A Chemometric Strategy for Optimization of Solid-Phase Microextraction: Determination of Bisphenol A and 4-Nonylphenol with HPLC

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Abstract

A sensitive method for the analysis of bisphenol A and 4-nonylphenol is developed by means of the optimization of solid-phase microextraction using Uniform Experimental Design methodology followed by high-performance liquid chromatographic analysis with fluorescence detection. The optimal extraction conditions are determined based on the relationship between parameters and the peak area. The curve calibration plots are linear (r^2 ≥ 0.9980) over the concentration range of 1.25–125 ng/mL for bisphenol A and 2.59–202.96 ng/mL for 4-nonylphenol, respectively. The detection limits, based on a signal-to-noise ratio of 3, are 0.097 ng/mL for bisphenol A and 0.27 ng/mL for 4-nonylphenol, respectively. The validity of the proposed method is demonstrated by the analysis of the investigated analytes in real water samples and sensitivity of the optimized method is verified by comparing results with those obtained by previous methods using the same commercial solid-phase microextraction fiber.

Introduction

Bisphenol A (BPA) is a compound widely used as the monomer for the production of polycarbonate plastics, and as a major component of epoxy resin for food packaging materials, from which BPA penetrates into food (1). 4-Nonylphenol (4-NP) is the biodegradation metabolite of non-ionic surfactants that has been shown to exist in the environment, such as in river water and sewage sludge (2,3). It is observed in food or feed via permeation by either environmental pollution or potential bioaccumulation and transfer through the food chain.

BPA and 4-NP belong to a group of xenoestrogens (4), which can disrupt endocrine function and be linked to adverse effects on the reproductive systems of wildlife and humans. Their estrogenic effect in human endometrial carcinoma cell line was investigated (5) and the weak estrogenic activity has been confirmed in vitro and in vivo (6,7).

Several methods for determining BPA and 4-NP in various matrices have been proposed (8–12). Depending on the matrix, sample preparation including solvent extraction (8), solid-phase extraction (9–11), and solid-phase microextraction (SPME) have been used (12–16). Common analysis methods have included gas chromatography–mass spectrometry (17), high-performance liquid chromatography (HPLC) equipped with direct UV (12–14), direct fluorescence (15,16,18) or derivatization fluorescence (10,19,20), and HPLC–mass spectrometry (21–23).

In this paper, an SPME technique was adopted and its optimization performed by experimental design methodology. An optimal SPME–HPLC method was developed to determine BPA and 4-NP in tap water. The results showed that a higher degree of sensitivity was achieved compared to that obtained by previous SPME–HPLC methods for the determination of BPA and 4-NP.

Experimental

Materials

BPA, purity greater than 98%, was purchased from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China). 4-NP, a mixture of compounds with branched side chains, purity greater than 98%, was from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). Acetonitrile of HPLC grade was from Dima Technology Inc. (Richmond Hill, ON). The water purified by Milli-Q Water System (Millipore, Bedford, MA) was redistilled again for preparing solutions and mobile phase. Sodium chloride of ana-

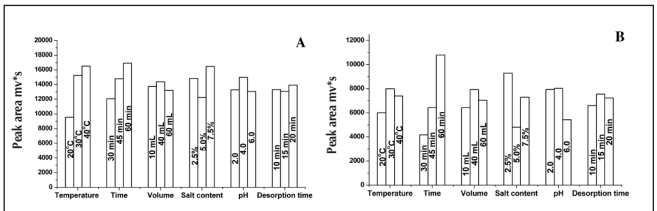

Figure 1. Extraction results of BPA (expressed as summation of chromatographic peak area at same level) at each level of six factors (A). Extraction results of 4-NP (expressed as summation of chromatographic peak area at same level) at each level of six factors (B). Chromatographic conditions: column, C18 (Hanbon Science & Technology Co., Ltd) (5 µm, 4.5 × 250 mm); mobile phase, A: water, B: acetonitrile. Time program: linear gradient (0–1 min A = 15%, B = 85%; 4 min A = 0%, B = 100%; 12 min stop); Fluorescence detector, Ex 275 nm, Em 315 nm; room temperature (14–17°C); flow-rate, 0.8 mL/min. Standard solutions, 0.808 µg/mL BPA; 1.148 µg/mL 4-NP.

