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# Coupling Liquid-Liquid Semimicroextraction with Micellar Electrokinetic Chromatography Through On-Capillary Decomposition for the Determination of 4-*tert*-Butylphenol and Bisphenol-A with Concentration Enhancement

JING TANG[1,*], SHENG-BIAO ZHENG[1], JIN-KUN ZHU[1] and TIAN-LIN WANG[2]

[1]Department of Science, Anhui Science and Technology University, Fengyang 233100, Anhui Province, P.R. China
[2]Department of Chemistry, Shanghai University, Shanghai 200444, P.R. China

*Corresponding author: E-mail: yayajing@shu.edu.cn

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The method of methyl chloroacetate as an extraction solvent for coupling liquid-liquid semi-microextraction with micellar electrokinetic chromatography through on-capillary decomposition was applied to the determination of 4-*tert*-butylphenol and bisphenol-A with concentration enhancement. Equilibration time of extraction for 4-*tert*-butylphenol and bisphenol-A tested was reached in a relatively short period of time of 4.5 min. The concentration factors of 4-*tert*-butylphenol and bisphenol-A were 72 and 97, respectively. Linearity and detection limit of the method were also evaluated. Recoveries of 4-*tert*-butylphenol and bisphenol-A in drink water were examined. The recovery was obtained in the range of 97.0-106.5 %. The established method will be applied to the determination of bisphenol-A and alkylphenols in environmental waters.

**Key Words: Micellar electrokinetic chromatography, Liquid-liquid extraction, Methyl chloroacetate, 4-*t*-Butylphenol, Bisphenol-A.**

## INTRODUCTION

Many man-made chemicals have been found in the environment, in increasing amounts in recent years, generating awareness regarding their potential impact. The alkylphenols including, 4-*tert*-octylphenol, 4-nonylphenol and 4-*tert*-butylphenol and bisphenol-A have been shown to exhibit endocrine disrupting properties in wildlife and laboratory animals[1,2]. Trace levels of these compounds can potentially cause adverse health effects in humans and there is an increasing demand to quantify these ultra trace contaminants. Many countries classify these alkylphenols and bisphenol-A as priority hazardous compounds[3,4]. These compounds are consumed on a large scale for their industrial use. Some of these compounds are still used in certain industrialized countries. For example, bisphenol-A is a monomer used to produce polycarbonate, epoxy resins and polyester-styrene resins, which are widely used in the canned food and beverage packing industries[5].

For the accurate assessment of human exposure of these compounds, it is important to develop simple analytical methods for those compounds. The regulated analytical method for bisphenol-A and 4-*tert*-butylphenol is gas chromatography-mass spectrometry (GC-MS) with the derivatization of these compounds. However, the derivatization procedure is complex and time consuming[6]. It is well known that micellar electrokinetic chromatography (MEKC) can provide higher resolution than that of high-performance liquid chromatography. Takeda *et al.*[7-9] studied the analysis of nonvolatile or thermally degradable chemicals in water by MEKC without any derivatization procedure. However, the sensitivity of the method was poor and the detection limit is still in the ppm level. Therefore, it is necessary to develop an effective concentration method for the application of MEKC to the determination of these chemicals in environmental samples. Trace enrichment can be performed by conventional techniques such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE) and equilibrium extraction techniques such as solid-phase microextraction (SPME) and liquid-phase microextraction (LPME). However, extraction for sample preparation in MEKC associates commonly with a 'dry-and-reconstitute' process which is time-consuming, unreliable and vulnerable to sample loss and contamination. Exceptionally, Zhan *et al.*[10] reported a novel means for coupling liquid-liquid semimicroextraction for sample preparation in MEKC based on the use of ethyl acetate as the extraction solvent which was able to decompose on capillary by basic catalysis in the presence of sodium hydroxide in MEKC. The results showed that an increase in extraction efficiencies of several tens to hundreds of times

could be obtained with this sample coupling method. However, introducing a plug of solution of sodium hydroxide for basic catalysis was tedious. Moreover, sodium hydroxide might also have catalytic effects on some solutes. The use of methyl chloroacetate as an extraction solvent of neutral compounds for sample preparation in MEKC is reported[11]. Introducing a plug of catalyst was not needed. The results showed that sample concentration of several tens to more than one hundred times for neutral compounds was readily obtained with this simple coupling method developed.

