Printed in the Republic of Korea

ANALYTICAL SCIENCE & TECHNOLOGY

Vol. 30 No. 1, 20-25, 2017

https://doi.org/10.5806/AST.2017.30.1.20

# Simultaneous determination of bisphenol A, chlorophenols and alkylphenols by solid-phase extraction and HPLC

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(Received October 28, 2016; Revised February 5, 2016; Accepted February 5, 2016)

**Abstract:** An analytical method for determining potential endocrine disruptors (bisphenol A, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, *p*-*t*-butylphenol, *p*-pentylphenol, *p*-hexylphenol, *p*-*t*-octylphenol, *p*-heptylphenol, nonylphenol) by solid-phase extraction (SPE) and High Perfomance Liquid Chromatography(HPLC) equipped with fluorescence and variable wavelength detector has been developed. The SPE process for sample concentration was performed on a commercially available Oasis HLB cartridge packed with polymeric sorbents. The effect of elution solvent and elution volume on the recoveries of the analytes were investigated with HPLC. Average recovery of >85% was achieved with 60mg sorbents using 5mL of methanol as elution solvent. Phenolic compounds in canned drinks, beverages and water samples were surveyed by this proposed method.

**Key words:** bisphenol A, alkylphenols, chlorophenols, high performance liquid chromathgraphy (HPLC), solid phase extraction (SPE)

## 1. Introduction

Much attention has been focused on the possibility of chemicals present in the environment interfering with the normal hormonal actions of animals disturbing their endocrine system, e.g. the glands of their reproductive system. These chemicals are also referred to as endocrine disruptors (EDs) or environmental hormones because they mimic hormones when introduced into the body. These EDs include a wide variety of chemicals, especially chlorinated organic compounds (COCs) including dioxins, phthalate esters, bisphenol A, and nonylphenol, which are suspected of having a hazardous impact on ecosystem causing reproductive impairment, deformations, growth impairment, and cancers in humans. Since comprehensive studies are being performed on the concentration or distribution of these EDs in the environment and their behavior when consumed, it is necessary to simultaneously analyze the multiple components at low concentrations to improve the efficiency of analysis.

Bisphenol A and alkylphenols are used in large quantities as stabilizers, plasticizers, surfactants, and

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raw materials for organic phenol and polycarbonate resins,[1,2] while chlorophenols are present as degradation products in disinfectants and pesticides, as pollutants in the aquatic environment,[3] or they can be formed from phenols that were not chlorinated during the chlorination of water. The primary analysis methods used for these substances are GC/MS[4,5] and HPLC.[4,6,7] Between the two, simultaneous multi-component analysis (MCA) using GC/MS has been the de facto method owing to its particularly high sensitivity and resolution. However, derivatization is required to analyze trace amounts of phenolic compounds (polar in nature) via GC and GC/MS analyses. Therefore, in order to overcome this disadvantage, the present study established an HPLC-based analysis using a fluorescence detector and used solid-phase extraction (SPE) as the method for sample concentration. SPE is used for concentrating trace amounts of organic compounds from the sample and for removing interfering components of the complex matrix for obtaining a clean extract that contains the analyte. Therefore, the selection of the stationary phase is the most important parameter to consider when extracting a compound from a complex matrix. Recently, SPE using $C_{18}$ or various polymeric substances has been applied extensively for the analysis of water-quality samples.[8,9]

The present study used bisphenol A, 4 types of chlorophenols, 6 types of alkylphenols, and phenol among suspected EDCs to establish a method for simultaneous MCA via HPLC using SPE. Moreover, this method was used to analyze raw surface water, purified surface water, landfill leachate wastewater, effluents from night soil treatment, and drinking water for the identification of residues in water. At the same time, the contents of bisphenol A, chlorophenols, and alkylphenols in commercially available canned beverage containers were also investigated.

## 2. Experiment

### 2.1. Reagent and apparatus

The experiment used dichloromethane, methane, acetone, acetonitrile, n-hexane, and *t*-butyl methyl ether for residual pesticide testing-use from Wako; methanol for HPLC-use from Fischer chemicals; and acetic acid for non-aqueous titration use from Wako. The standard materials 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol were procured from Supelco; *p*-*t*-butylphenol, and *p*-*t*-octyl phenol from Wako; *p*-pentylphenol, *p*-hexylphenol, and *p*-heptylphenol from KANTO CHEMICAL; and nonylphenol from Riedel-de Haen. Working standards suitable for the experiment were prepared by mixing methanol with 1.0 mg/mL of stock standard solutions to dilute them. The canned beverage container extraction test was performed by purchasing commercially available canned coffee, soft drinks, and ion beverages. The water quality samples were collected in glass bottles in accordance with water pollution process test standards and cold-stored before use.

