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Sensitive determination of bisphenol A in environmental water by gas chromatography with nitrogen–phosphorus detection after cyanomethylation

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Abstract

A new technique is proposed for the determination of bisphenol A in environmental water. The sample preparation consists of a single-step extraction of bisphenol A from a water sample with methylene chloride and the cyanomethyl derivatization of bisphenol A. 2,2′-Biphenol is used as an internal standard. Bisphenol A and biphenol can be quantitatively converted to their corresponding cyanomethyl ethers, which are then measured by gas chromatography with nitrogen–phosphorus detection. Peak shape and quantification of bisphenol A are excellent, with linear calibration curves over a range of 0.1–100 ng/ml. The detection limit is 0.1 ng/ml in water samples. The average recovery and RSD at a concentration of 5 ng/ml are 89.3 and 4.5%, respectively. The procedure is applicable to the quantification of bisphenol A in tap water, raw water and stream water.

Keywords: Water analysis; Environmental analysis; Derivatization, GC; Bisphenol A; Biphenol; Phenols

1. Introduction

Since the early 1950s, abnormalities in reproduction and development as well as disorders suspected of immunodeficiency or brain maldevelopment have been reported in several wildlife species all over the world [1]. Also in the human population, a number of abnormalities have been reported. They include reduced number and deterioration of sperm quality in males, abnormal or delayed development of male reproductive organs such as retained testis and hypospadias, increased incidence of prostate cancer, breast cancer and endometriosis with the associated infertility in females [2]. A considerable body of evidence indicates that many classes of environmental contaminants, including dioxins, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons, bisphenol A and 4-alkylphenols have the ability to interfere with normal hormonal activity by mimicking and blocking the action of natural hormones [3–8]. In South Korea, the Ministry of Environment has started monitoring for these compounds in water, sludge and fish.

Bisphenol A is the raw material used with epi-

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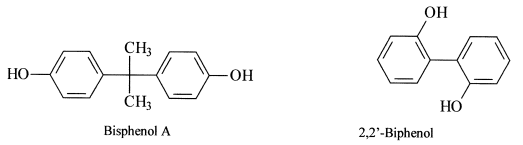

Fig. 1. Molecular structures of bisphenol A and 2,2′-biphenol.

chlorohydrin in the commercial synthesis of bisphenol A diglycidyl ether (BADGE). BADGE has been used as lacquer coating on cans and other storage vessels used for foodstuffs. Biotransformation of BADGE or the nonreacted raw material lead to the contamination of bisphenol A in water. We were concerned with the concentration of bisphenol A in environmental water.

Many methods based on different principles have been proposed for the determination of bisphenol A. For the determination of μg/l and ng/l concentrations of bisphenol A in environmental water the most frequently used methods are gas chromatography–mass spectrometry (GC–MS) [9–20] or high-performance liquid chromatography with ultraviolet (UV), mass spectrometric or electrochemical detection [21–25]. For the sensitive determination of bisphenol A by GC, a derivatization procedure such as methylation or silylation [20] is necessary.

For the selective and highly sensitive determination of bisphenol A in environmental water, we propose a novel derivatization method based on GC with nitrogen–phosphorus detection (NPD). The derivatization method can expand the usefulness of NPD to non-nitrogen- and non-phosphorus-containing compounds.

The structures of bisphenol A and 2,2′-biphenol (as internal standard) are shown in Fig. 1.

2. Experimental

2.1. Materials and water samples

Bromoacetonitrile (99% pure), bisphenol A (more than 99% pure) and 2,2′-biphenol (more than 99% pure) were obtained from Aldrich (Milwaukee, WI, USA). Potassium carbonate, potassium dihydrogenphosphate, sodium sulfate, sodium chloride, methylene chloride and acetone used in this experiment were purchased from Sigma (St. Louis, MO, USA). Water was purified in a Milli-Q system (Millipore, Milford, MA, USA) and used as blank. Tap water was sampled in Kongju University, raw water in Okreun water treatment utility in Kongju and stream water in Gum River in Kongju.

2.2. Extraction of bisphenol A from water

In a 250-ml separating funnel, 200 ml of the water sample were placed. About 8 g of KH_2PO_4, 40 g of NaCl and 50 μl of 2,2′-biphenol solution (100 μl/ml in acetone) were added to the solution, and the sample was extracted with 10 ml of methylene chloride by mechanical shaking for 10 min. The organic phase was transferred into a 20-ml glass stoppered test tube and evaporated in vacuum rotary and dried finally with a nitrogen stream.

