

New Ketoimine Sorbents in Solid Phase Extraction for HPLC Analysis of Bisphenol A and Other “Endocrine Disrupting” Residues in Drinking Water

A. Szymański*, I. Rykowska, W. Wasiak

Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland

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Abstract

Our study was undertaken to compare the behaviour and properties of two new ketoimine sorbents with a commercial sorbent C-18. The sorbents were used to concentrate the endocrine-disrupting compounds (EDCs) in the water, by the use at solid phase extraction (SPE). EDCs were analyzed by high performance liquid chromatography (HPLC) coupled to UV detection. The residues of bisphenol A, bisphenol A diglycidyl ether, bisphenol F and bisphenol F diglycidyl ether in the water having contact with polycarbonate plastic have been determined. The applicable concentration range was 0.5 to 100 µg/L in water samples. Detection limits were of about 0.20 µg/L for BPA and BPF, and 0.50 µg/L for diglycidyl ether derivatives. The recovery of bisphenol A introduced into water ranged from 93.3% to 97.0%, of BPF from 91.6% to 95.9%, of BADGE from 82.0% to 86.4%, and of BFDGE from 79.7 to 82.5%. The proposed method is simple and sensitive, and thus well suited for analysis of EDCs in the water.

Keywords: bisphenol a, water analysis, SPE, liquid chromatography

Introduction

There are a number of chemical substances that disturb regular performance of the hormonal system. They are referred to as endocrine disrupters [1, 2] and their undesirable effect is felt by both men and women. These substances disturb the hormonal equilibrium of organisms, which is particularly dangerous at developmental age, when changes are in most cases irreversible. Destabilization of the hormonal system can lead to a number of physiological effects, e.g. incorrect functioning of the thyroid gland hormones in the intrauterine life that can cause permanent brain damage. Endocrine disrupting compounds (EDCs) are responsible for neurological conditions, problems with reproduction and development, disturbances in the immunological system, and neoplas-

mic changes. The mechanisms of EDC activities in living organisms are of a different nature. However, the most common of them one the following: imitation of activity of natural hormones, acting as their antagonists, blocking of receptors, entering into reactions with the hormones, and modification of the synthesis of hormones [3, 4].

The EDCs are divided into three groups: pharmaceutical (e.g., contraceptive drugs and some therapeutic drugs), natural (estrogens found in plants), and some environmental pollutants. The compounds present in the natural environment and showing estrogenic properties are the following (among others): organochlorine pesticides, alkylphenols, phthalates, polychlorinated biphenyls and dioxins, organic tin compounds and bisphenol A [5].

Bisphenol A (4,4'-isopropylidene diphenol, BPA) has been used as a raw substance for mass production of epoxy resin, polycarbonate, polyester and polyacrylate

*Corresponding author; e-mail: arekszym@amu.edu.pl

plastics. BPA has been used as a fungicide, antioxidant, an agent suppressing inflammability in the rubber industry, and plastic production, and as a stabilizer in production of polyvinyl chloride. BPA is obtained in the reaction of condensation of phenol with acetone against ion-exchange resin as a catalyst. BPA and its derivatives are potentially hazardous to consumer health, thus their presence and concentration levels in food products should be monitored [6, 7].

Polycarbonate plastics (PC) are commonly used for production of food product packages, bottles for water, bottles for infant food, kitchen utensils, and some elements of medical equipment. The PC-made substances are able to release BPA when used for coverage of inner surfaces of tins for food products or in some dental fillings. BPA residues have been found in the water and other food products stored in PC packages. BPA is able to be liberated from a PC package and migrate into the food stored inside. Such a migration is favoured by acidity of the product stored, elevated temperature, mechanical cleaning and the use of detergents for cleaning bottles or other PC packages [6-8].

BPA was also recently re-evaluated by the Scientific Committee on Food (SCF), resulting in the establishment of a provisional tolerable daily intake (TDI) for BPA at 0.01 mg/kg body weight/day [9].

