

## Determination of Bisphenols in Water Samples by HPLC after Preconcentration with Multiwalled Carbon Nanotubes

Hideyuki Katsumata<sup>1</sup>, Hiromi Mizuno<sup>1</sup>, Satoshi Kaneco<sup>1</sup>, Tohru Suzuki<sup>2</sup> and Kiyohisa Ohta<sup>1</sup>

<sup>1</sup> Department of Chemistry for Materials, Graduate School of Engineering, Mie University, Tsu, Mie 514-8507, Japan

<sup>2</sup> Environmental Preservation Center, Mie University, Tsu, Mie 514-8507, Japan

Corresponding author: Hideyuki Katsumata

E-mail: [hidek@chem.mie-u.ac.jp](mailto:hidek@chem.mie-u.ac.jp)

**Abstract:** A sensitive and selective column adsorption method is proposed for the preconcentration and determination of bisphenols (BPs), namely bisphenol A (BPA), bisphenol E (BPE) and bisphenol F (BPF). BPs were preconcentrated on multiwalled carbon nanotubes (MWCNTs) as an adsorbent and then determined by high performance liquid chromatography (HPLC). Several parameters on the recovery of the analytes were investigated. The experimental results showed that it was possible to obtain quantitative analysis when the solution pH was 4 using 150 mL of validation solution containing 1.5 µg of BPs and 5 mL of methanol as an eluent. Recoveries of BPA, BPE and BPF were 71.2±1.3, 93.0±4.8 and 98.5±4.0% respectively at 95% confidence level under optimum conditions. Relative standard deviations for seven determinations were 1.9–5.2%. The maximum preconcentration factor was 180 for BPs when 900 mL of sample solution volume was used. The linear range of calibration curve was 5 to 40,000 ng mL<sup>-1</sup> with correlation coefficients of 0.995 (BPA), 0.990 (BPE) and 0.997 (BPF). The detection limits (3S/N) were 0.5 ng mL<sup>-1</sup> for all BPs. The proposed method was successfully applied to the determination of BPs in leaching water from plastic bottle with high precision and accuracy.

**Keywords:** bisphenols; preconcentration; adsorption; multiwalled carbon nanotubes; HPLC

### Introduction

Bisphenol A (BPA) is manufactured in high quantities, 90% or more being used as a monomer for the production of polycarbonate and epoxy resins, unsaturated polyester-styrene resins and flame retardants. The final products are used as coating on cans, as powder paints, as additives in thermal paper, in dental fillings and as antioxidants in plastics [2]. It is well known that BPA has an estrogenic activity, that is, it serves as an environmental endocrine disrupter [3]. Nearly 30% of world production is within EU, with 210,000 tonnes being reported to have been produced in Germany in 1995 [4]. The release into the natural environment as well as in surface water is possible during manufacturing process and by leaching from final products. In fact, leaching of BPA from polycarbonate baby bottle [5] and reusable container [6] has been reported. In addition, BPA was detected in plastic waste [7], in plasma stored in polycarbonate tube [8], in aquatic environment and air [9], and on land [9]. Moreover, high concentration of BPA can be contained in wastewater from its production factories because it is partially removed during wastewater treatment. The wastewater containing BPA can be source of contamination in aquatic environment [9,10].

The low concentrations at which bisphenols (BPs) are present in aqueous environmental samples and the complex and potential different composition of these samples make necessary the use of preconcentration and clean up steps prior to their

chromatographic analysis. GC/MS is the technique most commonly used to determine BPs at the ng/L level in water samples [11–14], its main drawback being the complex and time-consuming sample treatments required, which comprise at least three steps: extraction of the target analytes, evaporation of the extract to dryness and derivatization. A number of methods based on liquid chromatographic separation and mass spectrometric [15,16], fluorimetric [17] and electrochemical [17,18] detection, using simpler and faster sample treatments, have been recently reported.

Solid-phase extraction (SPE) is the technique commonly used for BPs extraction from environmental aqueous samples prior to LC analysis because of its advantages over liquid-liquid extraction: high preconcentration factors, low consumption of organic solvents, elimination of emulsions and easy automatization. C18-silica is the most widely used sorbent material, but it provides relatively low recoveries (about 80% for BPA at the ng/L level from 500 ml of water [15]), and, requires evaporation of eluates to reach the preconcentration factors demanded in environmental analysis (which greatly increases analysis time). Other sorbents such as polystyrene-divinylbenzene polymers present similar drawbacks [18].

