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Liquid chromatographic determination of bisphenols based on intramolecular excimer-forming fluorescence derivatization

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Abstract

A highly selective and sensitive fluorometric method for the determination of bisphenols has been developed. This method is based on an intramolecular excimer-forming fluorescence derivatization with a pyrene reagent, 4-(1-pyrene)butyryl chloride (PBC), followed by reversed-phase liquid chromatography (LC). The bisphenols, containing two phenolic hydroxyl groups in a molecule, were converted to the corresponding dipyrene-labeled derivatives by reaction with PBC. The derivatives afforded intramolecular excimer fluorescence (440–520 nm) which can clearly be discriminated from normal fluorescence (360–420 nm) emitted from PBC and monopyrene-labeled derivatives of monophenols. The PBC derivatives of bisphenols could be separated by reversed-phase LC on an octyl column with isocratic elution. The detection limits (signal-to-noise ratio = 3) for bisphenols were 3.0–5.0 fmol, for a 20 μl injection. The method was successfully applied to the determination of bisphenol A in hot water in contact with commercially available baby bottle samples after solid-phase extraction.

Keywords: Excimer fluorescence; Liquid chromatography; Bisphenols; 4-(1-Pyrene)butyryl chloride; Baby bottle

1. Introduction

2,2′-Bis(4-hydroxyphenyl)propane (bisphenol A (BPA)) is a condensation product of phenol and acetone, and is a major raw material of many resins (polycarbonate, epoxy, unsaturated polyester, polysulphone and polyacrylate). BPA is also used as flame-retardants, stabilizers or antioxidants.

In 1993, BPA was reported to be released from polycarbonate containers during autoclaving and to have a weak estrogenic activity [1]. Recently, it was reported that the exposure of pregnant mice to even low levels BPA (2 or 20 ng/g body weight) resulted not only in a reduction of daily sperm production, epididymal weight and an enlargement of prostate of male offspring [2,3], but also in an advanced puberty such as increase of body weight at weaning and shortness of time of first oestrus in female offspring [4]. Thus, a highly sensitive, selective and simple determination method is necessary for the monitoring of BPA levels in biological and environmental samples.

There are many analytical methods including gas chromatography–mass spectrometry (MS) [5–8] and liquid chromatography (LC) coupled with ultraviolet [9–11], MS [12–14], fluorescence [11,15–20], electrochemical [13,21,22] or chemiluminescence [23] detection system for the assay of BPA and its related compounds in environmental water, plastic waste, plasma, and so on. Among them, LC methods employing MS, fluorescence and electrochemical

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detections are relatively sensitive and selective, so they have been most widely applied to the assay of biological samples. Although MS methods are sensitive and highly reliable, its apparatus and operating cost are too expensive for routine analyses. Though the electrochemical detection methods are selective and highly sensitive, they tend to lack reproducibility mainly due to hysteretic degradation of the electrode. On the other hand, bisphenols have native fluorescence themselves but the fluorescence intensity is not so strong. Thus, fluorescence derivatization procedure to convert weakly fluorescent bisphenols into the strong fluorescent derivatives is needed to highly sensitive analysis [17–20]. However, the major defect of these methods in the highly sensitive assay is that monophenolic compounds are also fluorescence-derivatized to afford the fluorescent derivatives having the same fluorescence spectra as those of the bisphenols derivatives, which cause interfering peaks in the chromatogram. Therefore, they require highly sophisticated LC separation conditions and/or sample clean-up procedures are inevitable to eliminate the interferences from contaminated monophenolic compounds.

In our previous research, we developed a highly selective and sensitive determination method for polyamines [24] and basic amino acids [25] in LC based on intramolecular excimer-forming fluorescence derivatization. In these methods, all the primary and secondary amino moieties in polyamines and amino acids were labeled with a pyrene reagent, 4-(1-pyrene)butyric acid-*N*-hydroxysuccinimide ester (PSE), and the polypyrene-labeled derivatives afforded an intramolecular excimer to fluoresce in the wavelength region (440–520 nm) longer than that for usual pyrene derivatives (360–420 nm). By using these characteristics, polyamines and basic amino acids were determined selectively, even though the sample was contaminated with monoamino compounds. Recently, intramolecular excimer-forming fluorescence derivatization method was successfully applied to the determination of urinary histamine [26] and triethylenetetramine, which is a therapeutic drug for Wilson's disease and have polyamino structure in the molecule, in human and rabbit sera [27].

