DETERMINATION OF BISPHENOL A
IN WATER AND MILK
BY MICELLAR LIQUID CHROMATOGRAPHY

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SUMMARY

Polycarbonate plastics (PC) containing bisphenol A (BPA) are used for production of bottles for water, containers for storage of food products, bottles for feeding infants, kitchen utensils, and some components of medical equipment. Trace amounts of BPA have been detected in water and food products kept in PC containers. It has been established that BPA can be liberated from PC containers and migrate into the food products kept in them. Such migration is facilitated by use of detergents for washing such containers. This study was undertaken to establish a simple and rapid method for determination of BPA in powdered milk and mineral water commercially available in PC containers.

Residues of BPA in mineral water and milk were preconcentrated by solid-phase extraction and determined by micellar liquid chromatography. The micellar mobile phase was an aqueous solution of 0.2 M sodium dodecyl sulphate (SDS) and 10% 2-propanol. The linearity of the calibration plot was tested by use of water samples containing BPA at concentrations from 0.5 to 100 μg L^{-1} and 2,2-bis-(4-hydroxyphenyl)-propane-bis-(2,3-epoxypropyl) ether (BADGE) at concentrations from 1.0 to 100 μg L^{-1}. Recovery of BPA and BADGE from water was 92.3 and 84.2%, respectively. Detection limits for BPA and BADGE were 0.3 and 0.6 μg L^{-1}, respectively, and quantification limits were 1.0 and 2.0 μg L^{-1}, respectively. The method proposed is characterised by high accuracy and precision.

INTRODUCTION

It is known that in the natural environment there are compounds with the potential to disturb hormonal equilibria in living organisms. These compounds, mistakenly recognised by oestrogen receptors, are treated

the same as those naturally present in the organisms. The compounds cannot, however, perform the functions of those naturally present and, therefore, disturb regulatory mechanisms throughout the organism. Substances of this type are known as endocrine-disrupting compounds, EDC [1,2].

Negative effects of EDC have been observed in men and women. Their interaction is particularly threatening during development, when EDC can cause irreversible damage apparent only in adulthood. Endocrine disrupters have been shown to affect pituitary, thyroid, and adrenal glands, leading to destabilisation of the hormonal system and, eventually, several side-effects. They can cause neurological problems, anomalies in reproduction and development, even infertility, disturbances of the immunological system, and development of tumours (mainly breast and prostate). Although the mechanisms of action of xenobiotics vary in different organisms [3,4], there are, usually, basic similarities – imitation of the activity of natural hormones, antagonism of hormone activity, blocking of receptors, interaction with hormones, and modification of hormone synthesis.

It is impossible to predict the activity of individual EDC on hormonal systems on the basis of their molecular structure only. The problem is, moreover, even more difficult when the activity of two or three EDC combine; combination of a large number of disturbing substances, even at low concentrations, can substantially enhance the harmful effect. EDC can be divided into three groups: pharmaceuticals (e.g. contraceptives and some drugs), natural (estrogens occurring in plants), and some environmental pollutants.

During production processes and use, and as waste, synthetic chemicals penetrate the natural environment and can be assimilated by living organisms. Synthetic compounds with estrogenic properties which are found in the natural environment and in food include several pesticides and herbicides, organochlorine compounds, polycyclic aromatic hydrocarbons, alkylphenols, phthalates, polychlorinated biphenyls and dioxins, organic tin compounds, and bisphenol A and its derivatives [5].

Bisphenol A (2,2-di(*p*-hydroxyphenyl)propane, BPA) has been used for production of polycarbonates, epoxy resins, polysulphones, unsaturated polyesters, and polyacrylate resins [6–8]. In the production of glue and ink it is used as fungicide and antioxidant [9–11], in the production of rubber and plastics as a flame retardant, and in the production of poly(vinyl chloride) as a stabiliser [11,12]. BPA is obtained by condensation of phenol with acetone in the presence of an ion-exchange resin as a catalyst. Because

BPA and its derivatives can be harmful to living organisms their presence and concentrations in food products must be monitored.

