Optimized Stir Bar Sorptive Extraction Based on Self-Magnetic Nanocomposite Monolithic Kit for Determining Bisphenol A in Bottled Mineral Water and Bottled Milk Samples

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The current study describes a simple method to monitor bisphenol A (BPA) quantitatively through stir bar sorptive extraction (SBSE) coupled with high-performance liquid chromatography-ultraviolet (HPLC-UV) spectroscopy. The analyte was concentrated by SBSE based on self-magnetic nanocomposite monolithic (SMNM) kit. An experimental design based on the central composite design (CCD) was used to optimize factors affecting SBSE extraction. Limit of detection (LOD) of the BPA was 0.02 µg l^{-1} and 0.38 µg l^{-1} for bottled mineral water and bottled milk samples, whereas relative standard deviations (RSDs%) did not exceed 8.49% and 10.21 % for interday and intraday precisions, respectively. Interday and intraday precisions (n = 3) were obtained by extracting BPA at the concentration level of 0.10 µg l^{-1} for bottled mineral water and 0.50 µg l^{-1} for bottled milk sample. The calibration curve of BPA showed good linearity for bottled mineral water and bottled milk samples such that the coefficients of determination (R^2) were obtained to be 0.9976 and 0.9960, respectively. The proposed method was effectively used to analyze BPA in bottled mineral water and bottled milk samples.

Keywords: Stir bar sorptive extraction, Self-magnetic nanocomposite monolithic kit, HPLC, Bisphenol A

INTRODUCTION

Bisphenol A (BPA) is an organic synthetic compound with the chemical formula $(CH_3)_2C(C_6H_4OH)_2$ belonging to the group of diphenylmethane derivatives and bisphenols, with two hydroxyphenyl groups. This compound is used to produce certain plastics and epoxy resins. BPA-based plastic is clear and tough and is made into a variety of common consumer goods such as plastic water bottles [1], food storage containers, canned foods [2-4], toys, sports equipment, CDs, and DVDs. Epoxy resins containing BPA are used to line water pipes as coatings on the inside of many foods and beverage cans and in making thermal paper such as the one used in sales receipts [1].

BPA is considered as an endocrine disrupter that its determine action needs a very sensitive technique for evaluating all possible risks [5]. As the levels of BPA, in many cases, are very low in reusable drink containers, food storage containers, canned foods [2-4], and personal care products, a pre-concentration method should be used before its determination [6]. Since the development of stir bar sorptive extraction (SBSE) in 1999 [7], it has become one of the most common pre-concentration methods owing to its ease and robustness. In SBSE, analytes extractions are done using an adsorbent coated on a magnetic stir bar. Extraction can be performed directly by immersing the stir bar in the sample solution or in the headspace. The compounds trapped can be desorbed through thermal desorption (TD) before gas chromatography (GC), or through liquid desorption (LD) before liquid chromatography. SBSE has been successfully applied to environmental, food, and biological samples [8-11].

Composite materials consist of two or more diverse materials that are prepared *via* physical or chemical

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approaches showing new characteristics. The components of one composite material can complement the performance of each other causing a better total performance of the prepared composite than its original components. The excellent properties of composites have caused their wide application as adsorbents in methods of sample pretreatment, including solid phase extraction (SPE), solid phase microextraction (SPME) [12], and SBSE.

One of the difficulties involved in the use of SBSE technique is the bonding of the coating to the cover of the magnetic rod, which is generally glass [13]. SMNMSB kit is a tool that solves the problems in SBSE because it no longer requires difficult and timely bonding of polymer coating on the glass surface. The mentioned kit does not have a glass or magnetic rod core, which is one of its great advantages and is directly magnetized (due to the presence of Fe_3O_4 nanoparticles in the monolithic matrix). Besides, it does not require using the magnetic rod core or glass to create the character of the magnetic coating on for curing the polymer.

In this research, an SMNMSB kit based on the smart ratio of polyethersulfone (PES), Fe_3O_4 nanopowder, and graphite powder was prepared in our laboratory and then was successfully used for determining BPA in bottled mineral water and bottled milk samples using HPLC-UV. The field emission scanning electron microscopy (FESEM) coupled with energy dispersive X-ray (EDX) spectroscopy system was used to characterize the synthesized SMNMSB kit. Additionally, the effect of parameters on the extraction of the target analyte such as extraction time, stirring speed, salting-out effect, and pH was examined through a five-level four-variable central composite design (CCD). The performance of the suggested SMNMSBSE-LD method was assessed and it was effectively utilized to analyze BPA in bottled mineral water and bottled milk samples.

EXPERIMENTAL

Chemicals and Reagents

Bisphenol A (2,2-bis(4-hydroxyphenyl) propane, >98% purity) was purchased from Sigma-Aldrich Company (West Chester, PA, U.S.A.). Standard solution of BPA was prepared in methanol at a concentration of 1 mg ml^{-1}. Working standard solution was prepared by diluting the standard solution with high purity water to the required concentrations. Standard solutions were stored at -4 °C in the refrigerator. Methanol and acetonitrile (HPLC-grade) were purchased from Romil, LTD (Cambridge, UK). Dimethylsulfoxide (as a solvent for preparation of 15% (w/w) PES), ethyl acetate, n-hexane, dichloromethane, iron(III)-chloride hexahydrate, and polyethylene glycol (PEG, MW = 4000) were purchased from Merck Company (Darmstadt, Germany). Iron(II) sulfate heptahydrate was supplied from Scharlab (S.L., Spain). Ammonia solution was purchased from Chem-Lab (NV, Belgium). Graphite powder was supplied from Alfa Aesar Company (Ltd., the United States). Polyethersulfone (PES, ultrason E6020P with MW = 58,000 g mol^{-1}) was purchased from BASF Company. High purity water was obtained by a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.).

INSTRUMENTATION

In order to separate and detect BPA, A KNAUER HPLC model Smartline equipped with ultraviolet (UV) 2500 detector (Berlin, Germany) and a 20-µl sample loop was used. A reversed phase C18 HPLC Nucleodur column (5 µm, 250 × 4.6 mm, Macherey-Nagel Co., Düren, Germany) was used for separation. Water: acetonitrile solution with the ratio of 60:40 (v/v) was used as isocratic eluent. The flow rate, the volume of injection, and UV wavelength were adjusted at 1 ml min^{-1}, 20 µl and 230 nm, respectively. FESEM coupled with EDX spectroscopy system (TESCAN model MIRA3, Brno, Czech Republic) at accelerating voltage 20 kV was used to determine the size and morphology of prepared materials.

Preparation of Self-magnetic Nanocomposite Monolithic Kit

Table 1 illustrates the materials required to make the kit with different weight ratios. As can be seen, the fourth kit, which contains 0.05 g of graphite powder and presents the highest extraction efficiency for BPA, was selected for further analysis. To prepare the kit, a 15% (w/v) solution of polyethersulfone, Fe_3O_4 nanopowder and graphite powder were mixed with the weight ratio of 1:1:0.05 g in a clean and dry vial. The obtained mixture was mixed sonically for 30 min to disperse sufficiently Fe_3O_4 nanopowder and graphite powder in the polyethersulfone solution. Afterward,

Table 1. Extraction Efficiency of Various SMNMSB kit for Extracting Target BPA. [Concentration: C 0.10 μg l^{-1}; Extraction Step: Extraction Time, 40 min; Stirring Speed, 700 rpm; Salt Addition, 8% (w/v) and pH, 6.5; Desorption Step: Desorption Time, 10 min; Stirring Speed, 700 rpm; 2000 μl Acetonitrile (as Desorption Solvent)]

SMNMSB kit	PES solution (g)	Fe$_3$O$_4$ nano particle (g)	Graphite powder (g)	The relative recovery (%±RSD, n = 3)[a]
1	1	0.4	0.05	53.13 ± 9.82
			0.10	47.28 ± 10.12
2	1	0.6	0.05	69.04 ± 8.67
			0.10	63.71 ± 5.50
3	1	0.8	0.05	78.64 ± 7.45
			0.10	63.98 ± 8.89
4	1	1.0	0.05	87.80 ± 5.81
			0.10	72.31 ± 5.73

[a]The relative recovery is calculated from the equation: $E = (C_2/C_1) \times 100$, where C_2 and C_1 are the concentration of analyte after addition of known amount of standard in ultra-pure water after extracting by SMNMSB kit and the concentration of known amount of standard which was spiked to the ultra-pure water, respectively.

a black paste was obtained that was adequately kneaded and homogenized. In the next stage, the homogenized black paste was loaded into an uncapped tube template with an inner diameter of 5 mm. Based on the extraction of interest, the fill tube can be cut into different pieces. In this experiment, the template was cut into 15 mm pieces.

