Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1032 (2004) 45–49
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of bisphenol A in environmental water at ultra-low level by high-performance liquid chromatography with an effective on-line pretreatment device

Yoshiyuki Watabe [a], Takuya Kondo [b], Masatoshi Morita [b], Nobuo Tanaka [a], Jun Haginaka [c], Ken Hosoya [a,*]

[a] *Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan*
[b] *Endocrine Disrupters Research Laboratory, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan*
[c] *Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68, Koshien Kyuban-cho, Nishinomiya, Hyogo 663-8179, Japan*

Abstract

We have developed a simple HPLC method for the microanalysis of bisphenol A (BPA), which is often contained in environmental water and is known as an endocrine disrupter. HPLC coupled with electrochemical detection requires a simpler procedure of pretreatment compared to GC–MS. In this study, we analyzed BPA using molecularly imprinted polymer as an on-line pretreatment device. This polymer has molecular recognition sites and provides specific selectivity in extraction process. Due to this effect, the detection limit obtained with this HPLC was 0.36 ng/l. This method applied to environmental water and purified water samples containing 2–70 ng/l of BPA successfully. Furthermore, UV detection was performed in some actual analyses.

Keywords: Water analysis; Environmental analysis; Molecular imprinting; Column switching; Sample handling; Instrumentation; Bisphenol A; Endocrine disrupters

1. Introduction

Bisphenol A (BPA) is often contained in environmental water and is now attracting attention as an endocrine disrupter. It has recently been proposed that trace amounts of BPA also show estrogenic activity even at concentrations as low as 1 ng/l level [1–3]. In such ultra-low concentration range, a determination is interfered by many factors such as contaminations from glassware, container and injection syringe. Furthermore, purified water contains certain amounts of BPA due to some of resin parts in the water purification system.

We tried to determine BPA concentration in environmental water samples with simple HPLC. We do know that a GC–MS method is commonly used for BPA determination. However, the drawback to this method is that pretreatment requires approximately 1 day to be completed through complicated procedures. On the other hand, the HPLC–electrochemical detection (ED) method requires a simpler and quicker pretreatment method. This method has been applied to the practical application field such as the determination of BPA in serum [4,5].

In this study, we analyzed BPA using column switching concentration as a pretreatment procedure. To solve above-mentioned difficulties, auto-pretreatment excluding manual procedure as much as possible is quite effective. The column-switching HPLC system consisted of a pretreatment column connected to an analytical HPLC column via a six-port flow changeover valve. This HPLC system provided both large amounts of sample pretreatment (10–100 ml) and exclusion of BPA contamination from complicated manual pretreatment procedures.

To obtain highly reliable quantitative results of trace amounts of chemical substances in environmental samples, selective concentration of target component and exclusion of interference performed simultaneously on pretreatment column are important. Adsorbents with molecular recognition ability are effective for this purpose [6,7]. Molecularly imprinted polymer (MIP) can provide specific molecular recognition ability [8]. In traditional way for preparing a

* Corresponding author. Tel.: +81-757247828; fax: +81-757247710.
E-mail address: kenpc@ipc.kit.ac.jp (K. Hosoya).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.11.079

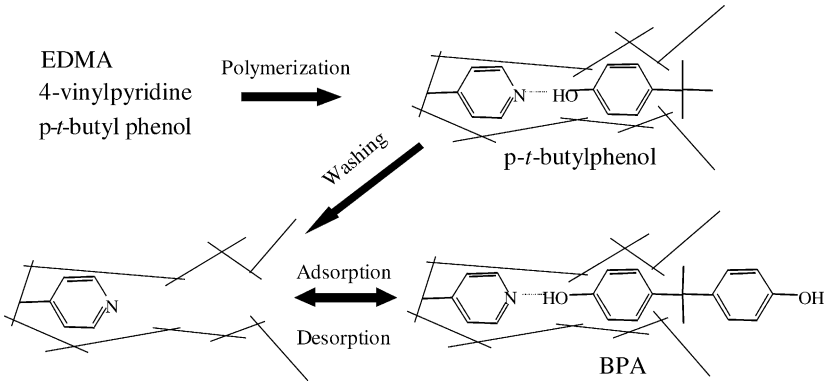

Fig. 1. Simplified schematics of imprinting procedures.

