한국환경분석학회지 제8권 (제2호) 67~71, 2005
J. of the Korea Society for Environmental Analysis

# Optimization of a Modified Solid-phase Microextraction Method for the Analysis of Alkylphenols in Water Samples

**Hyun-Mee Park, Soon-Min Hong, and Kang-Bong Lee†**

**Advanced Analysis Center, Korea Institute of Science and Technology, P. O. Box 131, Cheongryangri, Seoul 130-650, Korea*

Optimization of solid-phase microextraction(SPME) for the analysis of alkylphenols(4-t-butyl, 4-n-butyl, 4-n-hexyl, 4-n-heptyl, 4-t-octyl, nonyl) and bisphenol-A in water samples were investigated. Polyacrylate(PA) fiber was modified with silylating reagent Bis(trimethylsilyl)trifluoroacetamide (BSTFA)/ Trimethylcholro silane(TMCS)(100:1 v/v) before extraction. After extraction, the modified PA fiber was directly transferred to the injector of a gas chromatograph where the analytes are thermally desorbed and subsequently separated and quantified. Extraction parameters such as extraction time, pH of sample, concentration of salt added were optimised. The proposed method provided a good reproducibility less than 15%, and good linearity(R>0.9). Limits of detection(LODs) ranged between 0.2 and 1.7 μg/L and were almost equivalent to those by liquid-liquid extraction (LLE) (0.1~1.0 μg/L). The results obtained suggested that modified SPME was a rapid, simple and efficient technique for the analysis of alkylphenols and bisphenol A in water sample and it can simultaneously do derivatization and extraction. The formation of trimethylsilyl derivatives improves the selectivity, sensitivity and performance of the chromatographic properties.

**Key words** : Solid-phase microextraction, alkylphenols, water analysis, GC-MS, silylation

## 1. Introduction

Endocrine disruptors are of great environmental interest and have been found in water. Their release into the environment is of great concern because of their toxicity[1], effect in drinking water[2] and widespread use in industrial wastes[3]. Alkylphenols are present in the aquatic environment as a result of their industrial applications. Alkylphenols and bisphenol-A are included in EPA list of endocrine disruptors. Knowledge about their toxicity, even at low concentrations, has been increasing for the past few years. Therefore, it is essential to develop a rapid, sensitive and selective method of analysis for these compounds. Solid-phase microextraction (SPME) is a fast, simple, inexpensive and solvent free extraction technique4. It has been applied to the extraction of organic pollutants from different matrices at trace levels[5,6]. SPME has been applied to the analysis of phenols in environmental matrices, mainly in water samples[7-9]. Bulchholz and Pawliszyn[10] investigated the in situ acetylation of phenols followed by SPME-GC-MS analysis. They have shown the advantages of acetylation regarding the peak shape and the chromatographic separation of phenols. The methods based on GC are time-consuming and require a derivatization step[11,12]. An attempt at using a combination of solid-phase microextraction and on column silylation for the determination of bisphenol A was reported in the literature[13-16].

This paper describes the modification of SPME fiber with silylation reagent, bis(trimethylsilyl)trifluoroacetamide (BSTFA) before the extraction and the extraction of alkylphenols and bisphenol-A from environmental water samples. Direct SPME technique was used for this purpose. Different parameters affecting the analytical efficiency have been optimized. Detection limit, linearity and precision studies have been made with modified fiber.

†To whom correspondence should be addressed.

## 2. Materials and Methods

### 2.1. Reagents and Instrumentation

The compounds studied were 4-t-butylphenol, 4-n-butylphenol, 4-n-pentylphenol, 4-n-hexylphenol, 4-t-octylphenol, 4-n-heptylphenol, nonylphenol and bisphenol-A (BPA). 4-t-Butyl phenol, 4-n-pentylphenol, 4-t-octylphenol, 4-n-heptylphenol and bisphenol -A were supplied by Kanto (Japan) and 4-n-butylphenol was supplied by TCI chemicals (Japan). 4-n-hexylphenol was purchased from Wako chemical (Japan) and nonylphenol from Aldrich (WI, U.S.A.). Standard solutions of each phenol were mixed and prepared weekly in acetone (J.T. Baker, U.S.A.) and stored in a refrigerator. Working solutions were prepared weekly by diluting the stock solution with acetone and were later used to spike the water solutions. Bis(trimethylsilyl) trifluoroacetamide (BSTFA)(Aldrich, WI, U.S.A.) was used for the modification of fibers.