Several filled cylindrical templates with approximate sizes of 15 mm in 5 mm were obtained. Next, the cut templates were soaked into ultrapure water for 48 h. In this experiment, water plays the role of coagulant solvent for the kit. When water diffuses into the texture of the black nanocomposite paste from the two open template heads, it hardens the paste into a black bar; *i.e.*, the kit. Clean forceps were used to separate gently the kits from the templates. The separated kits were inserted in another vial containing ultrapure water for 24 h. Before using the kits as extraction sorbents for the first time, the kit was stirred in a vial containing 5 ml ultrapure water and then in another vial containing 5 ml acetonitrile for 30 min at 500 rpm. Eventually, the prepared kits were dried at room temperature and stored in a dry and clean place, before use.

For conditioning, kits were stirred in 5 ml acetonitrile using a magnetic stirrer at 500 rpm at ambient temperature and then washed with water, gently dried using a tissue paper, and kept at ambient temperature until performing the experiment.

Stir Bar Sorptive Extraction Procedure

The handmade kits with the dimension of 5 mm × 15 mm were applied for extraction and pre-concentration of BPA with SMNMSBSE process. First, a conditioned kit was placed inside a vial containing 60 ml of water sample. The aqueous sample was stirred using the magnetic stirrer at ambient temperature. The extraction and pre-concentration of BPA were performed under optimum conditions; *i.e.*, extraction time: 50 min, stirring speed: 700 rpm, 11% (w/v)

salt concentration, and pH = 6.50. The optimum experimental conditions were achieved through CCD with Design-Expert®version7 software (Stat-Ease, Inc., 2005). After extracting BPA in optimal conditions, the kit was removed from the specimen. Then, it was washed using Milli-Q water and slightly dried using a tissue paper. For the liquid desorption, the kit was put inside a vial containing 2000 µl acetonitrile and was stirred at 700 rpm for 20 min. After analyte desorption, the kit was removed and 100 µl of desorbed analyte was introduced to the sample loop (20 µl) for analyzing through the HPLC-UV.

RESULTS AND DISCUSSIONS

Preparation of Fe_3O_4 Nanoparticles

The Fe_3O_4 nanoparticles were synthesized through the method reported by Guo *et al.* [14]. A homogeneous mixture containing 90 ml 0.2 M $FeCl_3.6H_2O$ aqueous solution and 40 ml 0.3 M $FeSO_4.7H_2O$ aqueous solution was prepared. In the next step, 25 g PEG was added into this mixture and mixed sonically for several minutes to acquire a homogenous solution. This homogenous mixture was stirred at 70 °C and ammonia solution was added drop by drop to achieve the basic pH up to 9. Then, the reaction solution was stirred at the same temperature for 2 h and the Fe_3O_4 nanoparticles were separated using an external magnet. The resultant Fe_3O_4 nanoparticles were washed with distilled water several times and put in vacuum oven at 40 °C for 24 h for drying.

Characterization of Fe_3O_4 Nanoparticle and SMNM Kit

FESEM equipped with EDX spectroscopy was employed to determine size, morphology, and nanostructure of the produced kits. Figure 1a illustrates the FESEM image of Fe_3O_4 nanoparticles. According to this figure, the Fe_3O_4 particles are 5-250 nm in size. Figures 1b and 1c exhibit FESEM images of the width and length sizes of the kit that are about 5 mm in 15 mm. Also, Figs. 1d and 1e are associated with FESEM images of the kit's nanostructures that are illustrated with 14.7 kx and 89.2 kx magnifications, respectively.

FESEM images of Fe_3O_4 nanoparticle and the kit demonstrate that the presented matrix composition and morphology are consistent with EDX mapping results of Figs. 2a and 2b, considerably.

Optimization of Stir Bar Sorptive Extraction Procedure

The main parameters affecting extraction step, including extraction time, stirring speed, NaCl concentration as salting out effect, and pH, were optimized by central composite design (CCD) under surface methodology (RSM) and the numerical optimization function of the software. Unlike SPME, the influence of temperature on SBSE is not commonly assessed and the procedure is typically carried out at ambient temperature. There are only few reports on the influence of temperature [15-16]. But, in the present study, the effect of absorption temperature on extraction efficiency of BPA was investigated systematically (Fig. 3). Since no significant differences in sensitivity were observed between 25 to 35 °C and also at temperatures above 35 °C, the kit began to degenerate slowly and the Fe_3O_4 nanoparticles existing in the kit gradually began to leak inside the sample solution. Accordingly, we decided to set the extraction temperature at 25 °C.

Parameters affecting desorption step, including desorption time, stirring speed, and type of desorption solvent were optimized "one at a time". Chromatographic peak area related to BPA was considered as an empirical response.

Optimization by a Central Composite Design (CCD)

