

Letter to the Editor

**Simultaneous Determination of Bisphenols and Alkylphenols in Water by Solid Phase Extraction and Ultra Performance Liquid Chromatography-tandem Mass Spectrometry***SHAN Xiao Mei^{1,2,#,Δ}, SHEN Deng Hui^{2,Δ}, WANG Bing Shuang², LU Bei Bei³, and HUANG Fa Yuan¹

To establish an analytical method for determination of four bisphenols (BPA, BPB, BPF, and BPS) and two alkylphenols (4-n-OP, 4-n-NP) in water by ultra performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS). The water samples were extracted and condensed with solid-phase extraction (SPE) using C₁₈ cartridges and eluted by acetonitrile. Separation was carried out with Acquity BEH C₈ column and detection were performed by UPLC/MS/MS. Quantification was calculated by using the internal standard BPA-d₁₆ and 4-n-NP-d₈. The linear correlation coefficients of these compounds in the range of 1.0-100.0 μg/L were all over 0.999. The minimum detectable concentrations were 0.75-1.0 ng/L, and the recoveries ranged from 87.0% to 106.9%. Relative standard deviations (RSDs) were between 1.26% and 3.67%. Applying this method to detect the source water of Chaohu Lake and drinking water of Hefei, six target compounds were detected in different levels. This method is simple with high sensitivity and selectivity, could be suitable for the determination of these compounds in source and drinking water.

Bisphenols (BPs) and alkylphenols (APs) are extensively used as leading chemicals in production of epoxy resins with a variety of industrial applications, including food pack-aging, lubricants and coatings. In particular, the exposure of BPA, octylphenol and nonylphenol are the focus of a growing number of research studies, which have been mainly related to their estrogenic activity^[1]. As they are EDCs, so more and more countries and unions have established the laws and regulations to restrict their applications^[2-3]. Due to these restrictions, other bisphenolic compounds such as bisphenol B (BPB), bisphenol F (BPF), and bisphenol S (BPS), considered as substitutes for them in

industrial applications^[4], are starting to be used for the production of epoxy resins. However, no maximum residue levels or migration limits have been established to date for these compounds in water. In recent years' research, these compounds also have been found an estrogenic activity similar to BPA^[4]. It is possible to release of BPs and APs into the environment during manufacturing and by leaching from final products. So, the determination of these compounds in environmental water samples is demanded for environmental risk assessment. The trace level concentration of these contaminants and the complex matrix in environmental water samples makes it usually necessary to pretreat before instrumental analysis.

Methods for analyzing BPs and APs in water samples have been reviewed recently, showing that liquid-liquid extraction (LLE) and solid phase extraction (SPE) are the most common sample preparation treatments^[5-6]. However, SPE has many obvious advantages over traditional LLE, such as high recovery, high preconcentration factor, low consumption of organic solvents, simplicity, easy automation and operation and so on^[5-6].

For the determination of BPs and APs in water, liquid chromatography and gas chromatography coupled with mass spectrometry (LC-MS and GC-MS) are generally used. When these compounds are analyzed by GC-MS, a derivatization step is recommended in order to increase the volatility of the compounds and to enhance sensitivity in mass spectrometry^[7]. Since derivatization in GC-MS requires additional sample manipulation, thus increasing analysis time and reducing reproducibility, LC-MS has been used as an alternative technique in recent years for the analysis of these compounds^[6]. Until now, fewer researches have been reported about four BPs and two APs have been analyzed

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simultaneously in water, which coupling with ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and measured by the internal standard method with BPA-d₁₆ and 4-n-NP-d₈.

In this study, the preparation of SPE and its application in the pretreatment of four BPs (BPA, BPB, BPF, and BPS) and two APs (4-n-OP, 4-n-NP) from environmental water samples is reported. Coupling with UPLC-MS/MS, a highly selective, sensitive and simple analytical method was established which is suitable for the analysis of a large number of water samples.

For quantification of all compounds, water samples of 100 mL were spiked with 2 µg of 4-n-NP-d₈ and BPA-d₁₆ for use as internal standards. The enrichment and desalting were performed on C18 SPE cartridges (500 mg/6 mL, Waters, USA). Before use, the SPE cartridges were conditioned successively with 5 mL methanol, 5 mL water. The flow rate of the sample solution was 5 ml/min. After the sample was loaded on the cartridge and subsequently washed with 5 mL 5% methanol, the cartridges were dried on the vacuum system. The analytes were eluted from the SPE cartridges with 5 mL acetonitrile, and the eluent was concentrated to dryness under a gentle stream of nitrogen. Then the eluent was reconstituted with 70% methanol to a final volume of 1.0 mL for UPLC analysis. The analysis of all compounds was performed using an UPLC (Xevo TQ) (Waters Inc., USA), which contained data system software required for calibration, collection of UPLC-MS spectra and data processing for qualitative and quantitative analysis. Separation was achieved on a C8 column (50 mm×2.1 mm i.d., 1.7 µm particle diameter) from Waters Inc., USA. Mobile phases A and B were methanol and water, respectively. The system was run with a gradient program: 60%A-95%A (3 min)/95%A held for

