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Research Article

Highly sensitive determination of tetrabromobisphenol A and bisphenol A in environmental water samples by solid-phase extraction and liquid chromatography-tandem mass spectrometry

Using bamboo-activated charcoal as SPE adsorbent, a novel SPE method was developed for the sensitive determination of tetrabromobisphenol A and bisphenol A in environmental water samples by rapid-resolution LC-ESI-MS/MS. Important parameters influencing extraction efficiency, including type of eluent, eluent volume, sample pH, volume and flow rate, were investigated and optimized. Under the optimal extraction conditions (eluent: 8 mL methanol, pH: 7; flow rate: 4 mL/min; sample volume: 100 mL), low LODs (0.01–0.02 ng/mL), good repeatability (6.2–8.3%) and wide linearity range (0.10–10 ng/mL) were obtained. Satisfied results were achieved when the proposed method was applied to determine the two target compounds in real-world environmental water samples with spiked recoveries over the range of 80.5–119.8%. All these facts indicate that trace determination of tetrabromobisphenol A and bisphenol A in real-world environmental water samples can be realized by bamboo-activated charcoal SPE-rapid resolution-LC-ESI-MS/MS.

Keywords: Bisphenol A / Rapid resolution-LC-ESI-MS/MS / SPE / Tetrabromobisphenol A
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1 Introduction

Bisphenol A, 2,2-*bis*(4-hydroxyphenyl) propane (BPA), is a chemical contained in polycarbonate plastics and epoxy resins and is included in various products used in industry [1]. Tetrabromobisphenol A (4,4-isopropylidene-*bis*(2,6-dibromophenol), TBBPA) is used as a reactive or additive flame retardant in polymers, such as acrylonitrile butadiene styrene (ABS), epoxy and polycarbonate resins, high-impact polystyrene, phenolic resins, adhesives and others [2]. In printed circuit boards TBBPA content may be as high as 34% by weight [2]. The release of BPA and TBBPA into the environment from these products has attracted great attention all over the world because of their estrogenic activity or potential estrogenic activity [2, 3]. Therefore, it is crucial to develop rapid and simple analytical methods to monitor their concentration in the environment [3].

Chromatographic techniques, including gas chromatography and HPLC, have long been the most important methods for determining BPA and TBBPA. Gas chromatographic methods were developed based on the reaction of BPA and TBBPA with derivatizing reagents to form volatile compounds for chromatographic separation and detection, because they are compounds with strong polarity [4–5]. More recently, because HPLC/MS does not need prior derivatization, it has been shown to be superior to gas chromatography/MS, and is a frequently reported technique for the measurement of BPA and TBBPA [2, 6]. Low concentrations of TBBPA and BPA in real water samples make direct determination difficult. Suitable sample pretreatment methods are often necessary. An ideal sample pretreatment procedure should be simple, inexpensive, efficient, able to extract the largest number of target compounds and compatible with various determination techniques; that is the reason why these methods strive towards the simplification and miniaturization [7]. SPE has been developed in past decades and widely used for the concentration and measurement of many pollutants [8–10]. SPE has been an effective sample-handling technique with many obvious advantages such as high recovery, high pre-concentration factors, low consumption of organic solvents, simplicity, easy

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Abbreviations: BPA, bisphenol A**; RRLC-ESI-MS/MS,** rapid-resolution LC-ESI-MS/MS**; TBBPA,** tetrabromobisphenol A

automation and operation [11–12]. In SPE process, choice of adsorbents is the most important factor for obtaining higher enrichment efficiency of analytes. In recent years, bamboo-activated charcoal, a new material with special microporous and biological characteristics, has attracted great attention in environmental field. In addition, bamboo-activated charcoal is a kind of cheap material (about 0.002 US$/g), which was much lower than other adsorbents such as polymer, carbon nanotubes and other traditional adsorbents. The biggest advantage of the material is cheapness, which is very suitable for routine analysis of pollutants due to low consumption. And it has been successfully used as SPE adsorbents for the sensitive determination of triazine herbicides, perfluorooctanoic acid and phthalate esters in environmental water samples [13–15].

