

Field-Amplified Sample Injection-Capillary Electrophoresis for the Determination of Bisphenol A, α -Naphthol and β -Naphthol in Drinks and Lake Water

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Received: 17 September 2015 / Revised: 14 November 2015 / Accepted: 7 January 2016 / Published online: 2 February 2016
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Abstract To improve the sensitivity of capillary electrophoresis, field-amplified sample injection (FASI) was developed to determine bisphenol A (BPA), α -naphthol (α -NAP) and β -naphthol (β -NAP) in drinks and lake water. Parameters (sample matrix, concentration of NaCl, water plug length, sample injection time and voltage) affecting FASI have been systematically investigated. Under optimum conditions, the sensitivity was improved 17.1-, 15.8- and 9.9-fold for BPA, α -NAP and β -NAP, respectively. The detection limits of BPA, α -NAP and β -NAP were 0.071, 0.038 and 0.081 $\mu\text{g mL}^{-1}$ and the proposed method has been successfully applied to detect BPA, α -NAP and β -NAP in drinks and lake water with recoveries of 82.0–109.3 %.

Keywords Field-amplified sample injection · Capillary electrophoresis · Bisphenol A · α -Naphthol · β -Naphthol · Drinks · Lake water

Introduction

Bisphenol A (BPA), one of the major raw materials for synthesizing polycarbonate plastics and epoxy resins, has been widely used for household and industrial purposes [1]. However, BPA, even at low exposure level, can reduce sperm production, increase weight of prostate and

predispose breast cells to cancer [2], and thus has been declared as a toxic substance by US, EU, Canada and Norway [3]. α -Naphthol (α -NAP), one of the major precursors of broad-spectrum insecticide carbaryl, is known to have similar toxicity as naphthalene [4]. β -Naphthol (β -NAP), as a secondary product of many industrial activities, is a known hazardous substance to humans and the environment. Although α -NAP and β -NAP have their own properties and applications, they often coexist in drinking water and industrial waste water, and have been considered as ubiquitous environmental carcinogens [5]. BPA, α -NAP and β -NAP are all resistant to environmental degradation through chemical, biological, and photolytic processes, and hence have potential significant impacts on human health and environment [6]. Therefore, it is highly desirable to establish a simple and sensitive method for the determination of BPA, α -NAP and β -NAP.

Techniques such as gas chromatography (GC) [7–9], high-performance liquid chromatography (HPLC) [10] and capillary electrophoresis (CE) [11] have been developed for the determination of BPA, α -NAP or β -NAP. Among them, CE has been attracting the interest of analytical workers in pharmacy [12], environmentology [13], proteomics [14] and medicine research [15], etc., due to its fast speed of analysis, high resolution and sensitivity. However, the sensitivity of capillary electrophoresis-ultraviolet detection (CE-UV) is strictly limited by its small sample injection volume (nL grade) and narrow optical path length (id of the capillary). Such limitations can be overcome by incorporating either online concentration techniques or offline sample pretreatment techniques with CE-UV. Field-amplified sample injection (FASI), based on the discrepancy of electrical resistivity between sample solution and background electrolyte, is a powerful approach for substantially improving the sensitivity of CE-UV. For example, Sun [16] used FASI

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for the determination of isonicotinamide and nicotinamide in whitening cosmetics and supplemented foodstuffs by micellar electrokinetic capillary chromatography (MEKC), Wu et al. [17] successfully used FASI-MEKC for the determination of albumin and transferring in human urines, Gallart-Ayala et al. [18] also analyzed bisphenol A, bisphenol F and their derivatives in canned soft drinks with satisfactory results by using FASI-MEKC. However, FASI-CE-UV has been rarely used in simultaneous detection of BPA, α -NAP and β -NAP.

The purpose of this work was to develop and validate a sensitive and simple FASI-CE-UV method for determination of BPA, α -NAP and β -NAP in drinks and lake water. Influences of parameters such as sample solution matrix, injection voltage and time on the analysis were studied and the applicability of FASI was evaluated. The developed method was validated for analysis of BPA, α -NAP and β -NAP in drinks and lake water.

