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Gas Chromatographic Enantiomer Separation of Atropisomeric PCBs Using Modified Cyclodextrins as Chiral Phases

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Summary

Columns containing different types of cyclodextrin derivatives have been evaluated for chiral gas chromatographic separation of atropisomeric PCBs, *o,p′*-DDT and *o,p′*-DDD. Separation was attempted on columns containing mixed chiral selectors, and the performance of two closely related selectors was also examined. The cyclodextrins were: permethylated-β-CD (PM-β-CD), heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-CD (2,3-M-6-TBDMS-β-CD), heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethylsilyl)-β-CD (2,3-M-6-THDMS-β-CD), and heptakis(2,3-di-*O*-ethyl-6-*O*-*tert*-hexyldimethylsilyl)-β-cyclodextrin (2,3-E-6-THDMS-β-CD). The cyclodextrins were dissolved in OV-1701 or in a dimethylsiloxane/silarylene copolymer containing 5% phenyl in the backbone. The application of mixed chiral selectors led to improved separations, however; at most eleven PCB congeners were separated on a single column. Chiral resolution of *o,p′*-DDD was achieved. The use of a dimethylsiloxane/silarylene copolymer as a matrix for the cyclodextrins is a promising approach. With such a matrix, blocking of the CD cavities by silicone substituent groups can be avoided, and a reasonable CD solubility can be provided. The selectivity of heptakis(2,3-di-*O*-ethyl-6-*O*-*tert*-hexyldimethylsilyl)-β-CD and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethylsilyl)-β-CD was quite different, the former selector could separate four congeners, while the latter separated ten congeners.

1 Introduction

Polychlorinated biphenyls (PCB) are extremely persistent and they are found world-wide as environmental pollutants. Because of their potential health effects they need to be monitored. The importance and magnitude of the field is illustrated in recent reviews [1–3]. There are 209 possible PCB congeners and the separation of all these is not a trivial task. It has been predicted that 19 tri- or tetra-*ortho* substituted congeners have a sufficiently high energy barrier of internal rotation about the sp^2-sp^2 C—C single bond of biphenyl to exist in two enantiomeric forms at physiological temperatures [4]. Rotational barriers of atropisomeric PCB congeners have been examined [5, 6], and the thermal stability was found to be enough for gas chromatographic separation. The interest in chiral separation of environmental pollutants is mainly because the determination of enantiomer ratios allows the differentiation between abiotic and biotic degradation of organic xenobiotics [7]. The area has recently been reviewed [8, 9]. Similarly, *o,p′*-DDT and *o,p′*-DDD have a restricted rotation, and thus they may occur in two forms [9].

A number of different stationary phases has been used to separate the different atropisomers. This is summarized for PCB in **Table 1** and for DDT and DDD in **Table 2**. Vetter and coworkers used heptakis(2,3,6-tri-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (TBDMS-β-CD), synthesized according to Blum and Aichholz [10], as chiral selector. It was considered that this phase was indispensable for enantioselective analysis of chiral organochlorines [11]. However, a severe problem with this selector is that it cannot be synthesized in a reproducible manner [12]. Further, this type of modified CD is not stable, the large number of remaining unreacted OH groups leads to a slow degradation resulting in loss of any selectivity [12]. However, *in situ* crosslinking of the stationary phase in the column leads to stabilization. In summary, the separation performance will vary from batch to batch and the properties of the selector are changed during storage.

It was recently shown that the chiral selector 2,6-di-*O*-pentyl-3-trifluoroacetyl-γ-CD (2,6-P-3-TFA-γ-CD) could partly separate 15 PCB congeners [17]. However, it has also been shown that this selector is highly unstable. It was thus demonstrated with ^{19}F-NMR analysis that the trifluoroacetyl group was hydrolyzed [32].

Examination of Table 1 shows that none of the stable selectors can separate all atropisomers. It seems that it would be possible to separate a larger number of PCB atropisomers if more than one selector was employed. This can be accomplished in two ways, *viz.* by the use of columns coupled in

Table 1. Separations reported in the literature of enantiomers on different selectors and columns.