The analysis of BPA has been accomplished by chromatographic techniques, such as HPLC equipped with fluorescence [8, 10, 11], ultraviolet [7, 12, 13], electrochemical [14, 15] or mass spectrometry detection [16-18], gas chromatography [18-22], as well as micellar electrokinetic chromatography [23, 24].

The aim of the study was to propose a fast and simple method of determination of BPA and its derivatives in water having contact with packages made of plastic-containing BPA. To this goal, we tested bottled drinking water distributed through large networks in Poland. Analysis was performed with the preliminary isolation and concentration of the EDCs to be determined by the solid phase extraction (SPE) and followed by liquid chromatography on a chromatograph equipped with a UV detector.

Experimental

Chemicals and Reagents

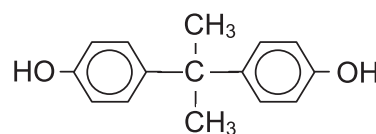
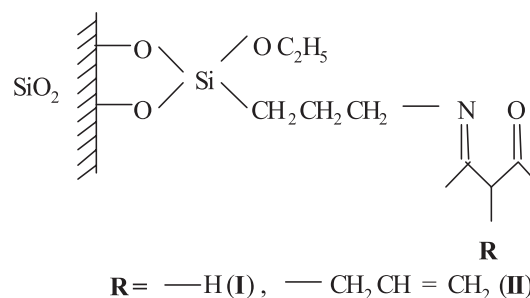
Methanol (MeOH) was a gradient grade for chromatography, dichloromethane HPLC grade (Merck, Germany), acetic acid was analytical grade (POCH, Poland).

Standards of bisphenol A (BPA), bisphenol A diglycidyl ether (BADGE), bisphenol F (BPF) and bisphenol F diglycidyl ether (BFDGE), purchased from Aldrich (USA), were used to prepare individual stock solution in methanol at the level of 1 mg/mL. Standard mixtures were made up in deionized water by dilution of stock solutions. Fig. 1 shows the molecular structures of target analytes.

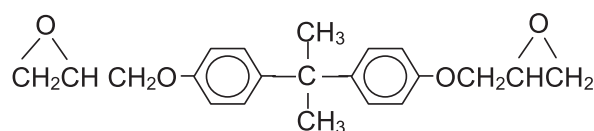
The water was distilled and then purified by a Milli-Q water purification system (Millipore, USA). Mineral water contained in 18.9 L polycarbonate plastic bottles made by three different producers was purchased in a supermarket.

Sample Preparation Procedures

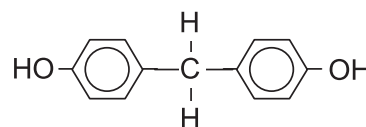
The solid phase extraction was performed with SPE-12G vacuum system (Baker SPE, Germany). For the purification and concentration of the samples, three types of sorbents were tested: C-18 (Baker), columns (I) and (II). The method of obtaining ketoimine sorbents is presented in [25].



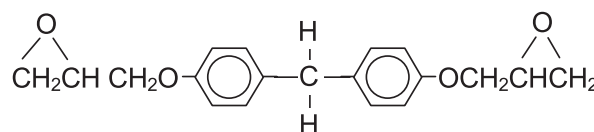
BPA 2,2-bis-(4-hydroxyphenyl)-propan



BADGE 2,2-bis-(4-hydroxyphenyl)-propane-bis-(2,3-epoxy) ether



BPF bis-(4-hydroxyphenyl)-methane



BFDGE bis-(4-glycidyloxyphenyl)-methane

Fig. 1. The molecular structures of the compounds studied.

Cartridges with sorbent were placed on a vacuum manifold, conditioned successively with 5 mL of MeOH (dichloromethane = 1/1, 5 mL of MeOH, and 10 mL of deionized water.

