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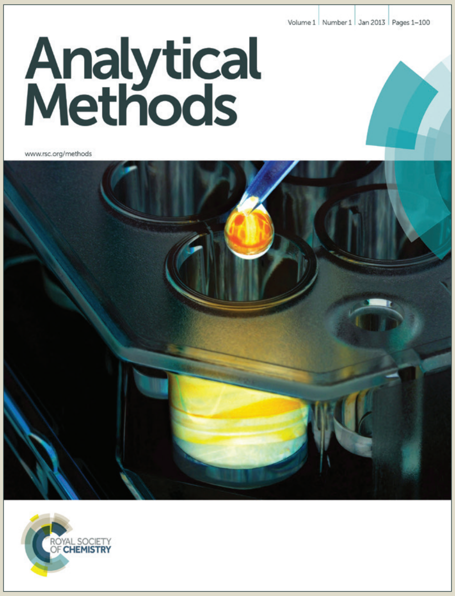

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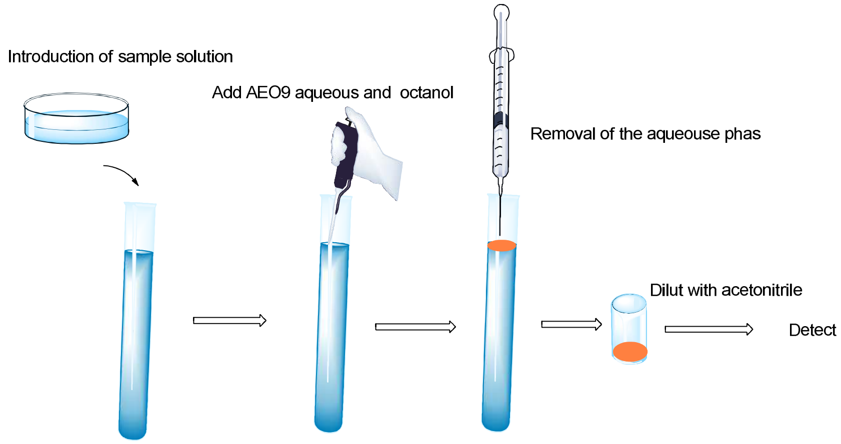

In this study, AEO9 was firstly applied for the extraction of BPA, BPAF and TBBPA, and then detected by high-performance liquid chromatography (HPLC).

Cloud point extraction for the determination of bisphenol A, bisphenol AF and tetrabromobisphenol A in river water samples by High–Performance Liquid Chromatography

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Abstract

In this work, cloud point extraction (CPE) was developed to preconcentrate and separation bisphenol A (BPA), bisphenol AF (BPAF) and tetrabromobisphenol A (TBBPA) prior to high-performance liquid chromatography (HPLC) analysis. As a first attempt, alcohol ethoxylate (AEO_9) was applied as the extractant and octanol worked as cloud point revulsant and synergic reagent for extraction, which decreased the cloud point temperature (CPT) of AEO_9 from 75 ℃ to 30 ℃ and assisted the subsequent extraction. The parameters influencing the CPE efficiency, such as AEO_9 concentrations, octanol volume, pH value, extraction time and temperature were systematically evaluated. Under the optimum conditions, the linear range of BPA, BPAF and TBBPA were from 0.05 to 25 mg L^{-1}. All correlation coefficients of the calibration curves were more than 0.9988. The relative standard deviations (RSD, n=5) were 1.6–4.7%. The limits of detection (LOD) were in the range of

0.27–0.32 μgL^{-1} and the limits of quantification (LOQ) were in the range of 0.71–0.98 μgL^{-1}. The proposed method was successfully applied to the extraction of BPA, BPAF and TBBPA in river water samples as a rapid, simple, and sensitive method.

