

DETERMINATION OF BISPHENOL-A IN DRINKING WATER USING NEW SPE SORBENTS WITH CHEMICALLY BONDED KETOIMINE GROUPS

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Key words: bisphenol-A (BPA), solid phase extraction (SPE), silica modified, gas chromatography (GC), water analysis

In the paper, a new approach is described to determine Bisphenol-A (BPA) in drinking water having contact with polycarbonate plastic. To determine the amount of BPA migrating from the plastic to the water, liquid-solid extraction was used connected with a gas chromatography (GC) analysis. In the presented work, a new SPE sorbent is proposed to determine Bisphenol-A contents. This sorbent contains chemically bonded ketoimine groups. This technique enabled detection limits of approximately $0.3 \mu\text{g/L}$. A recovery of Bisphenol-A introduced into the water was also performed, locating recovery results in the range of 92.6%–97.3%. The proposed method is simple and sensitive, thus highly suitable for the analysis of BPA in water. The new sorbent proposed is compared with popular C18 sorbent, to prove its usefulness in the chromatographic analysis

INTRODUCTION

Bisphenol-A (BPA) is widely used for mass production of plastic (polycarbonates PC) and epoxy resin [Morrissey *et al.*, 1987; Staples *et al.*, 1998]. For many years, BPA was treated as a non-toxic compound, with no negative impact on humans and animals. BPA-based products were commonly used, to mention lacquers for cans and vessels for storage of the food, drinking water, and medicines [Staples *et al.*, 1998; Kuo *et al.*, 2004; Coleman *et al.*, 2003; Lopez-Cervantes *et al.*, 2003; Liu *et al.*, 2004]. Since the second half of the 90-ties, numerous reports have arrived stating a negative influence of BPA on human health [Biles *et al.*, 1997; Olmo *et al.*, 1997; Casajuana *et al.*, 2003; Yoshida *et al.*, 2003; Gonzalez-Casado *et al.*, 1998]. The newest research [Moriyama *et al.*, 2002] classifies BPA as xenobiotic endocrine disruptor, disrupting the balance of the hormonal system of the humans and the animals. Since 1996, BPA has been classified by the European Commission as “external-derivative chemical influenced on human health and offspring”.

BPA was also recently re-evaluated by the Scientific Committee on Food (SCF), which resulted in the establishment of a provisional tolerable daily intake (TDI) for BPA at 0.01 mg/kg BW/day [Opinion of the Scientific Committee on Food on Bisphenol-A].

There are numerous research reported into determining remainders of BPA in environment and biological samples [Staples *et al.*, 1998; Yoshida *et al.*, 2003; Opinion of the Scientific Committee on Food on Bisphenol-A]. No information

is, however, available on determining BPA contents in natural environment samples in Poland, as well as Polish food and drinking water having contact with polycarbonate plastic.

In this work we propose a new method for the isolation and chromatographic detection of Bisphenol-A in drinking water having contact with polycarbonate plastic, as well as try to prove that BPA is migrating from the PC-made cans to the water they contain. The analysis of water samples for the contents of Bisphenol-A is performed with the following method: preconcentration of water samples using solid-phase extraction (SPE), drying the sorbent with an air stream, eluting the analyte by the use of methanol, evaporation of solvent excess, and finally GC analysis. We chose SPE because this is one of the most commonly used methods of sample preparation. Among different SPE sorbents, the modified silica-based ones (the so-called “silica with chemically bonded phases”) are still the most popular [Poole, 1991; Buszewski *et al.*, 2001; Unger, 1990]. These sorbents are under continuous improvement, towards better selectivity and greater recovery. Nowadays, such sorbents are usually modified by n-alkyl groups, including octyl- and octadecyl- groups. However, their further improvement is still needed. Thus, we performed some research and developed some new sorbents for SPE, differing from the actually used one in terms of ketoimine groups chemically bonded to silica’s surface.

To compare the quality of the method proposed, similar research was performed using standard C18 packing. It was proved that the newly proposed sorbent is characterized by higher recovery rates in comparison to the ones of C18.

MATERIALS AND METHODS

Chemicals and materials. All compounds investigated in the separations, including BPA, BADGE, and BFDGE, were purchased from Sigma – Aldrich, and had a purity of equal or greater than 98%. Water was purified in a Milli-Q apparatus (Millipore S.A. 67120 Molsheim, France). Structures of some of the compounds being tested are shown in Figure 1.