MIP, target molecule is used as a template. After polymerization, the template is removed and created specific recognition site plays an important role to increase adsorption ability towards the template molecule.

MIPs are often utilized in various fields, e.g. as stationary phase for HPLC [9], although one of the big problem of traditional MIP is that it is not possible to remove the utilized template molecule completely from the prepared MIP matrix even through tedious washing process with some organic solvents repeatedly. This can be a serious problem for quantitative microanalysis of chemical substances such as BPA at ppt level [10] because tiny amount of the template molecule might be continuously eluted out from the medium.

In such case, pseudo-template is most helpful. It can be separated from the real target compound in analytical process to avoid the interference. If the target compound is toxic or very rare, pseudo-template is greatly effective as well [11]. We prepared uniformly sized polymer particles for BPA trapping using *p-tert*.-butylphenol as a pseudo-template [12–15] through the two-step swelling and polymerization method [16]. A diagram of the imprinted site is illustrated in Fig. 1. This MIP for BPA was combined with column-switching HPLC and applied to the actual determination of BPA at low ng/l level in environmental water samples both with ED and typical UV detection.

2. Experimental

2.1. Materials

Monomers, ethylene glycol dimethacrylate (EDMA) as a cross-linking agent, and 4-vinylpyridine as the functional monomer, both from Wako (Osaka, Japan) were effectively purified by vacuum distillation techniques to remove polymerization inhibitor [17]. The template molecule, *p-tert*.-butylphenol was purchased from Nacalai Tesque (Kyoto, Japan). A polymerization initiator, 2,2′-azobis-(isobutyronitrile) (AIBN) was purchased from Wako.

A solvent realizing porous structure (porogenic solvent), toluene from Nacalai Tesque was of the highest grade. All chemicals for preparing HPLC mobile phase, sodium dihydrogen phosphate, disodium hydrogenphosphate and acetonitrile were purchased from Wako. Water for preparing BPA standard solution was obtained from Milli-Q water purification system of Millipore (Bedford, MA, USA).

2.2. Preparation of the molecularly imprinted polymer

The procedure for two-step welling, polymerization and clean up was performed as described in our previous work [8]. The feed ratio was as follows, EDMA: 3 ml, 4-vinylpyridine: 0.34 ml, toluene: 3 ml, *p-tert*.-butylphenol: 0.06 g, AIBN: 0.06 g (EDMA-4-vinylpyridine-*p-tert*.-butylphenol, 40:8:1 in mole ratio). Obtained polymer particles were 8 μm in diameter. The size uniformity of the polymer particles was excellent as reported previously [18]. This polymer was packed into a stainless steel column of 50 mm × 4 mm i.d. as a pretreatment device in column-switching HPLC for trace amounts of BPA determination.

2.3. HPLC analysis

The HPLC system including workstation software consisted of Shimadzu LC-VP series (Kyoto, Japan) except the ED system. A Coulochem II, electrochemical detector was purchased from ESA (Chelmsford, MA, USA). Fig. 2 shows flow diagram of the column-switching HPLC system employed in this study.

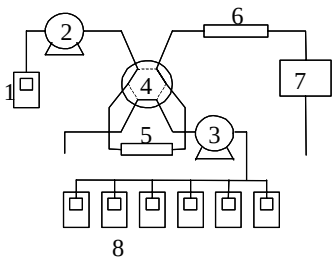

Fig. 2. Flow diagram of a column-switching HPLC system. (1) Mobile phase; (2, 3) pumps; (4) high-pressure flow changeover valve; (5) pretreatment column; (6) analytical column; (7) ED system; (8) samples (one for rinsing solvent).

One of the sample reservoirs was utilized for a rinsing solvent. The pump delivered 50 ml of BPA standard solution or environmental water sample and BPA was concentrated on a pretreatment column packed with the MIP. The column was washed with the rinsing solvent for 3 min consecutively. Then the mobile phase was delivered via six-port flow changeover valve and concentrated BPA was led to the analytical column and detected by ED and/or UV detection after the separation.

To create calibration curve and estimate a detection limit by utilizing statistical procedures, carefully prepared 1, 10, and 100 ng/l standard BPA solutions were analyzed five times repeatedly in respective concentrations. Water for preparing standard solution was obtained from water purification system, which consisted of Elix-UV and Milli-Q gradient, then treated with Empore disk of SDB-DX type (3 M, St. Paul, MN, USA). Actual environmental water samples were just filtrated with 0.45 μm membrane then analyzed immediately.