In this study, we have reported the determination method for bisphenols by using the above-mentioned intramolecular excimer-forming fluorescence derivatization. Bisphenols including BPA were converted to the corresponding dipyrene-labeled derivatives by reaction with 4-(1-pyrene)butyryl chloride (PBC), and the derivatives generated excimer fluorescence from the intramolecular dipyrene sites as well as monomer fluorescence (Fig. 1). The present method allowed highly selective and sensitive

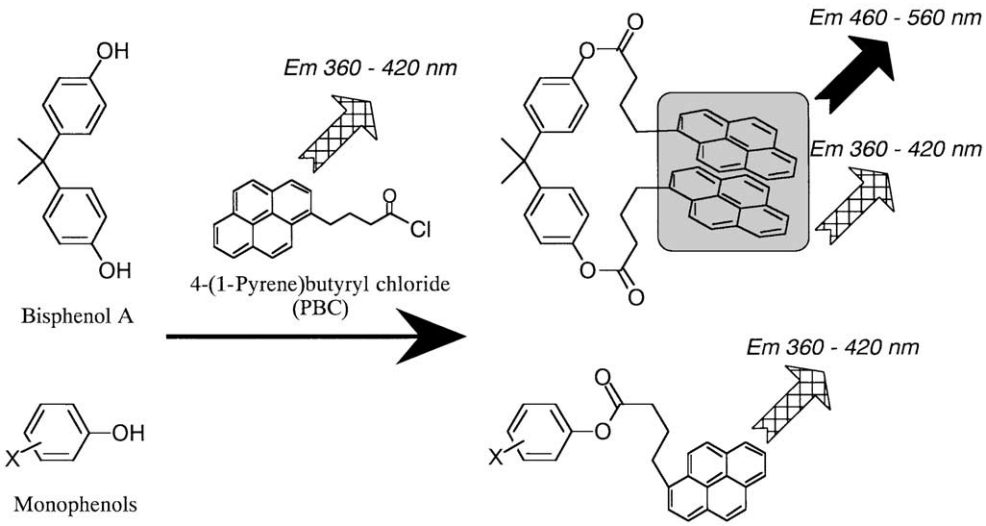


Fig. 1. Principle of intramolecular excimer-forming fluorescence derivatization of bisphenol A.

determination of bisphenols. The structures of dipyrene-labeled derivatives and the generation of intramolecular excimer fluorescence were confirmed by LC–MS and LC with three-dimensional (3D) fluorescence detection system, respectively. Furthermore, the method was successfully applied to the determination of BPA in hot water in contact with commercially available baby bottle samples made of polycarbonate.

2. Experimental

2.1. Reagents, solutions and materials

All chemicals and solvents were of the highest purity available and used as received. Distilled water, purified with a Milli-QII system (Millipore, Milford, MA, USA), was used to all aqueous solutions as the BPA-free water. An LC-grade acetonitrile obtained from Kanto Chemicals (Tokyo, Japan) was used to prepare the stock solutions and mobile phases. BPA, bisphenol B and all monophenols were purchased from Tokyo Kasei Kogyo (Tokyo, Japan), and bisphenols E, F, AP and P from Aldrich (Milwaukee, WI, USA). PBC was obtained from Toronto Research Chemicals (North York, Ont., Canada) and used without further purification. PBC and the organic solvents are toxic if exposed to lungs or skin and, therefore, should be carefully handled in accordance with the most current material safety data sheets.

Stock solutions (1.0 mM) of bisphenols (A, B, E, F, AP and P; Fig. 2) and monophenols (4-*n*-octyloxyphenol and 4-*n*-nonylphenol (NP)) were prepared in acetonitrile and stored at 4 °C. These phenol solutions were stable for at least 1 month and diluted further with acetonitrile to the required concentrations before use. The 5.0 mM solution of PBC dissolved in acetonitrile was usable for at least 3 days when stored at −20 °C. Both 1.0 M potassium carbonate and 1.0 M hydrochloric acid were prepared in water and stored at room temperature.

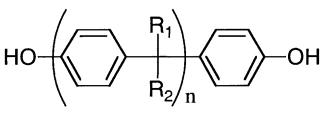
Oasis HLB (60 mg packing material) obtained from Waters (Milford, MA, USA) was used for solid-phase extraction. According to the previous methods [13,22], the cartridge was washed with 5 ml of ethanol and 10 ml of BPA-free water, successively. In every analysis, blank test was done to make sure there is no contamination with BPA, according to the following procedures.

2.2. Derivatization procedure

To a 200 μl aliquot of a standard solution placed in a 3.0 ml Reacti-vial (Pierce, Rockford, IL, USA), were added 200 μl of 5 mM PBC solution and 10 μl of 1.0 M potassium carbonate. The vial was tightly sealed and heated at 100 °C for 30 min in a block heater, Pierce Reacti-Therm (Model 18970). After cooling in ice-water, a 20 μl portion of the reaction mixture was injected into the chromatograph. To prepare the reagent blank, a 200 μl volume of acetonitrile in place of the standard solution was subjected to the same procedure.

2.3. Determination of migrated BPA

The 100 ml portions of boiling BPA-free water were transferred into a commercially available baby bottle. It was tightly capped and kept in an oven at 95 °C for 30 min [23]. After cooling to room temperature, the whole extract was transferred to a glass vessel, and 1.0 ml of 1.0 M hydrochloric acid for pH adjustment (ca. pH 3) and 50 μl of 1.0 nmol/ml bisphenol F (BPF) for an internal standard (IS) were added.



	n	R_1	R_2
Bisphenol A	1	CH_3	CH_3
Bisphenol B	1	CH_3	C_2H_5
Bisphenol E	1	H	CH_3
Bisphenol F	1	H	H
Bisphenol AP	1	CH_3	C_6H_5
Bisphenol P	2	CH_3	CH_3

Fig. 2. Bisphenols examined.