The main goal of this research was to develop a sensitive and cheap method for the determination of TBBPA and BPA at trace level in environmental water samples. Bamboo-activated charcoal as SPE adsorbents coupled to rapid resolution LC-ESI-MS/MS was used in this experiment. The important factors affecting the performance of SPE, such as type of eluent, eluent volume, amount of adsorbent, sample pH, volume and flow rate have been studied and optimized in detail.

2 Materials and methods

2.1 Reagents

HPLC-grade methanol and acetonitrile were purchased from Tedia company (Fairfield, OH, USA). Acetone was chromatographic grade, and purchased from Fuchen chemical reagent factory (Tianjin, China). BPA was purchased from Sigma-Aldrich Laborchemikalien Gmbh (Seelze, Germany). TBBPA standard (>98.5 %) was obtained from Shandong Morui company (Shouguang, China). Standard stock solutions (100 mg/L) containing these compounds were prepared in methanol and stored at 4°C. Fresh working solutions were prepared daily by appropriate dilution of the stock solution with purified water. Acetic acid and ammonia solution were used to adjust pH values of working solutions. All the other reagents and chemicals we are of analytical grade.

2.2 Instrument

Chromatographic separations were performed with a 1200 Binary SL Rapid Resolution series pump (Agilent Technologies, Palo Alto, CA, USA). An Agilent ZORBAX Eclipse® XDB-C18 column (4 mm × 50 mm, 1.8 μm particle size) as used held at room temperature with an Agilent 1200 series SL column compartment. A sample volume of 10 μL was injected with an Agilent 1200 series SL autosampler using a binary mobile phase composed of 90% v/v methanol and 10% v/v water containing 0.1% v/v acetic acid and 0.2% w/v ammonium acetate at a constant flow rate of 0.3 mL/min.

MS was performed on an Agilent 6410 triple quadrupole mass spectrometer fitted with an ESI MS source and controlled by Mass Hunter workstation. The ESI source conditions were established to obtain an average maximum intensity of the precursor ions. The nitrogen nebulizer pressure was set at 2.4×10^5 Pa and the nitrogen drying gas was set at 350°C with a 10 L/min flow rate. The capillary voltage was set at +3500 V. For MS-MS, N_2 of high purity was used as a collision gas. To optimize the multiple reaction monitoring transitions, direct injection of each individual compound in methanol was used. Optimal conditions are summarized in Table 1.

2.3 SPE

Bamboo-activated charcoal, commercially produced as an indoor air fresher, was purchased from Quzhou Minxin Charcoal Company (Zhejiang, China). It was triturated and sieved through 0.20 mm sieve, and dried at 80°C for 2 h prior to use as an SPE material. Bamboo-activated charcoal fibers with an average length of 3–15 μm were identified. The bamboo-activated charcoal used contained 81.21% carbon, 2.52% hydrogen and 16.27% other components.

Preparation of bamboo-activated charcoal-packed cartridges was similar to that of multiwalled carbon nanotube SPE-packed cartridges [16]. In brief, it was performed by modifying an Agilent Technologies AccuBond SPE ENV PS-DVB cartridge (1000 mg, 6 mL). After the PS-DVB packing was removed, 1.0 g of pre-treated bamboo-activated charcoal was packed in the SPE cartridge. The polypropylene upper frit was reset at the upper end of the cartridge to hold the bamboo-activated charcoal packing in place. The cartridge outlet was connected to an SHB-III vacuum pump (Great Wall Scientific and Trade, Zhengzhou, China), and the inlet was connected to a PTFE suction tube whose other end was inserted into sample solution. In order to reduce the interferences of organic contaminants, the entire SPE assembly needed to be washed with purified water and methanol before the first use.