For the studies of recovery and analytical precision, sample volumes of 500 mL of tap deionized water were chosen and spiked at 1 µg/L level with the EDCs investigated. After preconcentration, the sorbent was dried in a vacuum system for 20 min. The compounds retained were eluted with 2 mL of MeOH. The eluent was then evaporated to dryness and then reconstituted to 250 µL MeOH. The extracts were then injected onto the HPLC system.

Drinking water was analyzed by the following procedure: 500 mL of the water were extracted by SPE column. The samples were percolated through bonded phase silica extraction tube at a flow rate of approximately 1 mL/min, previously conditioned.

Liquid Chromatography Analysis

A Hewlett Packard liquid chromatograph, model 1050 (Waldbron, Germany) equipped with a quaternary pump, a variable-wavelength UV detector operated at 277 nm,

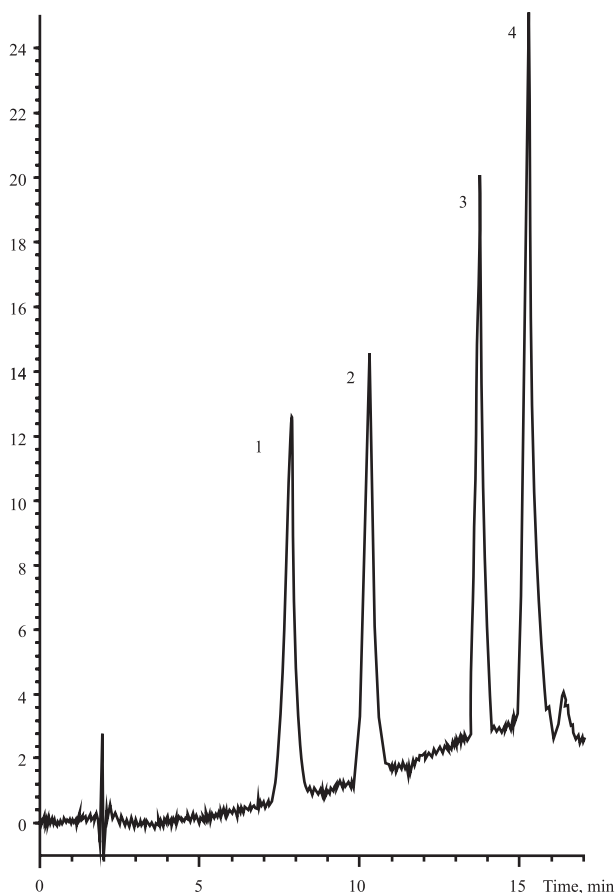


Fig. 2. Chromatogram obtained with a standard solution of the compounds analyzed. Peaks: 1. BPF, 2. BPA, 3. BADGE, 4. BFDGE. Chromatographic conditions are given in the section "Liquid chromatography analysis".

and an injector, model 7125 (Rheodyne) of a 20 µL sample loop was used, together with a LiChrospher 100 RP-18 column (Merck, Germany) (250 mm x 4 mm i.d., 5 µm particle size.

We used the following gradient elution: mobile phase A – MeOH/water containing 0.5% acetic acid (1:1 v/v), mobile phase B – MeOH. The separation was performed by the use of a gradient from 0% to 100% B in 14 min., and then isocratic for 1 min. The mobile phase was then returned to its initial composition in 1 min. The flow rate was 1 ml/min.

An example of the chromatogram obtained for a standard mixture of bisphenol F (0,5 mg/L), bisphenol A (0,5 mg/L), BADGE (4,0 mg/L), and BFDGE (4,0 mg/L) is shown in Fig. 2.

Results and Discussion

Chromatographic Optimization

Optimization was indispensable to provide complete separation of the compounds studied and to eliminate possible matrix interferences in the shortest time. A good separation for analysis of the applied compounds was obtained with a gradient elution. All of the peaks were completely separated within 14.5 min.

Calibration. Limit of Detection

Calibration was performed for a mixture of standard solutions of the analyzed compounds. To determine the linear range of the response to direct injection, 20 µL of standard solution in deionized water were injected. The calibration curves were constructed using the eight concentration levels. A simple solution at each concentration level was injected in triplicate. The calibration plots were approximated by the equation $y = ax + b$, where y is the peak area, and x is the concentration of determination compounds in mg/L. Good correlation coefficients, higher than 0.9984, were obtained.