Materials and Methods

Reagents and Solutions

1.0 mg mL⁻¹ stock standard solutions of BPA (Guaranteed grade, Aladdin Reagent, Shanghai, China), α -NAP and β -NAP (Analytical grade, Tianjin Kermel Chemical Reagents Development Center, Tianjin, China) were individually prepared with methanol (HPLC gradient grade), and stored at 4 °C in the dark, then diluted with doubly distilled water (DDW) to the desired concentration. All other chemicals, such as sodium hydroxide, hydrochloric acid, trifluoroacetic acid (TFA) and other additives are of analytical grade. Background electrolyte (BGE) of 0.5 M sodium tetraborate solution (pH 9.5) in 30 % (v/v) methanol were prepared daily. All other solutions were prepared with DDW, stored at 4 °C in the refrigerator, filtered through a 0.45 μ m membrane and degassed by ultrasonication for 15 min before use.

Instrumentation

CE experiments were performed on a Beckman P/ACE MDQ CE instrument (Fullerton, CA, USA) equipped with a UV detector. Uncoated fused-silica capillary tubing of 51 cm (43 cm to the detector, 75 μ m id \times 375 μ m od) with a polyimide outer coating was purchased from Yongnian Optical Fiber, Hebei, China. The reported pH of the solution was carefully measured with a PB-10 pH meter (Sartorius Scientific Instruments Co., Ltd., Beijing, China). A SK2200H type ultrasonic cleaning instrument (Shanghai Secco Ultrasonic Instrumental, Shanghai, China) and an

80-1 type centrifuge (Changzhou Guohua, Jiangsu, China) were used for real sample pretreatment.

Experiment Procedures

Procedure for CE

Conditions of CE process were similar to Zhong et al. [19]. All new capillaries were rinsed sequentially with ethanol, 1.0 M HCl, 1.0 M NaOH and DDW for 10 min, and then equilibrated with BGE for 10 min. Moreover, the capillary was washed between analyses with ethanol, DDW and BGE for 1, 2 and 4 min in sequence. Hydrodynamic injection was carried out at 0.5 psi for 15 s. All CE runs were implemented at 25 kV and 214 nm. The procedure was controlled by a Beckman 32 Karat 7.0 Software System.

Procedure for FASI-CE

A water plug was introduced into the capillary with hydrodynamic injection for 10 s at 0.5 psi. A high voltage (8 kV) was then applied to electrokinetically introduce the sample into the capillary for 50 s. When both electrode reservoirs were filled with BGE, the separation of BPA, α -NAP and β -NAP was performed by supplying 25 kV across the capillary.

Real Sample Preparation

10 mL of environmental water (from Qing Shan Lake), Sprite and Soda drinks (from school market) were separately kept in glass beakers through ultrasonication degassing for 30 min at room temperature, then filtered through a 0.45 μ m membrane prior to FASI-CE analysis.

Results and Discussion

Optimization of FASI Conditions

To improve the detection sensitivity of CE, FASI, an online preconcentration method was attempted. However, analytes, which can be separated successfully by CE, is not always suitable for FASI-CE. Thus, we should, in a univariate way, confirm that BPA, α -NAP and β -NAP can all be detected by FASI-CE. According to the principles of FASI, the stacking factors, including sample matrix, concentration of NaCl, water plug length, sample injection time and voltage, have been investigated one by one to get the best discrepancy of electrical resistivity between sample solution and background electrolyte.

Effect of TFA

Since the presence of a small amount of acid in sample solution can facilitate the protonation of analytes, the addition of an acid in sample matrix is usually applied to improve the stacking efficiency of FASI. In previous articles, acetic acid, formic acid, phosphoric acid, and TFA have been used for FASI [20–22], TFA was widely used due to the highest proton-donating capability (TFA > phosphoric acid > formic acid > acetic acid) [16], thus, we selected TFA and studied the effect of its concentration [0.1–1.0 % (v/v)] on stacking efficiency. The results demonstrated that the stacking efficiency increased with TFA, but when its content was over 0.5 % (v/v), the peak shape became broader. The reason is that a higher TFA content causes a lower pH and a bigger conductivity of the sample solution, resulting in insufficient sample stacking. Therefore, 0.5 % (v/v) TFA was chosen as the optimum.