Selectors	PM-β-CD[1]		Ch-Dex with 2,3,6-M-β-CD[2]		Chiraldex with PM-β-CD[3]		2,3-M-6-THDMS-β-CD[4]		2,3-M-6-TBDMS-β-CD[5]		2,3,6-TBDMS-β-CD[6]	
Analyte	Ref.	Rs	Ref.	Rs	Ref.	Rs	Ref.	Rs	Ref.	Rs	Ref.	Rs
PCB 45							[20]	bl	[19, 17]	1.8		
PCB 84	[13]		[17]	0.7	[17]	1.2	[20]		[21]		[24]	bl
	[14]	0.7			[6]	1.12						
					[19]	0.79						
PCB 88												
PCB 91	[13]	abl	[17]	0.9	[19]	1.1	[20]	bl	[19]	2.02		
	[14]	0.9							[17]	1.4		
PCB 95	[13, 15]	abl	[17]	1.3	[19]	1.25	[20]	bl	[19]	2.02	[40]	bl
	[14]	1.25							[17]	1.4		
PCB 131					[17]	0.6	[20]	abl	[20]			
PCB 132	[13. 15]	bl	[17]	1.5	[17]	1.1			[19]	1.2 (bl)	[24]	bl
	[14]	1.5			[6]	1.81						
					[19]	2.85						
PCB 135	[14]	0.8	[17]	0.8	[17]	1.2						
					[6]	0.97						
PCB 136	[13]	abl	[17]	0.8	[17]	0.91	[20]	bl	[19]	2.18		
	[14, 16]	0.8			[6]	1.16			[17]	1		
					[19]	1						
PCB 139												
PCB 144					[17]	0.5					[24]	ps
PCB 149	[13, 15]	abl	[17]	1.3	[6]	1.65	[20]		[22, 23]	0.9	[24]	ps
	[14]	1.25			[19]	1.47			[19]	1.03		
									[17]	0.6		
PCB 171									[21]		[24]	hh
PCB 174	[14]	0.8	[17]	0.8	[17]	1.3	[20]	abl	[19]	1.58	[24]	hh
					[6]	0.86						
PCB 175					[17]	0.5	[20]	hh	[19]	1		
PCB 176	[14]	0.8	[17]	0.8	[17]	0.6	[20]	abl	[19]	1.99		
					[6]	0.85			[17]	0.6		
					[19]	0.92						
PCB 183							[20]	abl	[19]	0.81	[24]	abl
									[21]			
PCB 196												
PCB 197												

Selectors	Chiraldex 2,6-P-3-TFA-γ-CD[7]		2,6-M-3-P-β-CD[8]		2,6-M-3-P-β-CD[9]		Chiraldex 2,3-M-β-CD[10]		Chiraldex 2-HPME-β-CD[11]		Chiraldex HPPMTFA-γ-CD[12]		2,3-M-6-TB-β-CD[6]	
Analyte	Ref.	Rs	Ref.	Rs	Ref.	Rs	Ref.	Rs	Ref.	Rs	Ref.	Rs	Ref.	Rs
PCB 45	[17]	1.1			[28.20]	abl	[17]	1.7						
PCB 84	[17]	0.8												
PCB 88	[17]	1.2			[20]									
PCB 91	[17]	0.7			[20]	bl	[17]	1.9						
PCB 95	[17]	0.4			[28.20]	abl	[17]	1.9						
PCB 131	[17]	1					[17]	0.6			[17]	0.5		
PCB 132	[17]	0.8			[20]				[17]	0.7			[31]	1.2
PCB 135	[17]	0.7	[20]											
PCB 136					[20]	bl	[17]	1.4	[17]	0.6	[17]	1		
PCB 139	[17]	1			[28.20]	abl								
PCB 144														
PCB 149	[17]	1					[17]	1	[17]	0.4			[31]	0.9
PCB 171	[17]	1												
PCB 174	[17]	0.7												
PCB 175	[17]	0.4							[17]	0.5				
PCB 176							[17]	0.9			[17]	1.2		
PCB 183	[17]	0.6							[17]	0.8				
PCB 196	[17]	0.7							[17]	0.4				
PCB 197											[17]	0.7		

Rs = enantiomeric resolution; abl = almost baseline; bl = baseline; hh = half height; ps = partly separated

[1] Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin.
[2] Chirasil-Dex with immobilized permethyl 2,3,6-tri-*O*-methyl-β-cyclodextrin.
[3] Chiraldex with heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin.
[4] Heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethylsilyl)-β-cyclodextrin.
[5] Heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin.
[6] Heptakis(2,3,6-tri-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin
[7] Chiraldex with 2,6-di-*O*-pentyl-3-trifluoroacetyl-γ-cyclodextrin.
[8] Heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin.
[9] Octakis(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin.
[10] Chiraldex with 2,3-di-*O*-methyl-β-cyclodextrin.
[11] Chiraldex with (S)-2-hydroxypropyl-methyl ether-β-cyclodextrin.
[12] Chiraldex with hydroxypropyl-permethyltrifluoroacetyl-γ-cyclodextrin.
[13] Heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyl)-β-cyclodextrin.

Table 2. Separations reported in the literature of enantiomers on different selectors and columns.

Selectors	2,3-M-6-TBDMS-β-CD[1]		2,3,6-TBDMS-β-CD[2]		2,6-M-3-P-β-CD[3]		2,3,6-E-γ-CD[4]		Ch-Dex with PM-β-CD[5]	
Analyte	Ref.	Rs	Ref.	Rs	Ref.	Rs	Ref.	Rs	Ref.	Rs
o,p′-DDT	[21]	bl	[26]	1.5–1.6	[28]	abl	[30]	1.2	[18]	0.5
			[27]	2.8						
o,p′-DDD			[27]	1.4	[28]	ps	[30]	1.14	[18]	0.6

Rs = enantiomeric resolution; bl = baseline; abl = almost baseline; ps = partly separated.

[1] Heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin.

[2] Heptakis(2,3,6-tri-*O*-tert-butyldimethylsilyl)-β-cyclodextrin.

[3] Chiraldex with 2,6-di-*O*-pentyl-3-trifluoroacetyl-γ-cyclodextrin.

[4] Octakis(2,3,6-tri-*O*-ethyl)-γ-cyclodextrin.

[5] Chirasil-Dex, heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin.

Table 3. List of columns.