This paper reports the method of methyl chloroacetate as an extraction solvent for coupling liquid-liquid extraction (LLE) with micellar electrokinetic chromatography (MEKC) through on-capillary decomposition applied to the determination of 4-*tert*-butylphenol and bisphenol-A with concentration enhancement.

## EXPERIMENTAL

4-*tert*-Butylphenol and bisphenol-A were purchased from Aldrich (Milwaukee, MI, USA). Sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate dodecahydrate of analytical grade and methyl chloroacetate of chemical pure grade were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Sodium dodecyl sulfate (SDS) of analytical grade was a product of Shanghai Haoshen Chemical Reagent Ltd (Shanghai, China). Drink water was purchased from Wahaha Group Co., Ltd. (Shanghai, China). Deionized water was used throughout the experiments.

Micellar electrokinetic chromatography was performed with a CE-L1 instrument (CE Resources Pte Ltd, Singapore) equipped with a Linear UVIS 200 detector (Alltech, Deerfield, IL, USA). Electropherograms were recorded with the CSW (Chromatography Station for Windows) (DataApex, Prague, Czech). Fused-silica capillaries of 55 cm total length and 43 cm effective length and of 375 μm OD and 50 μm ID used in the micellar electrokinetic chromatography (MEKC) experiments were purchased from Yongnian Optical Fiber Factory (Hebei, China). Nylon filters with pore size of 0.45 μm were obtained from Quandao Technical Company (Shanghai, China).

**Extraction procedure:** Extraction was carried out in volumetric flasks of 100 mL at 25 ºC. 2.5 mL of methyl chloroacetate was added to 100 mL of aqueous sample solution of 4-*tert*-butylphenol and bisphenol-A. Then, the samples were extracted by hand-shaking the flasks up and down for specified period of time. Actually, the contents of the flask could be agitated in any manner that ensures adequate mixing of the organic and aqueous phases, which often results in a much shorter equilibration time.

**Micellar electrokinetic chromatography operating procedures:** The running buffer for MEKC was prepared by dissolving 50 mmol/L SDS in 25 mmol/L phosphate buffer (pH = 7.0). Buffer used for MEKC was ultrasonicated for 10 min before use. Stock solution of the alkyl phenol was prepared by dissolving 4-*tert*-butylphenol (10 mg) and bisphenol-A (10 mg) in 1 mL methanol first and then topping up to 100 mL with deionized water. Methanol was used to dissolve them because of its fewer disturbances to the extraction and the UV detection. The aqueous sample solutions at desired concentrations were obtained by appropriately diluting the stock solution with deionized water. Before running the MEKC separations, the capillaries were conditioned by rinsing them with 0.1 mol/L NaOH for 10 min, deionized water for 5 min and the buffer for 10 min in sequence. Between runs, the capillaries were flushed with 0.1 mol/L NaOH and deionized water for 2 min of each and the buffer for 5 min in the same sequence above. Sample introduction in the MEKC was made by applying pressure of 0.30 psi for 12 s. The set-up voltage of the sample inlet side and temperature of the capillary cartridge were 20 kV and 25 ºC, respectively, throughout all experiments. On-capillary UV detection was conducted at 214 nm in the MEKC experiments.

## RESULTS AND DISCUSSION

**Selection of an extraction solvent:** Methyl chloroacetate meets the requirements of high rate of hydrolysis and low solubility in water basically. At 25 ºC, the solubility of methyl chloroacetate in water is 1.8 % v/v, much lower than that of ethyl acetate, which is about 7.7 % w/w in water. The decomposition products of methyl chloroacetate by hydrolysis are methanol and chloroacetic acid. Both can dissolve in the separation buffer in MEKC at any proportions. Thus, introducing samples with methyl chloroacetate as extraction solvent will not interrupt the current during the MEKC separation. Indeed, it was found that on-capillary hydrolysis of methyl chloroacetate catalyzed by NaOH was quite fast in the MEKC in our preliminary experiments. Stable current was reached immediately. We attempted to use methyl chloroacetate as an extraction solvent for coupling LLsME with MEKC through on-capillary decomposition for the separation of 4-*tert*-butylphenol and bisphenol-A with concentration enhancement without introducing the plug of NaOH solution. The experimental results showed that methyl chloroacetate was decomposed without introduction of any additional catalysts to a sufficient extent to conduct current in the MEKC.