Effect of Organic Solvent Type and Concentration

Adding an organic solvent into the sample solution is an immediate way to reduce its conductivity and enhance the sample stacking efficiency [23]. Effects of two common organic solvents, methanol (1.0–6.0 %) and acetonitrile (0.3–1.0 %), were investigated. The results revealed that acetonitrile had no obvious stacking effect, however, methanol produced a significant improvement. When methanol was over 4.0 % (v/v), separation was not stable even the process of separation was interrupted. Thus, 4.0 % (v/v) methanol was selected as the optimum.

To evaluate visually the effects described above, Fig. 1 shows the actual electropherograms of effects of sample matrix (effect of TFA and effect of organic solvent type and concentration). From Fig. 1b, c, the addition of 0.5 % (v/v) TFA and 4.0 % (v/v) methanol can improve obviously the detection sensitivity and separation efficiency of the three analytes as compared with Fig. 1a.

Effect of NaCl

FASI stacking efficiency relies on the different conductivity between the sample solution and running buffer. The lower conductivity the sample solution has, the higher the sensitivity will become [24]. Thus, we investigated the effect of NaCl [0–1.0 % (m/v)] on stacking efficiency. The results revealed that the stacking efficiency had no obvious variation within 0.2 % (m/v) NaCl. In our experiment, salt content of all real samples is lower than 0.2 % (m/v).

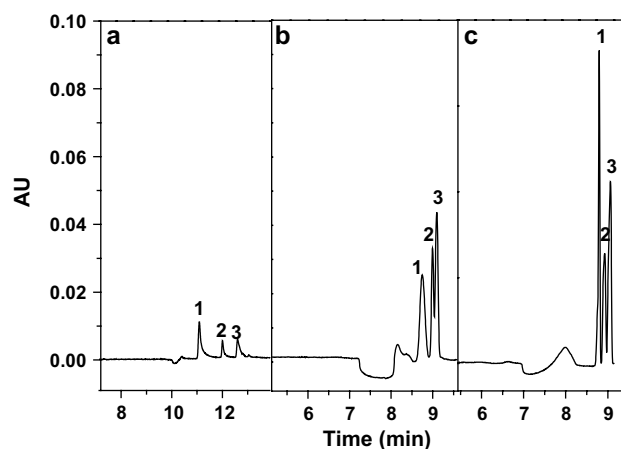


Fig. 1 Electropherogram of 10.0 $\mu\text{g mL}^{-1}$ BPA, 5.0 $\mu\text{g mL}^{-1}$ α -NAP, β -NAP obtained by FASI-CE. Sample matrix is *a* water; *b* water + 0.5 % (v/v) TFA; *c* water + 0.5 % (v/v) TFA + 4.0 % (v/v) methanol. The conditions are as in Table 1. 1 BPA, 2 β -NAP, 3 α -NAP

Optimization of the Water Plug Length

It has been proposed that the preinjection of a short water plug before sample injection in FASI can usually obtain higher sample stacking efficiency [23, 25]. Along with the increasing length of water plug, the total amount of analyte introduced into capillary by FASI becomes less. Therefore, the length of the water plug should be kept as short as possible to achieve the best efficiency. As demonstrated in Fig. 2a, an optimal water plug length is 10 s (hydrodynamic injection of water at 0.5 psi).