The cartridge packed with bamboo-activated charcoal was pretreated by washing with 5 mL methanol and 10 mL purified water prior to each SPE procedure. Then 100 mL

Table 1. Multiple reaction monitoring conditions for BPA and TBBPA

Compound	RT (min)	Prec. ion	Prod. ion (P1/P2)	Frag. (V)	Collision energy, E1/E2 (V)	ESI mode
BPA	1.93	227.1	212.1/132.9	120	10/20	Negative
TBBPA	3.08	542.8	419.8/80.80	220	50/50	Negative

purified water sample spiked with two compounds was passed through the pre-conditioned cartridge at the optimum flow rate. After the sample solution had passed through, the cartridge was washed with 5 mL purified water to remove co-adsorbed matrix materials from the cartridge. Then the bamboo-activated charcoal column was dried by negative pressure for 10 min. Subsequently the analytes retained on the SPE cartridge were eluted with 8 mL methanol. The resulting eluate was blown to 1.0 mL with a gentle N_2 flow. Finally, the extract was then analyzed by rapid resolution LC-ESI-MS/MS with an injection of 10 μL.

2.4 Water samples

In this experiment, three real-world environmental water samples, including tap water and waste water, were used for evaluating the feasibility of the developed method. Tap water was collected from our lab. Waste water samples were collected from one waste water treatment plant in Jinan. Before the environmental water samples were used, they were filtered through 0.45 μm micropore membrane filter and stored in brown glass bottles at 4°C, respectively.

3 Results and discussion

In order to obtain better extraction performance with bamboo-activated charcoal as SPE absorbent for concentrating TBBPA and BPA, their recoveries were used to assess their extraction efficiency. Related important factors influencing the extraction efficiency, such as type and volume of the eluent, flow rate, sample pH and sample volume, were optimized in detail.

3.1 Influence of type and volume of the eluent

First, a suitable eluent should be found to ensure that the target compounds can be eluted completely from bamboo-activated charcoal SPE cartridge because different desorption efficiency of target compounds would be achieved due to the different elution power of the various solvents. As TBBPA and BPA were concerned, in this experiment, three solvents differing in polarity, methanol, acetonitrile and acetone were tested. Experiment conditions are as follows: volume of the sample, 200 mL; concentration for two compounds, 10 ng/mL; pH of the sample, 7; flow rate of the sample, 4 mL/min; volume of the eluent, 10 mL. Trial results indicated that methanol (98.8% for BPA and 108.3% for TBBPA) can achieve much higher recoveries than acetonitrile (98.0% for BPA and 82.2% for TBBPA) and acetone (89.0% for BPA and 83.0% for TBBPA) for two target compounds. Therefore, in the following experiments, methanol was selected.

The volume of methanol also affected the extraction efficiency of target compounds. To obtain the appropriate volume without losts of extraction efficiency, a series of experiments was designed and investigated through changing the volume of eluent methanol from 4 to 12 mL. In the operation process of SPE, another 8 mL methanol and 8 mL purified water were employed, passing through the bamboo-activated charcoal packed cartridge before the next SPE extraction in order to get rid of possible residual analytes. The experimental results obtained are shown in Fig. 1. From Fig. 1, it can be observed that the recoveries of two target compounds increased with the increase of the volumes of methanol between 4 and 8 mL. When the volume of methanol was more than 8 mL, the recoveries of TBBPA and BPA remained constant. Therefore, in subsequent experiments, 8 mL methanol was selected.

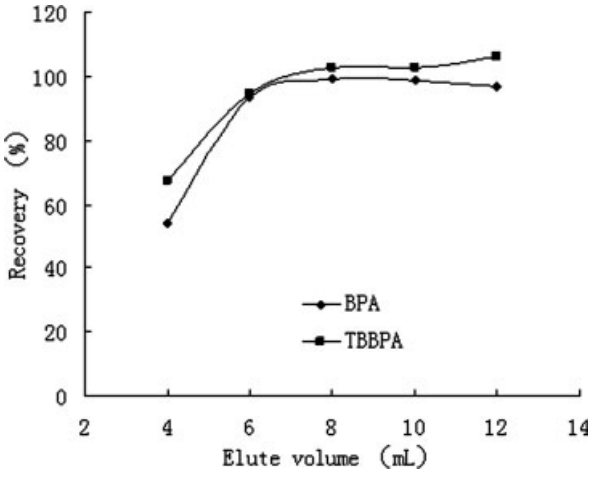

Figure 1. Influence of volume of eluent on the recoveries of TBBPA and BPA. Conditions: volume of the sample, 200 mL; concentration for two compounds, 1.0 ng/m L; pH of the sample, 7; flow rate of the sample, 4 mL/min.