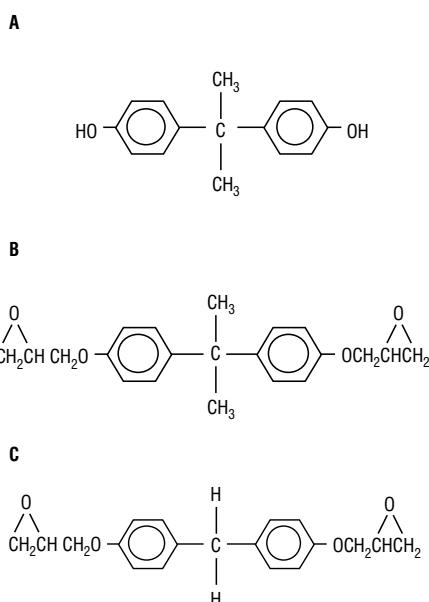


FIGURE 1. Structures of some of the compounds being tested: (A) 2,2-bis-(4-hydroxyphenyl)-propane (BPA); (B) 2,2-bis-(4-hydroxyphenyl)-propane-bis-(2,3-epoxypropyl) ether (BADGE); (C) bis-(4-glycidyloxyphenyl)-methane (BFDGE).

All standard stock solutions were prepared in methanol and used after proper dilution with the same solvent.

Mineral water contained in polycarbonate (PC) bottles was purchased from the city market.

Silica gel (Baker Analyzed[®]) was purchased from J.T.Baker. 3 pentano-2,4-dione derivatives, which were used for the modification of the silica surface, were obtained from the Metalloorganics Department of the Adam Mickiewicz University, Poznań, Poland. C18 silica gel (Si 100/C18) was purchased from Merck. Solvents used for the modification reactions (xylene and hexane – analytical grade) were purchased either from POCh (Gliwice, Poland), or Fluka (Buchs, Switzerland).

Apparatus. The chromatographic separation was performed using the following hardware: (1) Gas chromatography VARIAN CP-3380 equipped with flame ionization detector (FID), and a CP-SIL 5 CB capillary column

(10 m × 0.53 mm; DF=2.0). The analysis was performed at a temperature of 250°C (column) and 280°C (detector). Helium was the carrier gas. All the work was carried out in a constant flow mode set at 3.5 mL/min. (2) A Bakerbond spe vacuum manifold was used for the elution of SPE columns.

Elemental analysis was performed on a 2400 CHN Elemental Analyzer (Perkin-Elmer, Norfolk, USA).

Nitrogen adsorption/desorption isotherms at the temperature of liquid nitrogen were obtained with an ASAP 2010 sorptiometer (Micrometrics, Narcross, GA, USA).

Sorbent preparation. Dry silica (5 g) was immersed in a mixture of anhydrous xylene and 3-aminopropyltriethoxysilane. The mixture was boiled for 12 h in a vessel equipped with a reflux condenser. The contents were continuously stirred and carefully protected against the moisture. Unreacted silane was extracted with xylene and hexane in a Soxhlet apparatus. Next it was dried under vacuum and finally subjected to the so-called “end capping” reaction with hexamethyldisilazane in order to deactivate free silanol groups remaining at its surface.

The second step was bonding amino groups using an appropriate derivative of 3-pentano-2,4-dione. As previously, the reaction was performed under continuous stirring in anhydrous xylene and lasted 12 h. The system was protected against the moisture. The final product was extracted subsequently with xylene and hexane in a Soxhlet apparatus. Finally, silica was dried under vacuum.

A scheme in Figure 2 presents the steps of silica surface modification.

Properties of modified silica. To investigate physicochemical properties of the packings obtained, they were subjected to the elemental analysis which involved determining the contents of carbon, hydrogen and nitrogen and estimating the specific surface area. The surface concentration of bonded siloxane molecules (denoted by α) in $\mu\text{mol}/\text{m}^2$ was calculated from carbon content according to the following equation:

$$\alpha = \frac{\%C \cdot 10^6}{(100 \cdot n \cdot 12 - \%C \cdot M) \cdot S_{BET}}$$

where: $\%C$ denotes the percent of carbon contribution, n – number of carbon atoms in the molecule of bonded silane, M – molecular weight of the siloxane, and S_{BET} – specific surface area (m^2/g). The results are presented in Table 1.