Moreover, the SPE cartridge used was an Oasis HLB 3 cc/60 mg cartridge from Water and the device used to extract components adsorbed on the cartridge was Vacuum Manifold (12 points) from Alltech. For the analysis, HP1100 series HPLC system from Hewlett Packard connected to a fluorescence detector and UV excitation was used, while the column used was ADSORBOSPHERE XL C8 300A 5U (ID4.6 mm, length 250 mm) from Alltech. The analysis was performed according to the conditions shown in *Table* 1.

### 2.2. Solid-phase extraction

The samples were filtered with 0.45-mm filter and phosphoric acid was used to control the samples to pH 3 to prevent dissociation of the compounds. A specific amount was drawn for use as sample for analysis. SPE cartridge was washed with 5 mL of dichloromethane and activated with 5 mL of methanol and 5 mL of distilled water after which the sample was passed through the cartridge at a flow rate of 5–10 mL/min. After thorough drying, extraction was performed using 5 mL of methanol and the extract was concentrated to 1 mL using nitrogen gas in a 40 °C constant-temperature water bath, from which

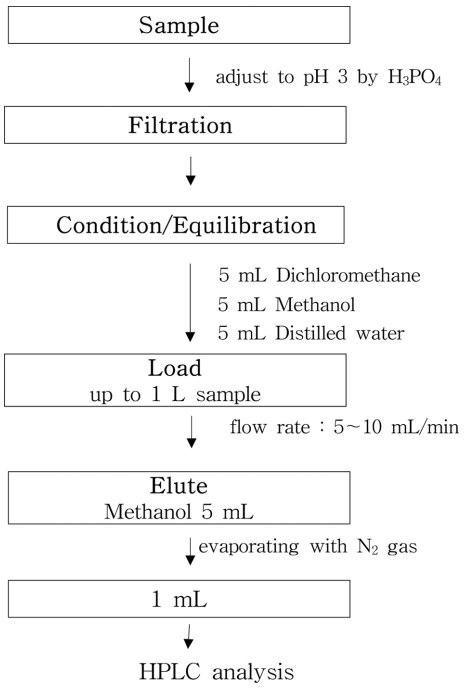


*Fig*. 1. Flow sheet of sample preparation using solid phase extraction.

10 mL was injected for HPLC analysis (*Fig*. 1).

### 2.3. Container extraction test via food simulants

After filling a canned beverage container with beverage-like solvent to the capacity of the container, the top was covered with a watch glass and left for 24 h at 40 °C. The solvent used for extraction was distilled water for ion beverages, sikhe, or coffee; 20 % ethanol for beers; and 4 % acetic acid for carbonated beverages. After adding the solvent, the extract solution was pretreated as shown in *Fig.* 1, and then used for analysis.

## 3. Results and Discussion

### 3.1. Analyses of phenols and bisphenol A via HPLC

To simultaneously analyze phenol, bisphenol A, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, *p*-*t*-butylphenol, *p*-pentylphenol, *p*-hexylphenol, *p*-heptylphenol, *p*-*t*-octylphenol, and nonylphenol, UV and fluorescence detector were used to establish the conditions for obtaining the optimal analysis results (*Table* 1). A reversed-phase octyl ($C_8$) column was used. Since using only water and methanol can result in a phenomenon of the peaks for 2,4,6-trichlorophenol and pentachlorophenol spreading out, acetic acid was added to the mobile phase. Among the analytes, fluorescence detector was used on phenol, bisphenol A, *p*-*t*-butylphenol, *p*-pentylphenol, *p*-hexylphenol,

*Table* 1. Operating conditions for HPLC

| | | | |
|---|---|---|---|
| Column | ADSORBOSPHERE XLC8 300A 5U (250 mm × 4.6 mm) | | |
| Detector | UV (280 nm, 9 min 294 nm) Fluorescence (Ex. 215 nm, Em. 313 nm) | | |
| Mobile phase | A : 1 % Acetic acid in distilled water<br>B : 1 % Acetic acid in methanol | | |
| Gradient | Time (min.) | A(%) | B(%) |
| | 0 | 40 | 60 |
| | 2.0 | 40 | 60 |
| | 18.0 | 0 | 100 |
| | 19.0 | 0 | 100 |
| | 20.0 | 40 | 60 |
| Flow rate | 1.0 mL/min. | | |
| Column temperature | 40 °C | | |
| Injection amount | 10 μL | | |

