Enantioselective determination of toxaphene and other organochlorines on tert.-butyldimethylsilylated ß-cyclodextrin

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1. Introduction

High resolution gas chromatography with chiral stationary phases (CSPs) allows enantiomer separation of several chiral organochlorine compounds. The first enantiomer separation of an organochlorine compound in 1989[1)] was followed by an intense research activity[2)]. No CSP enabled enantiomer separation of all chiral organochlorines but application of different CSPs solved almost every enantiomer separation problem[2)]. A CSP which separated the enantiomers of several compounds of technical toxaphene (CTTs), chlordane-related compounds, and atropisomeric PCBs is tert.-butyldimethylsilylated ß-cyclodextrin (ß-BSCD) which was introduced by Blum and Aichholz[3)]. The enantiomeric resolution of organochlorines was often very high on ß-BSCD even at particular high temperatures. Goal of the present study was to investigate the upper temperature limits of the ß-BSCD with respect to enantiomer separation of organochlorines and to present results obtained from isolates and biological samples.

2. Experimental Methods

Enantiomer separations were performed on HP 5890 (Hewlett-Packard) gas chromatographs equipped with either ^{63}Ni electron capture detector (ECD), electron ionization mass spectrometry (EI-MS), or electron capture negative ionization mass spectrometry (ECNI-MS) using parameters recently described in detail[4-5)]. The ß-BSCD columns consisted of 20% or 25% tert.-butyldimethylsilylated ß-cyclodextrin diluted in PS086 (BGB Analytik, Adliswil, Switzerland). The column parameters were: 30 m length, 0.25 µm i. d., and 0.18 or 0.20 µm film thickness.

Table 1: Systematic codes, Parlar numbers, and structure of important CTTs

Code [6)]	Structure	Parlar No.[7)]
B7-515	2,2,5-endo,6-exo,8,9,10-heptachlorbornane	#32
B7-1453	2-exo,3-endo,5-exo,9,9,10,10-heptachlorobornane	-
B8-1413	2-endo,3-exo,5-endo,6-exo,8,8,10,10-octachlorobornane	#26
B8-1414	2-endo,3-exo,5-endo,6-exo,8,9,10,10-octachlorobornane	#40
B8-1945	2-exo,3-endo,5-exo,8,9,9,10,10-octachlorobornane	#41
B8-2229	2-exo,5,5,8,9,9,10,10-octachlorobornane	#44
B9-1679	2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonachlorobornane	#50
B9-1025	2,2,5,5,8,9,9,10,10-nonachlorobornane	#62

3. Results and Discussion

3.1 Enantiomer separation of organochlorines at high temperatures

Recently, we separated the enantiomers of several chiral organochlorines on ß-BSCD and found that some compounds eluted at particular high temperatures (>210°C)[4,8]. So, we studied the upper temperature limit for the enantiomer separation of organochlorine compounds (see Table 2), which had shown excellent enantiomeric resolution on ß-BSCD[4]. For this reason the oven temperature was raised with a fast ramp from 120°C to the final temperature which was reached during the first five minutes. The enantiomeric resolution decreases with increasing temperatures and enantiomer separations should be performed at low temperatures in praxis. On the other hand, these experiments resulted in an extended maximum operation temperature for ß-BSCD phases in routine analysis. Blum and Aichholz described a maximum temperature of 250°C for ß-BSCD[3]. A careful conditioning was applied before we used the ß-BSCD up to 275°C. During that procedure, the column bleed significantly decreased while the retention times (RT_1 and RT_2 = retention time of the first and second eluted enantiomer) and enantiomeric resolution values (R) were stable. RT and R were measured after conditioning of the column at 265°C and starting with an injection at 275°C. Among the investigated compounds, there are not only high boiling and late eluted organochlorines but also chiral compounds (e. g. oxychlordane) which can be enantiomer separated on this and other CSPs at low elution temperatures. For several other compounds (e. g. the major octachlorobornane in biota, B8-1413 (see Table 1), we obtained no enantiomer separation at 265°C while a partial resolution was obtained until 255°C. On the other hand enantiomer separation was still possible at particularly high temperatures. Plotting R ln RT_2/RT_1 against 1/T resulted in (almost) straight lines for oxychlordane, B9-1679, and B9-1025. The graphs of trans-chlordane and B8-1945 were curves as a result of non constant $\Delta_{R,S}(\Delta H)$. From the data, regression lines on a 95% confidence level were calculated. The intercepts which correspond to temperatures with $\alpha = 1$ were estimated at 284°C for oxychlordane, 289°C for trans-chlordane, 288°C for B8-1945, 290°C for B9-1679, and 298°C for B9-1025.

