118%, due to the fact that the same effect is observed for different

urine samples; or, on the other hand, employing the standard addition calibration. Finally, according to these results, it can conclude that matrix effects were not significant for BP determination in the investigated water and urine samples, except for BPP in urine samples.

3.5. Comparison with other methods

A comparison between this work and previously reported procedures combining different SPE techniques and sorbents for the determination of several BPs in urine and water samples is summarized in **Table S4** [16,39–43]. As shown, the proposed method provides LODs comparable with previous works using a shorter extraction time (i.e., 2 min compared to 10 [39,41], 15 [16] and 30 min [43]). The publications that presented slightly lower LODs [16,41,42] included additional time-consuming steps of solvent evaporation and reconstitution in the extraction procedure. On the other hand, it is important to highlight that LODs reported in this work are empirical [38] whereas LODs recorded for previous methods were obtained employing statistical methods (i.e., calculated using signal-to-noise ratio or the standard deviation of the blank), which provide commonly lower LODs. In addition, the LOD for BPA in this work is comparable to the one obtained using micro-QuEChERs extraction coupled to GC-MS [42] employing more economical analytical instrumentation (i.e., LC-DAD). Regarding to amount of sorbent, this work shows similar amounts of sorbent to previous works, except for methods based on conventional SPE (i.e., 200 and 300 mg) [39,40] or QuEChERS (i.e., 750 mg) [42], which consume sorbent amounts that are one order of magnitude greater. Furthermore, the extraction technique developed here presents advantages related to ease of sorbent handling (i.e., magnetic properties) versus other techniques such as SPE and dispersive solid-phase microextraction (DSPME) (called SPME in

publication [41] and micro-QuEChERS [42]), avoiding column preparation [39,40] and centrifugation steps [41,42], respectively. Between the different methods based on MDSPE, on the one hand, the magnetic composite employed as sorbent in this work affords easier magnetisation synthesis than the other composites [16,43] (i.e., solvothermal method) and, on the other hand, HDTMA-ZSM-5/Fe_2O_3 composite is more economical than graphene-based composites [16] (e.g., (3DG)/$ZnFe_2O_4$) since zeolites are considered low cost materials.

Finally, to compare our method with a method using micro-QuEChERS [42], BPA was extracted from a urine sample using both sample preparation methods, employing the same separation/detection (i.e., LC-DAD). The results showed that our method gives higher sensitivity than the micro-QuEChERS method. The peak areas of BPA are 38.0 ± 1.1 mAU min and 248 ± 5 mAU min for 1 mg L^{-1} (QuEChERS) and 100 µg L^{-1} (HDTMA-ZSM-5/Fe_2O_3), respectively. This significant difference in sensitivities is due to the fact that QuEChERS are used for analyte extraction commonly using acetonitrile and, extract clean-up by dispersive solid-phase extraction (dSPE), whereas our method simultaneously offers analyte extraction and preconcentration and sample clean-up.

4. Concluding remarks

A new MDSPE-LC-DAD method using a modified zeolite-based magnetic composite has been developed and applied successfully to determine trace levels of four BPs in water and urine samples. It is important to emphasize that the proposed sorbent, HDTMA-ZSM-5/Fe_2O_3, provided fast synthesis and simple modification, offering a high extraction capacity and rapid extraction. In addition, zeolites are inexpensive materials with unique sorbent properties that can be of synthetic or natural origin, thus being widely available. Finally, it should be mentioned that

although there are a wide variety of extraction and microextraction techniques nowadays, MDSPE presents two main advantages: (i) improvement of the large contact area between the sorbent and the sample compared to SPE and miniaturized techniques; and (ii) shorter sample preparation times compared to DSPME due to easy handling of sorbent and centrifugation free protocol.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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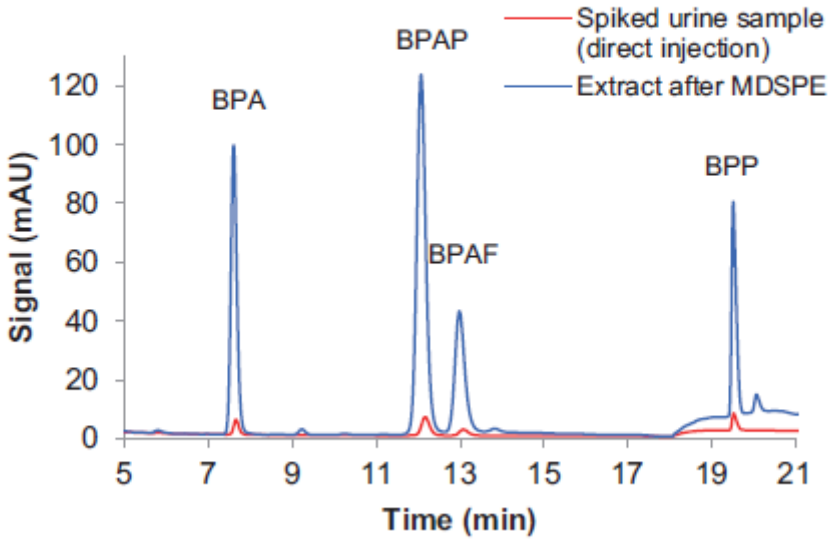

Fig. 1. LC-DAD chromatograms of (red line) urine sample spiked at 1 mg L^{-1} and (blue line) extract of urine sample spiked at 250 µg L^{-1} after MDSPE under optimized conditions.