Column	Column length and i.d.	Silicone matrix	Film thickness	HETP	Number of PCBs separated
B: PM-β-CD and 2,3-M-6-TBDMS-β-CD 25 : 25% (w/w)	15 m, 0.32 mm	OV-1701	0.25 µm	0.39 mm	9
C: PM-β-CD and 2,3-M-6-TBDMS-β-CD 10 : 40% (w/w)	15 m, 0.25 mm	OV-1701	0.25 µm	0.31 mm	9
D: PM-β-CD and 2,3-M-6-THDMS-β-CD 10 : 40% (w/w)	15 m, 0.25 mm	OV-1701	0.25 µm	0.71 mm	11
H: 2,3-E-6-THDMS-β-CD 30% (w/ww)	11 m, 0.25 mm	Sila	0.15 µm	0.78 mm	4
I: PM-β-CD and 2,3-M-6-TBDMS-β-CD 10 : 40% (w/w)	11 m, 0.25 mm	Sila	0.15 µm	0.44 mm	8
J: 2,3-M-6-THDMS-β-CD 30% (w/w)	11 m, 0.25 mm	Sila	0.15 µm	0.50 mm	10

Sila, dimethylsiloxane/silarylene copolymer containing 5% phenyl in the backbone.

series or by the use of a column having mixed selectors. A presupposition for successful results is, of course, that the different selectors act in the same direction, *i.e.* they should not counteract each other. Coupling columns in series is a quite simple approach. The advantages of such coupling in connection with separation of chlorinated pesticides were recently discussed by Baycan-Keller and Oehme [33]. In coupled columns, the chromatographic properties are the simple addition of the properties of the pure stationary phases. In columns coated with mixed phases synergistic or antagonistic effects may occur [34–36]. These effects are the simultaneous interaction between two types of host molecules and a solute molecule. Buser and Müller prepared chiral columns by mixing different chiral selectors [37]. However, they were not able to achieve enantioselectivity on the mixed phases for analytes that were resolved on the each of the phases in the mixture when used separately. On the other hand, Nie and coworkers [38] showed that enantioseparation was improved significantly for some compounds when using capillary columns containing mixtures of modified cyclodextrins. The aim of the present work was to evaluate the performance of mixed chiral selectors with regard to possible synergistic effects and the total number of PCBs that could be separated on a single column. In addition, the performance of two types of modified CDs was compared.

2 Experimental

2.1 Reference Compounds

The 19 stable atropisomers of PCB and *o,p′*-DDD were kindly provided by P. Haglund (Institute of Environmental Chemistry, Umeå University, Umeå, Sweden). Originally PCB 45, 84, 91, 95, 131, 132, 135, 136, 174, 175, 176, 196, and 197 came from Accustandard (New Haven, CT, USA), PCB 88 from Ultra Scientific (North Kingstown, RI, USA), PCB 149 from Cambridge Isotope Laboratories (Andover, MA, USA), PCBs 144, 177, and 183 from Institute of Applied Environmental Chemistry (Stockholm University, Stockholm, Sweden). The stock solution of PCB 139 was diluted in cyclohexane. The other PCBs and *o,p′*-DDD were diluted in isooctane. Standard solution concentrations were 100 pg/µL for PCBs and 94.5 pg/µL for *o,p′*-DDD. A standard solution of *o,p′*-DDT, 100 pg/µL, was prepared from solid *o,p′*-DDT, Riedel-de Haën (Seelze, Germany), using isooctane as solvent.

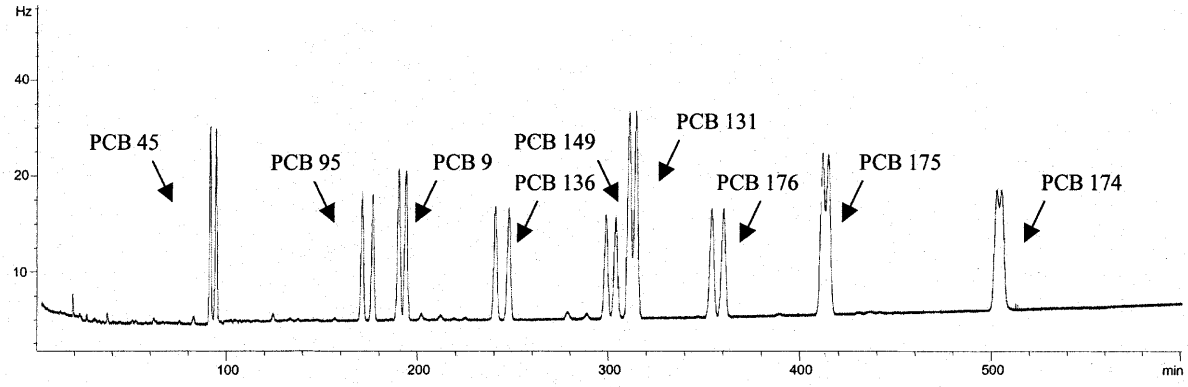

Figure 1. Gas chromatogram (ECD) of a mixture of PCB congeners. Column: fused silica 15 m × 0.25 mm i.d. coated with a mixture of permethylated-β-CD and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-CD and a home made silicone phase OV-1701, 10 : 40 : 50% (w/w/w), d_f = 0.25 µm (Column C). Conditions: splitless injection at 130 °C,isothermal for 1 min, then temperature programmed at 0.05 °/min to 180 °C. Carrier gas: H_2 at 40 cm/s. Peaks: PCB # 45, 95, 91, 136, 149, 131, 176, 175, and 174.