Table 2: Enantiomer separation of selected organochlorines on 20% ß-BSCD at high temperatures (Temperature program: 120°C, 2 min, 50°C/min to:)

		225°C	235°C	245°C	255°C	265°C	275°C
	RT_1	25.81	19.92	16.26	13.97	12.50	11.48
oxychlordane	RT_2	26.41	20.27	16.47	14.10	12.58	1153
	R	1.9	1.6	1.3	1.0	0.7	0.5
	RT_1	30.21	22.72	18.10	15.24	13.37	12.11
trans-chlordane	RT_2	31.52	23.49	18.56	15.51	13.56	12.26
	R	3.4	2.9	2.4	1.9	1.5	1.1
B8-1945*	RT_1	44.91	32.74	25.11	20.27	17.08	14.89
(Parlar #41)	RT_2	46.98	33.93	25.80	20.69	17.33	15.05
	R	3.5	3.0	2.4	2.0	1.4	1.2
B9-1679*	RT_1	49.62	35.87	27.26	21.79	18.17	15.70
(Parlar #50)	RT_2	50.77	36.53	27.65	22.03	18.31	15.79
	R	1.8	1.5	1.2	1.0	0.7	0.5
B9-1025*	RT_1	103.34	68.34	47.40	34.68	26.61	21.32
(Parlar #62)	RT_2	106.22	69.90	48.28	35.18	26.91	21.50
	R	2.1	1.8	1.5	1.3	1.1	0.8

These are among the highest temperatures for enantiomer separation ever reported and R values >1 above 250°C for all compounds is a further proof of the excellent properties of ß-BSCD for enantioselective HRGC. According to that, the ß-BSCD column can be used in temperature programmed runs at least up to 265°C without significant loss of efficiency. High final temperatures significantly reduce the total run time of an enantioselective analysis of biological samples which might contain high-boiling compounds difficult to elute from a CSP at temperatures below 250°C.

3.2 Enantiomer separation of a CTT isolated from the technical product Melipax.

Investigating several technical toxaphene products on a CSP using tandem mass spectrometry (MS/MS) in the *selective reaction monitoring* (SRM) mode, Buser and Müller found some chlorinated bornanes present in racemic and others in non-racemic composition[9]. However, interferences from other compounds present in these complex technical mixtures cannot be excluded even by MS/MS in the SRM mode. Anyhow, if the educt of the synthesis, technical camphene, is non-racemic, the chlorination products may also retain non-racemic composition. Recently, we isolated the persistent B7-1453 (for structure see Table1) from the technical mixture "Melipax"[10]. Melipax was produced and distributed until 1990 in the former German Democratic Republic and accounted for approx. 5% of the global toxaphene production[11]. B7-1453 was only a minor CTT in technical mixtures (<1% in Melipax). The mass chromatogram of the B7-1453 isolate from Melipax contained no significant impurities. The enantiomer separation of the B7-1453 isolate from Melipax was studied on ß-BSCD by GC/ECNI-MS and GC/EI-MS in the SIM mode by using several significant masses observed in the mass spectra. The first eluted enantiomer was significantly more abundant. All selected ions showed constant ratios of the two enantiomers and the ER of the B7-1453 enantiomers was established as 1.26±0.03 using both ionization techniques (see Figure 1, left)[12]. Several hepta-, octa-, and nonachlorobornane standards gained by photochlorination of lowly chlorinated bornanes[13-14], however, showed racemic composition[4]. Deviations of the observed ER from the expected value of 1.0 in these standards were in general < 0.05. Therefore, the experimental data of B7-1453 isolate exclude interferences and artifacts and confirms the non-racemic composition of the B7-1453 isolate from Melipax.

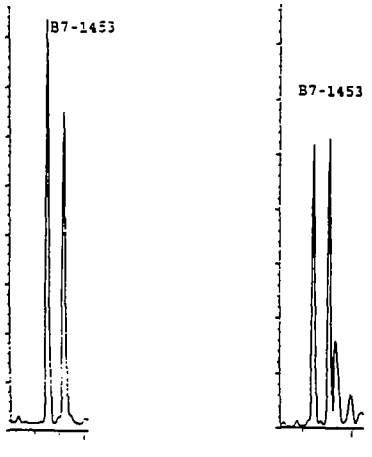

Figure 1: Enantiomer separation of B7-1453 on 25% ß-BSCD by GC/ECNI-MS.
left: non-racemic composition in the isolate from the technical product Melipax
right: isolate from cod liver extract

We also isolated B7-1453 from a Baltic cod liver extract and determined the ER (see Figure 1, right). Different to the isolate from Melipax, the cod liver isolate of B7-1453 showed an ER of 1.0. In the present case, i. e. application of an enantioenriched organochlorine compound, the alteration of the ER is owing to a faster degradation of the first eluted enantiomer and finally led to an ER of 1.0. This spectacular finding (enantioselective degradation leading to equal amounts of both enantiomers) is the result of biodegradation of enantiomers with different reaction speed which led by accident to an ER of 1.0. The interpretation of this result would be erroneous if synthetic (racemic) CTT single standards had been used as a reference contrary to B7-1453 isolate from Melipax. At the moment it is neither possible to decide if the non-racemic composition of B7-1453 observed in the product Melipax is also fact for other technical toxaphene products. Anyhow, ERs of CTTs determined in biological samples must be evaluated with care.