CCD was applied to optimize the extraction condition of BPA in bottled mineral water and bottled milk samples. For this purpose, four independent variables of extraction time (20-60 min), the speed of stirring (500-900 rpm), the addition of various concentrations of NaCl (0-16%, (w/v)), and pH (4.5-8.5) were considered. A total of 30 experiments with 6 repetitions at the central point were planned using the CCD. The central points were applied to check the reproducibility and stability of outcomes. A total of 5 levels (-1.68, -1, 0, +1 and +1.68) were considered for all factors. These values were designated by the codes shown in Table 2. Table 3 shows the real trial factors related to the design levels applied to create the model. The chromatographic peak area of BPA was selected as the response (Y) of the
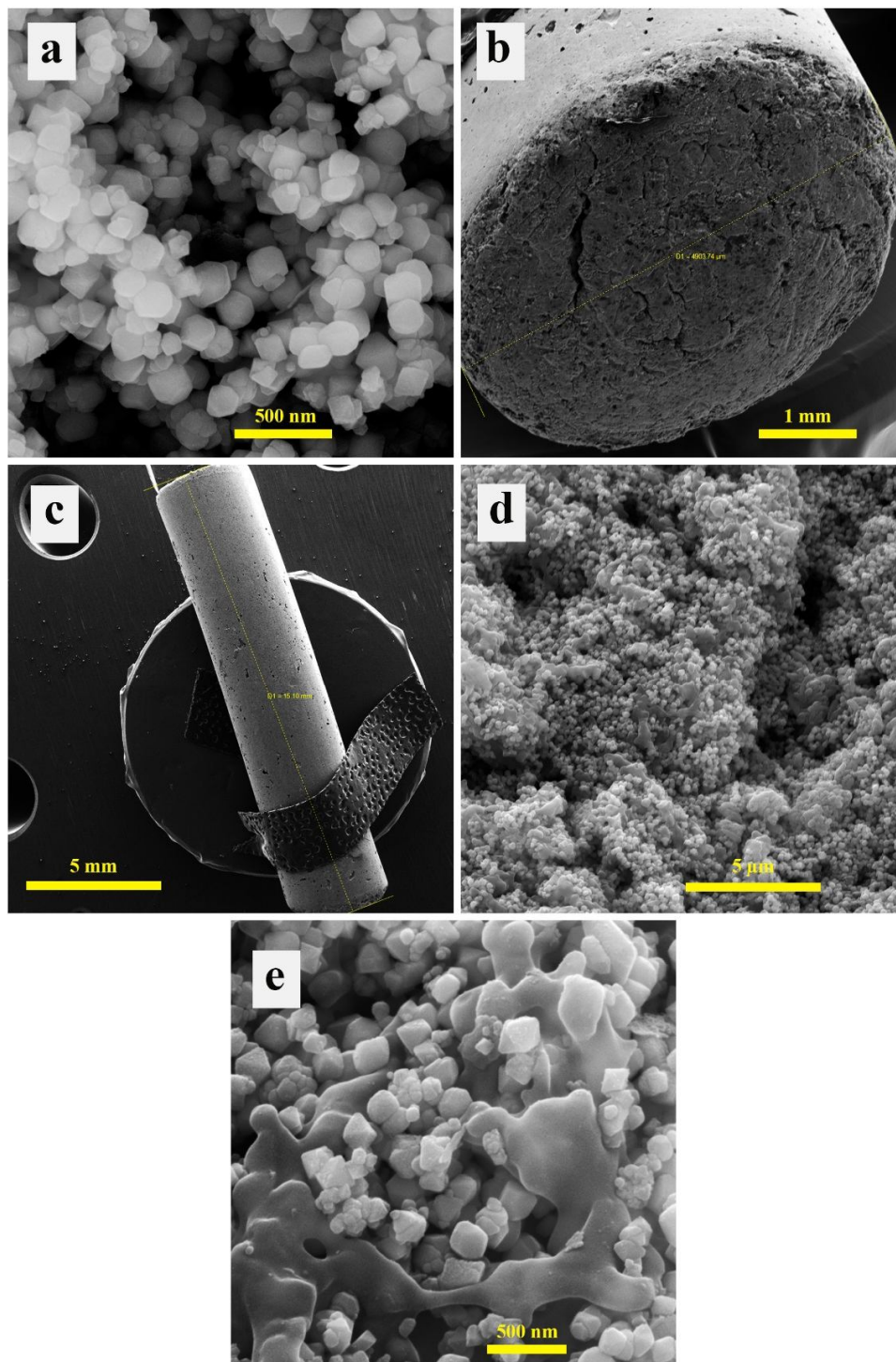
Fig. 1. FESEM images of (a) Fe_3O_4 nanoparticles; (b) the cross-section of the SMNM kit; (c) the length-section of the SMNM kit; (d) the kit's nanostructures with 14.7 kx magnification; (e) the kit's nanostructures with 89.2 kx magnification.
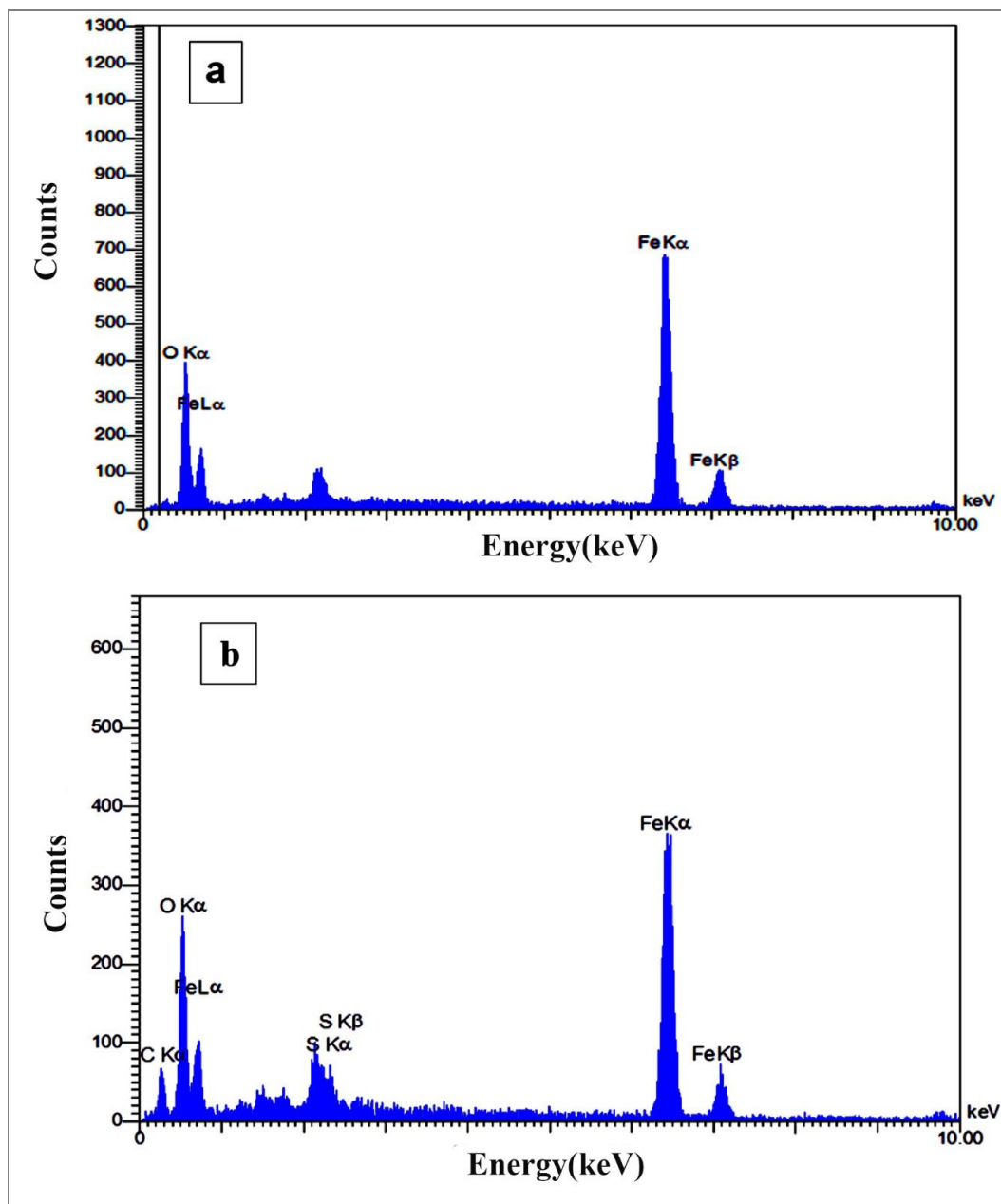
Fig. 2. EDX spectra of (a) Fe_3O_4 nanoparticle; (b) the magnetic nanocomposite monolithic stir bar kit.

experiments and the system behavior was described using the subsequent quadratic Eq. (1):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where Y is the expected response, β_0 represents intercept term, β_i, β_{ii} and β_{ij} are coefficients for the linear, quadratic, and interaction effects, respectively, X_i and X_j are independent variables, and ε represents residual. The responses resulted from the experimental design set (Table 3) were presented for multiplying nonlinear regressions
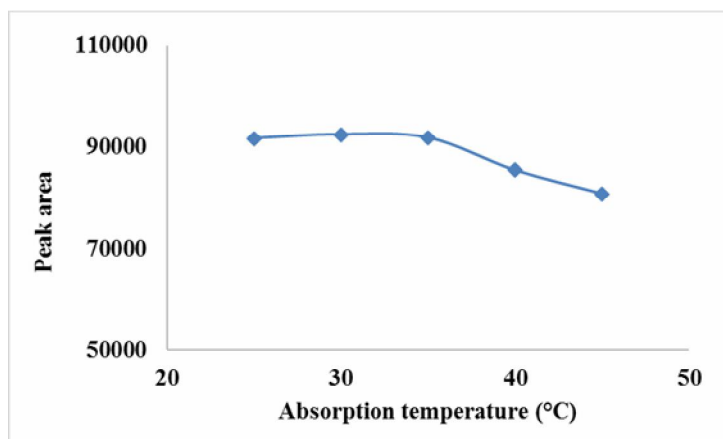
Fig. 3. Influence of the absorption temperature on the extraction efficiency of BPA.