Although as early as 1936 Doods and Lawson [13] reported the estrogenic properties of BPA, only in the nineteen-nineties did this compound attract particular attention, when several authors confirmed it was a xenobiotic disturbing the hormonal system of living organisms. In 1996 the European Commission classified BPA as a substance of external origin having harmful effect on the health of humans. The Scientific Committee on Food, (SCF) an independent board advising the European Commission on food safety, estimated a permissible level of BPA migrating to food products as 0.01 mg kg^{-1} body weight per day [14].

In addition to BPA, its derivative bisphenol A diglycide ether (BADGE), is a semiproduct used for production of epoxy resins widely used in production of food packaging, for example in the varnish coating the inside surface of cans. BADGE has been classified as a mutagenic substance i.e. a substance causing alteration of DNA.

Polycarbonates (PC) are characterised by great strength, stability, elasticity, and low density. For these reasons they have been widely used for production of food and pharmaceutical packaging, bottles for infants, kitchen utensils, medical equipment, computers, and electronic devices (CD) [15–17]. Substances capable of releasing BPA are used to coat the interior surfaces of cans for food or in the production of dental fillings [8,18]. BPA residues have been detected in water and other food products stored in packages made of PC. BPA can be released from PC and migrate to the food inside the package. This migration is promoted by acidity of the food stored, elevated temperature (e.g. on heating in microwave ovens), mechanical cleaning, and use of detergents for cleaning this packaging [12,19, 20].

Analysis of BPA has been accomplished by chromatographic techniques, for example HPLC equipped with UV [21–26], fluorescence [27–32], mass spectrometric [33–38], or electrochemical detection [39–41] and gas chromatography [26,42–46], and by electrochemical methods [47–49].

The objective of this study was to establish a rapid and simple method for determination of BPA and BADGE in water in contact with PC bottles containing BPA, and in powdered milk, either introduced during the manufacturing process or leached from containers [46]. Analysis was performed on drinking water distributed by large networks in Poland and on water stored in PC bottles for feeding infants. The method is based on preliminary extraction and concentration of BPA and its derivative by

solid-phase extraction (SPE) then determination by micellar liquid chromatography (MLC) with UV detection.

EXPERIMENTAL

Chemicals and Reagents

Gradient-grade methanol (MeOH) for chromatography and HPLC-grade 2-propanol and dichloromethane were from Merck (Germany) and sodium dodecyl sulphate was from Aldrich. Water used for preparations of solutions and mobile phase was deionised by use of a Milli-Q system (Millipore).

Bisphenol A (BPA) and 2,2-bis-(4-hydroxyphenyl)-propane-bis-(2,3-epoxypropyl) ether (BADGE) standards (Aldrich, USA) were used for preparation of individual stock solution in methanol (1.0 mg mL^{-1}). Standard mixtures (10 μg mL^{-1}) were prepared in the mobile phase by dilution of the stock solutions. The structures of the compounds are given below.

BPA

BADGE
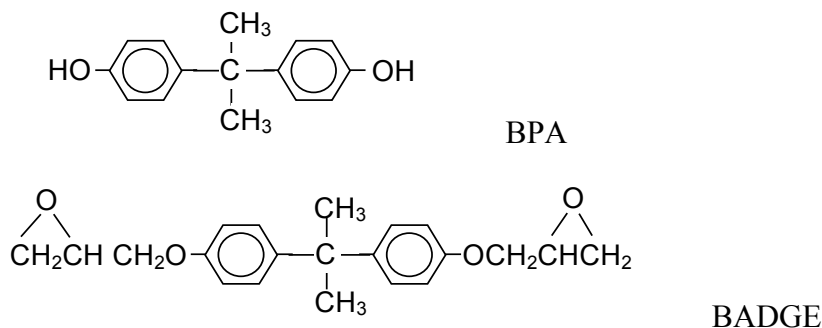
Powdered milk and mineral water in 18.9-L polycarbonate plastic bottles made by three different producers were commercial products.