The solutions were applied to the Oasis HLB cartridge at a flow-rate of approximately 1 ml/min, and subsequently washed with 2 ml of water. After drying for 10 min under vacuum, the compounds in the cartridge were eluted by passing 0.5 ml of methanol four times at a flow-rate of approximately 1 ml/min. All the eluates were combined and evaporated to dryness under a nitrogen stream. To the residue, 200 μl of 2.5 mM PBC solution and 5 μl of 1.0 M potassium carbonate were added and vortex-mixed for ca. 2 min to redissolve. The following derivatization procedure was treated according to the previous Section 2.2.

2.4. LC detection system and its conditions

An isocratic LC system consisted of a Jasco (Tokyo, Japan) PU-1580 chromatograph pump, a Rheodyne (Cotati, CA, USA) Model 7725i syringe-loading sample injector equipped with a 20 μl sample loop, a Jasco DG-1580-53 on-line degasser, a reversed-phase TSKgel SuperOctyl column (100 mm × 4.6 mm i.d., particle size 2 μm; Tosoh, Tokyo, Japan), and a Hitachi (Tokyo, Japan) L-7485 spectrofluorometer fitted with a 12 μl flow-cell. An aqueous 75% (v/v) acetonitrile was used as a mobile phase. The flow-rate of the mobile phase was set at 1.0 ml/min, and the column temperature was ambient (24 ± 4 °C). The fluorescence detector was operated at the excitation and emission wavelengths of 345 and 475 nm, respectively, and the slit-widths of both the monochromators were set at 15 nm. For comparative studies, monomer fluorescence was monitored at the excitation and emission wavelengths of 345 and 375 nm, respectively.

2.5. Characteristics of PBC derivatives

The structures of the LC peak components and the generation of intramolecular excimer fluorescence were confirmed by LC–MS and LC with three-dimensional fluorescence detection system, respectively.

To perform the LC–MS analysis, a Finnigan (San Jose, CA, USA) LCQ, ion-trap mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) interface, was used. A mixture of 0.1 M triethylamine (TEA)–0.1 M acetic acid–acetonitrile (1:1:6 (v/v/v)) was used as mobile phase instead of aqueous 75% (v/v) acetonitrile. Other separation conditions were the same as described in the previous Section 2.4. The effluent from the LC column was directly introduced to the LC–MS interface without splitting. The ion source voltage, temperatures of APCI vaporizer and the heated capillary were set at 6 kV, 450 and 200 °C, respectively. Nitrogen gas was used for sheath gas (80 psi).

An Agilent (Palo Alto, CA, USA) 1100 series LC system which consisted of a binary-pump, an on-line degasser, an auto-sampler, a column oven and a programmable 3D spectrofluorometer fitted with an 8 μl flow-cell was used to observe the generation of excimer fluorescence. Other separation conditions were stated previously (Section 2.4). The fluorescence detector operated with the excitation wavelength at 345 nm, and the on-line fluorescence emission spectra were monitored at 375–600 nm. The slit-width of excitation monochromator was set at 20 nm.

3. Results and discussion

3.1. LC separation conditions

An isocratic reversed-phase LC was investigated for the continual separation of the pyrene-labeled bisphenols and labeling reagent. When an ODS (C_{18}) column with aqueous (20–90% (v/v)) organic solvents (acetonitrile and methanol), which was the most commonly usable separation mode, was used, it took too long time to elute the PBC derivatives from the column. Whereas, the separation of the derivatives could not be achieved when phenyl, butyl (C_4) or TMS (C_1) columns were used. The octyl (C_8) column resulted in a satisfactory separation by the isocratic elution using an aqueous acetonitrile (75% (v/v)) as a mobile phase. The solvent composition was most suitable to the generation of intramolecular excimer fluorescence as in the case with polypyrene derivatives of polyamines [24].

Typical chromatograms obtained with standard mixtures of the bisphenols (A, B, E, F, AP and P) and monophenols (4-*n*-octyloxyphenol and NP) are shown in Figs. 3 and 4, respectively. Bisphenols gave the respective single peaks, and they were separated well from each other and from the intermolecular excimer fluorescence peaks of PBC and its hydrolyzate,