The detection limits (LOD), obtained from the peak height of the compounds analyzed were defined as the concentration of 3 SD of the baseline signal. The parameters of the resulting calibration lines and the limit of detection are listed in Table 1.

The Recovery Test

Table 2 shows the percentage recoveries together with relative standard deviations obtained for 4 selected EDCs from spiked tap water at concentration levels of 1.0 µg/L after SPE on C-18, and sorbents I and II. These tests were performed using the above method. The recovery of compounds added to water is high. The best results were obtained for the sorbent modified with ketoimine group (I).

Table 1. Parameters of the calibration curves and detection limits for the analyzed compounds.

| | BPF | BPA | BADGE | BFDGE |
|----------------|---------|---------|---------|---------|
| a | 24.52 | 20.38 | 10.34 | 6.92 |
| b | -37.59 | 12.33 | 13.19 | 13.76 |
| r ² | 0.9986 | 0.9984 | 0.9988 | 0.9969 |
| Range [µg/mL] | 0.5-100 | 0.5-100 | 2.0-100 | 2.0-100 |
| LOD [µg/L] | 0.20 | 0.20 | 0.50 | 0.50 |

Table 2. Recovery of analyzed EDCs extracted from 500 ml spiked tap water (1 µg/L) after SPE on C-18, I and II columns (n = 6).

| | BPF | | BPA | | BADGE | | BFDGE | |
|------|------|--------|------|--------|-------|--------|-------|--------|
| | [%] | R.S.D. | [%] | R.S.D. | [%] | R.S.D. | [%] | R.S.D. |
| C-18 | 91.6 | 4.59 | 93.3 | 3.86 | 85.4 | 4.23 | 82.1 | 4.58 |
| I | 95.9 | 3.60 | 97.0 | 3.35 | 86.4 | 5.80 | 82.5 | 6.81 |
| II | 93.2 | 4.26 | 94.9 | 4.24 | 82.0 | 7.23 | 79.7 | 7.95 |

Table 3. Results of drinking water analysis (n = 6).

| Water | C-18 | | I | | II | |
|-------|---------------|------------|---------------|------------|---------------|------------|
| | BPA [µg/L±SD] | R.S.D. [%] | BPA [µg/L±SD] | R.S.D. [%] | BPA [µg/L±SD] | R.S.D. [%] |
| I | 0.49 ± 0.03 | 4.78 | 0.51 ± 0.05 | 4.93 | 0.52 ± 0.03 | 5.82 |
| II | 0.54 ± 0.08 | 4.86 | 0.55 ± 0.07 | 4.64 | 0.53 ± 0.06 | 4.66 |
| III | 0.38 ± 0.05 | 3.92 | 0.38 ± 0.05 | 5.10 | 0.40 ± 0.08 | 6.92 |

Determination of BPA of the Water

Three types of mineral water samples contained in polycarbonate plastic bottles were purchased from a city market. Determination of the concentrated compounds was made using the sorbent with ketoimine group (I). The analysis was performed by the use of the standard

addition method. The concentration found for BPA in the different samples are summarized in Table 3. No presence of BADGE, BPF and BFDGE was detected in the water samples analyzed. Two exemplary chromatograms obtained as a result of the analysis performed are shown in Fig. 3.

