Selective determination of tetrabromobisphenol A by liquid chromatography following intramolecular excimer-forming fluorescence derivatization with pyrene-labeling reagent

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Introduction

A large number of compounds have been used as flame-retardants to protect different products from catching fire, and one of the most widely used flame-retardants is tetrabromobisphenol A (TBBPA). Trace amounts of TBBPA have been determined by gas chromatography (GC) with electron-capture detection or GC-mass spectrometry (MS) as reviewed in [1]. Only a few liquid chromatographic (LC) methods have been reported[2, 3].

Recently, we developed highly selective and sensitive determination methods for polyamines[4] and dicarboxylic acids[5] based on intramolecular excimer-forming fluorescence derivatization using pyrene reagents. By the derivatization, the resulting polypyrene-labeled derivatives of polyamines and dicarboxylic acids provided intramolecular excimer fluorescence at the wavelength region of 440 – 520 nm, which was shifted markedly to the higher emission wavelengths as compared to the wavelengths of the pyrene reagent itself and monopyrene-labeled concomitants (360 – 420 nm). This chemistry allowed to selectively analyzing poly-functional compounds even in the complex samples containing mono-functional compounds. More recently, we have found that 4-(1-pyrene)butanoyl chloride (PBC) reacts with not only polyamines, but also phenol compounds such as bisphenols, and the obtained PBC derivatives form strong intramolecular excimers[6].

The aim of this work was to develop an intramolecular excimer-forming derivatization method for fluorimetric determination of halogenated-bisphenols including TBBPA following their derivatization with PBC (Fig. 1). The new

method allows a highly sensitive and selective determination of. tetrabromobisphenol A.

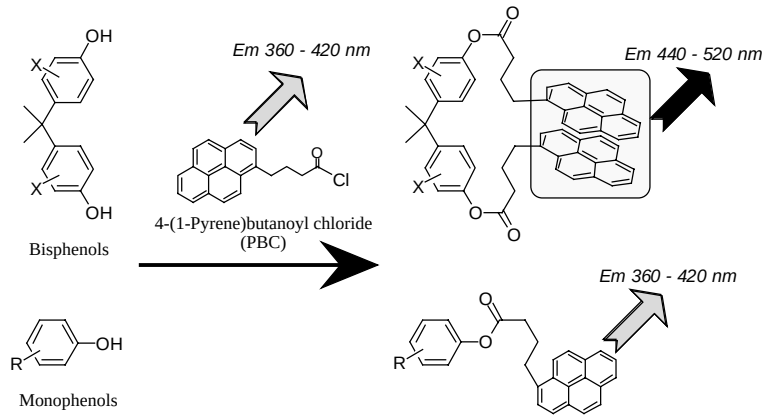

Fig. 1: Intramolecular excimer-forming fluorescence derivatization of bisphenols with 4-(1-pyrene)butanoyl chloride.

Methods and Materials

Reagents: All chemicals and solvents were of the highest purity available. PBC was obtained from Toronto Research Chemicals (North York, Ontario, Canada) and used without further purification. Stock solutions (1.0 mM) of bisphenols were prepared in acetonitrile and stored at -20 °C. These solutions were stable for at least 1 week and diluted further with acetonitrile to the required concentrations before use. The 5 mM solution of PBC dissolved in acetonitrile was usable for at least 3 days when stored at -20 ˚C.

Derivatization: To a 200-µL aliquot of standard solution placed in a 3.5-mL Reacti-vial, 10 µL of 2 M potassium carbonate and 200 µL of 5 mM PBC solution were added. The vial was sealed tightly and heated at 100 °C for 45 min. After cooling in ice-water, a 5-µL portion of the reaction mixture was injected into the chromatograph.

Apparatus and conditions: An isocratic LC system consisted of a Jasco (Tokyo, Japan) PU-1580 liquid chromatograph pump, a Rheodyne 7725i syringe-loading sample injector equipped with a 5-µL sample loop, a Jasco DG-1580-53 on-line

degasser, a reversed-phase L-column ODS-L (150 × 2.1 mm i.d.; particle size, 5 μm; Chemicals Evaluation and Research Institute, Tokyo, Japan), and a Waters (Milford, MA, USA) 2475 multi λ spectrofluorometer fitted with a 8-μL flow-cell. An aqueous 99 % (v/v) acetonitrile was used as a mobile phase. The flow-rate of the mobile phase was set at 0.2 mL/min, and the column temperature was ambient. The fluorescence detector was operated at excitation and emission wavelengths of 345 nm and 485 nm, respectively, and the slit-widths of both monochromators were set at 20 nm. For comparison, UV and native fluorescence detections were carried out without derivatization. Same apparatus and conditions were used for these examinations except mobile phase (an aqueous 50 % acetonitrile) and detection conditions (UV, 280 nm; native fluorescence, Ex/Em 275/305 nm).