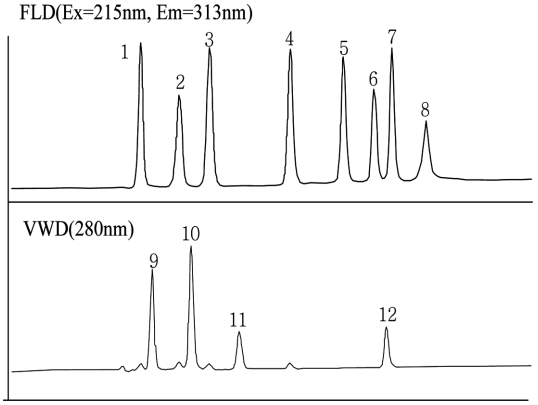


*Fig*. 2. HPLC chromatograms for standard mixture of bisphenol A and phenolic compounds. 1. phenol; 2. Bisphenol A; 3. *p*-*t*-butylphenol; 4. *p*-pentylphenol; 5. *p*-hexylphenol; 6. *p*-*t*-octylphenol; 7. *p*-heptylphenol; 8. nonylphenol; 9. 2-chlorophenoL; 10. 2,4-dichlorophenol 11, 2,4,6-trichlorophenol; 12. pentachlorophenol (※VWD : Variable Wavelength UV Detector)

*p*-heptylphenol, *p*-*t*-octylphenol, and nonylphenol, while UV was used on 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol for simultaneous analysis. Since the peak absorption wavelength of pentachlorophenol differs from that of other phenols, it was measured by changing the UV wavelength to 280 nm for 9 min and 294 nm for 9 to 20 min. *Fig.* 2 shows the chromatograms of the analytical standard solutions obtained from the analytical conditions shown in *Table* 1, which were established through a review of all the aforementioned conditions.

### 3.2. Recovery rates according to the extraction solvent

In the present study, SPE was used as the method for concentrating the analytes. The $C_{18}$ cartridge—the most commonly used cartridge—is not suitable for highly polar substances such as phenolic compounds, and thus, Oasis hydrophilic lipophilic balance (HLB) cartridge, which uses a polymeric adsorbent was used in the study. This uses a copolymer bonded to two monomers, lipophilic divinylbenzene and hydrophilic N-vinylpyrrolidone, which allows it to be used for both hydrophilic and lipophilic samples unlike typical cartridge fillers. First, to select the solvent that can most effectively extract the analytes adsorbed on the solid-phase, an experiment was conducted using each of *t*-butyl methyl ether, acetone, acetonitrile, and methanol. Among the solvents used, acetone, acetonitrile, and methanol showed a recovery rate ≥ 80 % for most analytes; on the other hand, acetone showed a poor recovery rate for phenol and 2-chlorophenol, and acetonitrile for bisphenol A and pentachlorophenol. Therefore, methanol with a generally good recovery rate for all analytes was selected as the extraction solvent, and the recovery rates according to the extraction solvent used in each analysis is as shown in *Fig*. 3.

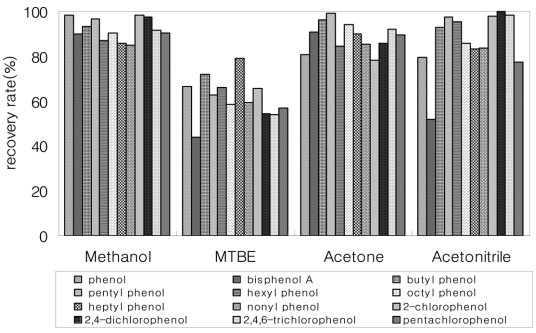


*Fig.* 3. Percent recovery as function of elution solvents (MTBE : *t*-butyl methyl ether)

To measure the volume of methanol needed to completely extract the analytes adsorbed on the solid-phase, 2, 3, 4, and 5 mL aliquots were analyzed. The results showed that chlorophenol was extracted completely with 3 mL and bisphenol A and alkylphenols with 4 mL. Based on these results, the present study used 5 mL of methanol to extract all