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lytical grade was from Tianjing Guangfu Chemical Reagent Co., Ltd. (Tianjing, China).

BPA or 4-NP (10.0 mg) were dissolved into 10 mL of acetonitrile to prepare the stock solutions, which were stored in a refrigerator at 4ºC. Working solutions were prepared by diluting the corresponding stock solutions with 2.5% NaCl (w/v) according to the different experimental procedures.

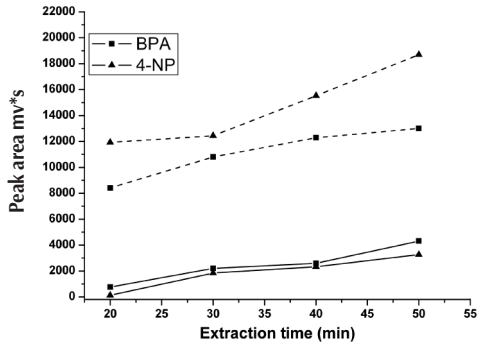

Figure 2. Effect of time on extraction (expressed as chromatographic peak area) in static extraction mode and dynamic extraction mode. SPME condition: fiber, CW/TPR, 50 µm; extraction temperature 40°C; sample volume 40 mL; desorption time 14 min; salt concentration 2.5%; pH 4.0; desorbing agent, 85% acetonitrile–water. Stirring rate 1080 rpm was adopted in dynamic extraction mode. --- Dynamic extraction mode; – the static extraction mode. Chromatographic conditions and standard solutions were same as in Figure 1.

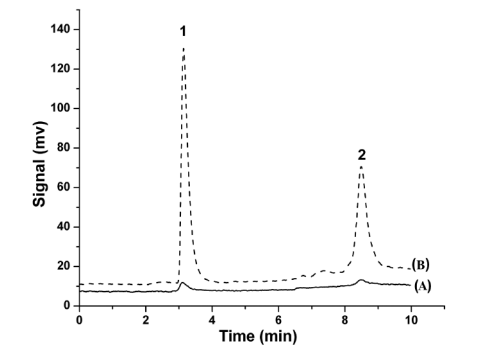

Figure 3. Chromatograms of synthetic sample containing BPA (100 ng/mL) and 4-NP (202.96 ng/mL) obtained by direct injection (20 µL) (A) and after SPME (B). Peak identification: BPA, 1; 4-NP, 2. Chromatographic and SPME conditions were same as in Figure 2. Dynamic extraction mode; extraction time: 40 min.

Table I. Six Factors and Six Levels (U_6 [6^6]) in Uniform Experimental Design and Extraction Results of BPA and 4-NP (Expressed as Chromatographic Peak Area)

Run	Extraction temperature* (°C)	Extraction time (min)	Sample volume (mL)	Desorption time (min)	Salt conc. (%)	pH value	Extraction results of BPA (mv*s)†	Extraction results of 4-NP (mv*s)†
1	RT	20	20	20	10.0	7.0	759	126
2	20	40	100	5	5.0	6.0	1787	549
3	25	60	10	25	0.0	5.0	2176	575
4	30	10	60	10	12.5	4.0	609	224
5	35	30	4	30	7.5	3.0	1224	113
6	40	50	40	15	2.5	2.0	4722	3696

* RT = Room temperature was maintained in the experiments between 14 to 17°C.
† Data represented the mean values of duplicate extractions from aqueous solutions with the same concentration levels (0.808 µg/mL BPA; 1.148 µg/mL 4-NP).

Apparatus

The modular LC used consisted of two high-pressure gradient pumps (Varian 210, Palo Alto, CA), SPME–HPLC interface (Supelco, Bellefonte, PA), RF-530 fluorescence detector (Shimadzu, Japan) and chromatographic workstation StarWS (Varian). The SPME–HPLC interface consisted of a six-port Rheodyne valve with the loop replaced by a desorption chamber.

The manual SPME device use was from Supelco. The SPME fiber used included polydimethylsiloxane/divinylbenzene (PDMS/DVB, 60 µm/partially crosslinked) and carbowax/templated resin (CW/TPR, 50 µm/partially crosslinked). Before use, the fibers were conditioned by the initial mobile phase in the desorption chamber until a flat baseline was obtained.

Hot plate stirrers from Corning Global Business Operations (Milan, Italy) and a Leici PHS-3B pHmeter from Shanghai Precision & Scientific Instrument Co., Ltd. (Shanghai, China) were used.