Table 2. Variables and Their Levels Employed in a Central Composite Design for the Evaluation of the Extraction Efficiency of BPA Using SMNMSB kit

Variables	Coded levels				
	Natural levels				
	-α (-1.68)	-1	0	+1	+α (+1.68)
Extraction time (min)	20	30	40	50	60
Stirring speed (rpm)	500	600	700	800	900
Salt addition (%w/v, NaCl)	0	4	8	12	16
pH	4.5	5.5	6.5	7.5	8.5

through Design Expert 7 software for obtaining the coefficients related to the second-order polynomial model and the responses surface. Optimum reaction factors for the highest efficiency were produced through RSM and the numerical optimization function software.

Fitting or Response Surface Model

RSM is a combination of mathematical and statistical methods that are beneficial to analyze problems in which a number of independent variables influence a dependent variable or response and the objective is optimizing the response [17]. According to the data analysis, the subsequent quadratic regression model (Eq. (2)) was suggested for the relationship between independent variables and response, which is the peak area related to BPA obtained from aqueous solution.

Response (the chromatographic area of BPA = $+8.42 \times 10^4 + 2.04 \times 10^4 A + 1.10 \times 10^3 B + 8.09 \times 10^3 C + 1.37 \times 10^3 D + 3.78 \times 10^3 AC - 2.41 \times 10^3 BD - 5.29 \times 10^3 A^2 - 1.04 \times 10^4 - 3D^2$ (2)

The evaluation of the fitted model is always necessary to ensure that this model offers an acceptable estimation of the real system and confirm that none of the least squares
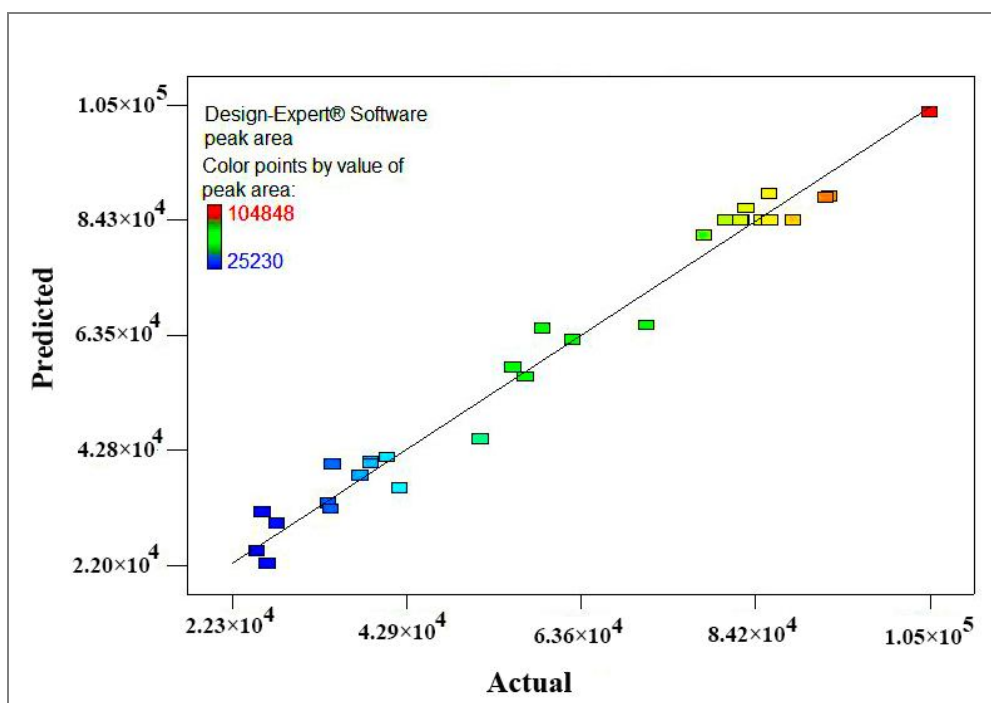
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Table 3. The Actual Experimental Parameters in Four-variable, Five-level Central Composite Design for Study and Optimization of BPA

SET	A	B	C	D	Chromatographic peak area of BPA		
	Extraction time (min)	Stirring speed (rpm)	Salt addition (%w/v, NaCl)	pH	Experimental	Predicted	Residual
1	30(-1)	600(-1)	4(-1)	5.5(-1)	25230	24564.38	665.62
2	50(+1)	600(-1)	4(-1)	5.5(-1)	55507	57724.04	-2217.04
3	30(-1)	800(+1)	4(-1)	5.5(-1)	25862	31588.13	-5726.13
4	50(+1)	800(+1)	4(-1)	5.5(-1)	59019	64747.79	-5728.79
5	30(-1)	600(-1)	12(+1)	5.5(-1)	33615	33169.21	445.79
6	50(+1)	600(-1)	12(+1)	5.5(-1)	78170	81462.88	-3292.88
7	30(-1)	800(+1)	12(+1)	5.5(-1)	34152	40192.96	-6040.96
8	50(+1)	800(+1)	12(+1)	5.5(-1)	92974	88486.63	4487.38
9	30(-1)	600(-1)	4(-1)	7.5(+1)	33942	32129.46	1812.54
10	50(+1)	600(-1)	4(-1)	7.5(+1)	71280	65289.12	5990.88
11	30(-1)	800(+1)	4(-1)	7.5(+1)	27602	29516.71	-1914.71
12	50(+1)	800(+1)	4(-1)	7.5(+1)	62531	62676.37	-145.37
13	30(-1)	600(-1)	12(+1)	7.5(+1)	38666	40734.29	-2068.29
14	50(+1)	600(-1)	12(+1)	7.5(+1)	85837	89027.96	-3190.96
15	30(-1)	800(+1)	12(+1)	7.5(+1)	37421	38121.54	-700.54
16	50(+1)	800(+1)	12(+1)	7.5(+1)	83110	86415.21	-3305.21
17	20(-1.68)	700(0)	8(0)	6.5(0)	26457	22309.75	4147.25
18	60(+1.68)	700(0)	8(0)	6.5(0)	104848	1.04E+05	1084.92
19	40(0)	500(-1.68)	8(0)	6.5(0)	38648	40336.92	-1688.92
20	40(0)	900(+1.68)	8(0)	6.5(0)	51669	44747.92	6921.08
21	40(0)	700(0)	0(-1.68)	6.5(0)	57008	55992.58	1015.42
22	40(0)	700(0)	16(+1.68)	6.5(0)	92553	88336.25	4216.75
23	40(0)	700(0)	8(0)	4.5(-1.68)	42059	35971.58	6087.42
24	40(0)	700(0)	8(0)	8.5(+1.68)	40610	41465.25	-855.25
25	40(0)	700(0)	8(0)	6.5(0)	82545	84210.83	-1665.83
26	40(0)	700(0)	8(0)	6.5(0)	82415	84210.83	-1795.83
27	40(0)	700(0)	8(0)	6.5(0)	80680	84210.83	-3530.83
28	40(0)	700(0)	8(0)	6.5(0)	85007	84210.83	796.17
29	40(0)	700(0)	8(0)	6.5(0)	88704	84210.83	4493.17
30	40(0)	700(0)	8(0)	6.5(0)	85914	84210.83	1703.17

Table 4. ANOVA Results of Design Expert 7 for Studied Response

Analyte	Probability for model	$R^{2\,a}$	Adj.$R^{2\,b}$	Pred.R^{2c}	Adeq. precision^d	SD^e	CV^f	PERESS^g	Probability for lack of fit
Bisphenol A	< 0.0001	0.9794	0.9685	0.9312	30.27	4444.37	7.39	1.25×10^9	0.1292

^{a}R^2: determination coefficient. ^{b}Adj. R^2: adjusted R^2. ^{c}Pred. R^2: predicted R^2. ^{d}Adeq. Precision: adequate precision. ^{e}SD: standard deviation. ^{f}CV: coefficient of variation. ^{g}PERESS: predicted residual error sum of squares.