*Table* 2. Recovery(%) of phenol, bisphenol A, alkyl phenols, chlorophenols in distilled water and repeatability (n=5) by the proposed method

| Compound | RT | run1 | run2 | run3 | run4 | run5 | RSD(n=5) |
|---|---|---|---|---|---|---|---|
| phenol | 3.752 | 89.0 | 86.0 | 87.1 | 86.0 | 87.0 | 1.2 |
| bisphenol A | 4.864 | 94.2 | 97.4 | 96.7 | 95.6 | 92.5 | 2.0 |
| *p*-*t*-butylphenol | 5.746 | 93.4 | 89.0 | 90.8 | 88.7 | 90.8 | 1.9 |
| *p*-pentylphenol | 8.074 | 96.8 | 98.8 | 99.5 | 96.9 | 98.9 | 1.2 |
| *p*-hexylphenol | 9.593 | 92.5 | 97.3 | 98.4 | 93.5 | 92.3 | 2.8 |
| *p*-*t*-octylphenol | 10.476 | 90.5 | 90.1 | 92.2 | 88.4 | 87.0 | 2.0 |
| *p*-heptylphenol | 10.988 | 85.6 | 85.3 | 90.5 | 86.3 | 85.7 | 2.2 |
| nonylphenol | 11.977 | 82.8 | 84.6 | 90.2 | 90.7 | 84.8 | 3.6 |
| 2-chlorophenol | 4.099 | 93.4 | 87.3 | 87.2 | 90.5 | 92.1 | 2.8 |
| 2,4-dichlorophenol | 5.232 | 95.9 | 90.2 | 87.7 | 90.3 | 95.8 | 3.7 |
| 2,4,6-trichloriphenol | 6.622 | 91.5 | 88.5 | 92.4 | 92.9 | 90.3 | 1.8 |
| pentachlorophenol | 10.864 | 93.8 | 96.2 | 96.2 | 94.2 | 95.0 | 1.1 |

analytes and used nitrogen gas to concentrate them to 1 mL for use as test solutions.

### 3.3. Reliability of analytic methods

To verify the reliability of the HPLC analysis method that used SPE in the present study, working standard solutions consisting of 5 mg/mL of chlorophenol, 0.5 mg/mL of bisphenol A, and 0.5 mg/mL of alkylphenols were added to distilled water for 5 repeated experiments. As shown in *Table* 2, the results showed that the overall recovery rate was 82-100% with standard deviation of 1.1-3.8 %, indicating good results. For the analyses of chlorophenols and alkylphenols, the limit of quantification was 0.5 mg/mL of chlorophenols and 0.05 mg/mL of alkylphenols. Therefore, when 1 L of water was used in SPE to concentrate by 1,000 folds to 1 mL, it was possible to detect up to limit of quantification of 0.0005 mg/mL.

### 3.4. Residue in water survey

Chlorophenols, alkylphenols, and bisphenol A are widely used in various industries, and as a result, they are introduced into water environments by a variety of routes. Consequently, 87 water purification plants were surveyed in two to examine the safety of water quality within Gangwon Province. Analysis results on 4 types of chlorophenols showed that they were undetectable. Moreover, a survey was also conducted on bisphenol A, 4 types of chlorophenols, and 6 types of alkylphenols in a total of 20 cases involving drinking water from polycarbonate containers, Lake Uiam, landfill leachate wastewater, and effluents from night soil treatment, along with 14 stations within Gangwon with a high population that use raw surface water as underground water. The results showed that trace amounts of *p*-heptylphenol and pentachlorophenol (0.9 mg/L and 12.4 mg/L, respectively) were detected only in landfill leachates. The 4 types of chlorophenols are designated as drinking water quality items required to be monitored, and the recommended level in Korea for chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol is 200 mg/L, 150 mg/L, 15 mg/L, and 9 mg/L, respectively, while there are still no regulations on levels of alkylphenols or bisphenol A in drinking water.