Sample preparation / Solid Phase Extraction. Enrichment preconcentration of the analyte from the water sam-

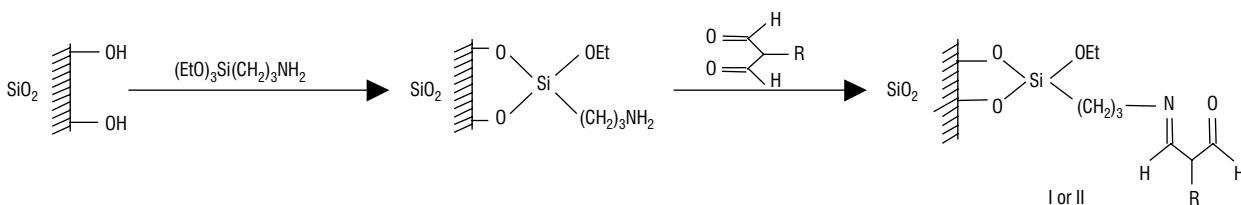


FIGURE 2. A scheme of the modification procedure for the packings.

TABLE 1. Surface properties of the modified silica support.

Sorbent	Elemental analysis (%)			Surface area (m ² /g)	Surface concentration of silane (μmol/m ²)	Pore diameter (nm)	Specific pore volume (mL/g)
	C	H	N				
Silica gel	0.05	0.99	-	552	-	6.18	0.88
(I)			10.70	2.99	2.25	376	4.04
(II)			13.44	3.34	1.96	326	4.37

ples was performed on little columns with constant sorbent C18 and silica modified with ketoimine groups. The columns were conditioned before introducing the samples by passing in turn: 5 mL of a mixture of the methyl chloride and methanol (1:1 v/v), 5 mL of methanol, and 15 mL of deionized water. Columns prepared this ways were injected with 500 mL of the water analyzed. Once the total amount of the sample was put through the column, the sorbent was dried for 10 min under vacuum, and the preconcentrated compounds were washed away with 3 mL of methanol. The extract was dried up and further dissolved in 0.5 mL of methanol. Determination of BPA in water was performed with the addition standard method. Two extractions were performed in parallel, on two identical columns. The first one was performed with 500 mL of the water under study, while the second one – with 500 mL of the water under study with an additional, known amount of BPA. The samples obtained were measured by means of GC systems.

RESULTS AND DISCUSSION

Calibration graph. Recovery

Dependencies between the peak area and the compound's concentration were determined as a result of a chromatographic analysis of water samples with added BPA, BADGE, and BFDGE. Based on these dependencies, the calibration graphs were prepared. To this end, some pattern solutions were used with the BPA concentration ranging from 1 to 100 μg/mL, and BADGE and BFDGE – from 2.5 to 100 μg/mL, in the methanol solution. The final peak area was taken as an average of three experiments in turn. Calibration plot was described with the general equation: $y = ax + b$, where y is the peak area, and x – the amount of determined compound in mg/mL. For the observed range of concentration values, a linear route is observed of the calibration curves for calibration coefficients higher than 0.9985.

The detection limits were defined as the amount of the compounds producing a peak three times higher than the noise level recorded for a matrix without the component

TABLE 2. Parameters of calibration curves and detection limits for the analyzed compounds.

Determined values	BPA	BADGE	BFDGE
a	29.05	12.23	3.96
b	-60.45	-10.36	0.86
r ²	0.9995	0.9997	0.9989
LOD μg/L	0.3	1.0	1.0

determined. The obtained parameters of the calibration curves for the analyzed compounds are reported in Table 2.

Recovery tests were performed for the deionized water, with significant, known amount of BPA added (0.5 μg/500 mL⁻¹). These tests were performed using the above described method, with the following results obtained: for C18 sorbent – 92.6%, and for sorbents with chemically bonded ketoimine groups – 97.3% and 95.5% (for I and II sorbent respectively, cf. Figure 1).

The calibration curves obtained are characterized by very good linearity. The method is characterized by good recovery rates, as well as low detection limits.