2.3. Derivatization

The dry residue was dissolved in 200 μl of dry acetone and then 20 μl of neat bromoacetonitrile were added. A 100-mg portion of K_2CO_3 was then added and the solution was heated for 60 min at 60°C in a heating block. A 2-μl sample of the solution was injected into the GC system.

2.4. Gas chromatography

All GC experiments were performed with a Hewlett-Packard (HP) 5890A gas chromatograph equipped with a nitrogen–phosphorus detector. A 30-m×0.2-mm I.D. fused-silica capillary column, coated with cross-linked 5% phenylmethylsilicone (0.33 μm film thickness), which was attached to the injection port. The carrier gas (helium) flow-rate was 1.2 ml/min, detector make-up gas (helium) flow-rate was 25 ml/min and the detector air and hydrogen flow-rates were 110 and 4 ml/min, respectively. The injection port temperature was 280°C and the detector temperature was set to 300°C. The oven temperature was programmed from 120 to 300°C at 20°C/min. A 2-μl aliquot of the final solution was injected in the split mode (split ratio 1:10). The split mode was used to prevent interference from a too large reagent peak.

2.5. Gas chromatography–mass spectrometry

All mass spectra were obtained with a Hewlett-Packard (HP) 5890/5971 B instrument. The ion source was operated in the electron ionization mode (EI; 70 eV, 150°C). Full-scan mass spectra (m/z 40–400) were recorded for analyte identification. An HP cross-linked 5% phenylmethylsilicone capillary column (SE-54, 25 m×0.2 mm I.D.), film thickness 0.33 μm was used. Samples were injected in the split mode with a split ratio of 1:10. The flow-rate of helium was 1.2 ml/min. The GC operating temperatures were: injector temperature, 280°C; transfer line temperature, 300°C; oven temperature, programmed from 100°C at 20°C/min to 300°C (held for 2 min).

2.6. Calibration and quantification

A calibration curve for bisphenol A was established by extraction and derivatization after adding 0.2, 0.5, 1.0, 2.5, 5.0, 10 and 20 μg of standards and 5.0 μg of internal standard (2,2′-biphenol) in 200 ml water. The ratio of the peak area of the standards to that of the internal standard was used in the quantification of the analyte.

3. Results and discussion

3.1. Derivatization

NPD is a detection method for the selective detection of nitrogen- and phosphorus-containing compounds. The nitrogen-specific response can be explained by the intermediate formation of cyano radicals. We reported the utility of iodoacetonitrile ($CNCH_2I$) and bromoacetonitrile ($CNCH_2Br$) as a means of improving detectability by GC–NPD of non-nitrogen- and non-phosphorus-containing compounds [26–28]. In the reaction, a cyano group was used as a chemical tag for the nitrogen-specific response, which is believed to play an important role in the detection mechanism of NPD.

Bisphenol A and biphenol were converted into their corresponding cyanomethyl ethers by treatment with bromoacetonitrile and potassium carbonate in acetone (Fig. 2).

The conventional methylation reaction of bisphenols with methylhalides also produces their corresponding methyl ethers. The hydroxy groups of bisphenol and biphenol act as nucleophiles in two reactions. Fig. 3 shows a typical chromatogram obtained from bisphenol A and biphenol standard after reaction with bromoacetonitrile.

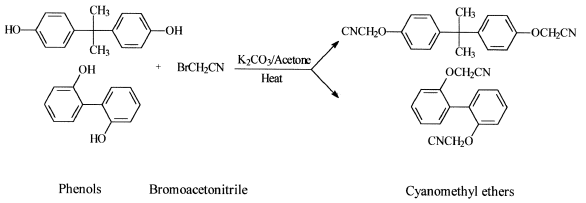

Fig. 2. Reaction of bisphenol A and 2,2′-biphenol with bromoacetonitrile.

The reaction rate of bisphenol A and biphenol with the derivatizing reagent was studied. This study was performed by varying the reaction temperature and observations of the formation rates of the derivatives. This sample was analyzed at reaction times of 5, 15, 30, 45, 60, 75 and 90 min (Figs. 4 and 5). Complete reaction takes place in about 60 min at 60°C. The derivative was stable in chromatographic system and minimum for 2 weeks in acetone at room temperature.