Sample-Preparation Procedures

Solid-phase extraction was performed with an SPE-12G vacuum system (Baker SPE). Cartridges containing C_{18} bonded-phase silica adsorbent (Baker SPE) were placed on the vacuum manifold and conditioned with 5 mL MeOH and 10 mL deionized water.

For studies of recovery and analytical precision, samples (500 mL) of deionized tap water were spiked with BPA and BADGE. After preconcentration the adsorbent was dried in air by use of the vacuum system for

20 min. The compounds retained were eluted with 2 mL MeOH. Extracts were then evaporated to dryness, reconstituted in 0.25 mL mobile phase, and analyzed by MLC.

For analysis of drinking water, samples (500 mL) were percolated through previously conditioned cartridges at a flow rate of approximately 1 mL min^{-1}. Retained bisphenol A was eluted with 2 mL methanol, the MeOH was evaporated to dryness, and the residue was reconstituted in 0.25 mL micellar mobile phase.

For analysis of powdered milk, an accurately weighed sample (0.5 g) was dissolved in 5 mL 50:50% (*v/v*) ethanol–water. The sample solution was mixed for 2 min in an ultrasonic chamber, centrifuged for 40 min at 5000 rpm, and finally filtered through a 3W membrane filter. These solutions were passed through previously conditioned columns, the adsorbent was then dried for 10 min under vacuum, and the retained compounds were eluted with 3 mL methanol. The extract was dried, dissolved in 0.25 mL mobile phase, and analysed by MLC.

Liquid Chromatography

HPLC was performed with a Hewlett–Packard HP 1050 chromatograph equipped with a UV–visible detector (190–600 nm) and a Rheodyne model 7125 injector with a 20-μL sample loop. Compounds were separated on a 250 mm × 4.6 mm, 5-μm particles, LiChrospher 100 RP-18 column (Merck) at 40°C. The mobile phase was an aqueous solution of the anionic surfactant sodium dodecyl sulphate, SDS (Aldrich); the flow rate was 1 mL min^{-1}. Before use the micellar mobile phase was filtered under vacuum through 0.45-μm cellulose acetate filters and degassed with helium. Detection of BPA was performed at 260 nm.

RESULTS AND DISCUSSION

Optimisation of the Chromatography

Optimisation was essential to achieve complete separation of the compounds in the shortest time and to eliminate possible matrix interferences. Good separation of the compounds was achieved with a micellar mobile phase. Figure 1 shows plots illustrating the dependence of retention coefficient on SDS concentration and on the amount of 2-propanol, as organic modifier, in the mobile phase.
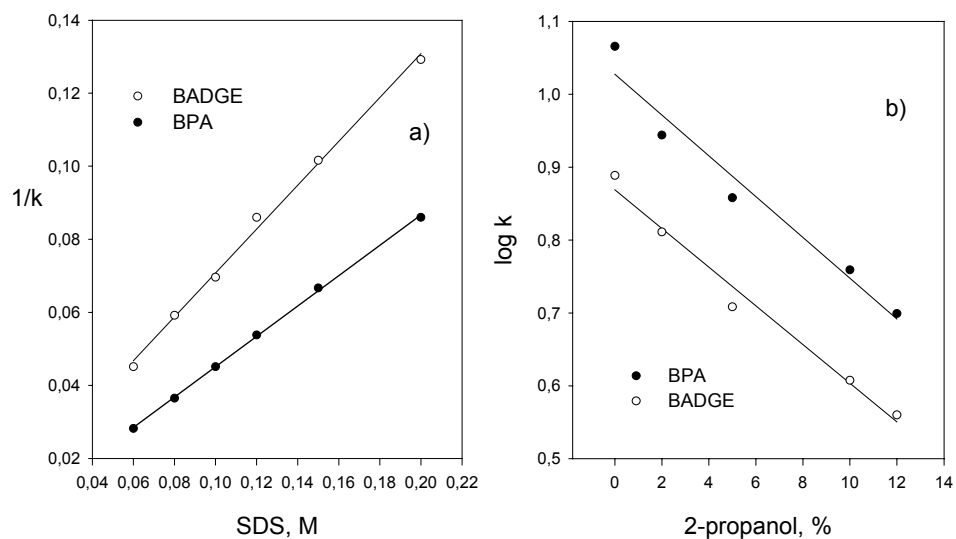
Fig. 1