2.0 min/95%A-60%A (0.1 min)/60%A held for 0.9 min. The flow rate was 0.3 ml/min, the column temperature was 40 °C, the sample temperature was 10 °C, the injection volume was 10 µL, and the whole analysis lasted for 6 min. Mass spectrometry was carried out on a triple quadrupole mass spectrometer using the negative electrospray ionization (ESI-), and with MRM scan mode.

During this study, bisphenols (BPA, BPB, BPF, and BPS), alkylphenols (4-n-OP, 4-n-NP) and the internal standards (BPA-d₁₆, 4-n-NP-d₈) were well separated under the conditions. The desirable linearity was obtained at a range from 1.00 to 100 µg/L with the correlation coefficient of no less than 0.999. The limit of detection (LOD) of BPs, OP, and NP in water samples were between 0.25 and 1.0 ng/L based on a signal-to-noise ratio (S/N) of 3, respectively (Table 1).

The recoveries were measured by analyses of the spiked water samples at three different concentration levels (low, middle, and high concentrations, the spiking levels ranged from 10 to 1 000 ng/L) with five parallels at each level. Also as shown in Table 2, satisfactory recoveries were obtained (89.2%-106.9%) with the proposed method.

Table 1. List of Linear Range, Calibration Curve, and Limit of Detection of Method

Compounds	Calibration Curve	Correlation Coefficient (r)	LOD (ng/L)
BPA	y=0.0491x-0.0087	0.9996	1
BPB	y=0.0635x-0.0094	0.9998	0.75
BPF	y=0.0407x-0.0104	0.9996	1
BPS	y=1.3163x+0.3614	0.9996	0.25
4-n-OP	y=0.0751x+0.0784	0.9997	0.75
4-n-NP	y=0.0515x+0.0337	0.9998	0.75

Note. Linear Range 1.0-100.0 µg/L.

Table 2. Recoveries of the Target Compounds in Water Samples

Compounds	Amount Spiked					
	10 ng/L (n=6)		100 ng/L (n=6)		1000 ng/L (n=6)	
	Average Recovery (%)	RSD (%)	Average Recovery (%)	RSD (%)	Average Recovery (%)	RSD (%)
BPA	90.5	3.57	103.1	1.26	103.1	1.81
BPB	89.2	3.67	99.6	1.66	99.6	1.85
BPF	91.2	2.71	103.9	2.37	106.9	1.61
BPS	89.8	3.51	90.2	2.30	91.4	2.05
4-n-OP	105.6	3.10	99.5	2.42	98.5	1.93
4-n-NP	106.9	2.02	103.6	2.03	101.2	1.93

In order to verify the reliability of the method, the SPE UPLC-MS/MS method was used for the simultaneous analysis of BPs and APs in twenty water samples. Half of them were collected from Chaohu Lake, which is the backup water source located in the southeast Hefei. And others were tap water samples, collected from the urban district. The largest RSD of every compound showed in Table 2 was selected to calculate the error range. The results indicated that BPA, BPS, 4-n-OP, and 4-n-NP were detected in all the lake water samples, at concentrations ranging from 7.3 to 224.9 ng/L, 0.3 to 2.2 ng/L, 10.9 to 38.2 ng/L and 38.6 to 86.1 ng/L. BPB was detected in only two source water samples, at a concentration of 1.92 and 13.23 ng/L, BPF was detected in three source water samples, at a concentration of 1.64 and 3.27 ng/L. As an example, Figure 1 shows a MRM chromatogram of UPLC-MS/MS corresponding to the No.3 of the lake water samples. It suggests that the source water of Chaohu Lake has been polluted in different levels by bisphenols and alkylphenols. In order to improve the quality of the water of Chaohu

Lake, which is the alternate source water for Hefei, it is essential that control measures be applied to reduce the levels of these estrogenic compounds in source water. In addition, the results shows that BPA was detected in all the tap water samples, at concentrations ranging from 1.9 to 2.7 ng/L, and other compounds were not detected in these samples. It indicated that the pollution of BPs and APs in drinking water of Hefei was at a low level.

