Enantioselective determination of 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane (B8-1412) in environmental samples

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Introduction

First reports on the enantioselective determination of compounds of technical toxaphene (CTTs) using high resolution gas chromatography (HRGC) were published in 1994 [1] [2]. These and some other follow-up studies focused on *tert.*-butyldimethylsilylated β-cyclodextrin (β-BSCD) as chiral stationary phase (CSP) [3]. This type of CSP was introduced by Blum and Aichholz [4] in 1990. The enantiomers of eight of the most important CTT standards were separated on this CSP [5]. Up to now, some papers reported about enantioratios (ERs) of the two major abundant CTTs B8-1413 and B9-1679 in biota (structures see Table 1) [1] [2] [6]. Only small deviations from the racemic composition (i.e. ER = 1.0) were reported for these two CTTs. On the other hand, the ERs of another persistent CTT in seal blubber (B8-2229) ranged from 2.6 to 4.3 [7]. Long it was thought, that β-BSCD is the only suitable CSP for enantioseparations of CTTs. However, enantioseparation of 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane (B8-1412), which was recently identified as a major CTT in biota [8], failed on β-BSCD. Enantioseparation of B8-1412 was obtained on heptakis(6-*O*-*tert.*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (β-TBDM) [9], introduced by Dietrich et al. [10]. Unfortunately, the major CTT in biota B8-1413 was not separated into its enantiomers on this CSP.
With the β-TBDM column ERs of a further main CTT, B8-1412, were established in different environmental samples. For quality control of enantioselective gas chromatography with electron capture detection (GC/ECD) the results were compared with those of achiral measurements of the respective samples.

Material and Methods

All measurements were performed on an HP 5890 II gas chromatograph (Hewlett Packard) equipped with two ^{63}Ni electron capture detectors (ECD).

For achiral measurements CP-Sil 8/C18 20% and CP-Sil 2 columns from Chrompack (Middelburg, The Netherlands) were applied. The samples were analyzed at the following GC conditions: 250°C injector temperature, 300°C detector temperatures, 1.2 bar helium (carrier gas); 60°C (1.5 min), 40°C/min to 180°C (2 min) 2°C/min to 230°C (25 min) 10°C/min to 270°C (15 min) (GC oven program).
For enantioseparations the CP-Sil 8/C18 20% column was replaced by a β-TBDM column (coated with 35% heptakis(6-*O*-*tert*.-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin diluted in OV1701; 30 m, 0.25 mm internal diameter, 0.15 µm film thickness).The CSP was synthesized by M. D. Müller, Swiss Federal Research Station Wädenswil, Switzerland and the column was made by G. Hottinger, BGB Analytik KG, Adliswil, Switzerland. The optimized GC conditions for B8-1412 were the following: 230°C injector temperature, 270°C detector temperatures, 1.0 bar helium (carrier gas); 120°C (1.5 min), 15°C/min to 185°C (68 min), 10°C/min to 200°C (35 min), 10°C/min to 230°C (40 min) (GC oven program).
A standard solution of eight CTTs (100 pg/µL each) was used (see Table 1).

Table 1: Systematic codes, Parlar numbers, and structures of important CTTs in biological samples and origin of the standard solutions

Code Nr. [11]	Parlar-Nr. [12]	Structure	Origin of the standard solution
B7-1453	-	2-*exo*,3-*endo*,5-*exo*,9,9,10,10-heptachlorobornane	1)
B8-1413	26	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane	2)
B8-1412	-	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane	3)
B8-1414	40	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10-octachlorobornane	4)
B8-1945	41	2-*exo*,3-*endo*,5-*exo*,8,9,9,10,10-octachlorobornane	4)
B8-2229	44	2-*exo*,5,5,8,9,10,10-octachlorobornane	4)
B9-1679	50	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane	2)
B9-1025	62	2,2,5,5,8,9,9,10,10-nonachlorobornane	2)

1) Calibrated solution after isolation from Melipax [13] (not commercially available)
2) BgVV three standard (5 mg/L) from Promochem (Wesel, Germany)
3) Isolate from seal blubber [8] (not commercially available)
4) single standard solutions (1 ng/µL) from Dr. Ehrenstorfer (Augsburg, Germany)

The sample clean-up was earlier described in detail [14]. Before quantification of the CTTs, PCBs and further aromatic organochlorines were separated. 1.0 mL of each sample extract was used for PCB/CTT group separation on 8.0 g activated silica gel (0.063-0.200 mm; activated 16 h at 130°C) in a glass column with an internal diameter of 10 mm [15]. Firstly, PCBs and other aromatic chlorinated compounds were eluted with 48 mL n-hexane (Fraction 1). CTTs were eluted in a second fraction with a more polar solvent. Instead of a mixture of n-hexane/toluene 65:35 (v/v, 50 mL), as suggested by Krock et al. [15], 50 mL of n-hexane/ethyl acetate 90:10 (v/v) were used. The elution profile was the same with the new solvent, but evaporation of the more volatile ethyl acetate is more convenient as compared with toluene.