Keywords: Cloud point extraction; High-performance liquid chromatography; Bisphenol A; Bisphenol AF ; Tetrabromobisphenol A; River water samples

Introduction

During the past years endocrine disruptors (EDs) have been attracting much more attention because of their possible negative effects on human health [1]. As important pollutants, bisphenol A, bisphenol AF and tetrabromobisphenol A are three synthetic endocrine disrupting compounds (EDCs) with a substantial use in consumer products and a widespread distribution in the environment [2-4]. BPA is a major industry product widely used in the production of resins and polycarbonate plastics [5]. As a cross-linking and curing agent, BPAF is mainly used in the synthesis of specialty elastomers to improve their chemical and thermal properties [6]. TBBPA is the most important individual brominated flame retardants (BFRs) used in industry [7]. The BPA, BPAF and TBBPA levels found in the aquatic environment were relatively low; hence, a simple, fast, low-cost, sensitive and selective analytical method is very important to assess their risk.

Different chromatographic techniques such as gas chromatography (GC), high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) and capillary electrophoresis (CE) with different detectors have extensive applications for simultaneous determination of various EDCs in liquid samples [8–11]. However, analysis of these compounds in the environment is difficult, due to their low concentrations and complicated matrix effects. A preconcentration and separation technique is usually required. Up to now, solid-phase extraction (SPE) [12], accelarated solvent extraction (ASE) [13], ionic liquid dispersive liquid-phase micro-extraction (ILDLPME) [14], vortex-assisted liquid–liquid microextraction (VALLME) [15] and dispersive liquid–liquid microextraction (DLLME) [16] have been applied to extract BPA, BPAF or TBBPA from the matrices. Cloud point extraction (CPE) on the basis of the micellar media is receiving considerable attention. CPE as an effective extraction method uses less solvent and only requires a very small amount of relatively nonvolatile and nonflammable surfactant that are environment friendly [17,18]. As such, CPE technique has been widely used for extracting trace metals and organic compounds coupled with atomic absorption spectrophotometry (AAS) [19–21], HPLC [22,23].

To our knowledge, CPE methods have been successfully applied to analyse BPA in water samples, and the non-ionic surfactants Triton X-114 and tricaprylmethylammonium chloride (Aliquat 336) were used as extractants [24, 25]. Triton X-114 and Aliquat 336 have aromatic group and would interfere with the analytes. Therefore, finding a new surfactant which would not interfere the determination of analytes is required.

In this study, AEO_9 was firstly applied for the extraction of BPA, BPAF and TBBPA. Compared with traditional CPE, AEO_9 which has no aromatic group would not interfere the detection of analytes, and extraction equilibration time was less than 10 min. With the addition of octanol, the CPT of AEO_9 was decreased from 75 ℃ to 30 ℃, thus the CPE procedure could be accomplished under lower temperature without adding salts. The optimized preconcentration and separation conditions were investigated in detail. The phenolic compounds of extraction phase were determined by high-performance liquid chromatography/diode array detection (HPLC/DAD). Finally, the proposed methods were successfully applied for the analysis of BPA, BPAF, TBBPA in water samples.

Experimental

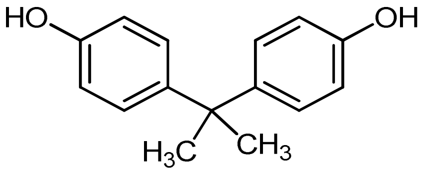
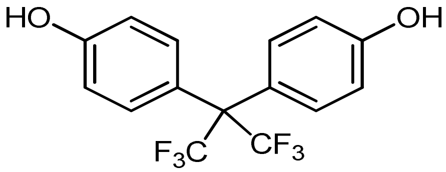
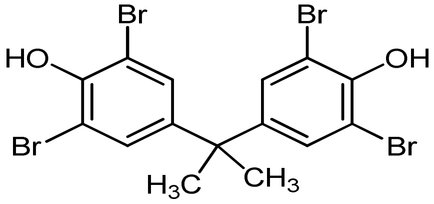
Reagents and solutions

Analytical grade BPA, BPAF and TBBPA (99%) were purchased from Aladdin (Shanghai, China). The stock solutions of BPA, BPAF and TBBPA with a concentration of 0.5 $mgmL^{-1}$ were prepared in methanol. All solutions were kept at 4 ℃ in the dark. The chemical structures of these compounds are depicted in Table 1.