Fig. 4. Actual *versus* predicted values of the responses for chromatographic peak area of BPA.

regression assumptions is disaffirmed. The statistical significance of the model was assessed through analysis of variance (ANOVA), with results listed in Table 4 and Table 5. Values of Prob > F < 0.0500 show the significant terms for the model. Moreover, according to the obtained outcomes, lack of fit (LOF) was not observed in the quadratic model (Prob > F for LOF > 0.0500). This result implies that the validity of the model suggested in the current research. Moreover, the model fitting was evaluated by coefficient of determination (R^2). The F-value of 90.20 shows the statistical significance of the model. The

probability of occurring this large amount of "Model F-Value" due to noise is only 0.01%. The Prob > F values of less than 0.0500 show that the terms of the model are statistically significant. In this situation, model terms A, C, AC, BD, A^2, B^2, C^2 and D^2 are statistically significant. Values more than 0.1000 show that the model terms are not significant. In case there are many non-significant model terms (not counting those required to support hierarchy), the model reduction may improve the model.

"Lack of Fit F-value" of 2.82 indicates the non-significant LOF of the pure error. The possibility of
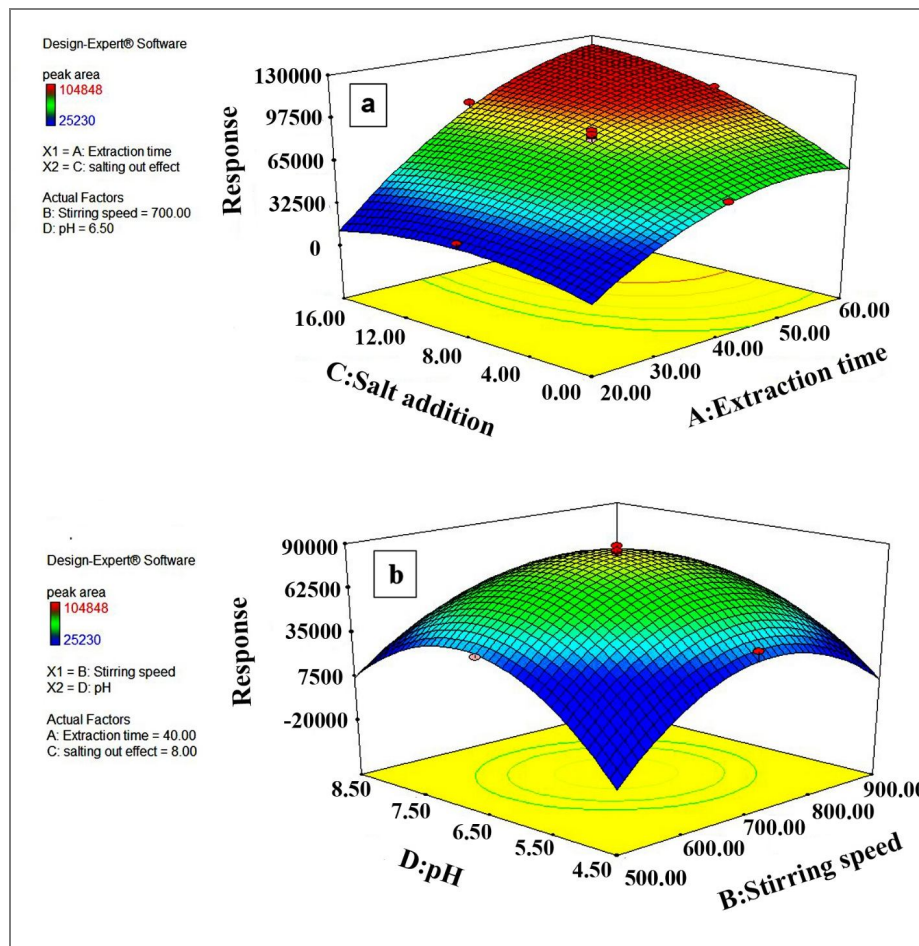
Fig. 5. Estimated response surfaces obtained using CCD for BPA by plotting (a) Extraction time *vs*. salt addition, (b) Stirring speed *vs*. pH.

occurring this value of "Lack of Fit F-value" due to noise is 12.92%. It is good to have insignificant LOF F-values. A "Pred R-Squared" of 0.9312 is reasonably consistent with the "Adj R-Squared" of 0.9685. "Adeq Precision" measures the signal-to-noise (S/N) ratio. A ratio of more than 4 is optimal in this regard. The ratio of 30.27 shows an acceptable signal. This model can be applied for navigating the space of the design. The small value of the coefficient of variation (CV; 7.39) shows excellent precision and reliability of the experiments. To compare the approximated outcomes with the experimental results, experimental rechecking was conducted under optimum conditions. The amount of chromatographic peak area for BPA resulted

from real test confirms the RSM model. Figure 4 shows the diagram of predicted response *versus* actual response for the objective analyte. As can be seen, the real values are dispersed near the straight line (y = ax + b) with a reasonably high coefficient of determination (R^2 = 0.9794). Figures 5a and 5b show the response surface charts achieved through plotting parameters.

Figure 5a illustrates the effect of extraction time and the addition of NaCl on the response (chromatographic peak area BPA) when pH and speed of stirring were set at the coded level of 0. According to this figure and Table 5, there was a significant interaction between these parameters considering the P-value and F-ratios of the ANOVA data.

Table 5. Coefficient and ANOVA Output for the Quadratic Model Proposed for the Evaluation of the Extraction Efficiency of BPA.

Analyte	Source	Coefficient estimate	Degree of freedom	Mean square	F-Value	P-Value
	Model	-	10	1.78×10^9	90.21	<0.0001[b]
	Intercept	84210.83	1	-	-	-
	A-Extraction time (min)	20363.33	1	9.95×10^9	503.83	< 0.0001[b]
	B-Stirring speed (rpm)	1102.75	1	2.92×10^7	1.48	0.2390
Bisphenol A	C-Salt addition (%, w/v)	8085.92	1	1.57×10^9	79.44	< 0.0001[b]
	D-pH	1373.42	1	4.53×10^7	2.29	0.1465
	AC	3783.50	1	2.29×10^8	11.60	0.0030[b]
	BD	-2409.12	1	9.29×10^7	4.70	0.0430[a]
	A^2	-5293.60	1	7.69×10^8	38.91	< 0.0001[b]
	B^2	-10417.10	1	2.99×10^9	150.69	< 0.0001[b]
	C^2	-3011.60	1	2.49×10^8	12.59	0.0021[b]
	D^2	-11373.10	1	3.55×10^9	179.61	< 0.0001[b]

[a]Significant with P-value < 0.05. [b]Significant with P-value < 0.01.