Effect of Sample Injection Time and Voltage

Effects of sample injection time and voltage on stacking efficiency were also investigated. When the injection voltage increased from +4 to +10 kV, the migration time gradually became short, but Joule heating and background noise were intensified. However, very low injection voltage would result in bad analytical sensitivity. As a compromise, an injection voltage of +8 kV, which gave rise to an efficient sample stacking, was selected. Similarly, the injection time was further optimized, and Fig. 2b shows that the optimum injection time was 50 s. However, when the injection time is longer than 50 s, the overloaded samples lead to the decrease of separation efficiency. Therefore, 50 s is selected as the optimum.

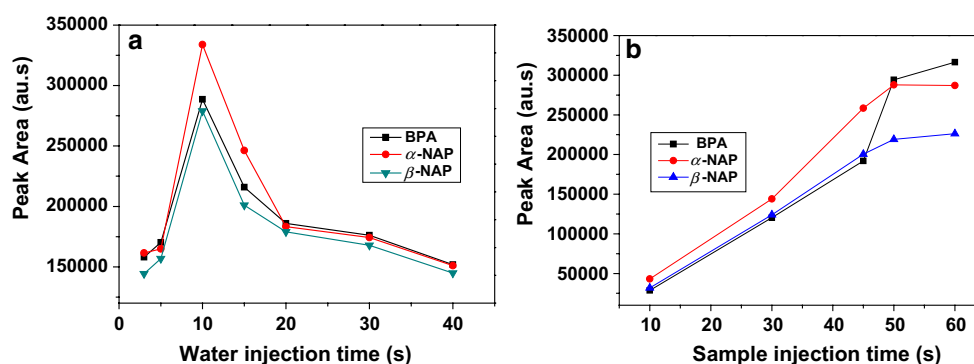


Fig. 2 Effects of water plug length (a), and sample injection time (b) on detection sensitivity. Sample conditions: $10 \mu\text{g mL}^{-1}$ BPA and $5 \mu\text{g mL}^{-1}$ α-NAP, β-NAP in 4 % (v/v) methanol with 0.5 % (v/v)

TFA. CE conditions are listed in Table 1. FASI conditions (a): electrokinetic injection of sample for 50 s at +8 kV. FASI conditions (b): pressure injection of water for 10 s at 0.5 psi

Table 1 Experimentally optimized parameters

CE conditions	
Capillary characteristics	75 μm id; 375 μm od; total length 51 cm; effective length 43 cm
BGE	50 mmol L^{-1} sodium tetraborate (pH 9.5); 30 % (v/v) methanol
Voltage	25 kV
Injection	Pressure-injected at the anodic side at 0.5 psi for 5 s
Detection	214 nm
FASI conditions	
Sample matrix	0.5 % (v/v) TFA; 4 % (v/v) methanol
Water plug length	Pressure-injected at the anodic side for 10 s
Sample injection time	Electrokinetic injection at the anodic side for 50 s
Sample injection voltage	8 kV

Separation Performance: Evaluation of the Combined Methodology

Under optimal conditions (summarized in Table 1), Fig. 3 shows the electropherograms of BPA, α-NAP and β-NAP by FASI-CE and direct CE. By comparing the peak intensity of the two electropherograms, an obvious enhancement in sensitivity is observed, indicating FASI has a remarkable preconcentration ability. The enhancement factors for BPA, α-NAP and β-NAP are 17.1-, 9.9- and 15.8-fold, respectively (enhancement factor $(A/C)/(A_0/C_0)$ where A and A_0 are the corrected peak areas of the analyte under stacking and normal conditions, respectively; C and C_0 are analyte concentrations under stacking and normal conditions, respectively). Analytical characteristic datas of the proposed FASI-CE method are summarized in Table 2. The calibration curves are established using peak area versus concentration. The linear ranges of BPA, α-NAP and β-NAP are $0.20\text{--}40.0 \mu\text{g mL}^{-1}$. LODs, calculated as the concentration of analyte that give rise to peak areas with three times of S/Ns, are 0.081, 0.038 and $0.071 \mu\text{g mL}^{-1}$ for BPA, α-NAP and β-NAP, respectively. Table 3 compares the analytical characteristics of this method with others.