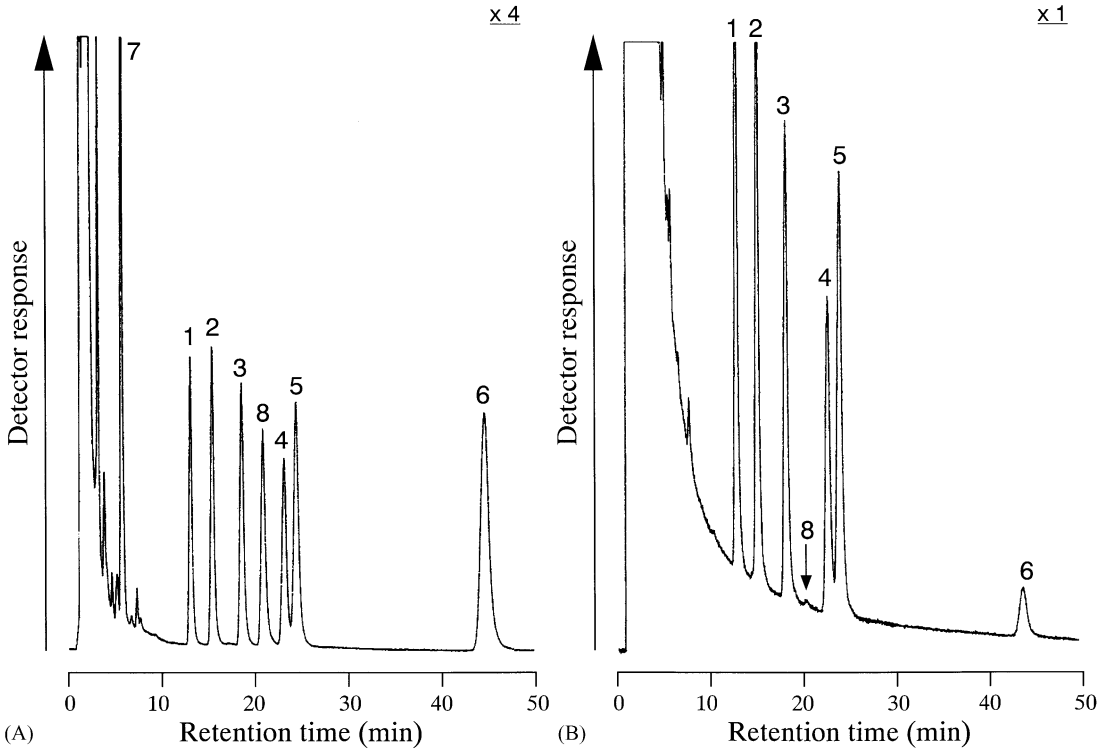


Fig. 3. Chromatograms obtained with the pyrene-labeled bisphenols detected in: (A) excimer fluorescence region (Em, 475 nm) and (B) monomer fluorescence region (Em, 375 nm) with the excitation wavelength at 345 nm. Amounts: 9.8 pmol each on column. Peaks: (1) bisphenol F; (2) bisphenol E; (3) bisphenol A; (4) bisphenol B; (5) bisphenol AP; (6) bisphenol P; (7) PBC; (8) unknown; others, reagent blank components.

4-(1-pyrene)butyric acid (Fig. 3A). When monitored at 375 nm (the monomer fluorescence of pyrene skelton), large and broad peaks for blank components partially overlapped with those for bisphenols (Fig. 3B) and interfered with the sensitive assay of bisphenols. Although the peak heights obtained with the excimer fluorescence detection are smaller than those with the monomer fluorescence detection, the noise level in the former is quite smaller than that in the later. So, the excimer fluorescence detection has high sensitivities (low detection limits) as described in the following Sections 3.4–3.6. On the other hand, monophenols could afford the respective monomer fluorescence peaks (Fig. 4B), but they gave none or very small peak in the excimer fluorescence detection (Fig. 4A). Thus, this method permits highly selective determination of bisphenols in the samples containing monophenols and other compounds.

3.2. Characteristics of PBC derivatives

The structures of pyrene-labeled BPA and BPF were analyzed by LC–MS with APCI interface (date not shown). A standard solution of BPA and BPF was treated as in the derivatization procedure (Section 2.2), and the resulting derivatives were applied to LC–MS analysis, in which volatile salt (a mixture of acetic acid and TEA) was added to the mobile phase. The addition of the salt did not affect the retention times of the derivatives in the chromatogram, but it increased the detection sensitivity of LC–MS analysis. The selected ion chromatograms ($[M + H]$) suggested that dipyrene-labeled derivatives were formed from BPA and BPF. Mass spectra for the respective peaks also provided the corresponding quasi-molecular ions ($[M + TEA + H]^+$) as base peaks. When detected at m/z ($[M + TEA + H]^+$ and $[M + H]^+$) corresponding