**Microextraction:** Because of operating convenience and minimizing solvent consumption, solvent extraction has been performed in volumetric flasks. Another merit in using volumetric flasks for solvent extraction is that vigorous agitation is allowed in order to hasten extraction equilibrium and consequently improve reproducibility and shorten analysis time. To extract 4-*tert*-butylphenol and bisphenol-A in aqueous matrix, 2.5 mL of methyl chloroacetate was added into 100 mL of aqueous sample of 4-*tert*-butylphenol and bisphenol-A in a 100 mL volumetric flask. Extraction was promoted by simply turning the volumetric flask up and down at a moderate speed of 60 times per minute. The results show that equilibrium for 4-*tert*-butylphenol and bisphenol-A tested was reached in a relatively short period of time of 4.5 min. When equilibrated, the organic phase was transferred by using a syringe to a sample vial for sample introduction in the MEKC.

**Concentration enhancement of LLsME:** Fig. 1 shows an electropherogram of 4-*tert*-butylphenol and bisphenol-A obtained in the MEKC before extraction, while Fig. 2 shows a typical electropherogram of 4-*tert*-butylphenol and bisphenol-A after extraction with methyl chloroacetate. The identity of 4-*tert*-butylphenol and bisphenol-A peaks was assigned on the

basis of spiking the standards individually. It is noted that there were some interference peaks (peaks 1 and 2) caused by impurities in the extraction solvent on trace b in Fig. 1. The interference peaks were identified by comparing trace b in Fig. 1 with the electropherogram of a blank (not shown). By comparing corresponding normalized peak areas of the two electropherograms, concentration factors using the LLsME for 4-*tert*-butylphenol and bisphenol-A were 72 and 97, respectively. These concentration factors were greater than the volume ratio of the aqueous sample solution to the extraction solvent because of the volume reduction of methyl chloroacetate recovered after being partially dissolved in the aqueous sample solution. The results shows that sample concentration of several tens to more than one hundred times for 4-*tert*-butylphenol and bisphenol-A was readily obtained with this simple coupling method developed.