In recent years, carbon nanotubes (CNTs), a novel nanomaterial, have been studied for removal of organic pollutants and metal ions. It has shown excellent extraction efficiency in the procedure of

SPE because of its large specific surface areas. It has been successfully applied to the determination of some organic pollutants in environmental samples. Cai et al. [17,19,20] used multiwalled carbon nanotubes (MWCNTs) as adsorbents for preconcentration of phthalate esters, BPA, 4-*n*-nonylphenol and 4-*tert*-octylphenol and chlorophenols in environmental water samples before chromatographic analysis, and favorable achievements were obtained. In addition, Zhou et al. [21,22] also indicated that MWCNTs showed its excellent enrichment efficiency as SPE adsorbents.

As described above, SPE procedure is useful for the preconcentration of BPs in water samples. Therefore, it is worthwhile to develop the preconcentration method (SPE-like) for BPs based on adsorption onto MWCNTs as SPE material. This technique would provide a viable alternative to existing SPE method. In this paper, a column method was proposed for the preconcentration of BPs, namely BPA, bisphenol E (BPE) and bisphenol F (BPF) in water by using MWCNTs as an adsorbent. The several parameters affecting the adsorption of BPs onto the adsorbent were evaluated by HPLC, which was able to separate these compounds. This proposed method was applied to the determination of BPs in leaching water from plastic bottle.

## Materials and Methods

### Reagents

BPA, BPE and BPF were obtained from Wako Pure Chemical Industries (Osaka, Japan) and were used without further purification. Stock standard solutions ( $20 \mu\text{g mL}^{-1}$ ) were prepared by dissolving the appropriate amounts of BPs in water. Working standard solution was prepared daily by diluting with water. All other organic solvents used were of analytical grade and were used without further purification.

MWCNTs (an average external diameter of 40–70 nm) as an adsorbent were purchased from Wako Pure Chemical Industries. Before use, MWCNTs were washed with deionized water and dried in an oven at  $80^\circ\text{C}$  for 2 h.

### Apparatus

Concentrations of BPs were determined by a Model GL-7410 HPLC pump (GL Science, Tokyo,

Japan) with a Model GL-7450 UV spectrophotometer (GL Science, Tokyo, Japan). The separation column used was an Inertsil ODS -2 (150 mm length  $\times$  4.5 mm i.d., GL Science, Tokyo, Japan). The mobile phase was acetonitrile/water (50/50) and a flow rate was  $1 \text{ mL min}^{-1}$ . Injection volume of solution was 20  $\mu\text{L}$ . pH of solution was measured by a Model D-21 digital pH/mV meter equipped with a glass electrode (Horiba Ltd., Kyoto, Japan).

### Preconcentration procedure

50 mg of MWCNTs was packed in a glass SPE cartridge (50 mm  $\times$  10 mm i.d.). The sample solutions (150 mL) spiked with  $10 \text{ ng mL}^{-1}$  of BPs were adjusted to suitable pH, and then, passed through the glass SPE cartridge at a constant flow rate. The pH of the solution (2 – 10) was adjusted with  $\text{H}_2\text{SO}_4$  or NaOH. Next, the retained BPs were eluted from MWCNTs with 5 mL methanol. The analytes in the eluate obtained were determined by HPLC. The absorbances of BPs were monitored at 220 nm. The quantifications of BPs were made by comparison of peak areas with those of standards. The results obtained were expressed in terms of recovery percentages of BPs, and were calculated as follows:

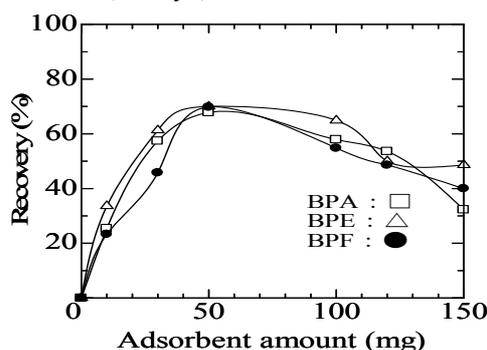
$$\text{Recovery (\%)} = (C / C_T) \times 100$$

where  $C$  and  $C_T$  were BPs concentrations after preconcentration found by HPLC and concentrations calculated theoretically, respectively. Blank and control experiments were performed in the same manner without BPs or MWCNTs.