The effects of pH and salt on the SPME fiber were examined. Citric acid(Yakuri pure chemicals, Kyoto, Japan) was used to control the pH of the saturated salt solution.

SPME fiber holder, with 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 65 μm carbowax/divinyl benzene (CW/DVB), 85 μm polyacrylate (PA) was used.

The GC-MS system used in this experiment consisted of Varian 3400 gas chromatograph with Magnum (Finnigan, U.S.A.) ion trap mass spectrometer. The chromatographic column was DB-5MS (30 m × 0.25 mm × 0.25 μm) made by J & W Scientific (U.S.A.). Helium gas was used as carrier gas(purity 99.999%). The splitless mode was used with the split valve closed for 5 min. Initial oven temperature was 120°C which was maintained for 2 min and then raised to 280°C at the 10 °C/min rate and maintained for 5 min. Injector temperature was held constant at 270°C. Trap, manifold and transferline temperatures were 250, 50 and 280°C, respectively. The mass spectrometer was used in the positive electron impact mode at 70 eV. A mass range of 30~450 amu was scanned. The automatic gain control was selected and the electron multiplier was set at 1350 eV.

### 2.2. SPME extraction procedure

For the modification of fiber with BSTFA-TMCS, fiber was immersed for 30 min in the mixture of BSTFA and 1% TMCS (trimethylcholrosilane) solution before the sample extraction, which make the fiber absorb the derivatization reagent. After then, fiber is exposed in the sample and alkylphenols and bisphenol-A are simultaneously derivatized and extracted on the fiber. 5 mL of water containing the target phenols was placed in a 8 mL vial. After the addition of sodium chloride (10%) and citric acid (pH 2), the vial was sealed with a cap with a PTFE-faced septum. The fiber was exposed in the water for 5~250 minutes depending on the experiment. The sample was magnetically stirred. The fiber was then immediately inserted into the GC injector and thermally desorbed for 2 minutes on the injector at 270°C.

## 3. Results and Discussion

### 3.1. GC-MS analysis

Total ion chromatogram and mass spectrum of alkylphenols and BPA are shown in Fig. 1. Peaks of nonylphenols have overlapped because of the many isomers. Quantitation ions of phenols and its trimethylsilyl (TMS) derivatives are listed at Table 1. -OH group was displaced with trimethylsilyl (TMS) group by derivatization reaction in which molecular weight was increased by 72 ($(3 \times CH_3)$+(Si)-(H)=(45)+(28)-(1)=72). Molecular weight of bisphenol A has increased by 144 because of two OH groups.

### 3.2. Type of fiber, extraction time and salt effect

This experiment was performed with the purpose of selecting the best extraction conditions. For this study, a spiked water sample with individual phenols concentration of 10 μg/L was employed. The extraction time was 60 min. To choose the proper fiber, 100 μm PDMS, 65 μm PDMS/DVB, 65 μm CW/DVB and 85 μm PA were employed. For the extraction of polar alkylphenols, polar PA and CW/DVB fibers had shown good