*Table* 3. Recovery(%) of bisphenol A, chlorophenols, alkylphenols from spiked aqueous food simulants

| Compound | Distilled water | 4% acetic acid | 20% ethanol |
|---|---|---|---|
| phenol | 87.9 | 75.8 | 65.5 |
| bisphenol A | 95.2 | 99.1 | 99.8 |
| *p*-*t*-butylphenol | 90.5 | 95.7 | 91.8 |
| *p*-pentylphenol | 98.4 | 98.7 | 98.5 |
| *p*-hexylphenol | 94.4 | 86.4 | 85.2 |
| *p*-t-octylphenol | 90.5 | 93.2 | 91.0 |
| *p*-heptylphenol | 87.7 | 88.7 | 91.9 |
| nonylphenol | 89.8 | 88.3 | 90.8 |
| 2-chlorophenol | 90.6 | 99.5 | 90.3 |
| 2,4-dichlorophenol | 95.9 | 96.5 | 90.0 |
| 2,4,6-trichloriphenol | 92.6 | 90.7 | 90.8 |
| pentachlorophenol | 95.1 | 89.3 | 87.5 |

### 3.5. Spike recovery test on food simulants

The epoxy resins used to coat the inside of cans are prepared with bisphenols as one of the major reactants.[10] In the present study, to determine the level epoxy resin transferred from the containers of various canned beverage products that use an epoxy resin for coating, an extraction experiment was performed using the contents and stimulants. First, the analytes were added to distilled water, 4 % acetic acid, and 20 % ethanol used in the canned beverage container experiment, and subsequently purified and concentrated by the method shown in *Fig.* 1. The recovery rates from HPLC analysis are shown in *Table* 3. A recovery rate of ≥85 % was found for most of the components, whereas phenol showed large deviations based on the solvent used and the recovery rate was also low. It was determined that such findings can be attributed to the chemical nature of phenol. Thus it was used only for qualitative analysis and excluded from quantitative analysis in the present study.

## 4. Conclusions

SPE of suspected EDCs 2-chlorophenol, 2,4-

dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, bisphenol A, *p*-*t*-butylphenol, *p*-pentylphenol, *p*-hexylphenol, *p*-heptylphenol, *p*-*t*-octylphenol, and nonylphenol was used to establish a simultaneous analysis method via HPLC.

1. SPE was used as the method for extracting and concentrating the analytes, while methanol was used as the extraction solvent with 5 mL as the volume of extraction solvent used. Moreover, the cartridge used was Oasis$^{TM}$ HLB (60 mg/3 cc) with polymeric substances adsorbed.

2. To verify the reliability of the analytic method established in the present study, recovery rates from 5 repeated experiments were compared. The results showed a recovery rate of 82-100 % with a standard deviation of 1.1-3.8 %.

3. To examine the safety of water quality within the Gangwon Province, 4 types of chlorophenols were analyzed by surveying (over 2 surveys) 87 purification plants within the province. The results showed that none of the chlorophenols was detected. Moreover, a total of 20 cases involving drinking water from polycarbonate containers, Lake Uiam, landfill leachate wastewater, and effluents from night soil treatment, along with 14 stations from 7 cities that use raw surface water as underground water were surveyed. The results showed that trace amounts of *p*-heptylphenol and pentachlorophenol (0.9 mg/L and 12.4 mg/L, respectively) were detected only in landfill leachates.

4. An extraction experiment on canned beverage containers showed that a trace amount of *p*-*t*-butylphenol (1.5-2.2 mg/L) was detected in ion beverage, sikhe, and coffee containers.

## References

1. J. S. Gandara, S. P. Abuin, P. P. Losada, and J. S. Lozano, *J. Chrom. Sci*., **31**, 450-454 (1993).
2. W. Summerfield, A. Goodson, and I. Cooper, *Food Addit. Contam*., **15**, 818-830 (1998).
3. J. E. Biles, T. P. McNeal, T. H. Begley, and H. C. Hollifield, *J. Agric. Food Chem*., **45**, 3541-3544 (1997).
4. H. Bagheri, A. Mohammadi, and A. Salemi, *Anal. Chim. Acta,* **513**, 445-449 (2004).
5. O. Jauregui and M. T. Galceran, *Anal. Chim. Acta,* **340**, 191-199 (1997).
6. C. Lambert and M. Larroque, *J. Chromatogr. Science*, **35**, 57-62 (1997).
7. K. Miyabe, N. Nomura, F. Morishita, S. Kurata, H. Asamura, and Y. Imada, *Anal. Sci.* **14**, 355-360 (1998).
8. D. Barceló and M. C. Hennion, *Anal. Chim. Acta,* **318**, 1-14 (1995).
9. V. Pichon and M. C. Hennion, *J. Chromatogr. A*, **665**, 269-281 (1994).
10. J. E. Bils, K. D. White, T. P. McNeal, and T. H. Begley, *J. Agric. Food Chem.,* **47**, 1965-1969 (1999).