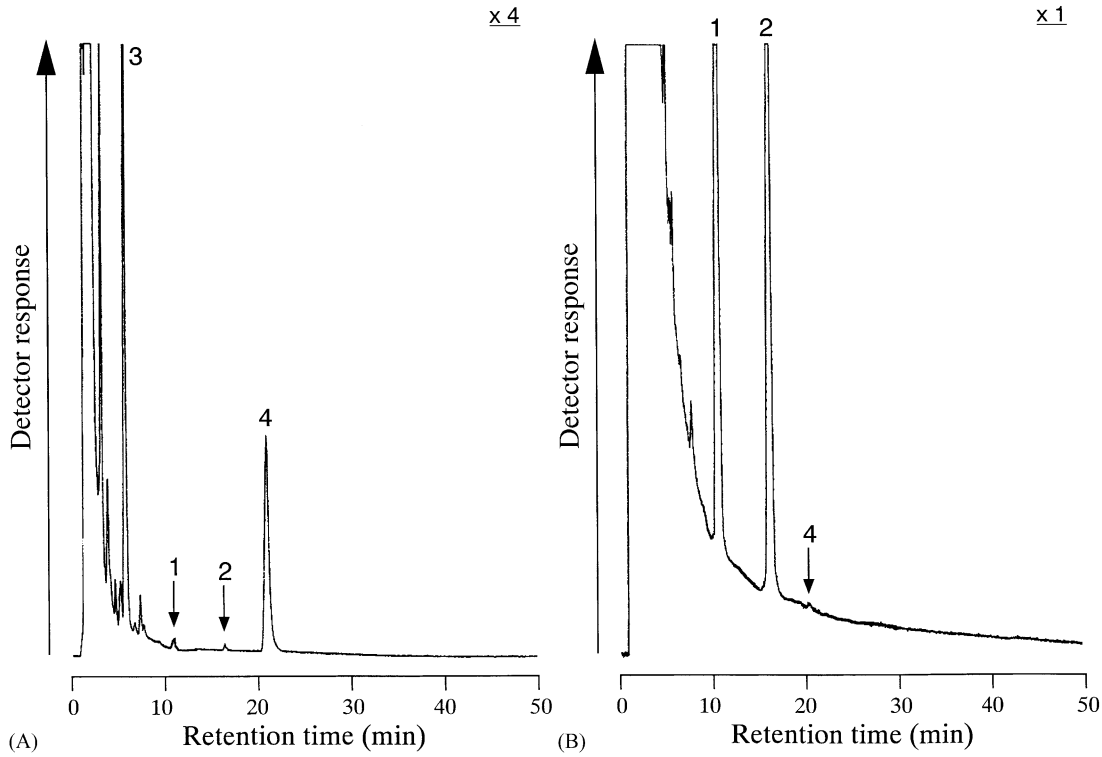


Fig. 4. Chromatograms obtained with the pyrene-labeled monophenols detected in: (A) excimer fluorescence region and (B) monomer fluorescence region. Amounts: 9.8 pmol each on column. Peaks: (1) 4-*n*-octyloxyphenol; (2) 4-*n*-nonylphenol; (3) PBC; (4) unknown; others, reagent blank components.

to the monopyrene-labeled derivatives of BPA and BPF, no significant peak was observed in the respective ion chromatograms. From these observations, we conclude that both the phenolic hydroxyl groups of bisphenols are labeled with PBC under the present derivatization conditions (Fig. 1).

Fig. 5 illustrates the three-dimensional fluorescence emission chromatogram of BPA and NP with the excitation wavelength at 345 nm. Dipyrene-labeled BPA resulted in fluorescence emission in both excimer (440–520 nm) and monomer (370–420 nm) fluorescence regions. On the other hand, the monopyrene-labeled derivative of NP and other early-eluting components emitted only monomer fluorescence; exceptionally highly concentrated PBC and 4-(1-pyrene) butyric acid exhibited excimer fluorescence because of the formation of intermolecular excimer and/or the tailing of the monomer fluorescence spectrum. Moreover, background fluorescence levels in monomer region were relatively high mainly due to the stray light from light source. From these observations, intramolecular excimer fluorescence derivatization method should permit high sensitivity and selectivity.

3.3. Optimum derivatization conditions

Optimization studies of derivatization were carried out to maximize the excimer fluorescence peak area for bisphenols. Some derivatizing reagents containing pyrene structure for phenolic hydroxyl groups are commercially available: PBC, PSE, 1-pyrenesulfonyl chloride and 1-pyrenecarbonyl fluoride. PBC afforded intense excimer fluorescence. Optimum derivatization conditions were examined concerning PBC concentration (0.1–7.5 mM), water–miscible organic solvents (acetone, acetonitrile, dimethylsulfoxide, *N*,*N*-dimethylformamide, methanol and tetrahydrofuran), potassium carbonate (0.01–2.0 M), reaction temperature