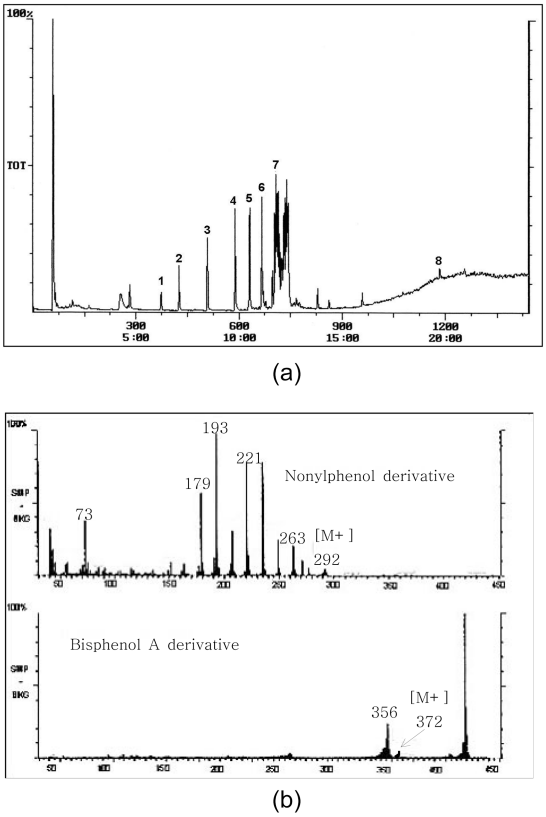


**Fig. 1.** Total ion chromatogram (a) and mass spectrum of alkylphenols and bisphenol-A(b). 1) 4-t-butylphenol, 2) 4-n-butylphenol, 3) 4-n-pentylphenol, 4) 4-n-hexyl phenol, 5) 4-t-octylphenol, 6) 4-n-geptylphenol, 7) nonylphenol, 8) Bisphenol-A.

**Table 1.** Quantitation ions of phenols and its TMS-derivatives

| Compounds | | Quan. Ions (m/z) | |
|---|---|---|---|
| | | Phenols | TMS-Derivatives |
| 1 | 4-t-butylphenol | 107, 135, 150 | 151, 207, 222 |
| 2 | 4-n-butylphenol | 107, 150 | 207, 179, 222 |
| 3 | 4-n-pentylphenol | 107, 164 | 179, 221, 236 |
| 4 | 4-n-hexylphenol | 107, 178 | 179, 235, 250 |
| 5 | 4-t-octylphenol | 107, 135, 206 | 263, 207, 278 |
| 6 | 4-n-heptylphenol | 107, 192 | 179, 249, 264 |
| 7 | nonylphenol | 107, 135, 220 | 193, 221, 292 |
| 8 | bisphenol-A | 119, 213, 228 | 207, 357, 372 |

– : characteristic ion.

efficiency (not shown). Fig. 2 shows the peak areas for the CW/DVB, PA and modified-PA fibers. Modified-PA fiber showed higher peak area than other fibers.

The extraction time profile was studied by monitoring the area counts as a function of the extraction time using modified-PA fiber. The standard solution was exposed for times which ranged from 5 to 250 min (Fig. 3). The temperature of extraction was set at room temperature and NaCl was not added to the sample. The sample was continuously stirred to decrease the time required for the analytes to reach the equilibrium. After the extraction, the compounds were desorbed from the fibers which had been kept throughout the run at 270°C in the injector of the gas chromatograph. The absorption equilibrium in modified-PA fiber was reached at 180 min for all compounds, but an extraction time of 60 min was selected for subsequent analysis. The shorter extraction time than the equilibrium time can

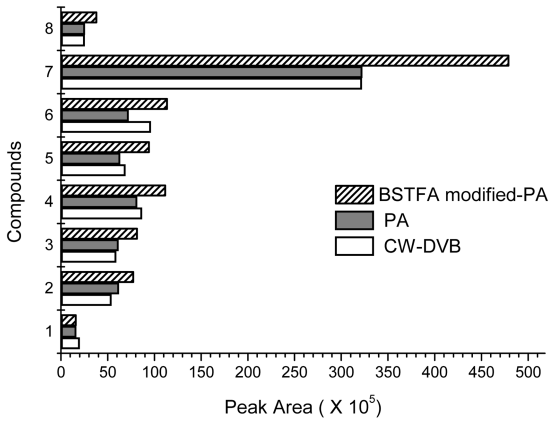


**Fig. 2.** Comparison of peak area of alkylphenols and bisphenol-A. 1) 4-t-butylphenol, 2) 4-n-butylphenol, 3) 4-n-pentylphenol, 4) 4-n-hexyl phenol, 5) 4-t-octylphenol, 6) 4-n-heptylphenol, 7) nonylphenol, 8) Bisphenol-A.