Determining BPA in drinking water having contact with polycarbonate plastic

The drinking water for the analysis of BPA concentration was collected from a 19-L can made of PC plastic. Such cans are widely used by several companies for distributing large amounts of drinking water for the human staff. The analysis was performed with the addition standard method. Sample chromatograms obtained as a result of the analysis performed are presented in Figures 3a and 3b. Six parallel determinations of BPA concentration were performed for three water samples. The results are presented in Table 3, as arithmetical-average results for the interval confidence of 95%.

Based on the results obtained, it was proven that BPA is migrating from the PC-made cans to the drinking water they contain. The measured concentration of BPA in drinking water accounts for 0.38–0.61 μg/L. Such a concentration is

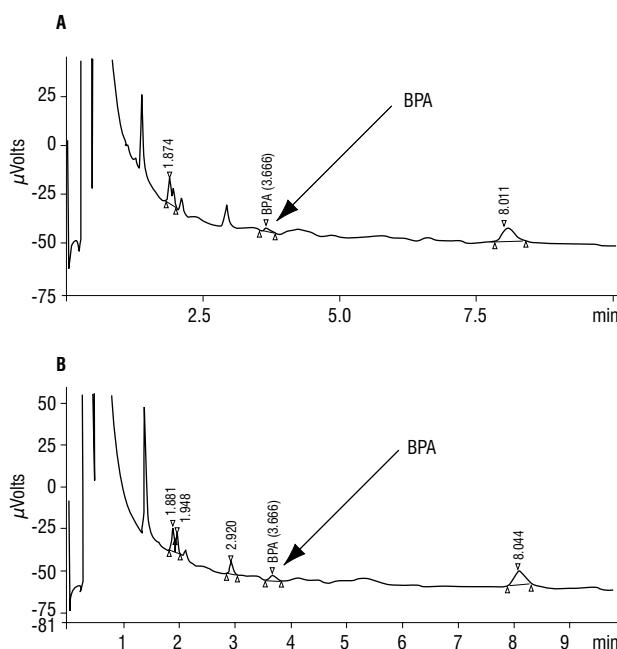


FIGURE 3. GC chromatograms of the analyzed compounds: A) with certain amount of BPA added; B) without artificially added BPA.

below the recommended limits of the European Commission (0.01 mg/kg BW/day) [Opinion of the Scientific Committee on Food on Bisphenol-A].

As for the analyzed water samples, the remaining two compounds (BADGE and BFDGE) were not detected at all.

CONCLUSIONS

The manuscript reports on the results concerning the synthesis and usage of new packings with ketoimine groups bonded to the silica surface. These packings were used for preconcentration of Bisphenol-A, originally from drinking water having contact with polycarbonate plastics. To determine the usefulness of the proposed method, similar preconcentration was performed using a common C18 sorbent.

A method based on solid-phase extraction followed by gas chromatography with FID detector has been established

to determine BPA in drinking water with a detection limit of 0.3 µg/L.

The proposed method of extraction and preconcentration of the analyte is characterized by high recovery of 92%÷97%. As proven by our experiments, newly synthesized sorbents with chemically bonded ketoimine groups may be used for preconcentration of BPA (originally from the tap water samples) by the use of the SPE method. These sorbents are characterized by better chromatographic properties in comparison with a common C18 sorbent.

It is worth emphasizing that the experiment involved analyses of drinking water from the Polish market. No similar research has been reported so far. We proved that our method, based on a popular FID detector, may replace the methods based on GCMS [Biles et al., 1997; Casajuana et al., 2003; Gonzales-Casado et al., 1998; Liu et al., 2004;] that need more expensive and thus not so popular hardware.