3.2 Influence of flow rate

For SPE process, the sample flow rate is another critical factor that affects both the retention of target compounds on the SPE cartridge and the time of passing through SPE cartridge. In this experiment, flow rate was investigated in the range of 1–5 mL/min. Experimental conditions are as follows: volume of the sample, 100 mL; concentration for two compounds, 1.0 ng/mL; pH of the sample, 7; volume of methanol, 8 mL. The experimental results indicated that the flow rate in the range of 1–4 mL/min has no obvious influence on the recoveries of TBBPA and BPA. Therefore, in the following experiments, 4 mL/min was selected to save sample pretreatment time.

3.3 Influence of pH of sample solution

The pH value of sample solution plays a critical role in the SPE procedure, because the pH value of sample solution determines the existing state of the target compounds, and

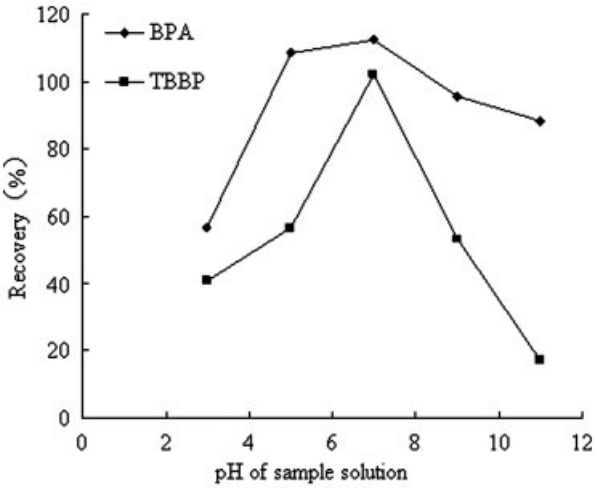

Figure 2. Influence of pH of sample solution on the recoveries of TBBPA and BPA. Conditions: volume of the sample, 100 mL; concentration for two compounds, 1.0 ng/mL; volume of methanol, 8 mL; flow rate of the sample, 4 mL/min.

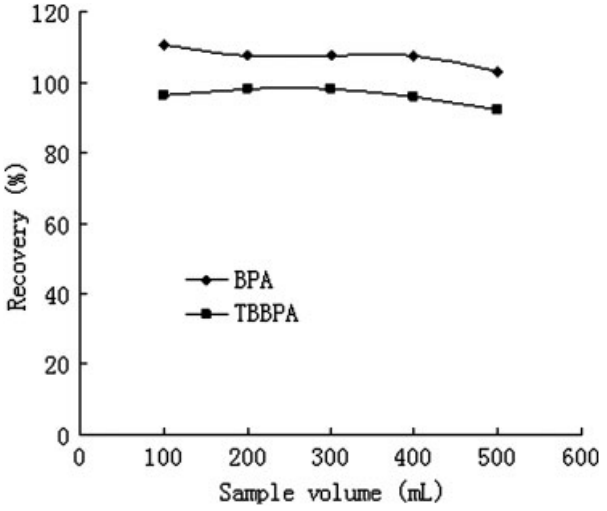

Figure 3. Influence of volume of the sample on the recoveries of TBBPA and BPA. Conditions: pH of the sample, 7; concentration for two compounds, 1.0 ng/mL; volume of methanol, 8 mL; flow rate of the sample, 4 mL/min.

thus influences the extraction efficiency of the target compounds. In order to higher extraction efficiency of TBBPA and BPA, different pH values of sample solutions ranging from 3 to 11 were studied. The experimental results are shown in Fig. 2. From Fig. 2, it is clear that with the increase of pH of sample solution, the recoveries of TBBPA and BPA increased, and the maximum recoveries (102.0% for TBBPA and 112.1% for BPA) were obtained at pH 7. Then the recoveries of the two objectives decreased with the increase of pH of sample solution. The lowest recoveries were obtained for the two objectives when the sample solution pH was equal to 3 or 11, and the main reason is that protonation or deprotonation of the hydroxyl of TBBPA and BPA increases their solubility in water. Based on the above consideration, the pH of sample solution was adjusted to 7 in subsequent experiments.