Results and Discussion

LC separation: We used TBBPA, tetrachlorobisphenol A (TCBPA), hexafluorobisphenol A (HFBPA), and bisphenol A (BPA) as the model compounds of bisphenols. An isocratic reversed-phase LC was investigated for the continual separation of the pyrene-labeled bisphenols and labeling reagent. The best separation of the PBC derivatives of bisphenols and reagent blank components (intermolecular excimer fluorescence peaks of PBC and its hydrolysate, 4-(1-pyrene)butanoic acid) was achieved within 50 min on an ODS column using aqueous 99 % (v/v) acetonitrile as the mobile phase. A typical chromatogram obtained with a standard mixture of bisphenols is illustrated in Fig. 2A. 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 hydrolysate.

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, 4-(1-pyrene)butanoic acid *N*-hydroxysuccinimide ester, 1-pyrenesulfonyl chloride and 1-pyrenecarbonyl fluoride. PBC afforded intense excimer fluorescence. Optimum derivatization conditions were examined concerning PBC concentration (0.1 – 10 mM), water-miscible organic solvents (acetone, acetonitrile, dimethylsulfoxide, *N*,*N*-dimethylformamide, methanol, and tetrahydrofuran), potassium carbonate (0.01 – 4.0 M), reaction temperature (4 – 120 °C) and time (1 – 120 min); the conditions described in the Methods and

Materials section were selected. The content of water must be kept less than 5 % in sample solution because the addition of water to the reaction mixture inhibited the derivatization reaction between acid chloride and phenolic hydroxyl group.

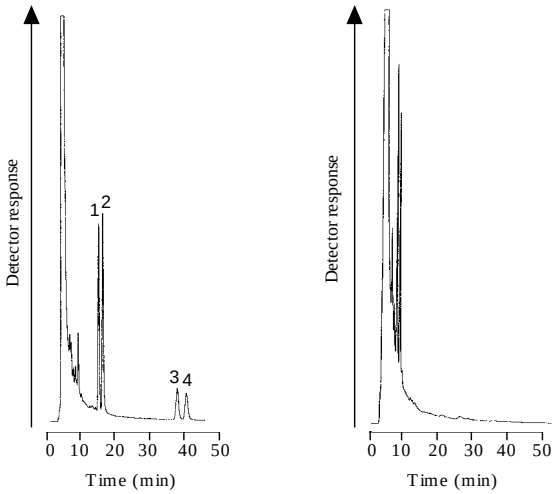

Fig. 2: Chromatograms obtained with (A) the pyrene-labeled bisphenols (2.4 pmol each on column) and (B) the reagent blanks.
Peaks: 1, BPA; 2, HFBPA; 3, TCBPA; 4, TBBPA; others, reagent blanks.

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 the daylight at room temperature and for 3 days in the dark at 4 °C.

Calibration graph, precision and detection limits: The relationship between the amounts of individual bisphenols and the peak heights were linear over the concentration range of 10 nM – 50 μM in the standard solution (24 fmol – 122 pmol per 5 μL injection volume); the linear correlation coefficients were more than 0.999 (n = 6). The between-day precision values were established by repeated determinations (n = 6) using the mixtures of standard compounds (1.0 μM each in a sample solution, 2.4 pmol each per 5 μL injection volume); the relative standard deviations were within 3.8 %.

Table 1 lists the detection limits of bisphenols using various detection methods, UV detection and native fluorescence detection (without derivatization) and excimer fluorescence derivatization method (the present method). The UV

detection method could be detected all bisphenols examined but its sensitivity was poor for trace analysis. Though native fluorescence detection was relatively highly sensitive for BPA and HFBPA, it did not work on TBBPA and TCBPA. By the excimer fluorescence derivatization, not only BPA and HFBPA, but also TBBPA and TCBPA could be detected highly sensitively.

Specificity: In the preliminary study by thin layer chromatography and reversed-phase LC with other separation conditions, some polyamino compounds, other polyphenolic compounds (catechol, 4,4'-biphenol and catechin), and aminophenols also reacted with PBC to afford the corresponding polypyrene-labeled products generating the intramolecular excimer fluorescence[4–7]. 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.

Table 1: Detection limits[a)] (fmol) of bisphenols using various detection methods in LC analysis.

Compound	Without derivatization		Excimer fluorescence derivatization method[d)]
	UV[b)]	Native fluorescence[c)]	
TBBPA	2300	>1000000	6.3
TCBPA	2000	>1000000	4.7
HFBPA	2800	230	2.7
BPA	1300	180	2.9

a) Defined as the amount in the injection volume (5 µL) giving a signal-to-noise ratio of 3.
b) UV detection: 280 nm.
c) Fluorescence detection: Ex 275 nm, Em 305 nm.
d) Fluorescence detection: Ex 345 nm, Em 485 nm.

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

Acknowledgements

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