HPLC conditions employed were as follows; mobile phase: 20 mM (sodium) phosphate buffer (pH 7)–acetonitrle (65:35, v/v), rinsing solvent: 20% (v/v) of acetonitrile aqueous solution, flow rate for analysis: 0.8 ml/min, flow rate for pretreatment: 2.5 ml/min, concentrated volume: 50 ml, analytical column: Shim-pack VP-ODS (150 mm × 4.6 mm i.d.), temperature: 40 °C, ED: at +0.35/+0.55 V (analytical cell, CH1/CH2, 1nAF.S.) and +0.6 V (guard cell), UV detection: at 275 nm.

3. Results and discussion

For ultra-low concentration of BPA analysis, BPA contamination from manual pretreatment procedures as well as adhesive for fixing needle of manual syringes affects the determination seriously. This means manual syringes cannot be applied to BPA microanalysis. To obtain reliable quantitative results at ppt concentration level, a column switching auto pretreatment system is essential.

Even employing this HPLC system, we unexpectedly encounter serious contamination problem due to BPA existing in purified water. A water purification system cannot remove BPA completely. A few ng/l level of BPA contamination often found in the purified water is not negligible and the contamination level varied daily. Consequently, there is no reliable way to correct a degree of contamination by calculation.

We have to obtain BPA-free water for preparing BPA standard solution otherwise reliable calibration curve cannot be created. BPA-free water was obtained by filtrating the purified water through Empore disk. Then the contamination was suppressed below the detection limit afforded by this HPLC system. Standard aqueous solution of BPA at 1, 10, and 100 ng/l were injected repeatedly ($n = 5$) to estimate the repeatability of peak area and the results were shown in Table 1. A linear calibration curve with a correlation coefficient ≥0.999 was obtained. The recovery at 100 ng/l was 100.5% and even a trace of leaking and/or carry-over of BPA was not observed at same concentration. The detection limit estimated by utilizing standard deviation of *y*-intercept of calibration curves was 0.36 ng/l, which is quite low concentration.

Table 1
Repeatability of column-switching HPLC in trace BPA analysis

BPA concentration (ng/l)	Repeatability (R.S.D.%, $n = 5$)
1	9.3
10	3.4
100	0.5

A column-switching HPLC has two profits for microanalysis. One is automatic pretreatment and the other is concentration effect. By using this system, some of manual procedures such as evaporation or transferences from glassware to others are not necessary to be done analyses. Then the loss of actual sample can be minimized [19]. When the sample volume is enough large, on-column concentration can be expected [20].

Proper combination of pretreatment column and sample solvent can provide more than 1000-fold concentration. Based on our preliminary investigation with manual concentration procedures, the R.S.D. value of 20 ng/l standard treatments ($n = 5$) was around 10% and overall BPA contamination was higher than 1.4 ng/l. These results presumably suggest that manual pretreatment is not suitable for BPA determination when an anticipated concentration is below 10 ng/l. Moreover, this kind of manual treatment generally requires manipulative skills and the operator should be well experienced.

Compared to these results, column-switching HPLC system can provide quite excellent repeatability and reliability. With the use of this HPLC system coupled with molecularly imprinted polymer [8] as a pretreatment column, we can determine trace amounts of BPA in actual environmental samples or purified water reliably. Fig. 3 shows

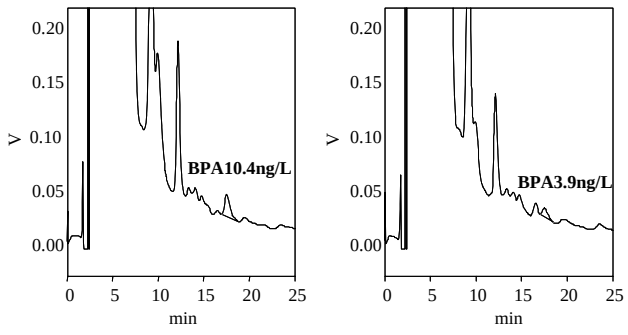

Fig. 3. Chromatograms of lake water samples obtained with column-switching HPLC. 50 ml of lake water was concentrated onto a pretreatment column packed with molecularly imprinted polymer. HPLC conditions; mobile phase, 20 mM (sodium) phosphate buffer (pH 7)–acetonitrle (65:35, v/v); flow rate, 0.8 ml/min; column, Shim-pack VP-ODS (150 mm × 4.6 mm i.d.); electrochemical detection at +0.55 V; temperature, 40 °C.

chromatograms of BAP in different actual lake water samples. The repeatability ($n = 5$) of actual environmental water samples containing approximately 20 ng/l was 2.4% R.S.D. In the preliminary investigation, the peak purity of BPA in water same sample obtained from the same river as that from Fig. 3 was confirmed by MS spectrometry after preparation with manual extraction.