Effect of (a) SDS concentration (M) and (b) the amount of 2-propanol (%, *v/v*) in 0.10 M SDS on the retention behaviour of BPA and BADGE
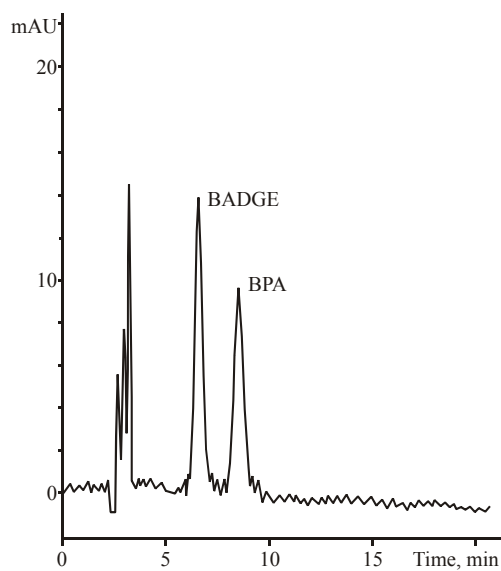

Fig. 2

Chromatogram obtained from a standard solution of BADGE (retention time 7.15 min) and BPA (9.08 min)

After optimisation, determination of BPA and BADGE was performed with a micellar mobile phase containing 0.2 M SDS and 12% (*v/v*) 2-propanol at a flow rate of 1 mL min^{-1} and a separation temperature of 40°C. An example of a chromatogram obtained under these conditions from a standard mixture of bisphenol A (1.0 µg L^{-1}) and BADGE (2.0 µg L^{-1}) is shown in Fig. 2.

Calibration, Limits of Detection and Quantification, and Recovery

Calibration was performed with mixed standard solutions of the compounds. To determine the range in which response was a linear function of amount injected, standard solutions containing 0.5; 1.0; 2.0; 5.0; 10.0; 20.0; 50.0; 100.0 µg L^{-1} BPA and BADGE in the mobile phase were injected (20 µL injection). Each solution was injected in triplicate. Good correlation coefficients (>0.9976) were obtained.

Limits of detection (LOD) and quantification (LOQ), respectively, were defined as the concentrations giving peak heights three and ten times the standard deviation of the baseline signal. The resulting calibration plots, regression data, and correlation coefficients are presented in Fig. 3. The limits of detection and quantitation, and the linear range, are listed in Table I.
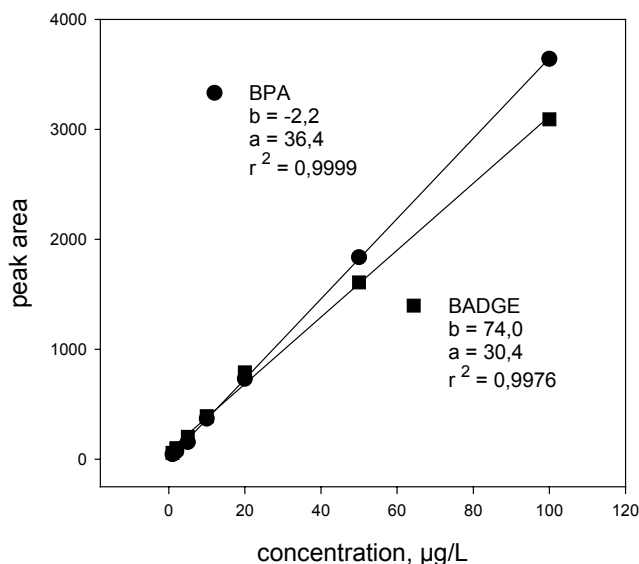
Fig. 3

Calibration plots for BPA and BADGE

Table I

Limits of detection and quantitation, and the linear range

	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)
BPA	0.50 – 100	0.30	1.0
BADGE	1.0 - 100	0.60	2.0

Recovery of BPA and BADGE from water, with relative standard deviations, are given in Table II. The compounds were added to the water at a concentration of 1 $\mu g\ L^{-1}$. The method proposed is characterised by high recovery.