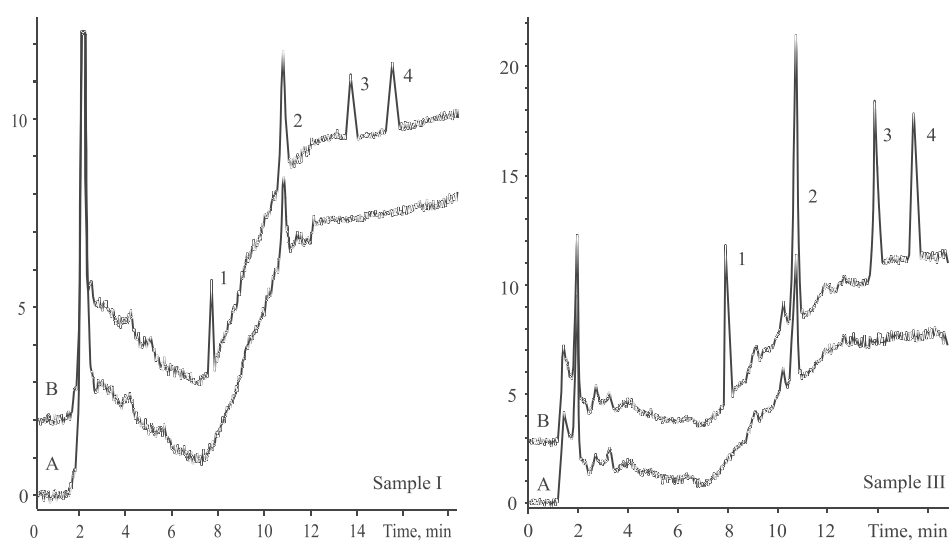


Fig. 3. Sample chromatograms obtained by the analysis of (A) two samples of mineral water (samples I and III in Table 3), and (B) the same samples spiked with a standard mixture of the analytes at 1 µg/L. Peaks as in Fig. 2.

Conclusions

The method proposed involves concentration of selected EDC residues in drinking water by the solid phase extraction method and their analysis by the gradient high performance liquid chromatography with UV detection. The method is simple, sensitive, fast and characterized by high recovery, e.g. for BPA the recovery varies from 93.3 to 97.0%. The detection limit is 0.2 µg/L of the water sample studied. The optimization of SPE was performed using three different column packings. Better results were obtained for ketoimine sorbent (cartridges I) than with C-18.