Table 6. Optimum Condition Derived by RSM and Optimization Function of the Design Expert

Optimal conditions				Chromatographic peak area of BPA (Response)		
A	B	C	D	Actual yields (mAU)	Predicted yields (mAU)	Residual
50	700	11	6.50	104315 ± 8.23[a]	105404	-1089

[a]Mean ± relative standard deviation (n = 3). A = Extraction time (min); B = Stirring speed (rpm); C Salt addition (%, w/v); D = pH.

Based on Fig. 5a, the response related to the analyte was enhanced by increasing the time of extraction and addition of the salt concentration in the above-mentioned condition. Therefore, the best response was estimated for the extraction time of more than 40 min. Similarly, an increase in salt addition at a constant time of extraction causes slow enhancement in the response.

Figure 5b presents the effect of stirring speed and pH on the extraction of BPA. The outcomes show that the response is increased with a simultaneous increase at stirring speed and pH in central points of experimental design (700 rpm and 6.5) at constant values of extraction time and salt concentration. Overall, after assessing the key parameters and their interactions, the optimal conditions to extract the target BPA were predicted using RSM and the optimization function of the Design Expert. Table 6 lists the optimal

Table 7. The Effect of Desorption Solvents on Desorption Efficiency in Experiment Conditions: [Analyte Concentration: C = 0.10 µg l^{-1}; Extraction Step: 40 min, 700 rpm, 8% (w/v) Salt Addition and pH 6.5; Desorption Step: 10 min, 700 rpm, 2000 µl Desorption Solvent]

Desorption solvent	Desorption efficiency (%±RSD, n = 3)
Methanol	79.02 ± 5.65
Acetonitrile	87.80 ± 5.81
Ethyl acetate	71.73 ± 3.19
Dichloromethane	90.85 ± [a]-
n-Hexane	13.14 ± 8.79

[a]The SMNMSB kit was dissolved after one desorption step in dichloromethane solvent.

Fig. 6. Effect of desorption time (10-50 min) on the extraction of BPA obtained with SMNMSBSE-LD method. Extraction step in optimum condition: extraction temperature, 25 °C; extraction time, 50 min; stirring speed, 700 rpm; salt addition, 11% (w/v); pH, 6.5; desorption step condition: stirring speed ,700 rpm; desorption time, 10-50 min; 2000 µl acetonitrile as desorption solvent and BPA concentration: 0.10 µg l^{-1}.

condition; *i.e.*, extraction time of 50 min, stirring speed of 700 rpm, salt concentration of 11% (w/v), and pH 6.50. Since the effects of interaction between extraction time *vs.*

stirring speed, extraction time *vs*. pH, stirring speed *vs*. salting-out effect, and salting out effect vs. pH were not fitted by the model in Eq. (2), their effects were not studied.

Optimum Conditions

RSM and the optimization function of the Design Expert were applied to predict optimal conditions of extraction and pre-concentration of BPA in the experimental range examined. Table 6 shows the results and the expected and real values. The analysis showed (Table 6) that the highest value of response was obtained at extraction time of 50 min, a stirring speed of 700 rpm, 11% (w/v) salt addition, and pH of 6.50. The comparison performed between the expected and experimental values showed their excellent agreement confirming the good capability of experimental models derived from the RSM and the numerical optimization function to define the relationship between the parameters and response in extraction and pre-concentration of BPA.

Desorption Condition Optimization

Liquid desorption (LD) was used for desorption of BPA from the self-magnetic nanocomposite monolithic stir bar kit. For this purpose, the parameters including desorption solvent, desorption time and stirring speed affecting desorption recovery were optimized systematically. After extraction of BPA by MNMSB kit and for LD of the analyte, the kit was put inside a glass vial containing 2000 µl of desorption solvent and it was then stirred. Chromatographic peak area related to the target BPA was chosen as the experimental response.

Effect of Desorption Solvent

The effect of desorption solvents on desorption efficiency was examined. Methanol, acetonitrile, ethyl acetate, dichloromethane, and n-hexane were studied as the desorption solvent to desorb BPA (Table 7). As can be observed, dichloromethane provides a better desorption recovery for target BPA compared to the rest of the solvents, but SMNMSB kit is dissolved after one desorption step in dichloromethane solvent. Thus, dichloromethane was not a good option as the desorption solvent. According to Table 7, acetonitrile is selected as the optimal desorption solvent, because a higher desorption efficiency for BPA was obtained using this solvent, which provided a higher desorption efficiency than other solvents for the target BPA.

Effect of Desorption Time

In order to study the effect of desorption time on desorption efficiency of BPA by SMNMSBSE-LD, this parameter was varied in the range of 10-50 min. According to Fig. 6, the extraction efficiency for BPA increases quickly with increasing desorption time from 10 to 20 min and then remains almost constant with a further increase in the extraction time to 50 min. Therefore, the desorption time of 20 min was selected for the next experiments.

Effect of Stirring Rate in Desorption Step

The effect of stirring speed on desorption of BPA was also examined in the range of 500-900 rpm. According to Fig. 7, the responses were enhanced with increasing the stirring rate from 500 to 700 rpm and then were slowly reduced with a further increase in the stirring rate to 900 rpm. Thus, a stirring rate of 700 rpm was adopted as the optimal value for the next experiments.

Reproducibility and Reusability of the Prepared SMNMSB Kit

The preparation reproducibility and reusability of SMNMSB kit were evaluated. The data listed in Table 8 indicate adequate preparation reproducibility with relative standard deviations (RSDs) of 7.14- 8.94% (bar to bar) and 13.85-15.67% (batch to batch). The reusability of homemade SMNMSB kit has been also examined and found that, according to the results in Fig. 8, the reusability of the prepared SMNMSB kit is 9 times for bottled mineral water sample and 6 times for bottled milk sample, with no considerable reduction in the extraction efficiency. Therefore, the reusability of produced SMNMSB kit in water and milk samples is obtained about 6-9 times.

Analytical Performance

The analytical performance of SMNMSBSE-LD/HPLC-UV was investigated under optimal conditions in order to determine BPA (Table 9). Limit of detections (LODs) of the BPA, based on S/N = 3 were 0.02 µg l^{-1} and 0.38 µg l^{-1} for bottled mineral water and bottled milk samples, respectively. Also, the limit of quantifications (LOQs) of the BPA, based on S/N of 10 were 0.06 and 1.24 µg l^{-1} for bottled mineral water and bottled milk sample, respectively. Interday and intraday precisions (n = 3) were obtained by extracting BPA at the concentration level of 0.10 µg l^{-1} for bottled mineral water and 0.50 µg l^{-1} for bottled milk
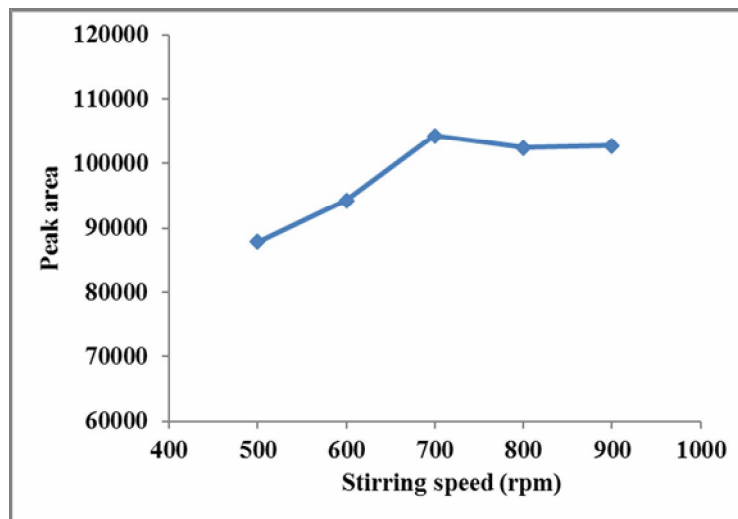
Fig. 7. Effect of Stirring speed (500-900 rpm) on the extraction of BPA obtained with SMNMSBSE method. Extraction step in optimum condition: extraction temperature, 25 °C; extraction time, 50 min; stirring speed, 700 rpm; salt addition, 11% (w/v); pH, 6.5; desorption step condition: stirring speed, 500-900 rpm; desorption time, 20 min; 2000 μl acetonitrile as desorption solvent and BPA concentration: 0.10 μg l^{-1}.