Table II

Recovery of BPA and BADGE from 500 mL tap water spiked at 1 $\mu g\ L^{-1}$ after SPE on C_{18} ($n = 6$)

|	Recovery (%)	RSD (%)
BPA	92.3	3.97
BADGE	84.2	4.56

Determination of BPA and BADGE in Water

Three types of mineral water in polycarbonate bottles were purchased at a city market and the amount of BPA was determined by the standard-addition method after SPE on C_{18}. The results are presented in Table III. Typical chromatograms obtained from analysis of the water are shown in Fig. 4a. No BADGE was detected in the mineral water samples analysed.

Table IV

Results from analysis of drinking water ($n = 6$)

Sample	BPA ($\mu g\ L^{-1} \pm$ SD)	RSD (%)
I	0.47 ± 0.04	4.18
II	0.52 ± 0.07	4.26
III	0.40 ± 0.06	4.70
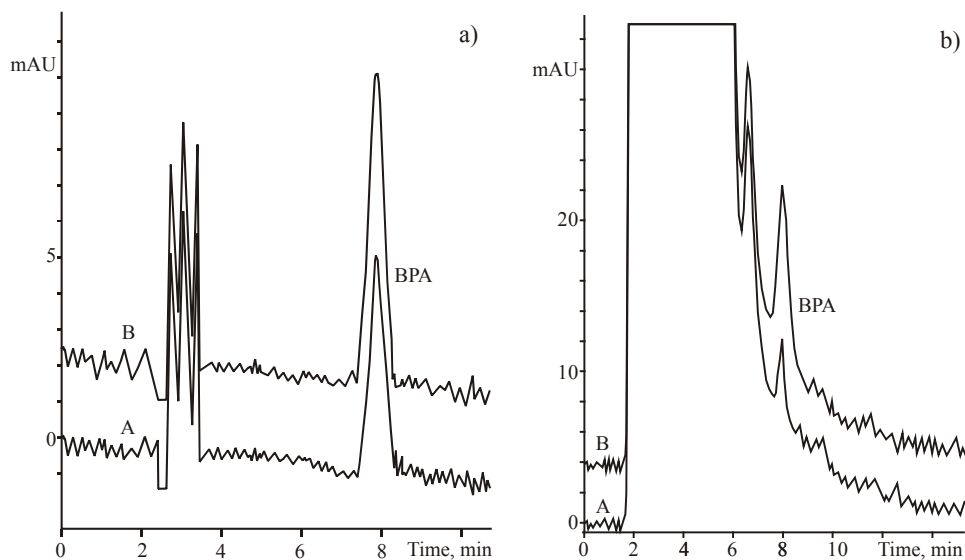
Fig. 4

Typical chromatograms obtained from analysis of (a) mineral water and (b) powdered milk without (A) and with (B) added BPA

Determination of BPA and BADGE in Powdered Milk

Results from analysis of BPA in two samples of powdered milk by the method of standard addition, according to the procedure described in the experimental section, are listed in Table IV. No BADGE was detected in the samples. Typical chromatograms obtained from analysis of the powdered milk are shown in Fig. 4b.

Table IV

Results from analysis of powdered milk ($n = 6$)

Sample	BPA ($\mu g\ L^{-1} \pm SD$)	RSD (%)
A	0.19 ± 0.02	6.18
B	0.24 ± 0.04	5.82

CONCLUSIONS

The proposed method involves concentration of BPA residues in drinking water and powdered milk samples by solid-phase extraction then

analysis by micellar liquid chromatography with UV detection. The method is simple, sensitive, rapid, and characterised by high recovery; for BPA, for example, recovery was 92.3%. The detection limit for BPA was 0.3 $\mu g\ L^{-1}$ in the water and milk samples studied. This combination of SPE and MLC enables determination of BPA at ppb levels. The experiment proved that BPA is present in the powdered milk at concentrations in range 0.19 to 0.24 $\mu g\ g^{-1}$.

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