REFERENCES

- Biles J.E., Mc Neal T.P., Begley T.H., Determination of bisphenol A in reusable polycarbonate food-contact plastics and migration to food-simulating liquids. *J. Agric. Food Chem.*, 1997, 45, 3541–3544.
- Buszewski B., Jezierska-Świtała M., Kalisz R., Wojtczak A., Albert K., Bachmann S., Matyska M.T., Pesek J.J., Selectivity tuning and molecular modeling of new generation packings for RP HPLC. *Chromatographia*, 2001, 53, 204.
- Casajuana N., Lacorte S., Presence and release of phthalic esters and Rother endocrine disrupting compounds in drinking water. *Chromatographia*, 2003, 57, 649–655.
- Coleman K.P., Toscano W.A., QSAR models of the *in vitro* estrogen activity of bisphenol A analogs. *QSAR Comb. Sci.*, 2003, 22, 78–88.
- Del Olmo M., Gonzalez-Casado N.A., Navas J.L., Determination of bisphenol A (BPA) in water by gas chromatography-mass spectrometry. *Anal. Chimica Acta*, 1997, 346, 87–92.
- Gonzalez-Casado A., Navas N., Del Olmo M., Vilchez J.L., Determination of bisphenol A in water by micro

TABLE 3. Average concentrations of BPA in mineral water stored in PC bottles (n=6).

	Concentration µg/L		
	water A	water B	water C
C18	0.55 ± 0.07	0.61 ± 0.07	0.38 ± 0.06
	0.59 ± 0.04	0.64 ± 0.03	0.41 ± 0.05
	0.58 ± 0.06	0.63 ± 0.05	0.40 ± 0.04

- liquid-liquid extraction followed by silylation and gas chromatography-mass spectrometry analysis. *J. Chromatogr.*, 1998, 36, 565–567.
7. Kuo H.W., Ding W.H., Trace determination of bisphenol A and phytoestrogens in infant formula powders by gas chromatography-mass spectrometry. *J. Chromatogr.*, 2004, 1027, 67–74.
8. 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.*, 2004, 1022, 179–189.
9. Lopez-Cervantes J., Paseiro-Losada P., Determination of bisphenol A in, and its migration from, PVC stretch film used for food packing. *Food Addit. Contam.*, 2003, 20, 596–606.
10. Moriyama K., Tagami T., Akamizu T., Usui T., Saijo M., Kanamoto N., Hataya Y., Shimatsu A., Kuzuya H., Nakao K., Thyroid hormone action is disrupted by Bisphenol A as an antagonist. *J. Clin. Endocrinol. Metab.*, 2002, 87, 5185–5190
11. Morrissey R.E., George J.D., Price C.J., Tyl R.W., Marr M.C., Kimmel C.A., The developmental toxicity of bisphenol A in rats and mice. *Fundam. Appl. Toxicol.*, 1987, 8, 571–582.
12. 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].
13. Poole C.E., Poole S.K., *Chromatography Today*, 1991. Elsevier, Amsterdam, pp. 142–170.
14. Staples C.A., Dorn P.B., Klecka G.M., O'Block S.T., Harris L.R., Quantitative determination of bisphenol-A in river water by cool on-column injection-gas chromatography-mass spectrometry. *Int. J. Environ. Anal. Chem.*, 1998, 69, 83–98.
15. Packings and Stationary Phases in Chromatography, 1990 (ed. K.K. Unger). Marcel Dekker, New York-Basel, pp. 249–269.
16. Yoshida T., Harada H., Nohta H., Yamaguchi M., Liquid chromatographic determination of bisphenols based on intramolecular excimer-forming fluorescence derivatization. *Anal. Chim. Acta*, 2003, 488, 211–221.

Received November 2004. Revision received and accepted June 2005.

OZNACZANIE BISFENOLU A W WODZIE PITNEJ Z ZASTOSOWANIEM NOWYCH SORBENTÓW DO SPE Z CHEMICZNIE ZWIĄZANYMI GRUPAMI KETOIMINOWYMI

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W pracy zaprezentowano wyniki oznaczeń bisfenolu A w wodzie pitnej mającej kontakt z tworzywem poliwęglanowym z wykorzystaniem do zatężania ekstrakcji ciecz-ciało stałe (SPE-solid phase extraction), a do analizy ekstraktu metody chromatografii gazowej. W pracy zaproponowano wykorzystanie do zatężania oznaczanego związku nowego sorbentu z chemicznie związanymi grupami ketoiminowymi. Wykazano, iż bisfenol A migruje z opakowań poliwęglanowych do zawartej w nich żywności (tab. 3, rys. 3). W oparciu o analizy roztworów wzorcowych bisfenolu A przeprowadzono badania nad optymalizacją warunków oznaczeń ilościowych metodą chromatografii gazowej.