2.2 Gas Chromatography

Separations were performed on an HP6890 (Hewlett Packard, Wilmington, USA) gas chromatograph equipped with an auto-sampler, HP 7673 (Hewlett Packard). A split/splitless injector was used (200 °C in methods 3 : 8 and 3 : 10, 250 °C in the other methods). A micro-cell electron capture detector, µ-ECD, G2397A (Hewlett Packard) was used. Detector temperatures were 250 °C in method 3 : 8 and 3 : 10, 300 °C in the other methods. The system was controlled by Hewlett Packard Chemstation software. The temperature programs are given in **Table 4** and in the Figure legends. Modified cyclodextrins were synthesized under controlled conditions according to Ref. 25. The CDs were purified by means of liquid chromatography and recrystallized twice. The purity of the cyclodextrins was >99% as controlled by ^{1}H-NMR, ^{13}C-NMR, and MALDI/MS. Fused silica capillaries were from Polymicro Technology (Phoenix, AZ). Silicones, OV-1701, and a dimethylsiloxane/silarylene copolymer containing 5% phenyl in the backbone [39] were synthesised in house. Six columns were used; column properties are given in **Table 3**. Hydrogen was used as carrier gas with different linear velocity in the different methods that were used. The electronic pressure control (EPC) was operated in the constant flow mode. The µ-ECD detector was operated with nitrogen as makeup gas with a constant flow of 60 mL/min. Injection of 1 µL aliquots of the standard solutions were made in the splitless mode (2 min splitless) and the temperature of the oven was then programmed.

3 Results and Discussion

3.1 Effects of Mixing Different CD-Selectors

Heptakis(2,3,6-tri-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (TBDMS-β-CD), prepared according to Ref. 10, is a mixture of a number of isomers. The problem is, as mentioned above, that the phase is not synthesized under controlled conditions and can therefore not be reproduced. Thus, different batches of the selector may have different compositions, leading to different chromatographic selectivities. For example, the elution order of α-HCH enantiomers was inverse on some different batches of the selector [11, 40]. It was suggested that one group of the synthesis products of TBDMS-β-CD elutes (+)-α-HCH in front of (−)-α-HCH and one group elutes the (−)-α-HCH first. The ratio of the two groups determines the overall elution order of α-HCH on the respective TBDMS-β-CD column. Both groups partly cancel each other and this may be the explanation of the poor enantiomeric resolution of α-HCH observed on some batches of TBDMS-β-CD [40]. This explanation suggests that selectivity is due to the addition of the properties of the different selectors present in the mixture. However, the relatively high number of chiral PCB congeners chirally separated on some batches of TBDMS-β-CD suggests the presence of synergistic effects. An attempt was made to improve the reproducibility and performance of heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (2,3-M-6-TBDMS-β-CD) by "purification" [41], but the chromatographic separation was, in general, inferior as compared to the results obtained with the unpurified selector. Purification may thus lead to improved selectivity in some cases while selectivity will be impaired in other cases. Major batch-to-batch variations were also reported for octakis(2,3,6-tri-*O*-ethyl)-γ-cyclodextrin [30, 42]. In our opinion, it is of fundamental importance that the selectors can be prepared in a reproducible way and that they are sufficiently stable.

The resolutions of the chiral PCBs obtained on four different columns are summarized in Table 4. Eleven out of nineteen examined PCBs could be resolved on column D. Separation on column C is shown in **Figure 1**, on column J in **Figure 2**, and on column D in **Figure 3**. In two cases, PCB 196, **Figure 4**, and *o,p′*-DDD, **Figure 5**, separation has been achieved on CD-mixtures where separation has not been reported before on any of the two CDs in the mixture alone. It should