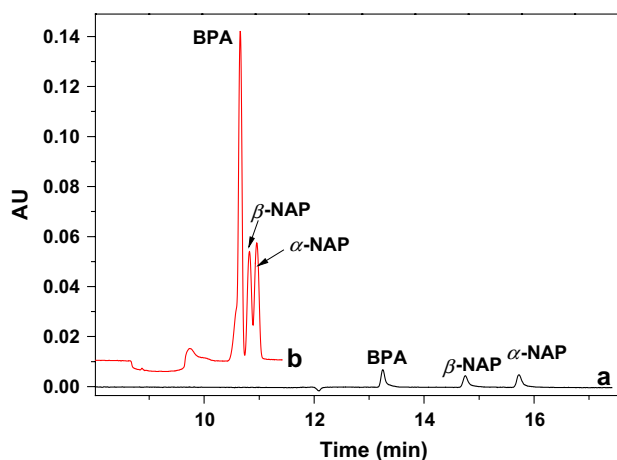


Fig. 3 Electropherograms of $10 \mu\text{g mL}^{-1}$ BPA and $5 \mu\text{g mL}^{-1}$ α-NAP, β-NAP obtained by a direct CE and b FASI-CE. The conditions are as in Table 1

It is obvious that LODs of this method are lower than the studies by Wu et al. [28] and close to that of Gallart-Ayala et al. [18]. However, as compared with others, although our LODs of FASI-CE are inferior, FASI, just based on the

Table 2 Figures of merit of the method

Compound	Regression equation	Correlation coefficient	Linear range ($\mu\text{g mL}^{-1}$)	RSD(%) ^a		EF ^b	Detection limits ($\mu\text{g mL}^{-1}$)	
				Peak area	Migration time			
FASI-CE							FASI-CE	CE
BPA	y=16274x+6534	0.9974	0.2~40	8.4	1.4	17.1	0.071	1.44
α -NAP	y=29787x+13844	0.9970	0.2~40	5.3	1.9	15.8	0.038	0.89
β -NAP	y=14185x+3751	0.9963	0.2~40	7.3	2.4	9.9	0.081	1.45
^a C= 0.2 $\mu\text{g mL}^{-1}$, n=5; ^b enhancement factor.								

^a $C = 0.2 \mu\text{g mL}^{-1}$, $n = 5$; ^b enhancement factor.^a $C = 0.2 \mu\text{g mL}^{-1}$, $n = 5$ ^b Enhancement factor**Table 3** Comparison of the proposed method with reported methods

Sample	Analytes	Methods	LODs ($\mu\text{g L}^{-1}$)	Enrichment factor	References
Canned soft drinks	Bisphenol A	FASI-MECC-DAD	54	50	[18]
River water	Bisphenol A	CPE-CE-DAD	0.5	50	[19]
	α -Naphthol		0.2		
	β -Naphthol		0.24		
Aqueous cosmetics	Bisphenol A	DLLME-CE-UV	5	60	[26]
	α -Naphthol		8	49	
	β -Naphthol		5	52	
Ground water	Bisphenol A	SPE-MEKC-UV	9.1	121	[27]
Sewage	Bisphenol A	SPE-MEKC-AD	3500	—	[28]
Water	Bisphenol A	CPE-HPLC-UV	0.1	11	[29]
Ground water	Bisphenol A	SPME-GC-MS	0.01	—	[30]
Drinks and Lake water	Bisphenol A	FASI-CE-UV	71	17.1	This work
	α -Naphthol		81	15.8	
	β -Naphthol		38	9.9	

CPE cloud point extraction, DLLME dispersive liquid–liquid microextraction, SPME solid-phase micro-extraction

discrepancy of electrical resistivity between sample solution and background electrolyte, is more convenient and simpler. Moreover, the preconcentration technique, such as CPE [19, 29], DLLME [26], SPE [27, 28], SPME [30], is time consuming and the operation costs of HPLC and GC-MS are much more expensive than FASI-CE-UV.

