CHROMATOGRAPHIC ISOLATION AND ENANTIOSELECTIVE SEPARATION OF ATROPISOMERIC POLYBROMINATED BIPHENYLS (PBBs)

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Introduction

Technical mixtures of polybrominated biphenyls (PBB) have been extensively used as flame-retardants in textile and electronic industries and as additives in plastics[1]. Despite a continuous reduction of the worldwide annual production in the last decades, the presence of PBBs in the environment was recently confirmed in a wide range of samples[2-4].

Likewise polychlorinated biphenyls (PCB), PBBs exist in a theoretical variety of 209 congeners[1]. Under environmental conditions, many PBB congeners containing two to four *ortho*-bromine substituents cannot fully rotate about the interannular phenyl-phenyl-bond due to steric hindrance. Depending on their substitution pattern, such rotation hindered PBB congeners can be axially chiral forming two atropisomers[5]. 19 axially chiral PCBs proved to be stable (high rotation barrier) at physiological temperatures[6,7]. However, due to the more bulky bromine substituent it can be assumed that more than 19 environmentally stable atropisomeric PBBs exist.

The purpose of this work was to evaluate different chromatographic tools for the enantioselective separation of atropisomeric PBB congeners. Since such PBB congeners are not commercially available, the components of a technical product (Firemaster BP-6®) had to be identified and relevant PBBs isolated prior to enantioselective separation.

Materials and Methods

Chemicals. Technical hexabromobiphenyl (Firemaster BP-6®, Michigan Chemicals) was obtained from LGC Promochem (Borås, Sweden). 2,2',4,5,5'-pentabromobiphenyl (PBB 101) and 2,2',4,4',5,5'-hexabromobiphenyl (PBB 153) were from Dr. Ehrenstorfer (Augsburg, Germany).

High performance liquid chromatography (HPLC). The HPLC system consisted of a GT-104 degasser, an LC-10AT VP gradient pump (both Shimadzu, Jena, Germany), a 6-port injection valve equipped with a 20 µL loop (Rheodyne, Rohnert Park, CA, USA), a CTO-10AS VP column oven, an SPD-6A UV detector (both Shimadzu) and a HP 3395 integrator (Agilent, Waldbronn, Germany). Separation of Firemaster BP-6® was performed at room temperature using three C_{18} columns coupled in series (2 × Prodigy ODS(3), 1 × Luna C_{18}(2), all 250 mm × 4.6 mm i.d., 5 µm particles, Phenomenex, Torrance, CA, USA). An isocratic flow of 1.2 mL/min acetonitrile/water (94/6; v:v) was employed. 50 µg Firemaster BP-6® were injected per run and fractions of eluting PBB congeners were collected manually. The following HPLC columns were tested for

enantioselective separation of PBBs: A Nucleodex [α]-PM, a Nucleodex [β]-PM and a Nucleodex [γ]-PM (permethylated α-, β- and γ-cyclodextrin, respectively, on silica, all 200 mm × 4 mm i.d., Macherey-Nagel, Düren, Germany). Optimised conditions for the Nucleodex [β]-PM column were isocratic separation with 0.5 mL/min acetonitrile/water (60/40; v:v) at 5 °C.

Gas chromatography (GC). Firemaster BP-6® was analysed on a Mega II 8065 gas chromatograph (Fisons, Milan, Italy) coupled to an MD800 quadrupole mass spectrometer (Finnigan, San Jose, CA, USA) operated in the electron-capture negative ion mode (ECNI-MS). Samples (2 µL, solvent *n*-hexane) were injected on-column with an AS800 auto-injection system (Fisons) onto a DB5MS capillary column (15 m × 0.25 mm i.d., 0.25 µm film, J&W Scientific, Folsom, CA, USA). Separation was performed using helium carrier gas. A Hewlett-Packard 5890 series II gas chromatograph (Agilent) equipped with two capillary columns (CP-Sil 2 and 80 % CP-Sil 8/20 % octadecylmethyl polysiloxane, respectively, both 50 m × 0.25 mm i.d., 0.25 µm film, Chrompack, Middelburg, The Netherlands) and two ^{63}Ni-electron capture detectors (ECD) was used for analysis of Firemaster BP-6® and fractions from HPLC separations[8]. Enantioselective separation of PBBs was studied using GC/ECD and GC/MS systems previously described in detail[8]. A 20 m × 0.25 mm i.d. fused-silica capillary column coated with 0.15 µm 35% impure heptakis(6-*O*-*tert.*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (β-TBDM) in OV 1701 (BGB Analytik, Adliswil, Switzerland) was applied. Atropisomeric PBBs were eluted isothermally.