HPLC grade acetonitrile was obtained from Merck (Darmstadt, Germany). Pure analysis methanol was purchased from Aladdin (Shanghai, China).

Octanol (98%) was purchased from Kedi (Tian Jin, China).The non-ionic surfactant AEO_9 (Shang Hai, china) was used without any purification. All other chemicals were of analytical reagent grade and used as received.

Table 1 Chemical and physical parameters of BPA, BPAF and TBBPA considered in this work

Analyte	Structure	Formula	Molecular weight (g mol^{-1})
BPA		$C_{15}H_{16}O_2$	228.29
BPAF		$C_{15}H_{10}F_6O_2$	336.23
TBBPA		$C_{15}H_{12}Br_4O_2$	543.87

Apparatus

Chromatographic evaluation and separation were performed on an HPLC system (consisting of a quatpump, an auto sampler, a vacuum degasser, and a diode-array detector; Agilent 1100 Series, Agilent Technologies, Calif., U.S.A.) equipped with a reversed phase C18 analytical column of 150 × 4.6 mm (Agilent TC-C18). Empower software was used for spectra recording of the studied BPA, BPAF and TBBPA, and used for spectra confirmations of peaks in the studied samples. A vortex agitator (Jiangsu, China) was used for vortex-assisted extraction. A water-bathing constant temperature vibrator (Jintan crystal glass experimental instrument, SHY-2A, JinTan, China) was used to implement CPE. A centrifuge (Shanghai, China) was used for complete phase separation.

HPLC conditions

The HPLC separation was performed on a reversed-phase system with the gradient elution using acetonitrile and water. The gradient elution was performed as follows: 42% acetonitrile (0–8.0 min), ramped to 85% acetonitrile (8.0–16.0 min). The prepared mobile phase was filtered and degassed using ultrasonic agitation. The flow rate was set at 1 mL min^{-1}. The column temperature was maintained at 25 ℃ and the injection volume was set to 10.0 μL. BPA, BPAF and TBBPA were recorded at the wavelength of 280 nm.

Cloud point extraction procedure

For CPE, 160 μL AEO$_9$ aqueous and 100 μL octanol were added to 8.0 mL sample or standard solution. Then, the mixture was vortex-shaken immediately for 2.0 min and heated for 8.0 min in a thermostatic bath at 30 ℃. Separation of the two phases was accelerated by centrifuging at 4000 rpm for 5 min. Finally, the coacervate formed a layer at the top of the aqueous sample. The aqueous phase was removed and the coacervate was deposited at the bottom of the tube. Then the coacervate was diluted to 0.5 mL with acetonitrile, and 10.0 μL of solution was injected into the HPLC system for analysis.

Results and discussion

CPE condition optimization

There are different factors that affect the extraction process such as AEO_9 concentration on CPE, volume of octanol, sample pH, temperature and time required to reach equilibrium conditions to obtain free particle extracts were investigated. It is very important to optimize these parameters in order to obtain high recovery and enrichment factor.

In this experiment, 8.0 ml of prepared sample solution of water were used in the study which spiked with different amounts of BPA, BPAF and TBBPA. All the experiments were performed five times and the averages of the results were used for optimization.

Effect of AEO_9 concentration

In CPE, AEO_9 was the extractant, and octanol worked as cloud point revulsant and synergic reagent for extraction, which decreased the CPT of AEO_9 below 30 ℃. The concentration of AEO_9 and volume of octanol remarkably affected the extraction efficiency of CPE, so careful attention should be paid to the two factors. In this section, the concentrations of AEO_9

were investigated. As shown in Fig. 1 A, the extraction efficiency of BPA, BPAF and TBBPA increased with the AEO_9 concentration increasing from 0.0% to 5.0% (v/v). However, the volume of the surfactant-rich phase increases from approximately 0.2 to 0.5 mL with increasing concentration of AEO_9 from 2.0% to 5.0% (v/v). To avoid the decrease of the concentration factor, AEO_9 concentration of 2.0% (v/v) was used in the following experiments.