3.4 Influence of sample volume

In order to obtain a high enrichment factor, breakthrough volume is another important parameter that should be considered for SPE. In this experiment, the influence of sample volume on the recoveries of two compounds was investigated in the range of 100–500 mL while other extraction conditions were kept constant. For this purpose, 100, 200, 300, 400 and 500 mL sample solution containing 100 ng TBBPA and BPA were passed through the bamboo-activated charcoal SPE cartridge with the optimal flow rate, respectively. The experimental results are shown in Fig. 3. Recoveries almost kept constant for SPE when sample volume went up to 500 mL. In order to save sample pretreatment time, sample volume of 100 mL was adopted in further experiments.

3.5 Analytical performance

Some important parameters such as linearity range, precisions and LODs were carefully investigated to evaluate whether the method could be suitable for the determination of TBBPA and BPA in real-world environmental samples. Under the above optimal conditions, linear ranges were investigated over the range of 0.10–10 ng/mL. The repeatability was determined by extracting spiked water samples containing 0.300 ng/mL of TBBPA and BPA. The RSDs were calculated to be in the range of 6.2–8.3% for the two compounds. The experimental results are presented in Table 2. The LODs, based on an S/N of 3, ranged from 0.01 to 0.02 ng/mL. Linear range, LODs and precision were obtained. Linearity was achieved over the range of 0.10–10 ng/mL. Correlation coefficients (R) ranged from 0.9985 to 0.9998. Compared with previous SPE-HPLC-UV, SPE-MEKC and SPE-LVSEP-NACE methods, the proposed SPE-RRLC-MS/MS method can achieve much lower LODs [17–19]. These experimental results proved that the present method was very sensitive and useful in the trace analysis of TBBPA and BPA in water samples.

3.6 Real environmental water sample analysis

The proposed method was then applied to analyze three real environmental water samples including tap water and two wastewater samples. Real water samples were directly analyzed, and then analyzed by spiking with standard concentration at the concentration of 0.10 and 0.30 ng/mL, respectively. Figure 4 shows the chromatogram obtained from real water samples by spiking TBBPA and BPA standard solution. The experimental results proved that

Table 2. Linearity range, precisions and LODs of proposed method

Compound	Linear range (ng/m L)	*R*	RSD (%)	LODs (ng/mL)	SPE-HPLC-UV [17] LODs (ng/mL)	SPE-MEKC [18] LODs (ng/mL)	SPE-LVSEP-NACE LODs (ng/mL) [19]
BPA	0.1–10	0.9998	8.3	0.02	0.17	9.1	–
TBBPA	0.1–10	0.9985	6.2	0.01	–	–	0.119