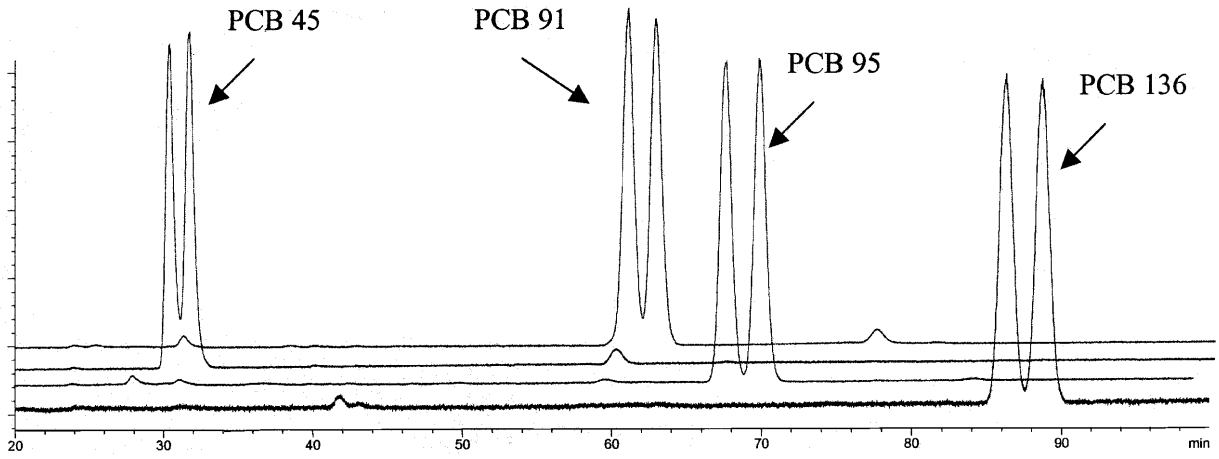

Figure 2. Gas chromatogram (ECD) of PCB congeners. Column: fused silica 11 m × 0.25 mm I.D. coated with heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethylsilyl)-β-CD, 30% (w) in a dimethylsiloxane/silarylene copolymer containing 5% phenyl in the backbone, d_f = 0.15 µm (Column J). Conditions: splitless injection at 130 °C, isothermal for 1 min, then temperature programmed at 0.1 °/min to 180 °C. Carrier gas: H_2 at 50 cm/s. Peaks: PCB # 45, 91, 95, and 136.

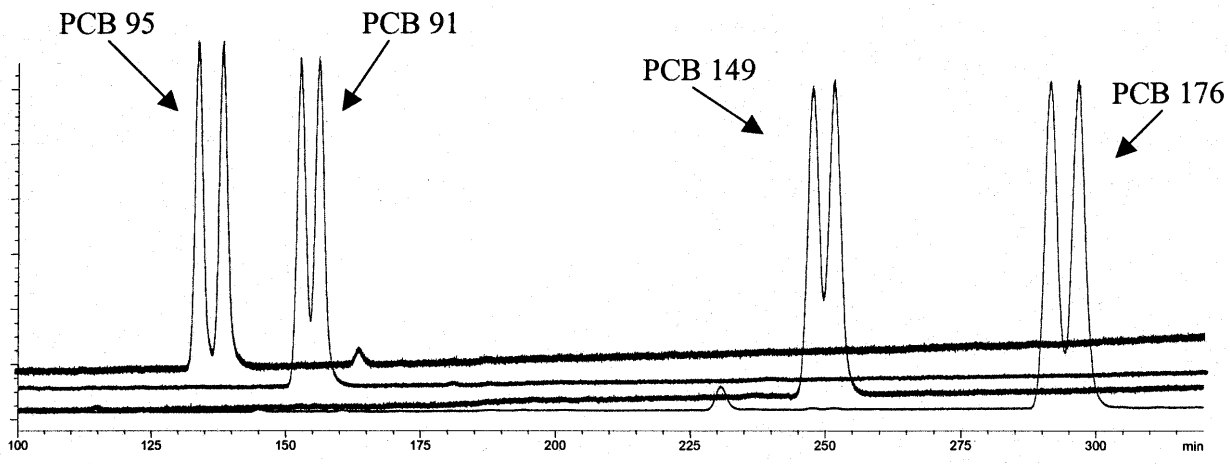

Figure 3. Gas chromatogram (ECD) of PCB congeners. Column: fused silica 15 m × 0.25 mm I.D. coated with a mixture of permethylated-β-CD, heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethylsilyl)-β-CD and a homemade silicone phase of OV-1701 type, 10 : 40 : 50% (w/w/w), d_f = 0.25 µm (column D). Conditions: splitless injection at 130 °C, isothermal for 1 min, then temperature programmed at 0.05 °/min to 180 °C. Carrier gas: H_2 at 40 cm/s. Peaks: PCB # 95, 91, 149, and 176.

be noted, however, that comparisons with literature data must be made with caution. This is because, in general, commercially available modified CDs consist of mixtures and the composition of a particular batch may strongly influence the selectivity. Moreover, other researchers may not have tried such slow temperature programming as applied here. Separation of PCB 196 by means of GC was recently demonstrated [17], and this congener has earlier been separated on HPLC [43]. Further, it is worth noting that the selectivity of bonded and dissolved CDs differs [44, 45]. Here bonded CDs means the case when CD moieties are bonded as substituent groups to the silicone backbone. It was recently demonstrated that Chirasil-Dex (chemically bonded PM-β-CD) provided separation of a number of atropisomeric PCBs while a corresponding polymer dissolved selector did not [45].

Column C contains the same selectors as column B, PM-β-CD and 2,3-M-6-TBDMS-β-CD. However, in C the content of 2,3-M-6-TBDMS-β-CD has been increased to 40% and PM-β-CD has been decreased to 10%. On this column, the resolutions were higher or about equal as compared to column B, this indicates that 2,3-M-6-TBDMS-β-CD is more powerful for the present application than the polymer dissolved PM-β-CD, but PCB 132 is an exception from this, Table 4.