3.2. Mass spectrometry

The mass spectra of the cyanomethyl ethers of bisphenol A and 2,2′-biphenol are shown in Fig. 6. The molecular ion at m/z 306 and the diagnostic ions at m/z 211, 251 and 291 in spectrum A indicated that bisphenol A was converted to the corresponding bis(cyanomethyl) ether by reaction with bromoacetonitrile. The base peak m/z 291 is due to the loss of a CH_3 group from the molecular ion. The ions at m/z 251 and 211 were from the losses of one or two CH_2CN group, respectively, from the base peak m/z 291. The molecular ion at m/z 264 and the diagnostic ions at m/z 224, 197 and 184 of spectrum B indicate that 2,2′-biphenol was converted the corresponding bis(cyanomethyl) ether with bromoacetonitrile. The base peak m/z 197 is due to the loss of $[CH_2CN+CN+H]$ from the molecular ion. The ion at m/z 224 was from the loss of one CH_2CN group from the molecular ion and the ion at m/z 184

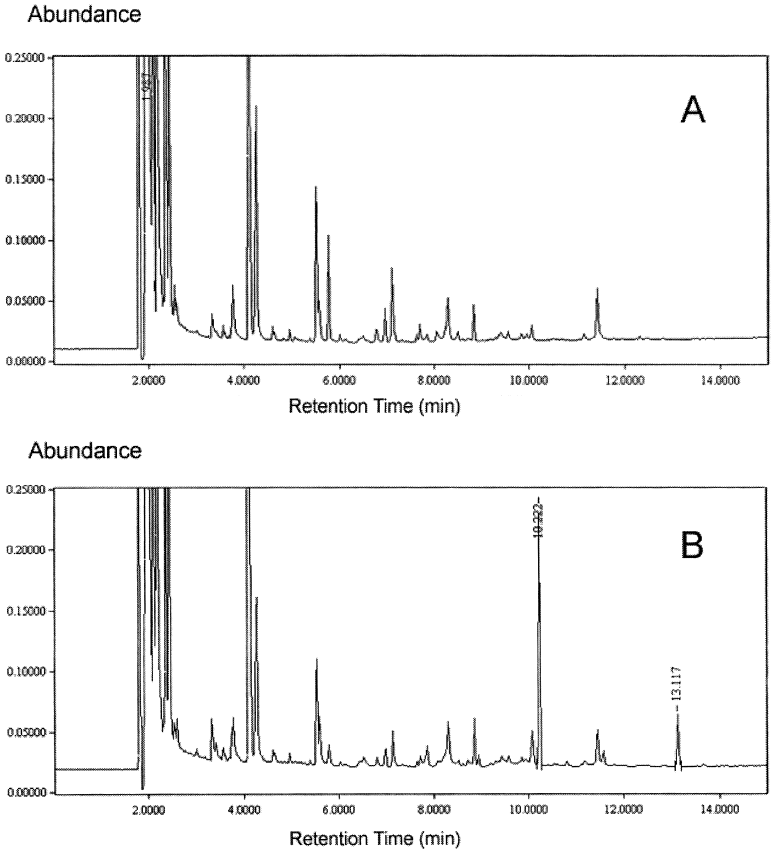

Fig. 3. (A) Reagent blank, (B0 GC chromatogram of bisphenol A (t_R=13.117 min) and 2,2′-biphenol (t_R=10.222 min) standard after reaction with bromoacetonitrile. The concentration of bisphenol A and 2,2′-biphenol is 10 ng/ml in water.

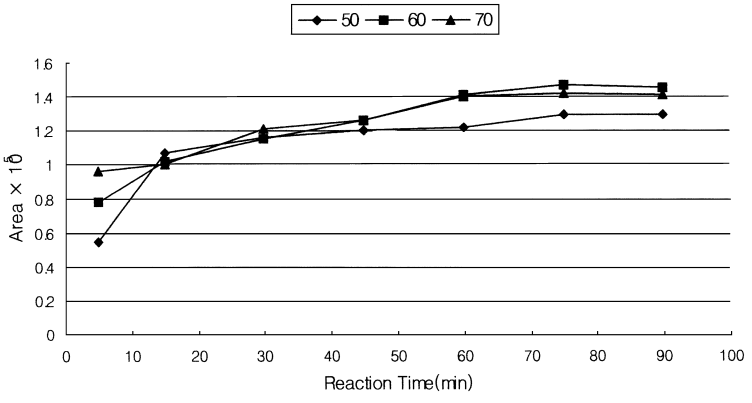

Fig. 4. Time course of the reaction of bisphenol A with bromoacetonitrile.