Analytical conditions

Chromatographic conditions

Chromatographic conditions included the use of a C18 column (5 µm, 4.5 × 250 mm, Hanbon Science & Technology Co., Ltd, Jiangsu, China) and gradient elution (A: water, B: acetonitrile; 0–1 min A = 15%, B = 85%; 4 min A = 0%, B = 100%; 12 min stop) with a flow-rate of 0.8 mL/min. Fluorescence detector was set with Ex 275 nm and Em 315 nm.

SPME procedure

Simulant solution containing BPA and 4-NP was prepared by diluting the appropriate stock solutions with NaCl solution. SPME fiber was directly immersed into the previously mentioned aqueous solution for the extraction. Analytes were separated by the HPLC system and the chromatographic peak area was used to evaluate extraction efficiency.

Experimental design

First, the experiments were performed and parameters of extraction were subjected to preliminary screening based on Table I (U_6 [6^6]) (as Un [q^s], n = number of runs; s = number of factors; q = number of levels) (24). Second, the experiments were carried out according to Table II. Table II (U_{15} [3^6]) was constructed according to the results of Table I, the theory of design (25), and the Table (U_{15} [15^6]) in http://www.math.hkbu.edu.hk/UniformDesign/. Third, the optimal operational conditions were predicted by the mathematical models, which were established according to the experimental results by the software Metlab (6.5) and SPSS (11.0 production facility).

Results and Discussion

Evaluation of fiber coating

The properties (physical and chemical) of the coating are crucial for the extraction

Table II. Six Factors and Three Levels (U_{15} [3^6]) in Uniform Experimental Design and Extraction Results of BPA and 4-NP (Expressed as Chromatographic Peak Area)

Run	Extraction temperature (°C)	Extraction time (min)	Sample volume (mL)	Desorption time (min)	Salt conc. (%)	pH value	Extraction results of BPA (mv*s)*	Extraction results of 4-NP (mv*s)*
1	20	45	40	10	5.0	6.0	1725	375
2	20	60	10	20	7.5	4.0	2667	1341
3	30	45	40	10	7.5	2.0	3155	1125
4	30	45	60	20	5.0	4.0	2935	1730
5	30	30	60	20	2.5	2.0	1923	1320
6	40	30	10	10	5.0	2.0	2286	985
7	40	45	60	10	2.5	4.0	3311	1921
8	30	60	10	10	2.5	6.0	3826	2190
9	40	45	10	15	5.0	2.0	3666	1275
10	30	60	60	15	7.5	6.0	3404	1630
11	20	30	10	15	2.5	4.0	1282	631
12	40	60	40	20	7.5	4.0	4775	2405
13	20	30	60	20	5.0	6.0	1618	441
14	20	60	40	15	2.5	2.0	2244	3223
15	40	30	40	15	7.5	6.0	2471	793

* Data represented the mean values of duplicate extractions from aqueous solutions with the same concentration levels (0808 µg/mL BPA; 1.148 µg/mL 4-NP).

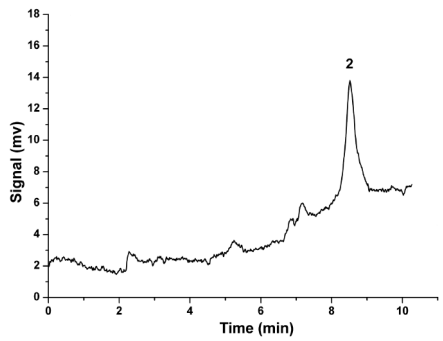

Figure 4. Chromatogram of tap water. Peak identification: 4-NP (2). Chromatographic and SPME conditions were the same as in Figure 2. Dynamic extraction mode; extraction time: 40 min.

process. In the referenced paper (15), four microextraction fiber samples were tested with superior results obtained on the polar coatings (65 µm CW and 60 µm PDMS-DVB). In our experiments, the polar coatings PDMS-DVB and CW/TPR fibers were tested with the CW/TPR selected for further analysis.

Selection of desorption

The desorption step was completed in the static mode. Static desorption of the fiber depended on the time and composition of the desorption solution. The initial mobile phase was fixed as the desorption solvent because it not only simplified the procedure but also could desorb completely within a suitable time. The desorption time was evaluated based on the obtained mathematical models (in the "Evaluation of microextraction" section). A blank analysis performed regularly showed no memory effects under the experimental conditions.