Low concentration of BPA has been determined successfully. After concentration process, concentrated matrix including BPA was washed with 20% of acenotitrile solution. When acetonitrile content was higher than 30%, peak broadening was observed due to excess elution power of the rinsing solution. Certain level of interference was removed from the pretreatment column whereas BPA was still retained due to large retention provided by MIP packed in the pretreatment column.

To succeed this procedure, specific large retention of BPA should consist with small pretreatment column size. Otherwise the interference cannot be removed from the pretreatment column during limited washing term. Long term washing leads obscured peak shape of BPA due to diffusion effect in the pretreatment column. Commercially available pretreatment column was also applied to different river water analysis preliminarily and gave a rater worse removal of interferences eluted in the front part and around BPA elution position of the chromatogram. It might be improved if rinsing sequence could have been inserted. But due to the small retention capacity and poor selectivity, it was impossible.

This column-switching HPLC system coupled with MIP pretreatment column provided even UV detection of standard BPA solution at 10 ng/l as is shown in Fig. 4. Then we tried to compare ED and UV detection on actual environmental water analysis. The result is shown in Fig. 5. Around 70 ng/l of BPA in actual sample was detected with even UV detection. This result suggests a possibility that trace amounts of BPA in actual environmental samples can be determined using much simpler HPLC system with UV detection.

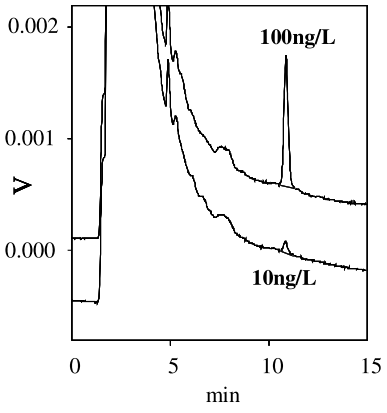

Fig. 4. Chromatograms of 10 and 100 of ng/l of BPA standard solutions with UV detection. HPLC conditions were same as in Fig. 3 except 40% (v/v) of acetonitrile mixing ratio in mobile phase.

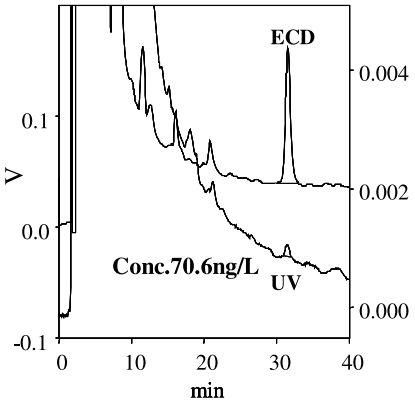

Fig. 5. Comparative chromatograms of river water sample detected with electrochemical and UV detection. HPLC conditions were as in Fig. 3 except 30% (v/v) of acetonitrile mixing ratio in mobile phase.

For improving a detection limit in UV detection, a removal of interference must be important. One solution may be a surface modification of MIP. An exclusion of interference can consist with a specific retention of target compound provided by molecularly imprinting technique on polymer media. The combination of hydrophilic surface and rater hydrophobic internal area with molecularly imprinted sites or pore distribution control may provide better detection limit of UV detection as well as that of ED. These kinds of designations can be easily achieved during MIP preparation. Consecutive optimization will spread applicable field of this method besides environmental water analysis.

4. Conclusion

BPA determination at ultra-low concentration such as below 10 ppt can't be accomplished with ordinary HPLC procedure [21]. The most suitable HPLC for this purpose is on-line column switching system. The HPLC system provided highly reliable results both in the detection limit and recovery compared to those obtained by using the manual pretreatments. The former was 0.36 ppt and the latte was 100.5% when 100 ng/l of standard solution was treated. The use of MIP as a pretreatment medium provided specific retention of BPA, which improved the separation of BPA and interferences. It could contribute to the good repeatability of 2.4% R.S.D. for approximately 20 ng/l of BPA contained in actual water samples. The combination of MIP pretreatment column, column-switching HPLC and ECD have afforded ultra-low detection limit and highly reliable results when applied to actual environmental water samples.