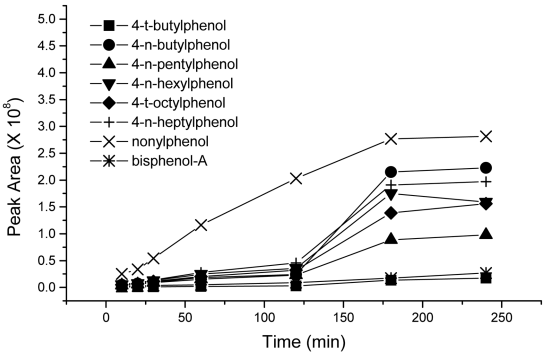


**Fig. 3.** Extraction time profile of alkylphenols and bisphenol-A by modified-PA fiber.

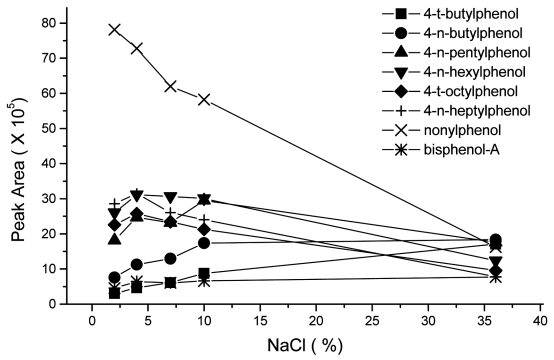


**Fig. 4.** Salt (NaCl) effect on the extraction efficiency.

be selected for the quantification if the stirring rate, the extraction time and the temperature are held constant[17].

NaCl is often added to the sample in order to increase the ionic strength and enhances the amount of analyte extracted by salt out effect[17]. Effect of salt concentration was investigated by 2, 4, 7, 10, 36% NaCl solution (Fig. 4). It was observed that 4-t-butylphenol, 4-n-butylphenol and bisphenol-A showed an increase in extraction yield with the addition of NaCl salt, but 4-n-pentyl, 4-n-hexyl, 4-t-octyl, 4-n-heptyl, and nonylphenols showed an decrease in extraction yield with the addition of NaCl. It seems that these behaviors are due to the difference in water solubility.

### 3.3. Linearity, detection limit and precision

To evaluate the linearity of the response, calibration studies were performed with modified-PA fiber. The concentration range was from 5 to 30 μg/L. The correlation coefficients (R) more than 0.9, shown in Table 2, demonstrated a directly proportional relationship between the extracted amount of phenols and its initial concentration in the sample. The detection limit was obtained by 3σ method, which gave 0.2 to 1.7 μg/L for TMS derivatives. Liqud-liquid extraction (LLE) with methylene chloride was also done to compare extraction efficiency with this method. Detection limit of this method is almost equivalent to liqud-liquid extraction (LLE) result (0.1 to 1.0 μg/L) (not shown). The precision of the experimental procedure was also evaluated. A series of five water sample with 10 μg/L of each phenol gave a relative standard deviations (RSDs) ranging from 1 to 11%.