In the cases when resolution was achieved, the resolutions were higher or as high on the mixed phases as reported for single selectors in earlier work. The theoretical plate height (H) was calculated for PCB 95 at 130 °C, Table 3. The highest efficiencies were found for columns B, C, and I, while col-

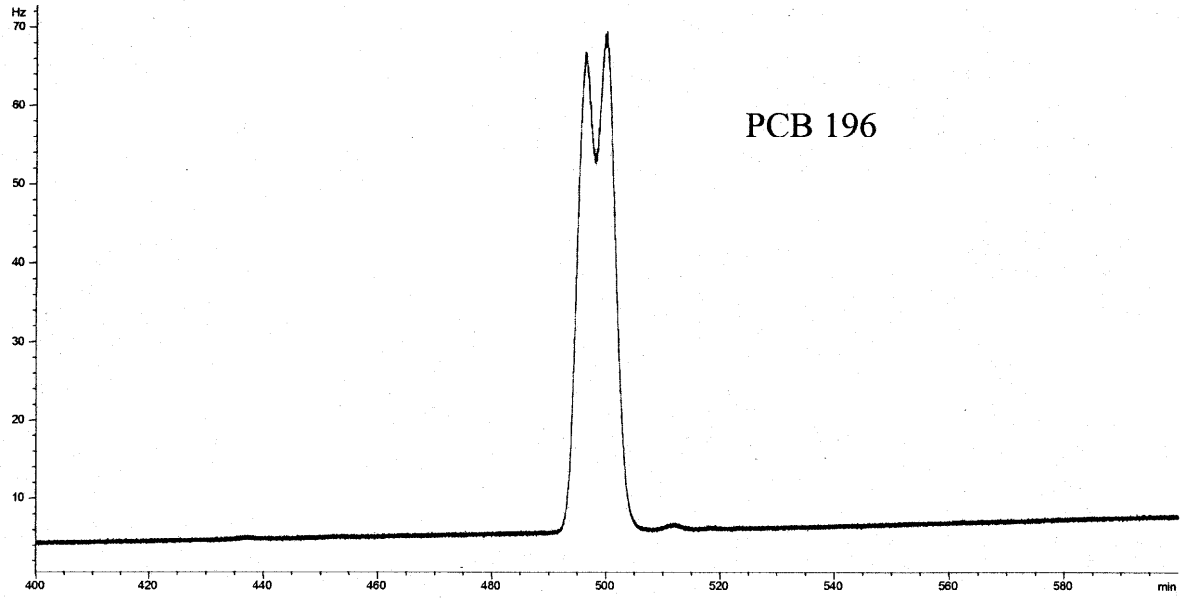

Figure 4. Gas chromatogram (ECD) of PCB # 196. Column: fused silica 15 m × 0.25 mm i.d. coated with a mixture of permethylated-β-CD, heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethylsilyl)-β-CD and a home made silicone phase OV-1701, 10 : 40 : 50% (w/w/w), d_f = 0.25 µm (Column D). Conditions: splitless injection at 130 °C, isothermal for 1 min, then temperature programmed at 0.05 °/min to 160 °C. Carrier gas: H_2 at 55 cm/s.

Table 4. Enantiomeric resolutions achieved on columns B (PM-β-CD and 2,3-M-6-TBDMS-β-CD, 25/25%, OV-1701, 50%), C (PM-β-CD and 2,3-M-6-TBDMS-β-CD, 10/40%, OV-1701, 50%), D (PM-β-CD and 2,3-M-6-THDMS-β-CD, 10/40%, OV-1701, 50%) and I (PM-β-CD and 2,3-M-6-TBDMS-β-CD, 10/40%, dimethylsiloxane/silarylene copolymer containing 5% phenyl in the backbone).

	Column B			Column C			Column D			Column I		
Analyte	Method	Rs	tR	Method	Rs	tR	Method	Rs	tR	Method	Rs	tR
PCB 45	5 : 2	1.5	74.9	04 : 11	1.8	83.0	04 : 18	1.4	43.7	04 : 22	0.8	34.3
PCB 84												
PCB 88												
PCB 91	5 : 2	0.7	143.0	04 : 11	1.3	159	04 : 15	1.1	153	04 : 22	0.6	74.7
PCB 95	5 : 2	1.9	130.2	04 : 11	2.4	146	04 : 15	1.7	134	04 : 22	1.4	66.9
PCB 131	5 : 2	0.7	216.5	04 : 11	0.8	237	04 : 13	0.7	162	04 : 22	0.6	130
PCB 132	5 : 2	0.7	260.6									
PCB 135												
PCB 136	5 : 2	1.8	175.6	04 : 11	2.0	194	04 : 15	1.7	187	04 : 22	1.4	96.6
PCB 139												
PCB 144												
PCB 149	5 : 2	1.3	208.9	04 : 11	1.4	229	04 : 15	0.9	248	04 : 22	1.0	123
PCB 171												
PCB 174				04 : 17	0.5	340	04 : 13	0.6	260			
PCB 175	5 : 2	0.5	273.1	04 : 11	0.6	297	04 : 15	0.7	352			
PCB 176	5 : 2	1.3	242.6	04 : 11	1.4	264	04 : 15	1.2	292	04 : 22	1.0	153
PCB 183							04 : 18	0.7	280			
PCB 196							04 : 18	0.6	460			
PCB 197												
o,p′-DDT												
o,p′-DDD	5 : 2	0.7	215.8	03 : 10	0.9	347	03 : 08	0.7	263	04 : 22	0.6	115