Effect of octanol volume

In the developed CPE, octanol worked as cloud point revulsant and synergic reagent, which decreased the CPT of AEO_9 to 30 ℃ and assisted the subsequent extraction. The CPT of AEO_9 was decreased from 75 ℃ to 30 ℃ by adding octanol, thus the CPE procedure could be accomplished under lower temperature without adding any salts. During the optimization, the volume of octanol was investigated from 50 μL to 250 μL. When the volume of octanol was below 50 μL, the extraction was difficult to be realized, because this amount of octanol was not enough to decrease the CPT of AEO_9. As shown in Fig. 1 B, with the increase of octanol volume from 50 μL to 100 μL, the extraction efficiency was improved. However, the recovery would decrease when octanol volume was larger than 150 μL. Hence, 100 μL of octanol was chosen for the following experiments.

Effect of sample pH

The pH of the sample solution plays an important role in the extraction of the target analytes because it affects their existing forms [26]. BPA, BPAF and TBBPA show similar pKa values（≈10.1). Usually, three analytes may exist as molecular form in acidic environment. However these compounds are soluble in alkaline environment, so it is hard to be extracted. Therefore the extraction of BPA, BPAF and TBBPA was studied with the pH from 1.0 to 6.0. As shown in Fig. 1C, the recoveries of BPA, BPAF and TBBPA increased with pH increasing in the range of 1.0–3.0. However, the decrease of recoveries was observed, when pH was higher than 3.0. That due to lower pH value BPA, BPAF and TBBPA are neutral molecular form, they can be easily extracted into surfactant-rich phase. Thus, the optimum pH value of 3.0 was selected for the analysis.

Effect of the equilibrium temperature

It is desirable to have the lowest possible equilibration temperature which compromises the completion of the reaction and efficient separation of phase. If the temperature is lower than the cloud point, two phases cannot be formed. Higher temperatures lead to the decomposition of BPA, BPAF and TBBPA. To employ the lowest possible equilibrium temperature for the efficient separation of phases, the equilibrium temperature was studied in the range of 20 to 40 ℃. It is also evident from Figure. 1D that the recovery percentages of the BPA, BPAF and TBBPA increase from 75.0% to 103.1%, when temperature is raised from 20 to 30 ℃, and beyond 30 ℃, the extraction efficiency is almost constant. Based on these results, 30 ℃ was selected as the working equilibrium temperature.

Effects of equilibration time

The equilibration time play important role in the outcome of CPE performance and the shortest incubation time is ideal. Hence, the dependence of extraction efficiency on equilibration time was investigated from 4.0 to 20.0 min (Fig.1E). The extraction efficiency increased with the increase of the equilibrium time from 4.0 to 8.0 min and did not change from 8.0 to 20.0 min. In this work, the equilibrium time of 8.0 min was adopted.The procedure was then accelerated by centrifugation at 3000 rpm for 5.0 min, which was enough to get a complete phase separation.