**Table 2.** Calibration, detection limit(DL) and reproducibility(RSD) by direct SPME

| Compounds | Calibration | SPME (TMS-derivative) | |
|---|---|---|---|
| | | DL(μg/L) | RSD(%) |
| 4-t-butylphenol | y=-3891.3+30975.5x R=0.9973 | 1.3 | 8.8 |
| 4-n-butylphenol | y=22634.5+45505.3x R=0.9986 | 1.1 | 7.1 |
| 4-n-pentylphenol | y=-70209.5+78420x R=0.9943 | 1.0 | 6.5 |
| 4-n-hexylphenol | y=-148098.9+81968x R=0.9943 | 0.2 | 1.3 |
| 4-t-octylphenol | y=-128435.4+63017x R=0.9905 | 1.7 | 11.4 |
| 4-n-heptylphenol | y=-141780+6229.18x R=0.9896 | 1.7 | 11.6 |
| nonylphenol | y=-439431+146018x R=0.9892 | 0.8 | 5.5 |
| bisphenol-A | y=-3077.5+4650.8x R=0.9490 | 1.0 | 6.4 |

## 4. Conclusions

In this paper, PA fiber was modified with BSTFA-TMCS silylation reagents and the different parameters affecting the SPME extraction of alkylphenols have been studied. Phenols were partially derivatized by BSTFA modified-PA fiber in the extraction procedure. 60 min was selected as extraction time by considering the extraction efficiency and time consuming.

4-t-butylphenol, 4-n-butylphenol and bisphenol-A showed an increase in extraction yield with the addition of NaCl salt, but 4-n-pentyl, 4-n-hexyl, 4-t-octyl, 4-n-heptyl, and nonylphenols showed a decrease in extraction yield with the addition of NaCl at pH 2.0. This BSTFA modified-PA fiber showed good linearity and precision for TMS-derivatized phenols. This can simultaneously do derivatization and extraction and so make the analysis procedure simple and rapid. From these results, we can conclude that BSTFA modified-PA fiber can be used for the analysis of alkylphenols and bisphenol-A as TMS-derivatized form.

## References


1. Material Safety Data Sheet for Phenol, Genium Publishing Corp., **1985**, Schenectady, NY, U.S.A..
2. W. Fresenius, K.E. Quentin and W. Schneider, "*Water Analysis*", **1988**, Springer, Berlin, German.
3. W. Rowe, "*Evaluation Methods for Environmental Standards*", **1983**, CRC Press, Boca Raton, FL, U.S.A..
4. J. Pawliszyn, "*Solid Phase Microextraction : Theory and Practice*", **1997**, Wiley-VCH, New York, U.S.A..
5. S.A. Scheppers-Wercinski, "*Solid Phase Microcxtraction : A Practical Guide*", **1999**, Marcel and Dekker, New York, U.S.A..
6. J. Pawliszyn, "*Applications of Solid Phase Microextraction*", **1999**, Royal Society of Chemistry, London, U.K..
7. K.D. Buchholz and J. Pawliszyn, *Anal. Chem*., **1994**, 66, 160-167.
8. P. Bartak and L. Cap, *J. Chromatogr. A*, **1964**, 767, 171-175.
9. M.R. Lee, Y.C. Yeh, W.S. Hsiang and B.H. Hwang, *J. Chromatogr. A*, **1998**, 806, 317-324.
10. K.D. Buchholz and J. Pawliszyn, *Eviron. Sci. Technol*., **1993**, 27, 2844-2848.
11. P. Mussmann, A. Preiss, K. Levsen, G. Gunsch, J. Dfer and W. Engewald, *Bom wasser*, **1992**, 79, 145.
12. C.L. Arthur, L. Killam, K. Buchholz, J. Berg and J. Pawliszyn, *Anal. Chem*., **1992**, 64, 1960-1966.
13. Z. Zhang and J. Pawliszyn, *Anal. Chem*., **1993**, 65, 1843-1852.
14. P. Braun, M. Moeder, P. Popp and W. Engewald, *J. chromatogr. A*, **2003**, 988, 41-51.
15. C. Basheer and H.K. Lee, *J. chromatogr. A*, **2004**, 1057, 163-169.
16. M. del Olmo, A. Zafra, A. Gonzalez-Casado and J.L. Vilchez, *J. chromatogr. B*, **2005**, 817, 167-172.
17. E. Penalver, E. Pocurrull, and R.M. Marce, *J. chromatogr. A*, **2000**, 872, 191-201.