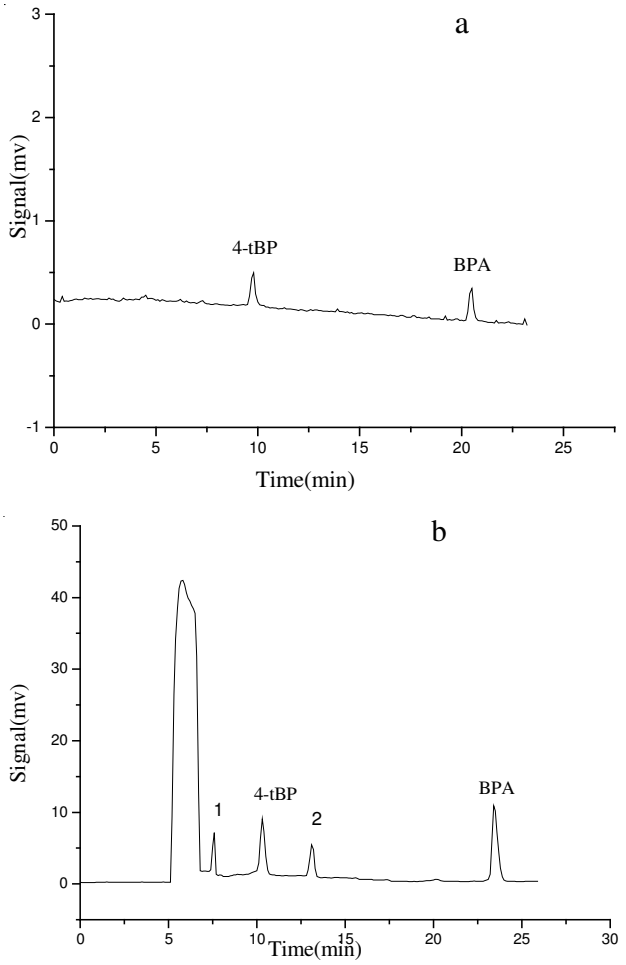


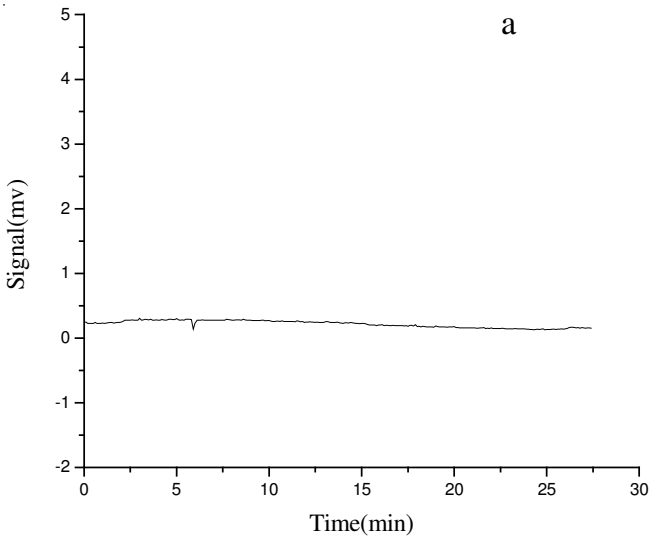

Fig. 1. (a) Electropherogram of 4-*tert*-butylphenol (4-tBP) and bisphenol-A (BPA) (2.0 mg/L) obtained in the MEKC before extraction. (b) Electropherogram of 4-*tert*-butylphenol and bisphenol-A obtained in the MEKC coupling with liquid-liquid semi-microextraction using methyl chloroacetate as extraction solvent. Concentrations of 4-*tert*-butylphenol and bisphenol-A in the original aqueous solution were 2.0 mg/L. Note that there was a large peak that was the undecomposed methyl chloroacetate and there were some interference peaks (peaks 1 and 2) caused by impurities in the extraction solvent. 4-*tert*-Butylphenol, bisphenol-A-sample peaks. Injections were made hydrostatically at 12 s. Buffer was 25 mmol/L phosphate with 50 mmol/L SDS, pH 7.0; capillary, 50 μm I.D., with a total length of 55 cm (effective length, 43cm); applied voltage, 20 kV

**Quantitative analysis:** Quantitative data are shown in Table-1. The extraction and determination of 4-*tert*-butylphenol and bisphenol-A was performed with the optimal LLsME conditions and MEKC. Under these optimum conditions, the LLsME acceptor phase was directly compatible to MEKC. Linearity was observed over the range of 0.1-4.0 mg/L for the analytes. Coefficients of correlation ($r^2$) were all above 0.99. The detection limits for 4-*tert*-butylphenol and bisphenol-A were slightly different from each other. The detection limit of 4-*tert*-butylphenol was 0.05 mg/L, while 0.04 mg/L for bisphenol-A.

TABLE-1
LINEAR EQUATION AND CORRELATION COEFFICIENT

| | 4-*tert*-Butylphenol | Bisphenol-A |
|---|---|---|
| Calibration line | y = 74.105x + 1.2179 | y = 102.3x + 1.2957 |
| Linear range (mg/L) | 0.1-4.0 | 0.1-4.0 |
| Correlation coefficient | 0.9909 | 0.9919 |
| LOD (S/N = 3) (mg/ L) | 0.05 | 0.04 |