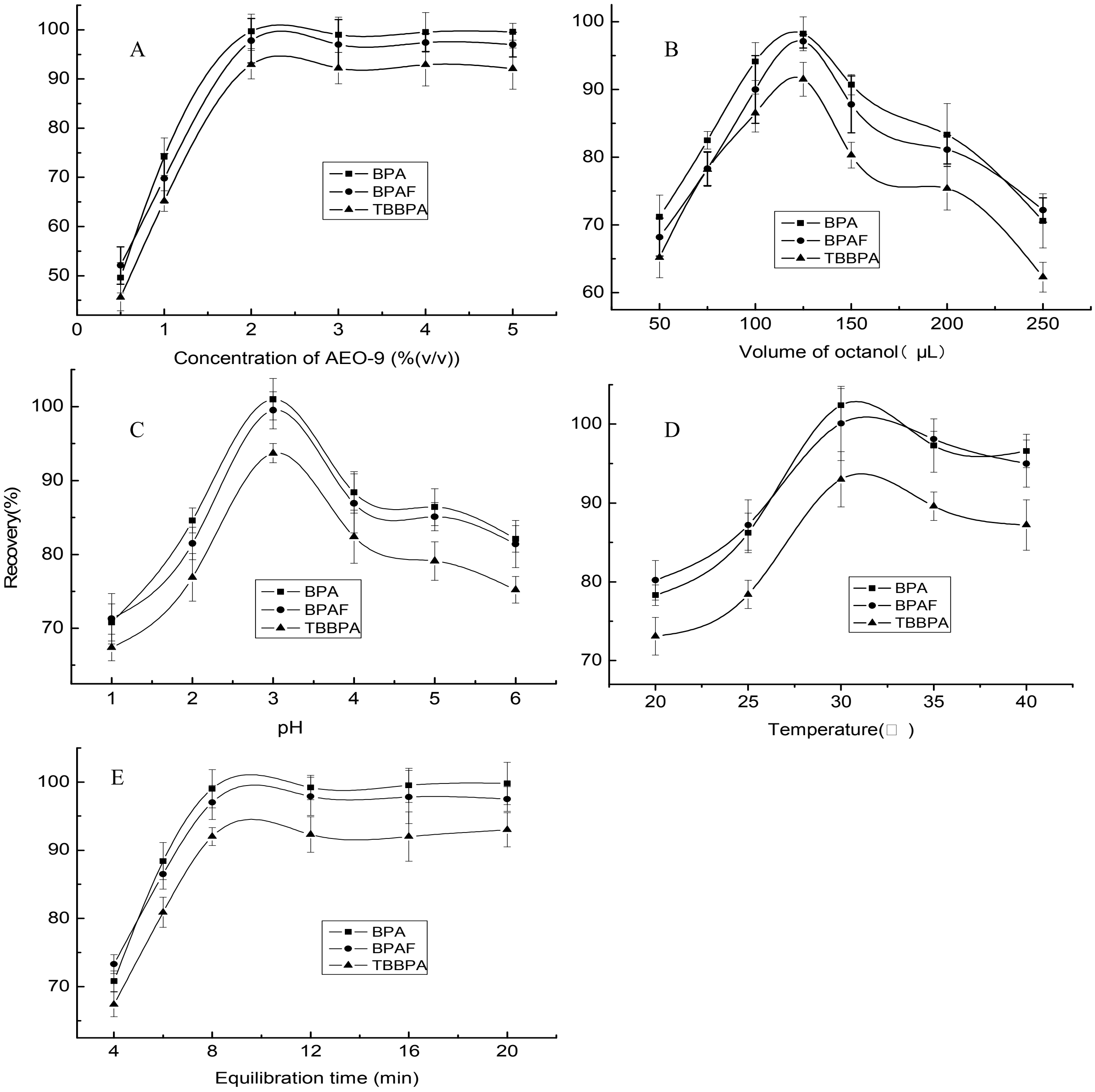


Fig. 1. Optimization of the CPE procedure. (A) Effect of AEO_9 concentration on the extraction of BPA (4 mgL^{-1}), BPAF (4 mgL^{-1}) and TBBPA (5 mgL^{-1}). CPE conditions: pH 3.0; cloud point temperature 30 ℃; octanol volume 100 μL; equilibration time 8.0 min. (B) Effect of octanol volume on the extraction of BPA (4 mgL^{-1}), BPAF (4 mgL^{-1}) and TBBPA

(5 mgL^{-1}). CPE conditions: pH 3.0; cloud point temperature 30 ℃; 2.0% (v/v) AEO$_9$; equilibration time 8.0 min. (C) Effect of sample pH on the extraction of BPA (4 mgL^{-1}), BPAF (4 mgL^{-1}) and TBBPA (5 mgL^{-1}). CPE conditions: octanol volume 100 μL; cloud point temperature 30 ℃; 2.0% (v/v) AEO$_9$; equilibration time 8.0 min. (D) Effect of the equilibrium temperature on the extraction of BPA (4 mgL^{-1}), BPAF (4 mgL^{-1}) and TBBPA (5 mgL^{-1}). CPE conditions: pH 3.0; octanol volume 100 μL; 2.0% (v/v) AEO$_9$; equilibration time 8.0 min. (E) Effect of the equilibrium time on the extraction of BPA (4 mgL^{-1}), BPAF (4 mgL^{-1}) and TBBPA (5 mgL^{-1}). CPE conditions: pH 3.0; octanol volume 100 μL; cloud point temperature 30 ℃; 2.0% (v/v) AEO$_{9.}$