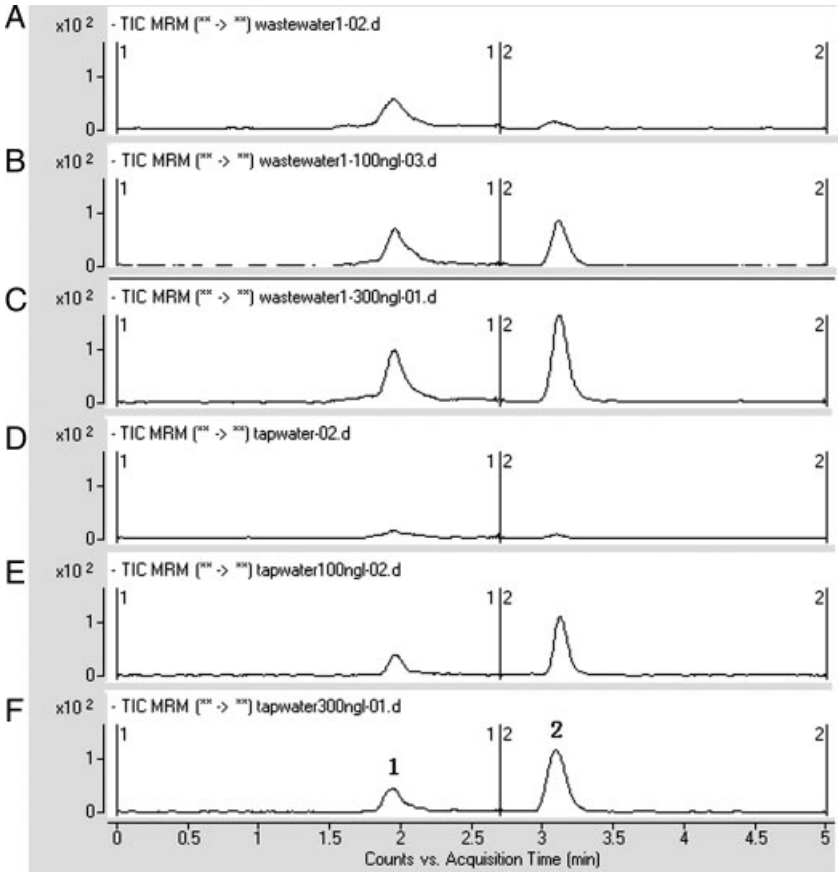

Figure 4. SPE-RRLC-MS/MS chromatograms of environmental samples. (A) Wastewater sample; (B) wastewater sample spiked with 0.100 ng/mL; (C) wastewater sample spiked with 0.300 ng/mL; (D) tap water sample ; (E) tap water sample spiked with 0.100 ng/mL; (F) tap water sample spiked with 0.300 ng/mL 1. BPA 2. TBBPA.

Table 3. Analytical results of BPA and TBBPA in tap water and wastewater samples

Water samples	Compounds	Found (ng/mL)	Added (ng/mL)	Recovery (%)	Added (ng/mL)	Recovery (%)
Tap water	BPA	ND	0.100	106.3	0.300	95.0
	TBBPA	ND	0.100	108.8	0.300	103.6
Wastewater 1	BPA	0.319	0.100	86.9	0.300	91.6
	TBBPA	0.031	0.100	80.5	0.300	85.3
Wastewater 2	BPA	0.291	0.100	119.8	0.300	105.9
	TBBPA	0.013	0.100	82.8	0.300	95.5

there was no TBBPA and BPA in tap water samples. Different concentrations of TBBPA and BPA were present in the wastewater samples (see Table 3). The experimental results indicated that the spiked recoveries of environmental water samples were in the range of 80.5–119.8% for TBBPA and BPA, and these data demonstrated that the proposed method was successfully used for highly sensitive determination of TBBPA and BPA in complex sample matrices.

4 Concluding remarks

This paper describes a simple, cheap and sensitive method for the analysis of BPA and TBBPA in environmental water samples. The method was based on SPE and RRLC-ESI-MS/MS. Bamboo-activated charcoal, an inexpensive material, exhibited excellent merits as a SPE adsorbent for the enrichment of BPA and TBBPA. More importantly, the

obvious advantage of the developed SPE method is that it can allow the simultaneous enrichment and determination of BPA and TBBPA without any complicated pretreatment procedure. At the same time, BPA and TBBPA packed cartridge can be reused more than 200 times without an obvious decrease of its adsorbent recoveries. The LODs for this method ranged from 0.01 to 0.02 ng/mL, which can satisfy analytical needs. The developed sample pretreatment procedure offered many obvious advantages such as economy, simplicity, rapidity of use, easy operation, sensitivity and good repeatability. All of these proved that bamboo-activated charcoal is a good adsorbent for the enrichment of BPA and TBBPA at trace levels in environmental water samples. In a word, it can be concluded that bamboo-activated charcoal has great potential for the enrichment and determination of BPA and TBBPA in environmental water samples.

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