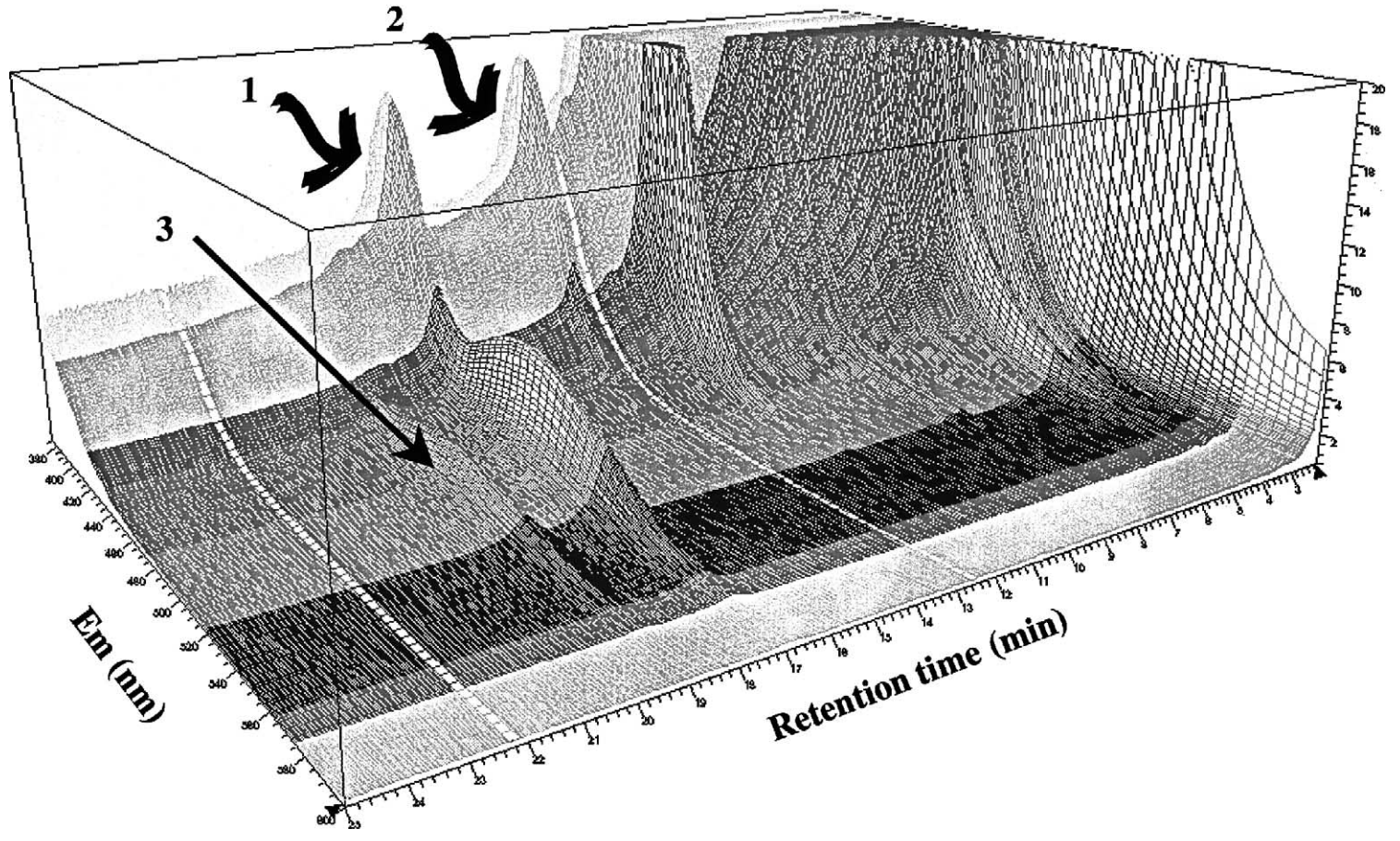


Fig. 5. Three-dimensional fluorescence emission chromatogram obtained with the pyrene-labeled bisphenol A and 4-*n*-nonylphenol with the excitation wavelength at 345 nm. Amounts: 200 pmol each on column. Peaks: (1) bisphenol A; (2) 4-*n*-nonylphenol; (3) unknown.

(25–120 °C) and time (1–120 min); the conditions described in Section 2.2 were selected. The content of water must be kept <5% in sample solution because the contamination of water to the reaction mixture inhibited the derivatization reaction between acid chloride and phenolic hydroxyl group.

The pyrene-labeled bisphenols in the final reaction mixture were stable, and still gave the constant fluorescence intensities after standing for at least 8 h in daylight at room temperature and for 3 days in the dark at 4 °C.

3.4. Calibration graph, precision and detection limits

The relationships between the amounts of individual bisphenols and the peak heights were linear over the concentration range of ca. 0.01–10 pmol per 20 μl injection volume, which corresponded to 1.0–1000 pmol/ml in a sample solution. The linear correlation coefficients were more than 0.999 ($n = 3$) for bisphenols A, B, E, F, AP and P. The between-day precisions were established by repeated determinations ($n = 5$) of the standard mixtures of bisphenols (1.0 and 100 pmol/ml each in a sample solution, ca. 10 and 1000 fmol each per 20 μl injection volume); the relative standard deviations were within 5.8 and 4.3%, respectively.