Results and Discussion

Recently, we reported about the enantioseparation of CTTs on β-TBDM [9]. β-TBDM was the first CSP which enantioseparated B8-1412. However, application of a 20 m column resulted in coelution of the resolved enantiomers of B8-1412 with the unresolved B8-1413 peak [9]. To establish ERs of B8-1412 in environmental samples, we applied a longer GC column. On the 30 m column B8-1412 eluted approximately 2 min after B8-1413 (see Figure 1). No interference was found for B8-1412 with this technique (see below). β-TBDM also enantioseparated B7-1453, B8-1945, B8-2229, and B9-1025. B8-1414 and B9-1679 were also enantioseparated, but the first eluted enantiomer of B8-1414 coeluted with the first eluted enantiomer of B9-1679.

PCBs and other aromatic chlorinated compounds were removed by PCB/CTT group separation before enantioselective analyses (see Material and Methods). Nevertheless, B8-1412 was the only CTT to be analyzed on β-TBDM in the present sample extracts. All further detected CTTs coeluted with unknown (aliphatic) compounds. All results from chiral measurements were compared with those from achiral measurements on two different columns. Without this quality control the ERs wouldn't be secured.

Table 2: Concentrations and ERs of B8-1412 in cod liver samples of the Baltic Sea

Sample	B8-1412 [µg/kg][1]		$ER_{(1/2)}$[2]
	CP-Sil8/C18 / CP-Sil2	β-TBDM	β-TBDM
1	23	18	0.4
2	31	31	0.7
3	12	10	0.3
4	15	16	0.6
5	24	26	0.5

[1] *concentrations of B8-1412 calculated with the ECD response factor of B8-1413*

[2] $ER_{(1/2)}$ = Peak height of the first eluted enantiomer / Peak height of the second eluted enantiomer

In all cod liver samples from different areas of the Baltic Sea, $ERs_{(1/2)}$ of B8-1412 were significantly < 1.0 (Table 2). In blubber of a grey seal (*Halichoerus grypus*) from Iceland we found an $ER_{(1/2)}$ of 0.4. The same result we obtained for a Weddell seal (*Leptonychotes Weddelli*) from the Antarctic.

Although the number of samples investigated so far is low, the results clearly indicate an enantioenriched B8-1412 in all samples. Figure 1 shows parts of GC-ECD chromatograms on β-TBDM with B8-1412 in sample extracts of a cod liver from the Baltic Sea and blubber of a Weddell seal from the Antarctic.

In contrast to this, the major abundant CTTs in biota, B8-1413 and B9-1679, showed only a slight predominance of one enantiomer in samples at a high trophic level [1][2][5][6][7]. At the moment we do not know, if B8-1412 is enantioselectively degraded. Metabolites are likely to be formed by reductive dechlorination of other CTTs [16]. According to that nonachlorobornanes might be the precursors of B8-1412. Although B8-1412 was detected in technical toxaphene [8], it might additionally be the metabolite of nonachlorobornanes with geminal Cl on the six-membered ring as well as a major CTT in biota B9-1679 by removing of a Cl on C10.

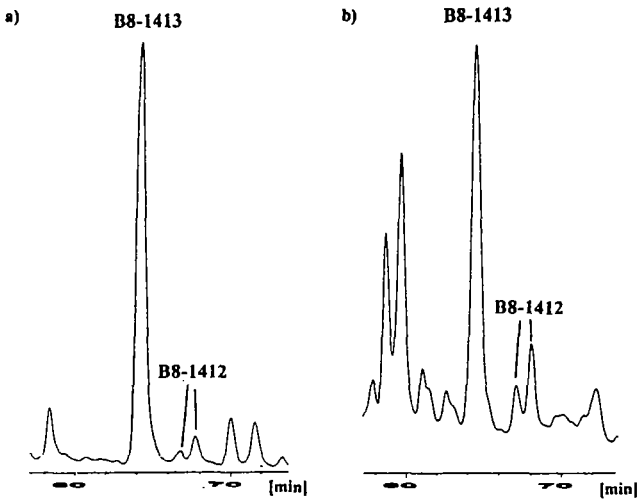

Figure 1 **GC-ECD chromatograms (parts) on β-TBDM**

a) Enantioseparation of B8-1412 in a blubber extract of a Weddell seal from the Antarctic

b) Enantioseparation of B8-1412 in a cod liver extract from the Baltic Sea

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