## Results and discussion

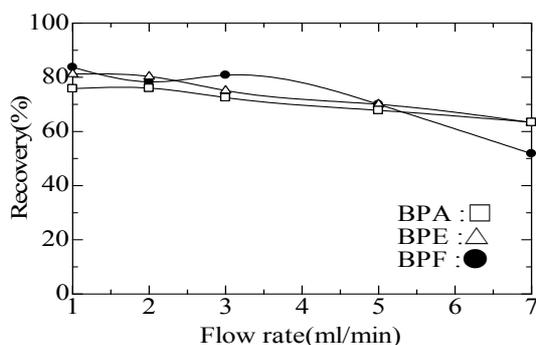
### Effect of variables on preconcentration of BPs

Effect of MWCNTs amount on preconcentration of BPs ( $10 \text{ ng mL}^{-1}$ ) was examined in the range of 0 – 150 mg. The results are shown in Fig. 1. The recoveries of BPs increased with increasing the adsorbent amount up to 50 mg, and then decreased. About 70% of recovery percentages for BPs were obtained upon 50 mg of MWCNTs. Therefore, 50 mg of the adsorbent was found to be optimum of the preconcentration method.



**Figure 1.** The effect of MWCNTs amount on the recovery of BPs. BPs solution:  $10 \text{ ng mL}^{-1}$ ,

The flow rate of water sample solution, always contributes a significant impact to the procedure because the sample flow rate affects both the recoveries of analytes and loading time in a solid-phase extraction system. The experiments indicated that the recoveries of BPs could reach approximately 70% even if the flow rate was at 5 mL min<sup>-1</sup>. The effect of flow rate on the recovery of the compound is shown in Fig. 2.

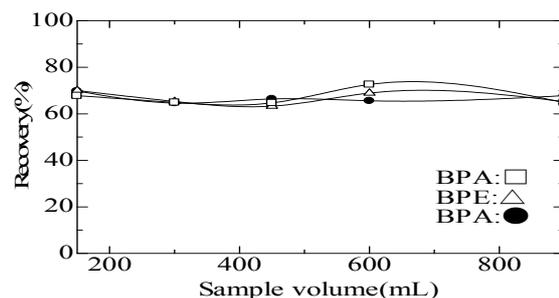


**Figure 2.** The effect of flow rate on the recovery of BPs by MWCNTs. BPs solution: 10 ng mL<sup>-1</sup>, 150 mL; MWCNTs amount: 50 mg; pH: 6; eluent: methanol, 5 mL

In a preliminary test, methanol was found to be an effective eluent for BPs. To find the required volume of methanol to elute the analytes from the glass cartridge, eluent volumes up to 10 mL were investigated. The experimental results indicated that sufficient recoveries (70%) could be achieved when the volume of methanol was in the range 5–10 mL. Because application of a smaller volume of eluent contributes to obtaining a higher preconcentration factor, 5 mL of methanol was adopted as the eluent throughout the experiments.

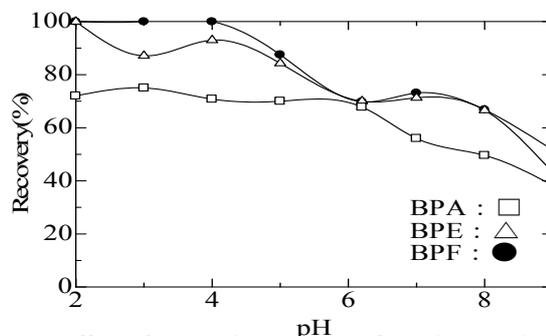
In order to determine the maximum applicable sample solution, the effect of changes in the volume of validation solution on the adsorption of BPs was investigated (Fig. 3). By varying the volume of validation solution (150, 300, 450, 600 and 900 mL) for the fixed amount of adsorbent (50 mg) and BPs (1.5 µg), no change was observed in the recoveries of BPs up to a volume of 900 mL. Therefore, sample volume up to 900 mL was recommended to use for the preconcentration of unknown sample. These results show that a preconcentration factor was obtained as 180 when we use 900 mL of sample volume.

Sample pH plays an important role in the SPE procedure, because the pH value of the solution determined the present state of analytes in solution as ionic or molecular form, and thus determined the extraction efficiency of the target analytes. For this experiment, a wide range of sample pH from 2 to 9 was evaluated. These results are shown in Fig. 4. The recovery percentage of BPA was almost constant over the pH range of 2 – 6, and then decreased with increasing pH above 6.



**Fig. 3.** Effect of sample solution volume on the recovery of BPs by MWCNTs. BPs amount: 1.5 µg; MWCNTs: 50 mg; flow rate: 5 mL min<sup>-1</sup>; pH: 6; eluent: methanol, 5 mL

On the other hand, those percentages of BPE and BPF rapidly decreased with increasing pH above 4. Therefore, the pH value of sample solution used for subsequent experiments was 4.



**Fig. 4.** Effect of pH on the recovery of BPs by MWCNTs. BPs solution: 10 ng mL<sup>-1</sup>, 150 mL; MWCNTs amount: 50 mg; flow rate: 5 mL min<sup>-1</sup>; eluent: methanol, 5 mL.