Calibration, precision and detection limit

To evaluate the quantitative applicability of the method by establishing the calibration curves. Three analytes in the different concentration were prepared by CPE and then analyzed in HPLC under optimum experimental conditions. Linear relationships between the concentration and the peak area of BPA, BPAF and TBBPA were investigated. As was shown in Table 2, the linear range of BPA, BPAF and TBBPA were from 0.05 to 25 mg L^{-1}. All correlation coefficients of the calibration curves were more than 0.9988. The relative standard deviations (RSD, n=5) were 2.4–3.9%. The limits of detection (LOD) were in the range of 0.27–0.32 μgL^{-1} and the limits of quantification (LOQ) were in the range of 0.71–0.98 μgL^{-1}. The HPLC method using the CPE to determine BPA, BPAF and TBBPA have very high sensitivities, which are much higher than those reported methods [10,21,22]. Therefore, this method is very suitable for the determination of trace amounts of BPA, BPAF and TBBPA.

Table 2 Results of linear range, regression data, RSD and concentration limits of detection for the analytes

Analyte	Regression equation	linear range(mg L^{-1})	R^2	RSD / % (n=5)	LOD (μg L^{-1})	LOQ (μg L^{-1})
BPA	y = 32.252x - 2.1	0.05-20	0.9992	2.4	0.27	0.71
BPAF	y = 56.856x - 5.575	0.05-20	0.9989	3.6	0.28	0.85
TBBPA	y=79.295x + 13.204	0.1-25	0.9988	3.9	0.32	0.98

Comparison this method with other extraction methods

The proposed method has been compared with other extracting methods, including CPE–CE [21], CPE–HPLC/UV [22] and ILDLPME–HPLC/FLD [10], and the datas were shown in Table 3. The proposed method has shorter extraction time and lower equilibrium temperature than ILDLPME–HPLC/FLD and higher than CPE-HPLC/UV. Higher recovery was obtained and LOD was better than CPE–CE and CPE–HPLC/UV. Although this method is not a sensitive determination method like ILDLPME–HPLC/FLD, it is worthy to note that the method under this study is more simple, rapid, inexpensive, and easier to use.

Table 3 Characteristic performance data obtained by using this method with other extraction methods for determination of BPA

Method	Extractant	Extraction time (min)	Equilibrium temperature (℃)	Recovery (%)	LOD (μgL^{-1})	Reference

CPE–CE	Triton X-114	>20	30	92.4–104.4	1.67	[21]
CPE–HPLC/UV	Aliquat 336	=31	25	90–108.	0.34	[22]
ILDLPME–HPLC/FLD	[C8MIM][PF6]	>30	80	85.1–116.0	0.23	[10]
CPE-HPLC/DAD	AEO_9	=15	30	92.6–103.1	0.29	This work

[C8MIM][PF6]: 1-oxyl-3-methylimidazolium hexafluorophosphate

Determination of BPA, BPAF and TBBPA in water samples

In order to validate the precision, accuracy, and reproducibility of the proposed method, the proposed method was applied successfully for the determination of BPA, BPAF and TBBPA in river water samples (Kunming, China) and spiked of BPA, BPAF and TBBPA in river water samples. The results are provided in Table 4, the relative recoveries for the analytes were in the range of 92.6–103.1% and the RSDs (n=5) ranged from 1.6 % to 4.7 % in river water samples. The chromatograms of river water samples and the river water samples spiked with BPA, BPAF and TBBPA were shown in Fig.2. According to the results, BPAF and TBBPA were not found in the river water, BPA was detected in river water, the concentration was 0.35 μg L^{-1}. Some materials were found in water samples but did not interfere with the determination of BPA, BPAF and TBBPA.