Results and Discussion

Characterisation of Firemaster BP-6® and isolation of potential atropisomeric PBBs

The composition of the technical hexabromobiphenyl mixture Firemaster BP-6® was first studied by GC/ECD, GC/ECNI-MS and reversed phase HPLC/UV on three serially coupled C_{18} columns (Figure 1). The following criteria were used for congener identification:

i) The identity and relative ratios of major PBB congeners in Firemaster BP-6® was derived from literature data[9,10]. ii) Two individual PBBs (PBB 101 and 153) were available for verification. iii) The elution order of PBB congeners on different GC columns was supposed to be similar to the corresponding PCB congeners on the same columns. iv) GC/ECNI-MS in the full scan mode revealed the degree of bromination for each congener. v) Assignment of signals in the HPLC chromatogram (Figure 1) was verified by analysing collected HPLC fractions by GC.

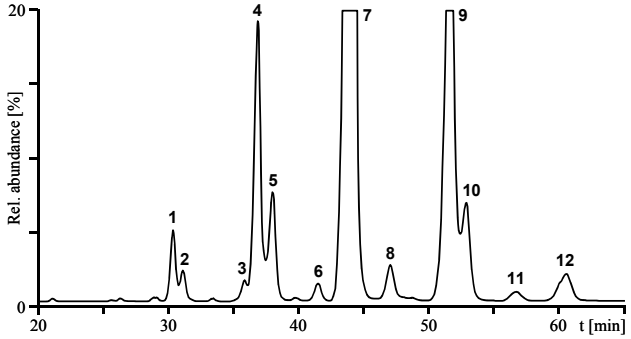

Figure 1. HPLC/UV (220 nm) chromatogram of 50 µg Firemaster BP-6®. Relative abundances of signals no. 7 (100 %) and no. 9 (25 %) exceed the shown range. For signal identification see Table 1

Table 1. Identification of PBBs in Firemaster BP-6® by HPLC/UV. Signal numbers refer to Figure 1

Peak no.	Tentative struct. IUPAC number*	Rt [min]	Area [%]
1	PBB 101 (5/2)	30.5	1.8
2	PBB 149 (6/3)	31.2	0.9
3	PBB 99 (5/2)	35.9	0.5
4	PBB 138 (6/2)	36.9	8.8
5	PBB 118 (5/1)	38.1	3.4
6	PBB 132 (6/3)	41.6	0.7
7	PBB 153 (6/2)	44.1	59.6
8	PBB 174 (7/3)	47.2	1.5
9	PBB 180 (7/2)	51.7	16.7
10	PBB 167 (6/1)	53.0	3.3
11	PBB 170 (7/2)	56.8	0.5
12	PBB 194 (8/2)	60.7	1.1

* (x/y) refer to: x, degree of bromination
y, number of *ortho*-substituents

Twelve PBB congeners in the technical product were studied (Table 1). Apart from signals no. 3 (PBB 99) and no. 11 (PBB 170) the identification of PBBs can be considered as certain. Since the potential atropisomeric tri-*ortho* substituted PBB congeners were of low relative abundance (see Table 1, peaks no. 2, 6 and 8), an initial fractionation of the technical product was carried out employing the HPLC separation shown in Figure 1. To obtain enough material of isolated congeners for enantioselective HPLC, 50 µg technical hexabromobiphenyl was injected twice and fractions of congeners 1 to 12 collected separately. Corresponding fractions of the two separations were pooled and fractionated once again. With this procedure, all congeners apart from no. 2 (PBB 149), no. 3 (PBB 99) and no. 12 (PBB 194) were obtained with purities >95 % (GC/ECD).