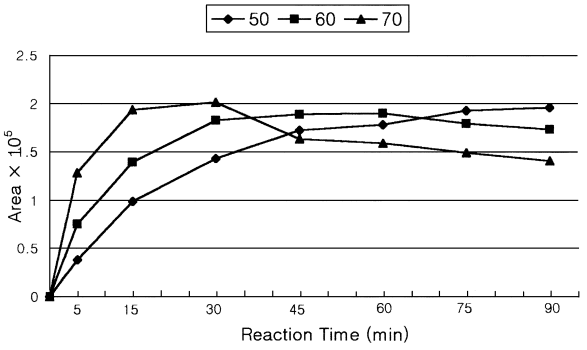

Fig. 5. Time course of the reaction of 2,2′-biphenol with bromoacetonitrile.

was from the loss of [CH] from the base peak *m*/*z* 197.

The general features of the spectra were essentially equivalent to those of methyl derivatives, but some additional features could be seen. Intense high-mass peaks were often seen at [M+-40] corresponding to the losses of CH_2CN from the base peak. These ions may have use for more sensitive strategies by GC–MS (selected ion monitoring mode).

3.3. Chromatography

For the GC separation of the derivative, the use of a non-polar stationary phase was found to be efficient. The column was stable over more than one thousand injections without a noticeable change of the separation characteristics. A chromatogram is shown in Fig. 3. As can be seen from the figure, the peaks of bisphenol A and internal standard are symmetrical and no tailing can be seen. The retention times of the cyanomethyl derivatives of bisphenol A and the internal standard were 13.117 and 10.222 min, respectively. Separation of the derivative and the internal standard from the background compounds in water was very good. There were no extraneous peaks observed in a chromatogram of blank water at the retention times of 13.117 and 10.222 min.

3.4. Linearity

Examination of a typical standard curve by computing a regression line of peak area ratios of bisphenol derivative to internal standard on concentration using a least-squares fit demonstrated a linear relationship with the correlation coefficients being consistently greater than 0.999. The line of best fit for bisphenol A is $y=0.0155x-0.0510$ ($r^2=0.9999$) over the range 0.1–100 ng/ml, where x is the analyte concentration (ng/ml) and y is the peak area ratio of the analyte to internal standard.

3.5. Recovery

Several water samples at the concentrations of 1, 5 and 10 ng/ml of bisphenol A were prepared and the recoveries were calculated from the percentage of derivative recovered from water samples. The mean recovery was about 89.3% at a concentration of 5 ng/ml of bisphenol A, and was found to be constant at other concentrations.

3.6. Precision and accuracy

The reproducibility of the assay was very good, as shown in Table 1. For five independent determinations at 1.0, 5.0 and 10.0 ng/ml, the RSD was less than 6%.

3.7. Sensitivity

The combination of low background, high extraction yield, high derivatization yield, and the high sensitivity of the derivative by the detector permits the determination of bisphenol A in water at concentrations well below those reported previously [14,18,19,24,25]. Detection limits were 0.1 ng/ml for bisphenol A based upon an assayed water volume of 200 ml. Limits were defined by a minimum signal-to-noise ratio of 3 and a RSD for replicate determinations ($n=5$) of 15% or less.

3.8. Application to environmental samples

Environmental water samples containing tap water, raw water and stream water were analyzed by the proposed method. No interfering peaks were observed near the peaks of bisphenol A and the internal standard. The data are given in Table 2. Bisphenol A was detected at a concentration of 0.6 ng/ml from tap water and 0.3 ng/ml from stream