Table 4 Determination of BPA, BPAF and TBBPA in river water samples

water samples	Added (μg L^{-1})	Found (μg L^{-1}) (RSD / %)^a			Recovery (%)		
		BPA	BPAF	TBBPA	BPA	BPAF	TBBPA

river water 1	0	0.35(4.1)	-	-	-	-	-
	100	99.6(3.6)	95.1(4.7)	92.9(1.6)	99.6	95.1	92.9
	300	309.5(3.2)	285.3(2.6)	279.4(3.8)	103.1	95.1	93.1
river water 2	0	-	-	-	-	-	-
	100	97.1(4.3)	96.6(2.8)	92.6(4.7)	97.1	96.6	92.6
	300	295.2(2.3)	301.2(3.9)	278.4(3.9)	98.4	100.4	92.8
river water 3	0	-	-	-	-	-	-
	100	98.9(2.3)	97.4(3.1)	91.6(2.7)	98.9	97.4	91.6
	300	307.2(4.5)	289.2(3.5)	278.5(2.0)	102.4	96.3	92.7

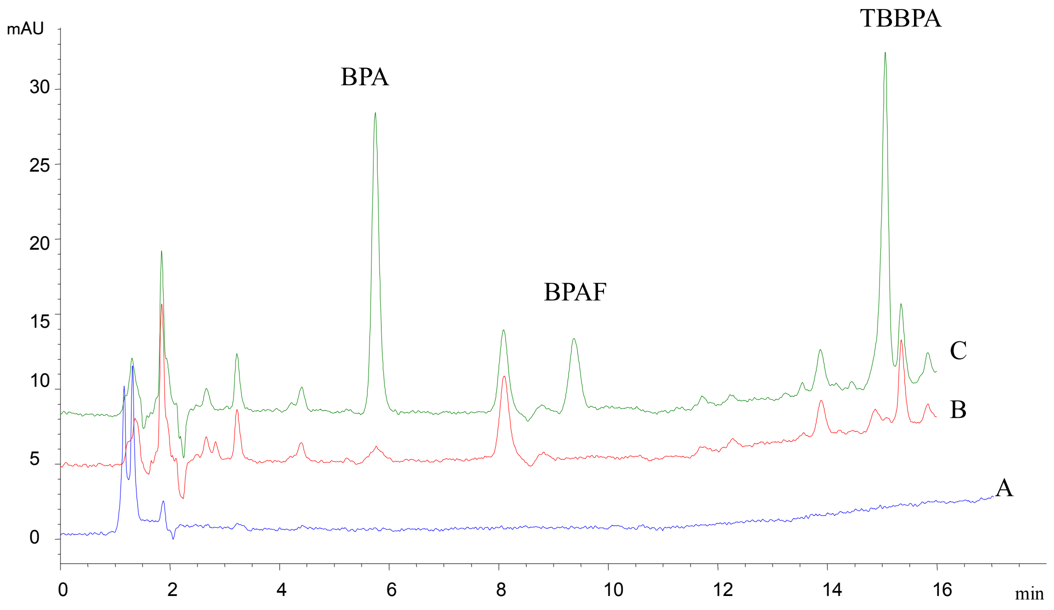


Fig. 2.. Typical chromatogram of river water: (A) blank river water; (B) river water after CPE; (C) river water spiked with BPA, BPAF and TBBPA (100 μgL^{-1}) after CPE.

Conclusions

In this work, the cloud point extraction method was combined with HPLC for the determination of of BPA, BPAF and TBBPA in river water samples. AEO_9 was applied as the extractant and octanol worked as cloud point revulsant and synergic reagent for extraction, which decreased the CPT of AEO_9 from 75 ℃ to 30 ℃ and assisted the subsequent extraction. This method offers various advantages, including efficacy, safety, rapid analysis and low cost. The proposed method was applied to detect the compounds of BPA, BPAF and TBBPA in river water at low level quantities with satisfactory results.

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