References

1. COLBORN T., DUMANOSKI D., MYERS J. P., *Our Stolen Future*, Peenguin Books, New York, **1996**.
2. MACZKA C., PANG S., POLICANSKY D., WEDGE R., Evaluating impacts of hormonally active agents in the environment; *Environ.Sci.Technol.* **34**, 136A, **2000**.
3. HOCK B., SEIFERT M., 5th European Workshop on Biosensors for Environmental Monitoring of Endocrine Disruptors, Weihstephan, Munich, May **1997**.
4. CASTILLO M., BARCELO D., Analysis of industrial effluents to determine endocrine-disrupting chemicals; *Trends in Anal. Chem.*, **16**, 574, **1997**.
5. KEITH L. H., *Environmental Endocrine Disruptors*; *Pure & Appl. Chem.*, **70**, 2319, **1998**.
6. HORIE M., YOSHIDA R., ISHII R., KOBAYASHI S., NAKAZAWA H., Determination of bisphenol A in canned drinks by LC/MS; *Bunseki Kagaku* **48**, 579, **1999**.
7. YOSHIDA T., HORIE M., HOSHINO Y., NAKAZAWA H., Determination of bisphenol A in canned vegetables and fruit by high performance liquid chromatography; *Food Addit. Contam.* **18**, 69, **2001**.
8. BILES J. E., MCNEAL T. P., BEGLEY T. H., Determination of bisphenol A migrating from epoxy can coatings to infant formula liquid concentrates; *J. Agric. Food Chem.* **45**, 4697, **1997**.
9. Opinion of the Scientific Committee on Food on Bisphenol A. SCF/CS/PM/3936 Final (Brussels: European Commission. Health and Consumer Protection Directorate-General) [http://europa.eu.int/comm/food/fs/sc/out128_en.pdf].
10. Del OLMO M., ZAFRA A., JURADO A. B., VILCHEZ J. L., Determination of bisphenol A (BPA) in the presence of phenol by first-derivate fluorescence following microliquid-liquid extraction (MLLE); *Talanta* **50**, 1141, **2000**.
11. NAASSNER M., MERGLER M., WOLF K., SCHUPHAN I., Determination of the xenoestrogens 4-nonylphenol and bisphenol A by high-performance liquid chromatography and fluorescence detection after derivatisation with dansyl chloride; *J. Chromatogr. A*, **945**, 133, **2002**.
12. PULGAR R., OLEA-SERRANO M. F., NOVILLO-FERRELLA., RIVAS A., PAZOS P., PEDRAZA V., NAVAJAS J. M., OLEA N., Determination of bisphenol A and related aromatic compounds released from bis-GMA-based composites and sealants by high performance liquid chromatography; *Environ. Health Perspect.* **108**, 1, **2000**.
13. BROSSA L., POCURULL E., BORRULL F., MARCE R. M., A rapid method for determining phenolic endocrine disrupters in water samples; *Chromatographia* **56**, 573, **2002**.
14. INOUE K., YOSHIE Y., KONDO S., YOSHIMURA Y., NAKAZAWA H., Determination of phenolic xenoestrogens in water by liquid chromatography with coulometric-array detection; *J. Chromatogr. A* **946**, 291, **2002**.
15. TOYO'OKA T., OSHIGE Y., Determination of alkylphenols in mineral water contained in PET bottles by liquid chromatography with coulometric detection; *Anal. Sci.* **16**, 1071, **2000**.
16. PEDERSEN S. N., LINDHOLST C., Quantification of the xenoestrogens 4-tert.-octylphenol and bisphenol A in water and in fish tissue based on microwave assisted extraction, solid-phase extraction and liquid chromatography-mass spectrometry; *J. Chromatogr. A* **864**, 17, **1999**.
17. RODRIGUEZ-MOZAZ S., LOPEZ DE ALDA M., BARCELO D., 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, **2004**.
18. JEANNOT R., SABIK H., SAUVARD E., DAGNAC T., DOHRENDORF K., Determination of endocrine-disrupting compounds in environmental samples using gas and liquid chromatography with mass spectrometry; *J. Chromatogr. A* **974**, 143, **2002**.
19. Del OLMO M., GONZALEZ-CASADO A., NAVAS N. A., VILCHEZ J. L., Determination of bisphenol A (BPA) in water by gas chromatography-mass spectrometry; *Anal. Chim. Acta* **346**, 87, **1997**.
20. BRAUN P., MOEDER M., SCHRADER ST., POPP P., KUSCHK P., ENGEWALD W., Trace analysis of technical nonylphenol, bisphenol A and 17 α -ethinylestradiol in wastewater using solid-phase microextraction and gas chromatography-mass spectrometry; *J. Chromatogr. A* **988**, 41, **2003**.
21. LIU R., ZHOU J. L., WILDING A., Simultaneous determination of endocrine disrupting phenolic compounds and steroids in water by solid-phase extraction-gas chromatography-mass spectrometry; *J. Chromatogr. A* **1022**, 179, **2004**.
22. KUO H. W., DING W. H., Trace determination of bisphenol A and phyestrogens in milk powders by gas chromatography-mass spectrometry; *J. Chromatogr. A* **1027**, 67, **2004**.
23. TAKEDA S., IIDA S., CHAYAMA K., TSUJI H., FUKUSHI K., WAKIDA S., Separation of bisphenol A and three alkylphenols by micellar electrokinetic chromatography; *J.Chromatogr. A* **895**, 213, **2000**.
24. TAKEDA S., OMURA A., CHAYAMA K., TSUJI H., FUKUSHI K., YAMANE M., WAKIDA S., TSUBOTA S., TERABE S., Separation and on-line concentration of bisphenol A and alkylphenols by micellar elektrokinetic chromatography with anionic surfactant; *J. Chromatogr. A* **1014**, 103, **2003**.
25. WASIAK W., RYKOWSKA I., VOELKEL A., Ketoimino groups as silica surface modifiers; *J. Chromatogr. A* **969**, 133, **2002**.