Enantiomer separation of atropisomeric PBBs by HPLC

Haglund successfully separated 14 of the 19 axially chiral PCBs on two serially connected HPLC columns containing permethylated β-cyclodextrin on silica (β-PMCD) as chiral stationary phase[11]. A similar β-PMCD column as well as an α-PMCD and a γ-PMCD column were employed in this study. In a first series of tests the enantiomer separation performance of the three columns with respect to the atropisomeric tri-*ortho* substituted PBBs 132, 149, 174 and the di-*ortho* substituted PBB 180 was evaluated at room temperature with an isocratic flow of 0.3 mL/min acetonitrile/water (75/25; v:v). β-PMCD was the only chiral stationary phase that at least partially resolved the atropisomers of all four investigated PBB congeners. Therefore, an optimisation of the column temperature, mobile phase flow rate and composition (acetonitrile-water ratio) was performed with the β-PMCD column. The optimised parameters are given in Materials and Methods. Figure 2 shows the enantioselective separation of six atropisomeric PBB congeners upon optimisation of the HPLC parameters. Successful enantiomer separation was confirmed by collecting fractions of the first and the second eluting atropisomer as well as a fraction in between (middle fraction) and analysis of these fractions by non-chiral GC/ECD. PBB congeners 101 and 153 were also investigated, but no separation of atropisomers was achieved. As can be seen from Figure 2, tri-*ortho* substituted PBBs (PBB 132, 149, and 174, Figure 2A-C) and di-*ortho* 2,2'-substituted PBBs with at least one bromine in an adjacent *meta* (3 and/or 3') position (PBB 138, 180, and 194, Figure 2D-F) were separated into atropisomers at 5 ºC. Di-*ortho* 2,2'-substituted PBBs without bromine in adjacent *meta* positions were not enantioselectively resolved (PBB 101 and 153). The effect of substituents in adjacent *meta* positions to increase the enantiomerization barrier has previously been observed for PCBs and was referred to as "buttressing effect"[11]. However, only di-*ortho* 2,2'-substituted PCBs with chlorine atoms in both 3 and 3' position have been separated into atropisomers at 0 ºC[11]. The larger covalent radius of bromine compared to chlorine seems to be responsible for the higher stability of PBB atropisomers with only one buttressing substituent.

Enantiomer separation of atropisomeric PBBs by GC

Enantiomer separation of axially chiral PBB congeners by GC is an analytical challenge. The high molecular weights of PBBs require high elution temperatures (typically >200 °C). These temperatures are close to the maximum operation temperatures for chiral stationary phases. Furthermore, enantiomerization of an axially chiral PBB congener is likely to occur at elevated temperatures, though the same congener may form stable atropisomers under physiological conditions. In this study, enantiomer separation of the atropisomeric tri-*ortho* PBBs 132, 149 and 174 was tested on several chiral stationary phases. β-TBDM (see Materials and Methods) was the only column, which partially separated the atropisomers of PBB 149 (Figure 3). None of the other

investigated PBBs could be enantioselectively resolved on any of the columns. Successful chiral resolution of PBB 149 was verified by injection of single PBB 149 atropisomers isolated by enantioselective HPLC (see before). This also allowed determining that the elution order of PBB 149 atropisomers on the β-TBDM GC column was the same as on the β-PMCD HPLC phase.

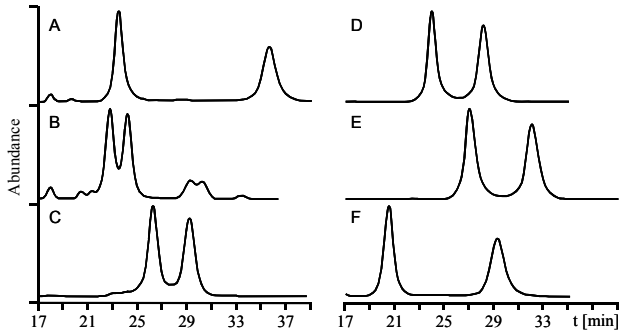

Figure 2. Enantioselective HPLC separation of PBB congeners on a β-PMCD column at 5 ºC. For separation details see Materials and Methods. A, PBB 132; B, PBB 149; C, PBB 174; D, PBB 138; E, PBB 180 (A-E: flow 0.5 mL/min); F, PBB 194 (flow 1 mL/min)

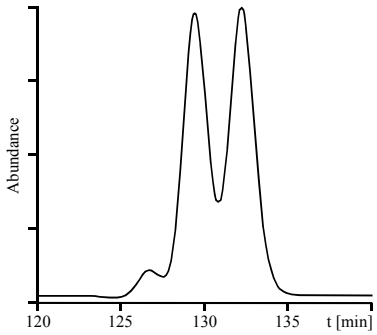

Figure 3. Enantioselective GC separation of PBB congener 149 on randomly modified heptakis(6-*O*-*tert*.-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (β-TBDM)

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