The detection limits (signal-to-noise ratio = 3, in fmol on per 20 μl injection volume) for bisphenols A, B, E, F, AP and P were 3.5, 5.0, 3.0, 3.2, 3.8 and 3.9, respectively, which corresponded to about 0.31–0.51 pmol/ml in a sample solution. The quantification limits (signal-to-noise ratio = 10, in fmol per 20 μl injection volume) were 12, 17, 10, 11, 13 and 13, respectively. The sensitivity of this intramolecular excimer-forming derivatization method is comparable to those of the most sensitive LC methods with electrochemical [13,21,22] or fluorometric [17–20] detection.

3.5. Specificity

In the preliminary study by thin layer chromatography on a silica gel plate (5553 aluminium sheets; Merck, Darmstadt, Germany) and reversed-phase LC with other separation conditions, some polyamino compounds (arginine, lysine, ornithine, glycyl-L-lysine, histamine, putrescine, cadaverine, spermidine, spermine, triethylenetetramine, 1,3-phenylenediamine and 2,3-diaminonaphthalene) and other polyphenolic compounds (catechol, 4,4′-biphenol and catechin) also reacted with PBC to afford the corresponding polypyrene-labeled products generating the intramolecular excimer fluorescence. However, no peak was observed within 60 min under the present LC conditions because most of them were eluted earlier from the column together with the reagent blanks and others were not eluted.

The following biological and environmental compounds having only one phenolic hydroxyl group or none in the molecule, at a concentration of 1.0 nmol/ml, did not afford any peak under the present conditions; the compounds tested were acidic and neutral amino acids, acids (acetic acid, palmitic acid, oxalic acid, 5-hydroxyindole-3-acetic acid, L-ascorbic acid, α-ketoglutaric acid and phenylpyruvic acid), sugars (D-glucose, D-fructose, D-ribose, *N*-acetyl-D-glucosamine, maltose and sucrose), nucleic acid bases (adenine, guanine, thymine, cytosine and uracil), other biological compounds (ammonia, acetylcholine, cholesterol, creatine, creatinine and urea) and environmental compounds (methanol, acetone, aniline, phenol, 4-chlorophenol, formaldehyde and acetaldehyde). Though some monophenols, i.e. 4-*n*-octyloxyphenol and NP, gave small peaks in excimer fluorescence region (Fig. 4A), these peaks disappeared by decreasing the concentrations and did not describe the calibration curves, because these peaks were ascribable to the intermolecular excimer fluorescence.

These observations suggest that the present derivatization method including LC separation conditions is usefully selective for bisphenols.

3.6. Determination of BPA migrated from baby bottles

In order to investigate the practicality of the intramolecular excimer-forming derivatization method in real sample analyses, the present method was applied to the determination of BPA in hot water in contact with commercially available baby bottle samples. A solid-phase extraction method using an Oasis HLB cartridge was tentatively employed for the concentration of BPA in water sample from baby bottle [13,21,22].

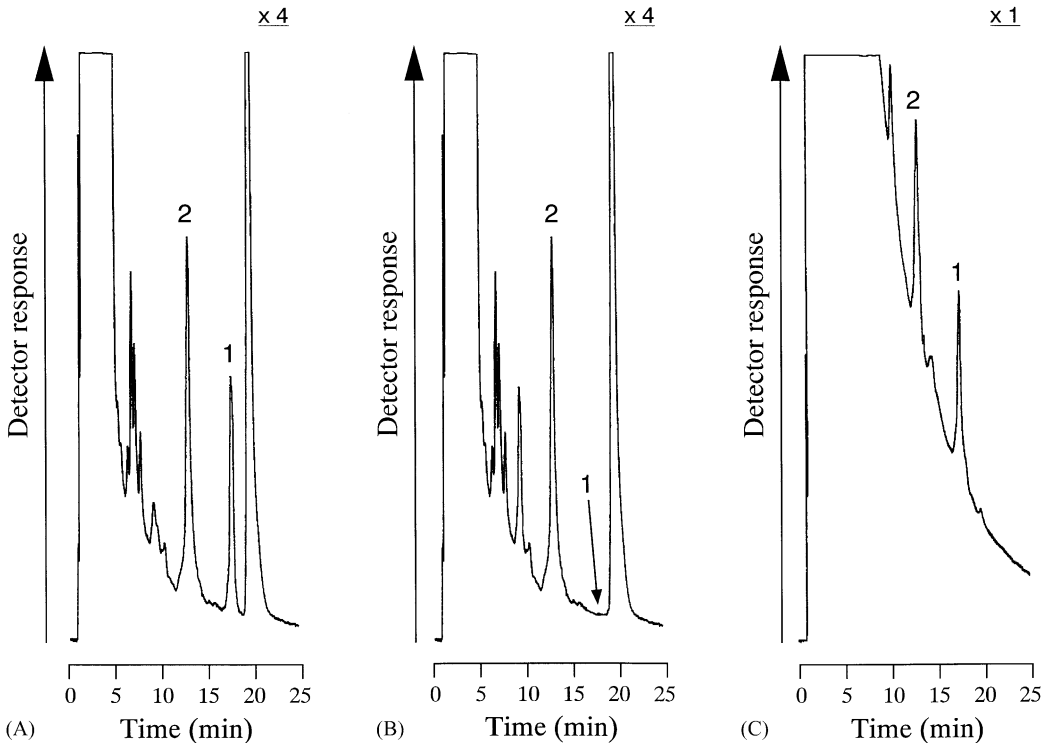


Fig. 6. Chromatograms obtained with the water contacted to the baby bottles detected in: (A) and (B) excimer fluorescence region and (C) monomer fluorescence region. Baby bottle samples: (A) and (C) sample 2, polycarbonate, extract with first migration (BPA, 89 ppt); (B) sample 5, glass, extract with first migration. Peaks: (1) bisphenol A; (2) bisphenol F (IS).