Evaluation of microextraction

As shown in Table I, the best extraction result was obtained from the sixth run. Further optimization around the sixth run was carried out in the subsequent process. In order to preserve the SPME fibers from high temperatures and low pH, 40°C as the highest temperature and 2.0 as the lowest pH value were selected for investigation. A salt concentration range of 2.5%–7.5% was selected for further study in order to optimize the extraction ratio due to salting out effects.

Based on the results in Table I, the optimal level of each factor was investigated. Figure 1A showed the results of each level of six factors of BPA, which were expressed as the summation of chromatographic peak area at the same level. Similarly, the results of 4-NP were exhibited in Figure 1B. Obviously, the optimal extraction parameters for BPA and 4-NP were as follows: extraction time, 60 min; sample volume, 40 mL; and a pH of 4.0. A salt concentration of 2.5% NaCl was chosen due to extraction yield effects that were significantly less for BPA with a 9.97% decrease versus the use of 7.5% NaCl solution. In contrast, when employing a 7.5% NaCl solution, a 21.4% decrease in 4-NP extraction yield was observed.

To obtain optimal extraction conditions and determining the parameters that effect extraction, several mathematical models were constructed considering all variables and their interaction.

Mathematic models which do not consider parameter interactions were first established based on the multiple linear regression values for the relationship between chromatographic peak area and all variables exhibited in Tables I and II:

For BPA:

$$S_1 = 1909.205 + 73.056x_1 + 55.484x_2 + 2.294x_3 - 18.177x_4 + 22.083x_5 + 7.137x_6 \quad \text{Eq. 1}$$

For 4-NP:

$$S_2 = 59.023 + 17.858x_1 + 40.023x_2 + 7.106x_3 - 13.099x_4 - 20.300x_5 - 200.033x_6 \quad \text{Eq. 2}$$

For the mean peak area of BPA and 4-NP:

$$S_3 = 984.114 + 45.457x_1 + 47.753x_2 + 4.700x_3 - 15.638x_4 + 0.891x_5 - 96.448x_6 \quad \text{Eq. 3}$$

Where S_1 is the peak area of the BPA, S_2 is the peak area of 4-NP, S_3 is the mean peak area of BPA and 4-NP, variables x_1 to x_6 corresponded to the extraction temperature, extraction time, sample volume, desorption time, salt concentration and pH. As the result of t-test, extraction temperature and time were found to be significant. Because the correlation coefficients (R^2) between experimental and calculated data were low (the highest correlation coefficient was 0.831), the non-linear regression equations were investigated further considering the interactive effect of all variables and quadratic effect ($x_1 \cdot x_2 \ldots x_5 \cdot x_6$, $x_1^2 \ldots x_6^2$) with stepwise method:

For BPA:

$$S_1 = 546.878 + 1.901x_1x_2 - 4.313x_4^2 + 121.547x_4 \quad \text{Eq. 4}$$

For 4-NP:

$$S_2 = 249.419 + 1.229x_1x_2 \quad \text{Eq. 5}$$

For the mean peak area of BPA and 4-NP:

$$S_3 = 81.256 + 1.591x_1x_2 \quad \text{Eq. 6}$$

The key parameters for the extraction and the optimal extraction conditions of BPA could be predicted from equation 4. Extraction temperature, adsorption and desorption time were the key factors for the extraction of BPA. As a result, extraction temperature, 40°C; adsorption time, 60 min; and desorption time, 14 min were predicted as the best conditions within the range tested herein. It agreed better with the validated experimental results. For 4-NP, the mathematical model (equation 5) showed that the extraction temperature and time were important and the predicted results were the same as those of BPA. So the optimal extraction conditions were as follows: extraction temperature 40°C; 40 mL of sample volume; pH 4.0; 2.5% NaCl; adsorption time 60 min; and desorption time 14 min.

Considering that stirring could improve the mass transfer of BPA and 4-NP in the extraction, some complementary experiments with stirring were done under the optimal conditions. The results are shown in Figure 2 (without stirring and with stirring). It could be seen that chromatographic peak response markedly increased under agitating mode, and extraction efficiency was improved greatly, especially for 4-NP. At last, stirring mode (1080 rpm) was used in the following experiments and the non-equilibrium extraction was adopted for saving analytical time (as the extraction of BPA was close to equilibrium). So adsorption time was shortened to 40 min.