3.3 Enantiomeric ratios of further CTTs in biological samples.

In 1994, the enantiomer selective determination of CTTs in biota was obtained[15-16]. Since that time most publications focused on the enantioselective determination of B8-1413 and B9-1679[2]. These most abundant CTTs in biological samples showed only a slight predominance of one enantiomer even in samples at high trophic level. Recently, we identified seven persistent CTTs (those in Table 1 except B7-515 which was absent in samples) in seal blubber and fish[5,17]. Enantiomer separation of these CTT standards was recently obtained on ß-BSCD in one GC run[4]. In blubber of seals, signals at the retention times of both enantiomers were detected for these persistent CTTs, and this was a further proof of their presence in environmental samples. Although we applied GC/ECNI-MS, determination of the ER of B8-1414 and B8-1945 was impossible due to coelution with further, still unknown octachlorobornanes detected in the samples[5]. However, enantiomeric ratio of B8-2229 which eluted late on ß-BSCD could be determined in blubber of seals. Surprisingly, the enantioselective accumulation of B8-2229 in blubber of seals was much more pronounced compared with B8-1413 and B9-1679 (see Table 3). In all samples the second eluted enantiomer of B8-2229 was below 40% of the first eluted enantiomer.

Table 3: ER of B8-1413, B9-1679, and B8-2229 in blubber of seals from the Antarctic

Sample age, sex	B8-1413[4]	B9-1679[4]	B8-2229
Weddell Seal, female, subadult	1.1	1.2	3.4
Weddell Seal, male, adult	1.2	1.3	2.6
Weddell Seal, male, adult	1.1	1.3	2.8
Leopard Seal, unknown, adult	1.3	1.6	4.3

For quality control, we quantified the two most abundant ions in the M-Cl fragment (m/z 376.9 and m/z 378.9 for octachloro- and m/z 410.8 and m/z 412.8 for nonachlorobornanes) and calculated the ratio of these respective ions (m/z 376.9 vs. m/z 378.9) separately for both signals. Since the ratios were within 5% of the theoretical ratio, interference with compounds other than isomers can be excluded. Figure 2 shows an example of the enantiomer separation of B8-2229. Due to the high content of B8-2229 in the samples[5], we can also exclude coelutions with other octachlorobornanes. Therefore, the ER of B8-2229 presented in Table 1 are established. Furthermore, B8-2229 was recently isolated from grey seal blubber together with another so far unknown major CTT in biota[18]. This B8-2229 isolate also showed an ER >2.0. At the moment we do not know if the breakdown of B8-2229 occurs more enantioseletively than for other CTTs or if the compound was present in non-racemic ratio in technical mixtures.

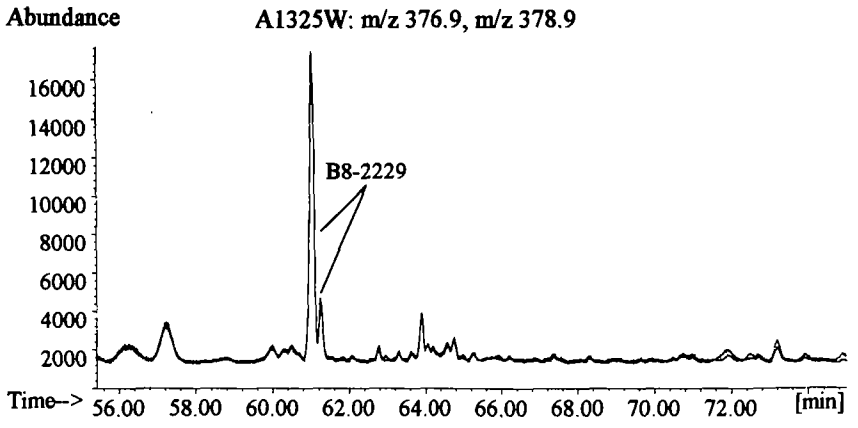

Figure 2: GC/ECNI-MS chromatogram of the enantiomer separation of B8-2229 (Parlar #44) in blubber of Antarctic Weddell seal (*Leptonychotes weddelli*)

4. References

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