#### Characteristics of the method

For the precision of this method, the optimum conditions described above were used. For this purpose, seven successive adsorption-elution cycles were carried out and BPs were determined in the solution by HPLC. The spiked solution (150 mL) was used to contain 10 ng mL<sup>-1</sup> of BPs. The recoveries of BPA, BPE and BPF were 71.2±1.3%, 93.0±4.8% and 98.5±4.0%, respectively, with a relative standard deviation for seven determinations of 1.87%, 5.18% and 4.03%. These results are sufficient for preconcentration purposes.

Calibration graphs for BPs determination were prepared by using a series of 150 mL BPs solutions (initial concentration: 0.5 – 40,000 ng mL<sup>-1</sup>) preconcentrated under the optimum conditions. The linear range of the calibration graphs was from 5 to 40,000 ng mL<sup>-1</sup> with correlation coefficients of 0.995, 0.990 and 0.997 for BPA, BPE and BPF, respectively. The detection limits (3S/N) were found to be 0.5 ng mL<sup>-1</sup> for all BPs.

*Determination of BPs in leaching water from plastic bottle*

The proposed method was applied to the determination of BPs in leaching water from plastic bottle. Leaching water sample was prepared by adding boiling water into plastic bottle for 2 h. The preconcentration procedure was carried out as soon as possible after sampling. The amount of MWCNTs used in this experiment was 50 mg. BPs were not found in the leaching water as shown in Table 1. The leaching water sample (150 mL) was spiked with 5, 10 and 15 ng mL<sup>-1</sup> of each BPs for evaluating the recoveries. The recovery tests were carried out under the optimum conditions. The recoveries of spiked-BPs were in the range of 92.0–101% and relative standard deviations were in the range of 1.1–5.7% for three replicates. As a result, the recoveries and precision of BPs determination were very satisfactory. Therefore, 50 mg of MWCNTs could be sufficient for the preconcentration of BPs in water samples, which contains various organic compounds. Thus, the present

method is suitable for the preconcentration and determination of BPs from water samples.

**Conclusion**

A simple and rapid procedure was developed for the determination of BPs in the range 5 – 40,000 ng mL<sup>-1</sup> after the preconcentration with MWCNTs as an adsorbent. BPs were quantitatively recovered with high precision. The maximum preconcentration factor was 180 for BPs when 900 mL of sample solution volume was used. Furthermore, the proposed method was successfully applied to the determination of BPs in leaching water from plastic bottle. The recoveries of spiked BPs in the leaching water were in the range of 92–103 %. In the preconcentration method, it was found that trace BPs in aqueous samples could be determined without interferences of matrix compounds and chemical treatment. This method not only applicable to successive analysis of BPs but also to on-line preconcentration. Furthermore, this technique could be adapted to *in situ* analysis.

Table 1. Determination of BPs in leaching water from plastic bottle

	Added ( $\mu\text{g L}^{-1}$ )	Found ( $\mu\text{g L}^{-1}$ )	Recovery [%] <sup>a)</sup>	
Leaching water	–	BPA	n.d.	
	–	BPE	n.d.	
	–	BPF	n.d.	
	5		BPA	4.89 [97.8±5.01]
			BPE	4.65 [92.9±1.08]
			BPF	4.60 [92.0±1.34]
	10		BPA	9.54 [95.4±4.15]
			BPE	10.1 [101±5.68]
			BPF	9.76 [97.6±1.40]
15		BPA	15.5 [103±2.70]	
		BPE	15.0 [100±2.92]	
		BPF	14.8 [98.9±1.08]	

n.d.; Not detected.

<sup>a)</sup> Average of three determinations.