Table 8. Preparation Reproducibility of Magnetic Nanocomposite Monolithic Stir Bars for the Determination of BPA

Bisphenol A	RSD (%)[a]	
	Bar to bar	Batch to batch
	(n = 3)	(n = 3)
Bottled mineral water	7.14	13.85
Bottled milk	8.94	15.67

[a]Calculated at a concentration level of 0.10 μg l^{-1} and 0.50 μg l^{-1} BPA in bottled mineral water and bottled milk, respectively.

sample. The relative standard deviations (RSDs%) did not exceed 8.49% and 10.21% for interday and intraday precisions, respectively. The obtained calibration curves illustrated satisfactory linearity in the ranges of 0.04-200 μg l^{-1} for bottled mineral water and 0.50-200 μg l^{-1} for bottled milk sample. Coefficients of correlation (r^2) for BPA in bottled mineral water and bottled milk sample were 0.9976 and 0.9960, respectively.

The enrichment factors calculated by the ratio of the slope of the calibration curves, obtained with and without SMNMSBSE, within the range of 20.24-22.13-fold.

Real Sample Analysis

The SMNMSBSE-LD/HPLC-UV method was used under optimal conditions to determine target BPA in bottled mineral water and bottled milk samples. The obtained
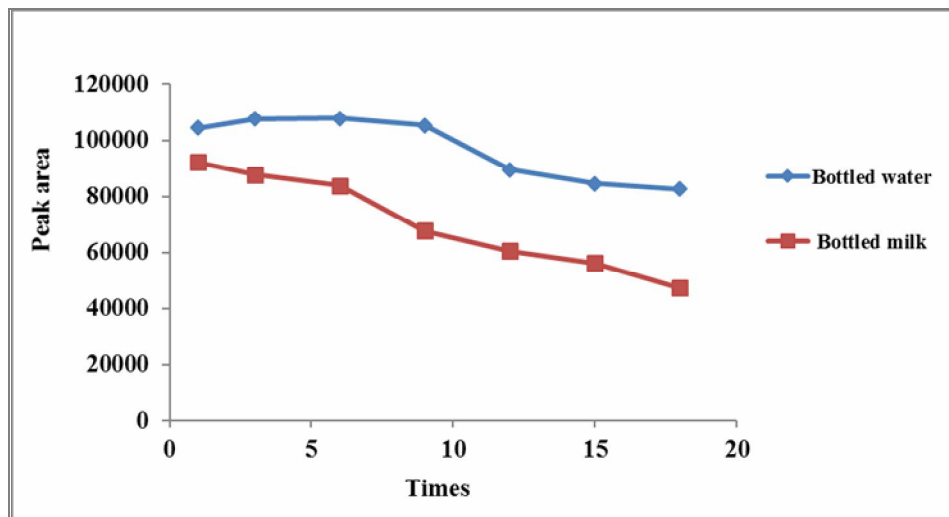
Fig. 8. The reusability of SMNMSB kit for extracting of BPA in bottled mineral water and bottled milk samples. Extraction step in optimum condition: extraction temperature, 25 °C; extraction time, 50 min; stirring speed, 700 rpm; salt addition, 11% (w/v); pH, 6.5; desorption step condition: stirring speed ,700 rpm; desorption time, 20 min; 2000 µl acetonitrile as desorption solvent and BPA concentration: 0.10 µg l^{-1} and 0.50 µg l^{-1} BPA in bottled mineral water and bottled milk sample, respectively.

Table 9. Analytical Performance of the Proposed MNMSBSE-LD/HPLC-UV for Preconcentration and Determination of BPA in Real Samples

Sample	Analytical performance of MNMSBSE–LD/HPLC-UV							
	Linear equation	Linear range (µg l^{-1})	Correlation coefficient (r)	Limit of detection (µg l^{-1})	Limit of quantification (µg l^{-1})	Relative standard deviation (%, n = 3)[a]		EF [b]
						Interday	Intraday	
Bottled mineral water	y = 1 × 10^{6}x + 7709.9	0.04-200	0.9976	0.02	0.06	6.98	7.68	22.13
Bottled milk	y = 85656x + 21064	0.50-200	0.9960	0.38	1.24	8.49	10.21	20.24

[a]Calculated at a concentration level of 0.10 µg l^{-1} and 0.50 µg l^{-1} BPA in bottled mineral water and bottled milk, respectively. [b]EF (Enrichment factor), calculated by the ratio of the slope of the calibration curves obtained with and without MNMSBSE.

Table 10. Analytical Performance of MNMSBSE-LD/HPLC-UV for BPA Analysis in Real Bottled Mineral Water and Bottled Milk Samples

Sample	Bottled mineral water			Bottled milk		
	Added	Found	Recovery	Added	Found	Recovery
	(μg l^{-1})	(μg l^{-1})	(%±RSD, n = 3)	(μg l^{-1})	(μg l^{-1})	(%±RSD, n = 3)
	BPA	BPA		BPA	BPA	
Brand A	0.00	ND[a]	-	0.00	ND	-
	0.10	0.09	90.00 ± 5.72	0.50	0.43	86.00 ± 8.12
	2.50	2.39	95.60 ± 6.16	5.00	5.03	100.60 ± 7.31
Brand B	0.00	ND	-	0.00	ND	-
	0.10	0.10	100.00 ± 6.20	0.50	0.45	90.00 ± 7.56
	2.50	2.41	96.40 ± 7.07	5.00	5.12	102.40 ± 7.38
Brand C	0.00	0.07	-	0.00	ND	-
	0.10	0.16	90.00 ± 6.34	0.50	0.42	84.00 ± 5.27
	2.50	2.47	96.00 ± 5.59	5.00	4.70	94.00 ± 8.35

[a]Not detect.

analytical outcomes are listed in Tables 9 and 10. As can be noted, using SMNMSBSE-LD/HPLC-UV, 0.07 μg l^{-1} of BPA was detected in the bottled mineral water sample (Brand C) and in the rest of the samples while BPA was not detected or quantified. The recoveries of BPA were in the ranges of 90.00%-100.00% and 84.00%-102.40% for spiked bottled mineral water sample and spiked bottled milk sample, respectively.

Bottled Mineral Water Sample Preparation for Determination of BPA

Three different brands of bottled mineral water were purchased from local shops of Sari, Iran, and kept in their packages at room temperature, before experiments. Extraction and determination of BPA were performed in accordance with the SMNMSBSE procedure**.**

Figure 9A shows the SMNMSBSE-LD/HPLC-UV chromatograms of bottled mineral water and its spiked sample, respectively.

Bottled Milk Sample Preparation for Determination of BPA

Three brands of milk were bought from local shops in Sari, Iran. They were preserved in their packages at ºC, until the time of the analysis. Before extraction and pre-concentration of BPA from milk samples, the proteins and fats existing in the milk should be removed since their presence blocks the pores of the kit and declines BPA extraction efficiency and method recovery.