Real Sample Analyses

The developed FASI-CE method was applied to determine BPA, α -NAP and β -NAP in drinks and lake water under the optimum conditions. The samples and their spiked recoveries were treated as in Section real sample preparation. Figure 4 shows the electropherogram of BPA, α -NAP, β -NAP in lake water obtained by FASI-CE. In Fig. 4, (a) is an unspiked sample solution; (b) is a spiked sample solution. The results are summarized in Table 4. The recoveries of BPA, α -NAP and β -NAP are 82.0–109.3 %, indicating that the method is suitable and acceptable.

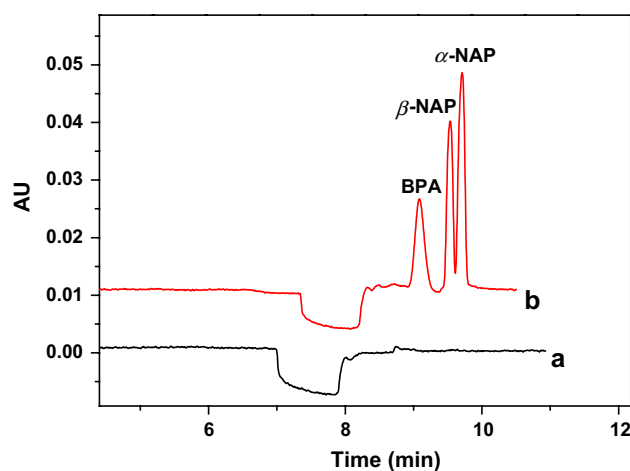
**Fig. 4** FASI-CE analysis of BPA, α -NAP and β -NAP in Qing Shan Lake water. *a* Unspiked lake water and *b* Spiked lake water. The conditions are as in Table 1

Table 4 Analytical results of BPA, α -NAP, β -NAP in sample solution

Sample	Added ($\mu\text{g mL}^{-1}$)	BPA		β -NAP		α -NAP	
		Measured ($\mu\text{g mL}^{-1}$) ^a	Recovery (%)	Measured ($\mu\text{g mL}^{-1}$) ^a	Recovery (%)	Measured ($\mu\text{g mL}^{-1}$) ^a	Recovery (%)
Lake water	0	ND	–	ND	–	ND	–
	4	3.88 \pm 0.07	97.0	3.72 \pm 0.14	93.0	3.92 \pm 0.03	98.0
	12	12.51 \pm 0.08	104.3	11.83 \pm 0.07	98.6	12.60 \pm 0.15	105.0
Sprite drink	0	ND	–	ND	–	ND	–
	4	3.35 \pm 0.06	83.8	3.74 \pm 0.13	93.5	4.37 \pm 0.04	109.3
	12	11.02 \pm 0.07	91.8	11.91 \pm 0.08	99.3	9.85 \pm 0.08	82.1
Soda drink	0	ND	–	ND	–	ND	–
	4	3.998 \pm 0.16	99.9	3.37 \pm 0.06	84.3	3.28 \pm 0.03	82.0
	12	13.13 \pm 0.09	109.4	11.28 \pm 0.05	94.0	13.12 \pm 0.06	109.3

Not detected

^a For three determinations

Conclusion

FASI, a simple online preconcentration technique, was successfully used for improving the sensitivity of CE-UV in determination of BPA, α -NAP and β -NAP in drinks and lake water. As compared with direct CE, the sensitivity of FASI-CE was improved by 17.1-, 15.8- and 9.9-fold for BPA, α -NAP, β -NAP, respectively. In comparison with other reported methods, the main advantages of this preconcentration method were more convenient, simpler and cheaper. The results indicated this method may be potentially used for the detection of BPA, α -NAP and β -NAP in real samples with low conductivity.

Acknowledgments This project was financially supported by National Nature Science Foundation of China (No. 20975031), Education Committee of Hubei Province (D20132501), Hubei Key Laboratory of Pollutant Analysis and Reuse Technique (Hubei Normal University) (KL2013M06).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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