Table III. Linear Regression Data and Detection Limits of Analytes by Optimized SPME–HPLC Method

Analytes	Linear range (ng/mL)	Equation of calibration curve*	R^2 value†	RSD (%)‡	LOD§ (ng/mL)	LOQ** (ng/mL)
BPA	1.25–50	$y = 15.169x + 5.9985$	0.9999 ($n = 6$)		0.097	0.34
	1.25–125	$y = 17.089x - 15.144$	0.9980 ($n = 7$)	6.62	–	–
4-NP	2.59–202.96	$y = 9.1165x + 43.201$	0.9996 ($n = 5$)	9.73	0.27	0.90

* y = Peak area (mv*s); x = analyte concentration (ng/mL).
† Correlation coefficient of calibration curve, n = the number of calibration points in the linear range.
‡ The relative standard deviation, evaluated at the 100 ng/mL BPA and 50.74 ng/mL 4-NP in the water, three replicates.
§ Limit of detection (S/N = 3).
** Limit of quantitation (S/N = 10).

Method validation

A synthetic solution containing the analytes of interest was prepared and analyzed using the optimal experimental conditions. It was observed that the enrichment ratio of BPA and 4-NP with SPME was greater than 60 and 70, respectively (Figure 3). Synthetic standard solutions were also used to determine linear range, detection limit, reproducibility, and quantitation limit. Table III shows these analytical characteristics. It can be seen that good linearity, detection, and quantitation limits were achieved.

The method proposed was compared with other SPME–HPLC methods (Table IV). According to Table IV, the commercial fiber CW/TPR employed in the referenced method (16) exhibited detection limits of 0.43 ng/mL and 0.29 ng/mL for BPA and 4-NP, respectively, which was greater than the values of 0.097 ng/mL and 0.27 ng/mL, respectively, obtained in the proposed procedure. Therefore, the chemometric strategy for optimization of SPME in the proposed procedure was successful.

The proposed method was applied for the determination of BPA and 4-NP in tap water. BPA was not detected and the concentration of 4-NP was found to be 10.73 ng/mL (Figure 4). The recoveries of BPA and 4-NP from tap water were 100.19 to 104.89% (n=3) and 95.38 to 113.29% (n = 3), respectively. It was

Table IV. Comparison Between Our Method and Other Methods

Methods (ng/mL)	Samples	SPME fiber	Analytes	Detection limit (ng/mL)	References
In-tube SPME–HPLC-UV	Liquid medicines and intravenous injection solution	Supel-Q PLOT capillary column (60 cm × 0.32 mm i.d., 12 µm film thickness, Supelco)	BPA, alkylphenols, and phthalates	0.1–4.0	(12)
In-tube SPME–HPLC-DAD	Food package	Supel-Q PLOT capillary column (60 cm × 0.32 mm i.d., 12 µm film thickness)	BPA 4-NP	0.1 0.6	(13)
In-tube SPME–HPLC-UV	Tap water and Donghu Lake (Wuhan, China)	Ordered mesoporous silica coated capillary	BPA	2.8–2.9	(14)
SPME–HPLC-FL	Food packages	Commercial CW fiber	BPA	1.1	(15)
SPME–HPLC-FL	Water samples	Electrochemical prepared CPANI fiber	BPA 4-NP	0.014 0.091	(16)
		Commercial CW/TPR fiber	BPA 4-NP	0.43 0.29	
SPME–HPLC-FL	Water sample	Commercial CW/TPR fiber	BPA 4-NP	0.097 0.27	Our method

shown that this method could be used to determine the analytes in tap water. Furthermore, it also could be applied for analysis these compounds in yellow river water sample (Lanzhou, China) (the data were not shown).

Conclusions

A useful optimization method based on uniform design was applied to determine BPA and 4-NP by SPME–HPLC. Optimization of the operational conditions using experimental design resulted in lower detection limits. The proposed method can be applied to determine the analytes in aqueous samples such as tap water, and may also be employed on a trial basis for the determination of other phenolic estrogens based on similar molecular structures. This optimization strategy can also be applied to other fields such as optimization of chromatographic conditions and derivatization conditions, etc.

Acknowledgments

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