First, 5 ml of the milk sample was diluted with 15 ml of dilute HCl (pH = 3; with 0.5% (w/v) NaCl). Then, the mixture was shaken manually and transferred to a centrifuge tube. The tubes were centrifuged 10 min at 11000 rpm. In this way, the proteins and fats of milk precipitated and 5 ml of the clear supernatant was diluted with 55 ml dilute NaOH, which contained 11.95% (w/v) NaCl. The pH and NaCl content (as a salting-out effect) of the resulted solution were 6.5 and 11% (w/v), respectively. Then, a conditioned kit was inserted in a vial containing 60 ml treated milk
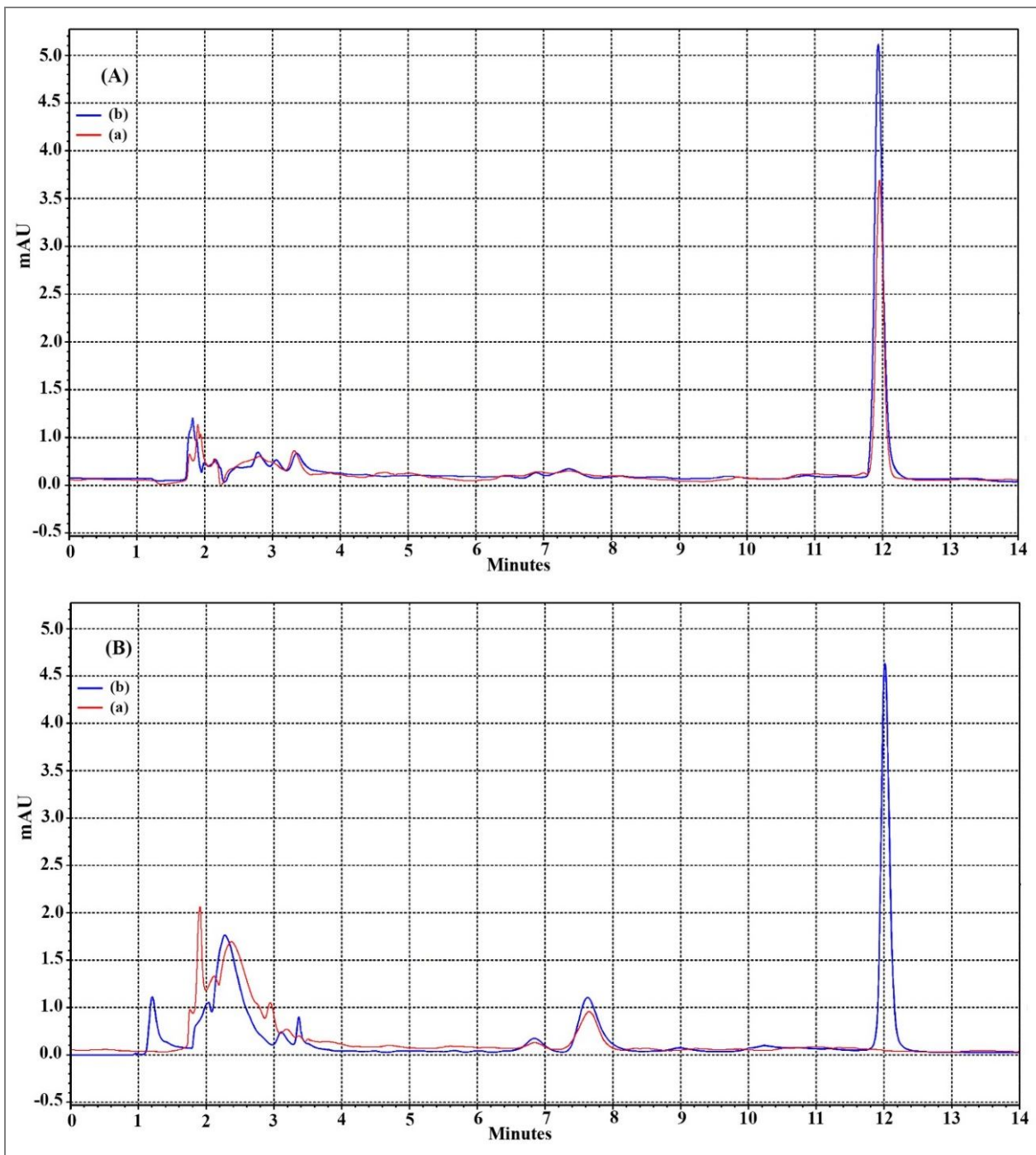
Fig. 9. HPLC-UV chromatograms of (A): Bottled mineral water sample (Brand C); (a) injection of the mineral water sample after extraction by SMNMSBSE-LD and (b) injection of mineral water sample spiked with 0.10 µg l^{-1} BPA after extraction by SMNMSBSE-LD. (B): Bottled milk sample (Brand C); (a) injection of the milk sample after extraction by SMNMSBSE-LD and (b) injection of the milk sample spiked with 0.5 µg l^{-1} BPA after SMNMSBSE-LD to the HPLC-UV instrument, respectively.

Table 11. Comparison of SMNMSBSE-LD-HPLC-UV with other Extraction Methods for Determination of BPA in Water and Milk Samples

Methods	Samples	Limit of detection ($\mu g\ l^{-1}$)	Linear range ($\mu g\ l^{-1}$)	Precision RSD (%)	Recovery (%)	Ref.
SMNMSBSE-LD-HPLC-UV[a]	Bottled mineral water	0.02	0.04-200	6.98-7.68	90.00-100.00	This work
	Bottled milk	0.38	0.5-200	8.49-10.21	84.00-102.40	
SPE-MLC-UV[b]	Mineral water	0.3	0.5-100	4.18-4.70	92.3	[18]
	Powdered milk	0.3	0.5-100	5.82-6.18	92.3	
DLLME–HPLC-UV[c]	Tap water River water	0.07	0.5-100	6.0	93.4-98.2	[19]
IL-DLPME-HPLC-MS[d]	Tap water Surface water	0.76	1-70	8.4	N.R[e]	[20]
SPE-LC-UV[f]	Drinking water	0.20	N.R	3.35-4.24	93.3-97.0	[21]

[a]Self-magnetic nanocomposite monolithic stir bar sorptive extraction-liquid desorption-high performance liquid chromatography-ultra violet spectroscopy. [b]Solid-phase extraction-micellar liquid chromatography-ultra violet spectroscopy. [c]Dispersive liquid-liquid microextraction-high-performance liquid chromatography-ultra violet spectroscopy. [d]Ionic liquid-dispersive liquid phase microextraction-high-performance liquid chromatography-Mass spectrometry. [e]Not reported. [f]Solid phase extraction-liquid chromatography-ultra violet spectroscopy.

solution. Extraction and determination of BPA were performed according to the section of the SMNMSBSE procedure. Figure 9B presents the SMNMSBSE-LD/HPLC-UV chromatograms of bottled milk sample and its spiked sample, respectively.

Comparison of SMNMSBSE-LD-HPLC-UV with Other Extraction Methods

Extraction and determination of the BPA existing in bottled mineral water and bottled milk sample through the application of SMNMSBSE-LD in combination with HPLC-UV detection is compared with some other methods in Table 11. The relative standard deviation and recovery values of the proposed method are similar to the other methods. Apparently, the performance of SMNMSBSE-LD-HPLC-UV is superior to the other methods, with respect to limit of detection and linear range of the calibration curves. Accordingly, LOD and linear range for determination of BPA are better than those for most of the other methods.

CONCLUSIONS

In the current study, a self-magnetic nanocomposite monolithic kit was produced and a new method of SMNMSBSE-LD/HPLC-UV was presented to determine BPA in two different samples of bottled mineral water and bottled milk.

Five-level-four factors CCD were used to optimize the factors affecting extraction, and parameters affecting desorption were optimized "one at a time".

The suggested method has several benefits including simplicity of the procedure to make SMNMSB kit, low detection limits, a high extraction efficiency, an excellent clean-up, and a wide linear range. Ultimately, the kit can be potentially commercialized and be easily utilized in the common microextraction procedures.

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COMPLIANCE WITH ETHICAL STANDARDS, CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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