## References

- Harries E., Runnalls T., Hill E., Harris C.A., Maddix S., Sumpter J.P. and Tyler C.R. (2000) Development of a reproductive performance test for endocrine disrupting chemicals using pair-breeding fathead minnows (*Pimephales promelas*). *Environ. Sci. Technol.*, **34**, 3003–3011.
- Staples C.A., Dorn P.B., Klecka G.M., O'Block S.T. and Harris L.R. (1998) A review of the environmental fate, effects, and exposures of bisphenol A. *Chemosphere*, **36**, 2149–2173.
- Krishnan A.V., Stathis P., Permuth S.F., Yokes L., Freldman D. and Sonoyama N. (1993) Bisphenol-A: An estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology*, **132**, 2279–2286.
- Leisewitz A. (1997) Stoffstrome wichtiger hormonell wirkender substanzen (Behavior of important hormonal active substances). Project No. 10601076, German Federal Environmental Agency, Berlin.
- Takao Y., Lee H.C., Kohra S. and Arizono K. (2002) Release of bisphenol A from food can lining upon heating. *J. Health Sci.*, **48**, 331–334.
- Biles J.E., McNeal T.P., Begley T.H. and Hollifield H.C. (1997) Determination of bisphenol-A in reusable polycarbonate food-contact plastics and migration to food-simulating liquids. *J. Agric. Food. Chem.*, **45**, 3541–3544.
- Yamamoto T. and Yasuhara A. (1999) Quantities of bisphenol A leached from plastic waste samples. *Chemosphere*, **38**, 2569–2576.
- Sajiki J., Takahashi K. and Yonekubo J. (1999) Sensitive method for the determination of bisphenol-A in serum using two systems of high-performance liquid chromatography. *J. Chromatogr. B*, **736**, 255–261.
- Staples C.A., Dorn P.B., Klecka G.M., O'Block S.T., Branson D.R. and Harris L.R. (2000) Bisphenol A concentrations in receiving waters near US manufacturing and processing facilities. *Chemosphere*, **40**, 521–525.
- Fürhacker M., Schaf S. and Weber H. (2000) Bisphenol A: emissions from point sources. *Chemosphere*, **41**, 751–756.
- Vilchez J.L., Zafra A., González-Casado A., Hontoria E. and Olmo M. (2001) Determination of trace amounts of bisphenol F, bisphenol A and their diglycidyl ethers in wastewater by gas chromatography-mass spectrometry. *Anal. Chim. Acta*, **431**, 31–40.
- Suzuki T., Nakagawa Y., Takano I., Yaguchi K. and Yasuda K. (2004) Environmental fate of bisphenol A and its biological metabolites in river water and their xeno-estrogenic activity. *Environ. Sci. Technol.*, **38**, 2389–2396.
- Fromme H., Kuchler T., Otto T., Pilz K., Müller J. and Wenzel A. (2002) Occurrence of phthalates and bisphenol A and F in the environment. *Water Res.*, **36**, 1429–1438.
- Liu R., Zhou J.L. and Wilding A. (2004) Simultaneous determination of endocrine disrupting phenolic compounds and steroids in water by solid-phase extraction-gas chromatography-mass spectrometry. *J. Chromatogr. A*, **1022**, 179–189.
- Rodríguez-Mozaz S., López de Alda M.J. and Barceló D. (2004) Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction-liquid chromatography-mass spectrometry. *J. Chromatogr. A*, **1045**, 85–92.
- Watanabe Y., Hosoya K., Tanaka N., Kondo T., Morita M. and Kubo T. (2005) LC/MS determination of bisphenol A in river water using a surface-modified molecularly-imprinted polymer as an on-line pretreatment device. *Anal. Bioanal. Chem.*, **381**, 1193–1198.
- Cai Y., Jian G., Liu J. and Zhou Q. (2003) Multiwalled carbon nanotubes as a solid-phase extraction adsorbent for the determination of bisphenol A, 4-*n*-nonylphenol, and 4-*tert*-octylphenol. *Anal. Chem.*, **75**, 2517–2521.
- Inoue K., Yoshie Y., Kondo S., Yoshimura Y. and Nakazawa H. (2002) Determination of phenolic xenoestrogens in water by liquid chromatography with coulometric-array detection. *J. Chromatogr. A*, **946**, 291–294.
- Cai Y., Jian G., Liu J. and Zhou Q. (2003) Multiwalled carbon nanotubes packed cartridge for the solid-phase extraction of several phthalate esters from water samples and their determination by high performance liquid chromatography. *Anal. Chim. Acta*, **494**, 149–156.
- Cai Y., Cai Y., Mou S. and Lu Y. (2005) Multiwalled carbon nanotubes as a solid-phase extraction adsorbent for the determination of chlorophenols in environmental water samples. *J. Chromatogr. A*, **1081**, 245–247.
- Zhou Q.X., Wang W.D. and Xiao J.P. (2006) Preconcentration and determination of nicosulfuron, thifensulfuron-methyl and metsulfuron-methyl in water samples using carbon nanotubes packed cartridge in combination with high performance liquid chromatography. *Anal. Chim. Acta*, **559**, 200–206.
- Zhou Q.X., Xiao J.P., Wang W.D., Liu G., Shi Q. and Wang J. (2006) Determination of atrazine and simazine in environmental water samples using multiwalled carbon nanotubes as the adsorbents for preconcentration prior to high performance liquid chromatography with diode array detector. *Talanta*, **68**, 1309–1315.