Method 3 : 8 = 70 °C 2 min, 1.0 °/min to 140 °C, 140 °C in 400 min. He 36 cm/s; **method 3 : 10** = 70 °C 2 min, 1.0 °/min to 140 °C, 140 °C in 400 min. H_2 35 cm/s; **method 4 : 11** = 130 °C 1 min, 0.1 °/min to 180 °C. H_2 40 cm/s; **method 4 : 12** = 130 °C 1 min, 0.08 °/min to 180 °C. H_2 40 cm/s; **method 4 : 13** = 130 °C 1 min, 0.1 °/min to 180 °C. H_2 55 cm/s; **method 4 : 15** = 130 °C 1 min, 0.05 °/min to 180 °C. H_2 40 cm/s; **method 4 : 16** = 130 °C 1 min, 0.05 °/min to 180 °C. H_2 55 cm/s; **method 4 :17** = 130 °C 1 min, 0.08 °/min to 180 °C. H_2 55 cm/s; **method 4 : 18** = 130 °C 1 min, 0.05 °/min to 180 °C. H_2 65 cm/s; **method 5 : 2** = 130 °C 1 min, 0.1 °/min to 180 °C. H_2 38 cm/s; **method 5 : 3** = 130 °C 1 min, 0.05 °/min to 180 °C. H_2 38 cm/s.

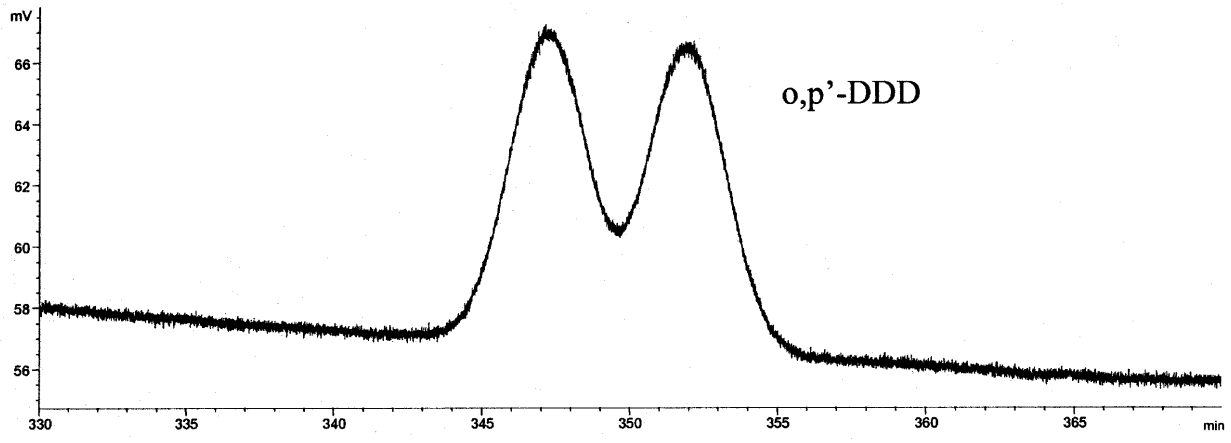

Figure 5. Gas chromatogram (ECD) of *o,p'*-DDD. Column: fused silica 15 m × 0.25 mm i. d. coated with a mixture of permethylated-β-CD, heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-CD and a home made silicone phase OV-1701, 10 : 40 : 50% (w/w/w), d_f = 0.25 μm (Column C). Conditions: splitless injection at 70 °C, isothermal for 2 min, then temperature programmed at 1.0 °/min to 140 °C. 140 °C was held for 400 min. Carrier gas: H_2 at 35 cm/s.

umns D and H were less efficient. Differences in plate heights may be due to slow interaction kinetics or insufficient CD solubility in the silicone matrix, see below.

Our intention was to be able to separate a broader range of chiral PCBs by the application of mixtures of selectors. Column D offers an example of the approach. Here selectors PM-β-CD and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethylsilyl)-β-CD are combined, and this facilitates the separation of PCBs 45, 131, 132, 175, and 183 on a single column, Table 3. Possibly, PM-β-CD separated PCB 132, while PCBs 45,131,175, and 183 were separated by the interaction with the other selector.

3.2 Influence of Elution Temperature and Stationary Phase Matrix on the Resolution

In general, chiral selectivity in GC is improved, as the elution temperature is decreased [16]. This has been demonstrated in practice, cf. Refs. 19 and 29. As it is known, elution temperature is affected by a number of factors. For example, type of silicone and concentration of selector may influence elution temperature as well as separation efficiency [19]. In general, non-polar stationary phases retain analytes less than more polar phases. Thus non-polar phases lead to lower elution temperatures. However, it should be noted that the solubility of modified cyclodextrins decreases as the polarity of the silicone matrix decreases. In order to avoid precipitation of the CDs, the concentration of a polar chiral selector has to be lower when using non-polar stationary phases as compared to more polar phases. Typically, selector precipitation leads to decreased plate numbers. The system used in column I was examined in a test tube. Crystals of CD could be observed, but these disappeared at temperatures above 120 °C. It seems that selector solubility puts a limitation to the application of non-polar matrixes, at least for more polar CDs.