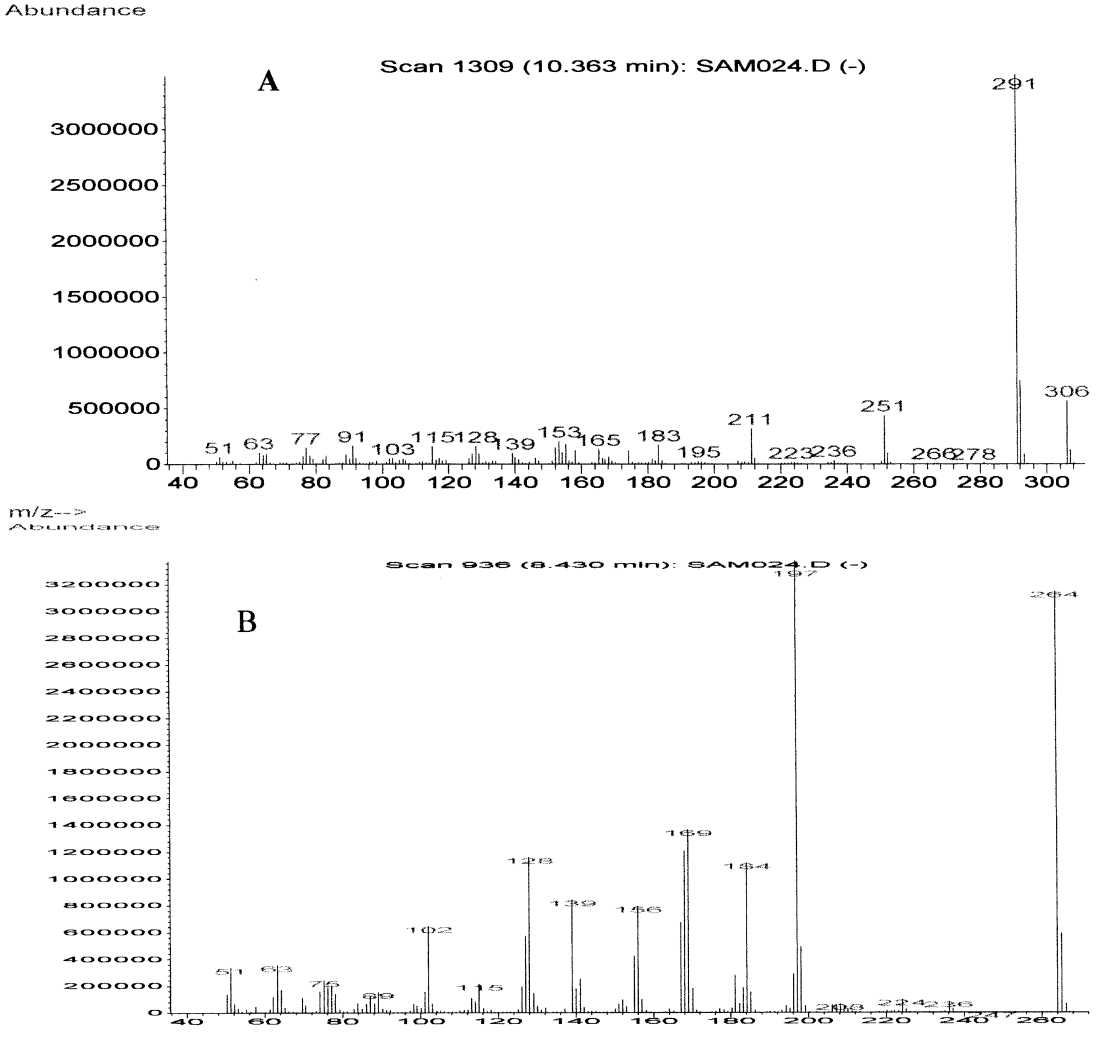

Fig. 6. The mass spectra of bisphenol A bis(cyanomethyl) ether (A) and 2,2′-biphenol bis(cyanomethyl) ether (B).

Table 1
Within-run precision and accuracy of bisphenol A in water sample (n=5)[a]

Added concentration (ng/ml)	Found (ng/ml)	
	Results	x±SD (RSD)
0.0	0.0, 0.0, 0.0	0.0±0.0 (0)
1.0	1.0, 0.9, 0.9, 1.0, 1.0	1.0±0.1 (5.1)
5.0	5.1, 5.0, 4.6, 5.2, 4.9	5.0±0.2 (4.2)
10.0	9.6, 9.7, 9.6, 9.9, 10.3	9.8±0.3 (2.7)

[a] x, mean value; SD, standard deviation; and RSD, relative standard deviation.

Table 2
Concentration (ng/ml) of bisphenol A in the analyzed samples (n=3)

Samples	Concentration (ng/ml)	
	Results	Mean±SD
Tap water	0.7, 0.6, 0.6	0.6±0.1
Raw water	0.0, 0.1, 0.0	0.0±0.1
Stream water	0.3, 0.2, 0.3	0.3±0.1

water. However bisphenol A was not detected in raw water. How bisphenol A was contaminated in tap water is questionable. Perhaps it may be from coagulant containers, liquid chlorine containers, or water treatment systems.

4. Conclusions

The cyanomethyl derivative of bisphenol A has good chromatographic properties and offers very sensitive derivative for the nitrogen element-specific detection. The single-step extraction of bisphenol A in water sample with methylene chloride also gave a very high recovery with small variation. Quantification of bisphenol A is excellent, with a linear calibration curve over the range of 0.1–100 ng/ml and a detection limit of 0.1 ng/ml. The present method may also be applicable to the analysis of bisphenol A in food.

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