**Demonstration of potential application of the method developed:** A drink water sample from wahaha factory was filtrated through a 0.45 μm nylon filter. The filtrated water was divided into three parts. One part was injected directly for the MEKC and the electropherogram obtained is shown as trace a in Fig. 2. No peak was observed. Another part of the filtrated water was extracted with methyl chloroacetate as did for 4-*tert*-butylphenol and bisphenol-A in the extraction procedure. The separated organic phase was injected for the MEKC under the same experimental conditions for trace a. The electropherogram obtained for the organic phase is shown as trace b in Fig. 2. Several large peaks could be seen clearly. Last part was spiked with the standard solution of 4-*tert*-butylphenol and bisphenol-A and subjected to the same extraction and separation. The electropherogram obtained for the organic phase is shown as trace c in Fig. 2. The average recoveries of 4-*tert*-butylphenol and bisphenol-A were between 97.0 and 106.5 %. Application potential of the method developed might be demonstrated by these experimental results.



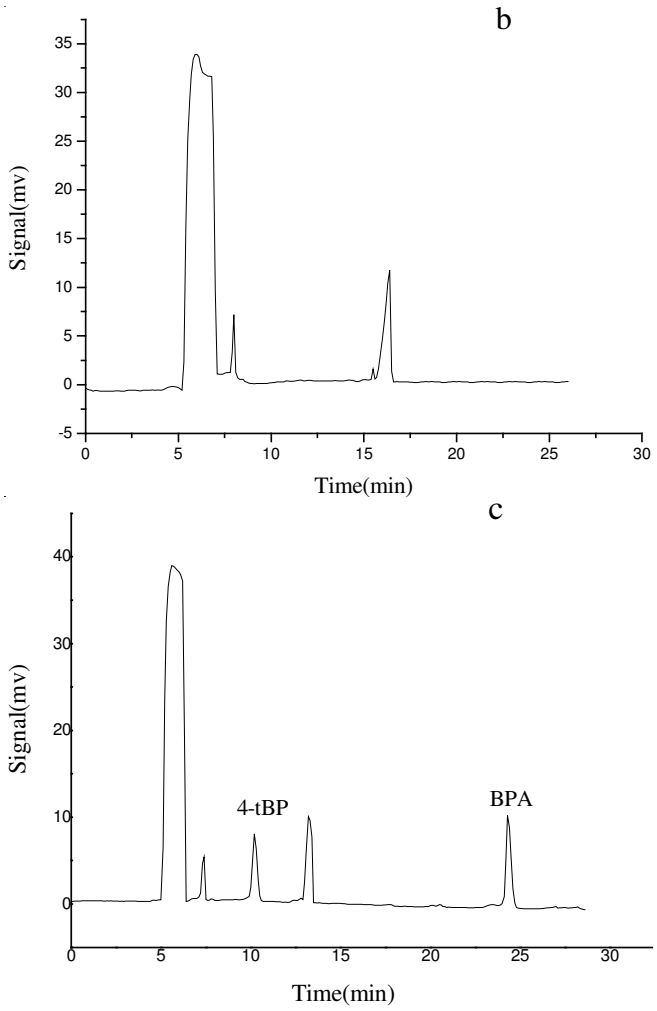



Fig. 2. (a) Electropherogram of drinking water before extraction without addition in MEKC. (b) Electropherogram of drinking water after extraction without addition in MEKC. Sample peak was not found. Note that there was a big peak that was the undecomposed methyl chloroacetate and there were some interference peaks caused by impurities in the extraction solvent. (c) Electropherogram of drinking water after extraction with addition in MEKC. 4-*tert*-Butylphenol, bisphenol-A-sample peaks. Note that there was a big peak that was the undecomposed methyl chloroacetate and there were some interference peaks caused by impurities in the extraction solvent. Other experiment conditions were the same as Fig 1.

## Conclusion

The relatively high concentration factors of 4-*tert*-butylphenol and bisphenol-A were achieved by coupling liquid-liquid semi-microextraction (LLsME) with micellar electrokinetic chromatography (MEKC) through on-capillary decomposition. The results shows that sample concentration of several tens to more than one hundred times for 4-*tert*-butylphenol and bisphenol-A was readily obtained with this simple coupling method developed. The established method will be applied to the determination of bisphenol-A and alkylphenols in environmental waters.

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