The advantage of more polar matrixes is that they provide a better solubility of the chiral selector. A high solubility is desirable because the separation power can be strongly influenced by the concentration of the CD. For example, Bicchi *et al.* [46] have shown that enantioselectivity can increase with increase of the content of chiral selector in the column. However, we must be careful what type of polar silicone we choose. In cases when the silicone has substituent groups that fit into the CD cavity, these groups may enter the CD cavity and this thereby becomes blocked. With such blocked CDs, chiral interaction is less likely [47]. Buda *et al.* [48] maintained that, using OV-1701 as matrix, the presence of an achiral component in the stationary phase reduces the effectiveness of cyclodextrin-analyte interactions. On the other hand, it has been shown that phenyl containing polysiloxane matrixes such as OV-35 can provide excellent separations of polychlorinated compounds [42]. It may then be speculated that it is the cyanopropyl substituent group of OV-1701 that can block CD-cavities. For the separation of relatively volatile compounds, it has been reported that enantioselectivity achieved in apolar matrixes usually are higher than those obtained with increased CD content in more polar polysiloxanes [49–51]. In the present work, we attempted to avoid CD blocking by the application of a matrix having only methyl substituent groups. Further, in order to provide improved CD solubility, as compared to a pure methyl silicone, we used a siloxane/silarylene copolymer having 5% phenyl in the backbone as the matrix. However, it seems that a somewhat higher phenyl content would have been beneficial since that would have led to improved solubility of the CD moieties, for example in column I.

Of course, long analysis times, *e.g.* as reported here for column C, are not suitable in practice, and in an attempt to decrease the analysis times, column I was prepared. This column contains the same selectors as column C, but the station-

Table 5. Enantiomeric resolution on column H and J and literature data (from reference 20).

Analyte	Column H: 2,3-E-6-THDMS-β-CD[1] t_{r1}	Rs	T_1 (°C)	Column J: 2,3-M-6-THDMS-β-CD[2] t_{r1}	Rs	T_1 (°C)	2,3-M-6-THDMS-β-CD[2] Rs
PCB 45	34.8	–	133.4	30.3	1.35	132.9	bl
PCB 84	78.9	0.49	137.8	74.3	–	137.3	?
PCB 88	65.7	–	136.5	58.0	–	135.7	
PCB 91	73.3	–	137.2	68.3	1.64	136.7	bl
PCB 95	67.8	–	136.7	60.9	1.34	136.0	bl
PCB 131	134.7	–	143.4	122.4	0.66	142.1	abl
PCB 132	152.7	1.03	145.2	150.4	–	144.9	
PCB 135	117.9	0.69	141.7	111.5	–	141.1	
PCB 136	99.0	–	139.8	86.3	1.41	138.5	bl
PCB 139							
PCB 144	120.3	–	141.9	112.1	–	141.1	
PCB 149	127.6	–	142.7	118.1	0.63	141.7	?
PCB 171	229.2	–	152.8	216.3	–	151.5	
PCB 174	217.2	1.10	151.6	*274.5	0.97	143.7	abl
PCB 175	188.4	–	148.7	*224.6	0.52	141.2	hh
PCB 176	160.0	–	145.9	142.7	0.86	144.2	abl
PCB 183	200.0	–	149.9	*245.9	0.54	142.2	abl
PCB 196	305.4	–	160.4	293.0	–	159.2	
PCB 197	234.7	–	153.4	213.3	–	151.2	
o,p′-DDT							
o,p′-DDD	117.4	–	141.6	104.7	0.48	140.4	

abl = almost baseline; bl = baseline; hh = half height; ? = separated but no data given.

[1] heptakis(2,3-di-*O*-ethyl-6-*O*-*tert*-hexyldimethylsilyl)-β-cyclodextrin.

[2] heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethylsilyl)-β-cyclodextrin.

Method used for column H and J: 50 cm/s, 1.3 mL/min. 130 °C, 0.1 °/min. *Rate = 0.05 °/min.

ary phase film is thinner, 0.15 µm, the matrix is a relatively non-polar silicone and column length is shorter, 11 m. All the changes contribute to shorten retention times. The results are given in Table 4. Column I gives considerably shorter retention times than column C. For example, PCB 136 was eluted after 194 min on column C but only 97 min were required on column I. For column I, conditions were selected to shorten separation times, and resolution can not be directly compared.

3.3 Comparison of Two Closely Related Selectors

Two closely related selectors were synthesized and evaluated for chiral PCB separation. The selectors were, heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethylsilyl)-β-CD (2,3-M-6-THDMS-β-CD) and heptakis(2,3-di-*O*-ethyl-6-*O*-*tert*-hexyldimethylsilyl)-β-CD (2,3-E-6-THDMS-β-CD). The results of the evaluation are presented in **Table 5.** Selector 2,3-M-6-THDMS-β-CD separated the same PCBs as earlier published by König and coworkers [20] with one exception, PCB 84 that could not be resolved here. Changing the methyl group of the selector to ethyl resulted in drastically changed selectivity. Now PCBs 84, 132, and 135 that were not separated on 2,3-M-6-THDMS-β-CD were separated. The PCB 174 was separated on both selectors, but the other PCBs separated on 2,3-M-6-THDMS-β-CD could not be separated on 2,3-E-6-THDMS-β-CD.

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