Fig. 6 shows typical chromatograms obtained with the bottle samples according to the procedure. The peak components of peaks 1 and 2 in Fig. 6 were identified as the PBC derivatives of BPA and BPF (IS), respectively, on the basis of their retention times by a comparison with those in Fig. 3 and also by co-chromatography using 65–85% (v/v) acetonitrile containing 0–2.0% (v/v) acetic acid as an eluent. Baseline separations among the peaks for BPA and the early-eluting blank peaks were attained in the excimer fluorescence detection (Fig. 6A). However, in monomer fluorescence detection, the BPA peak overlapped with the tailing of the blank peaks, and could not be used for the sensitive quantification (Fig. 6C). Thus, intramolecular excimer-forming derivatization method permitted quite selective and sensitive analyses of the real samples.

The recovery tests of BPA were performed by the addition of 100 μl of standard BPA solutions (0.01, 0.10 and 1.0 nmol/ml) to 100 ml of BPA-free water. As shown in Table 1, the recoveries were >85% in all concentrations. The main losses may be due to oxidative decomposition of BPA, adhesions to glassware and SPE cartridge. These results are acceptable, since these recoveries are superior or at least comparable to those of the previous reported methods [13,21,22]. The limit of quantification of BPA in water sample was 0.24 pmol/100 ml (0.54 ppt), which corresponds to ca. 20 fmol on column per 20 μl injection volume.

Table 1
Recovery tests of bisphenol A in examination water

Bisphenol A (pmol/100 ml)	Recovery (%) ($n = 6$) [R.S.D.] (%)
1	88.3 [3.6]
10	87.9 [3.5]
100	85.8 [3.3]

Table 2
Determination of migrated BPA from baby bottle samples

Sample	Raw material	Migration sequence	Assay of BPA (ppt)
1	Polycarbonate	First	190
		Second	52
		Third	18
		Fourth[a]	4
2	Polycarbonate	First	89
		Second	30
		Third	10
		Fourth[a]	3
3	Polycarbonate	First	60
		Second	15
		Third	4
		Fourth[a]	1
4	Polycarbonate	First	8
		Second	3
		Third	1
		Fourth[a]	Trace[b]
5	Glass	First	N.D.[c]
		Second	N.D.[c]
		Third	N.D.[c]
		Fourth[a]	N.D.[c]
6	Glass	First	N.D.[c]
		Second	N.D.[c]
		Third	N.D.[c]
		Fourth[a]	N.D.[c]

[a] Baby bottles were washed with a brush to facilitate the migration by creating scratches on the inner surface before the fourth migration test.
[b] Less than limit of quantification.
[c] N.D., not detected.

Commercial six baby bottles (four polycarbonate bottles and two glass ones) were subjected to the migration test. The migration test was successively repeated four times of each bottle as shown in Table 2. In the first migration test, BPA in polycarbonate bottles was found to migrate at 8–190 ppt, and the amount of migrated BPA decreased with increasing the repeated number of the test. Although several ppt levels of BPA were migrated from polycarbonate baby bottles, these values were within the criterion concentration of the Food Sanitation Law in Japan. After the third migration test, baby bottles were washed with a brush to facilitate the migration by creating scratches on the inner surface. But the brushing did not give any significant change in the migration of BPA. In the case of glass bottles, no BPA could be detected throughout all the tests (Fig. 6B and Table 2). These migration results are almost the same as other migration tests [23].

Recently, some easier procedures for the concentration of BPA have been reported [12,16,28]. The present derivatization method might be improved by using the concentration procedure, and should be used for routine use.

4. Conclusion

By an intramolecular excimer-forming derivatization, bisphenols were found to be converted to the respective dipyrene-labeled derivatives. They afforded intramolecular excimer fluorescence (440–520 nm), which can clearly be discriminated from normal fluorescence (370–420 nm) of PBC and other pyrene concomitants, with the irradiation of normal pyrene excitation wavelength (345 nm). This unique property as well as prolonged Stokes shift allowed highly sensitive and specific detection of bisphenols. In fact, this method had enough selectivity and sensitivity for the simple determination of migrated BPA from baby bottles. Furthermore, this intramolecular excimer-forming derivatization method might be applicable to not only the bisphenol monomers but also polyphenolic degradation compounds that are of increasing interest, nowadays.

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