To evaluate this method, relative reports were collected to compare. Previously, Xu Li, et al.^[7] reported a method for investigated the levels of 4-NP and BPA in bottled water and tap water in Guangzhou, China. The samples were derivated by pentafluorobenzoyl chloride and detected by GC-MS. LOD were 2.0 and 7.0 ng/L, respectively. Results show that 4-NP was present in all the bottled water while 17 out of 21 contained BPA. Their concentrations in bottled water ranged from 108 to 298 ng/L and 17.6 to 324 ng/L, respectively. The highest concentrations in tap water for 4-NP, BPA were 1987 and 317 ng/L, respectively. Vega-Morales

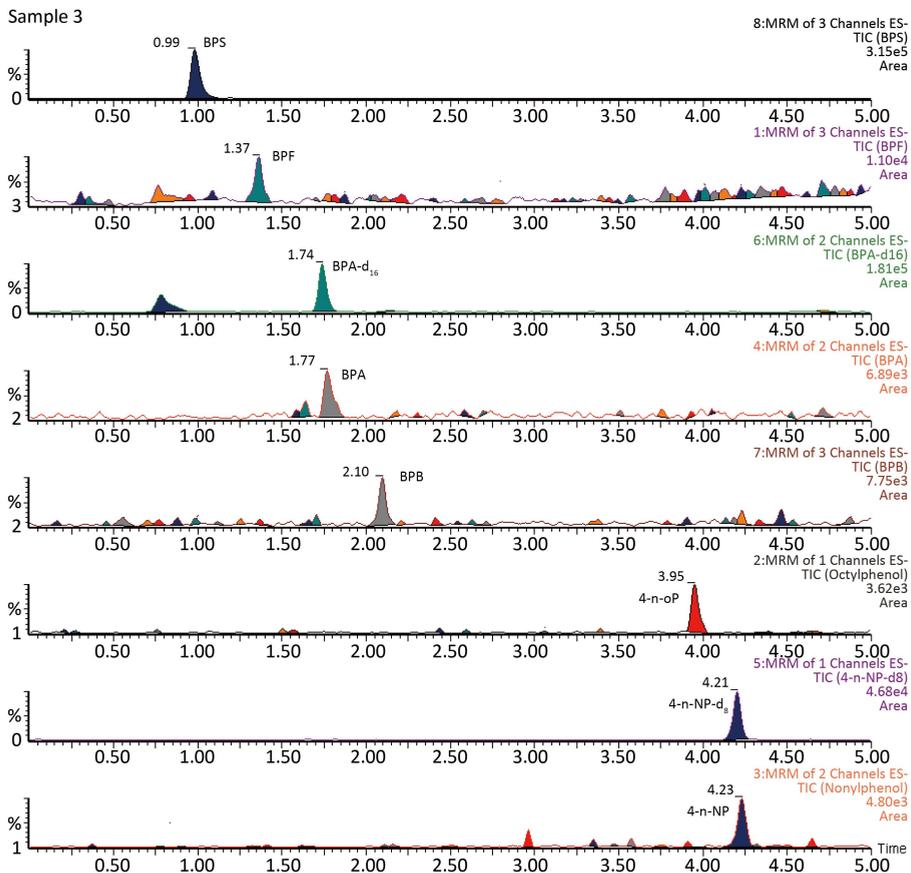


Figure 1. The MRM Chromatogram of a Water Sample.

T, et al.^[9] measured alkylphenols and BPA in sewage samples by SPE and LC/MS/MS. Satisfactory detection limits (between 1.3 and 1.9 ng/L) and analyte recoveries (between 93% and 97%) were achieved for these compounds. H.W. Chen, et al.^[10] developed a sensitive method for measuring NP, OP, and BPA in drinking water in Taiwan. The method was based on the use of automated SPE coupled with UPLC-MS /MS. The LOD of NP, OP, and BPA were 28.8, 3.3, and 1.4 ng/L, respectively. And the method was used to assess the occurrence of NP, OP, and BPA in water sources and treated water in Taiwan, to evaluate the treatment efficiencies of these compounds.

In this paper, an analytical method has been developed based on SPE-UPLC-MS/MS for the quantitative measurement of four BPs and two APs in water. Compared with the detection of GC/MS^[7], this method is free from tedious and toxic derivation procedures. Moreover, compared with the detection of LC-MS/MS, the UPLC-MS/MS spectrometry is faster with similar sensitivity^[9-10], which is suitable for the analysis of a large number of samples. In addition, the satisfactory results were obtained: high recovery (89.2%-106.9%), low detection limits (0.25-1.00 ng/L), and good repeatability (RSD <5%).

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