Commonly used UV detection has detected 10 ng/l of BPA standard solution and 70 ng/l of it in actual environmental samples. It was due to the specific retention of BPA on MIP, which improved the separation of BPA in actual water samples. Additional modification onto MIP will provide lower detection limit, higher reliability. Hopefully this methodology and HPLC system should provides a useful

tool for more careful, accurate investigation and determination of BPA levels in the full spectrum of samples ranging from environmental to food products and consequently research on endocrine disrupters in biological matrices.

Acknowledgements

This research was partly supported by the Nanotechnology Project of the Ministry of Environment and Grant-in-Aid for Scientific Research (Nos. 13640604 and 14042232) from the Ministry of Education, Science, Sport, and Culture of Japan. In addition, financial support for this work by Shimadzu Science Foundation and Hosokawa Foundation are gratefully acknowledged.

References

[1] F.S. von Saal, P.S. Cooke, D.L. Buchanan, P. Paianza, K.A. Thayer, S.C. Nagel, S. Parmigiani, W.V. Welshons, Toxicol. Ind. Health 14 (1998) 239.
[2] J.S. Fisher, K.J. Turner, D. Brown, R.M. Sharpe, Environ. Health Perpect. 107 (1999) 397.
[3] R. Steinmetz, N.A. Mitchner, A. Grant, D.L. Allen, R.M. Bigsby, N. Ben-Jonathan, Endocrinology 138 (1997) 1780.
[4] J. Sajiki, K. Takahashi, J. Yonekubo, J. Chromatogr. B 736 (1999) 255.
[5] J. Sajiki, J. Chromatogr. B 775 (2001) 9.
[6] S.A.S. Wercinski, Solid Phase Microextaction: A Practical Guide, Marcel Dekker, New York, 1999.
[7] E.M. Thurman, M.S. Mills, Solid-Phase Extraction, Wiley, New York, 1998.
[8] R.A. Bartsch, M. Maeda, Molecular and Ionic Recognition with Imprinted Polymers, American Chemical Society, Washington, DC, 1998.
[9] B. Sellergren, Molecularly Imprinted Polymers, Elsevier, Amsterdam, 2001.
[10] T. Kubo, K. Hosoya, Y. Watabe, T. Ikegami, N. Tanaka, T. Sano, K. Kaya, J. Chromatogr. A 987 (2003) 389.
[11] K. Hosoya, K. Yoshizako, H. sakai, K. Kimata, N. Tanaka, J. Chromatogr. A 828 (1998) 91.
[12] M. Quaglia, K. Chenon, A.J. Hall, E.D. Lorenzi, B. Sellergren, J. Am. Chem. Soc. 123 (2001) 2146.
[13] J. Matsuki, K. Fujiwara, T. Takeuchi, Anal. Chem. 72 (2000) 1810.
[14] J. Haginaka, H. Sanbe, Anal. Chem. 72 (2000) 5206.
[15] J. Jodlbauer, N.M. Maier, W. Lindner, J. Chromatogr. A 945 (2002) 45.
[16] J. Ugelstad, K.H. Kaggerud, F.H. Hansen, A. Perge, Makromol. Chem. 180 (1979) 737.
[17] D.D. Perrin, W.L.F. Armarego, D.R. Perrin, Purification of Laboratory Chemicals, Pergamon, Oxford, 1980.
[18] K. Hosoya, J.M.J. Frechet, J. Polym. Sci., Part A Polym. Chem. 31 (1993) 2129.
[19] A.K. Sakhi, T.E. Gundersen, S.M. Ulven, R. Blomhoff, E. Lundanes, J. Chromatogr. A 828 (1998) 451.
[20] H.S. Lee, C.K. Jeong, H. M Lee, S.J. Choi, K.S. Do, K. Kim, Y.H. Kim, J. Chromatogr. A 848 (1999) 179.
[21] H. Sanbe, J. Haginaka, K. Hosoya, Anal. Sci. 19 (2003) 715.