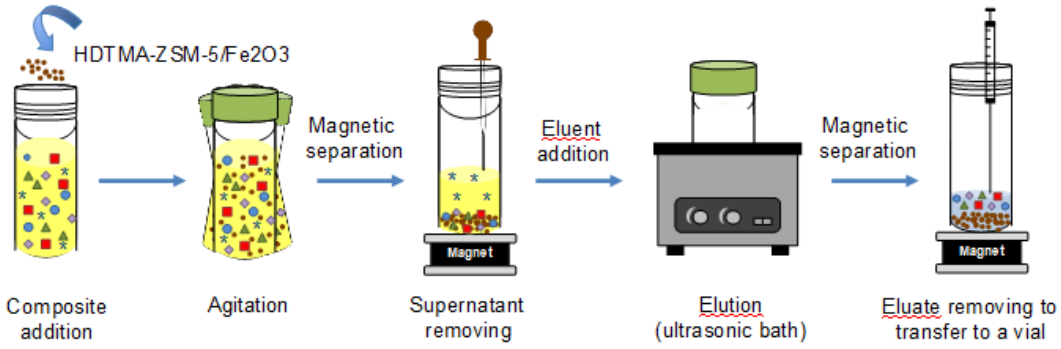

Fig. 2. Scheme of the overall procedure of the proposed MDSPE.

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

Analyte	Working range (μg L^{-1})	r[a]	Sensitivity[b] (mAU min $μg^{-1}$ L)	CV[c] (%)		LOD[d] (μg L^{-1})	EF[e]
				20 μg L^{-1}	100 μg L^{-1}		
BPA	2-250	0.999 (5)	1.89 ± 0.012	6	5	0.6	35.8 ± 4.0
BPAP	2-250	0.999 (5)	2.83 ± 0.04	6	4	0.6	32.5 ± 3.8
BPAF	5-250	0.999 (5)	1.34 ± 0.02	6	3	1.5	32.2 ± 2.3
BPP	2-250	0.995 (5)	1.96 ± 0.02	11	8	0.6	20.1 ± 1.8

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

[b] Slope of the calibration curves ± standard deviation.

[c] Coefficient of variation (CV): mean value for 6 replicate analyses of a 20 μg L^{-1} and 100 μg L^{-1} spiked solution.

[d] Limit of detection (LOD): determined by the empirical approach. The LODs were the lowest concentration whose signal could be clearly distinguished from blank [38].

[e] Enrichment factor (EF). Calculated as the ratio of the signal obtained at 250 μg L^{-1} with and without MDSPE.

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]					
	Drinking water		River water		Wastewater	
	20 μg L^{-1}	100 μg L^{-1}	20 μg L^{-1}	100 μg L^{-1}	20 μg L^{-1}	100 μg L^{-1}
BPA	94 (3)	92 (9)	98 (3)	91(5)	104 (2)	102 (5)
BPAP	105 (1)	93 (4)	95 (4)	91 (4)	103 (1)	96 (6)
BPAF	100 (4)	94 (4)	97 (5)	91 (4)	91 (2)	103 (10)
BPP	83 (2)	89 (3)	87 (1)	86 (3)	97 (6)	84 (5)

[a]Three replicate analysis at indicated levels.

Table 3. Relative recoveries and CV values (in parentheses) obtained for the analytes in the three studied real urine samples.

Analyte	Relative recoveries (%) and CV values in parentheses (%)[a]					
	Urine 1		Urine 2		Urine 3	
	20 μg L^{-1}	100 μg L^{-1}	20 μg L^{-1}	100 μg L^{-1}	20 μg L^{-1}	100 μg L^{-1}
BPA	97 (3)	94 (7)	87 (3)	91 (1)	91 (3)	93 (11)
BPAP	84 (9)	81 (7)	82 (8)	86 (1)	86 (6)	89 (6)
BPAF	88 (10)	83 (9)	108 (4)	92 (2)	88 (1)	83 (13)
BPP	47 (9)	52 (9)	57 (13)	59 (1)	51 (9)	50 (4)

[a]Three replicate analysis at indicated levels.