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Consequences of variable purity of heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin determined by liquid chromatography–mass spectrometry on the enantioselective separation of polychlorinated compounds

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Abstract

The composition of 10 batches of heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (PM-CD) from different suppliers was determined by high-performance liquid chromatography combined with atmospheric pressure chemical ionisation mass spectrometry (MS). Considerable differences were found. Some batches consisted of more than 95% pure PM-CD, whereas others were not completely derivatised or contained a significant amount of by-products. Some suggestions about the structures of these impurities are given though neither nuclear magnetic resonance spectroscopy nor MS–MS investigations could completely reveal their nature. Capillaries for high-resolution gas chromatography were coated with the batches of most differing composition. They demonstrated widely varying column performance and separation properties for selected chiral polychlorinated substances such as chlordane compounds, *o,p′*-DDT, *o,p′*-DDD, α-hexachlorocyclohexane and atropisomeric polychlorinated biphenyls. The best enantioselectivity was obtained with the purest PM-CD. Compared to separations reported in the literature, a broader enantioselectivity was observed and also *trans*-heptachlor epoxide and oxychlordane could be resolved into enantiomers.

Keywords: Enantiomer separation; Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin; Polychlorinated biphenyls; Pesticides; Organochlorine compounds

1. Introduction

Capillaries based on cyclodextrin derivatives are widely applied for enantioselective separations by high-resolution gas chromatography (HRGC). Until now, no universal phase has been found which is able to separate all the compounds of importance. Therefore, various substituted cyclodextrins are used for different applications such as the enantioselective analysis of essential oils, aromas and flavours [1,2] or polychlorinated pesticides [3].

However, commercially available cyclodextrin derivatives are not very pure and often are mixtures of differently substituted products [4,5]. Already small changes in the cyclodextrin composition can significantly influence the enantioselective properties of the stationary phase [6], which might lead to reproducibility problems. Polychlorinated pesticides

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are especially sensitive to such composition changes, and even reversed enantiomer elution orders on apparently identical phases have been observed [6–8].

Consequently, only cyclodextrin derivatives of reproducible composition should be used for HRGC capillaries, which requires a detailed characterisation of the synthesised products. In the past, several characterisation methods were introduced. For examples, the application of various mass spectrometry (MS) techniques such as fast atom bombardment MS [4,9,10], UV matrix-assisted laser desorption MS [11] or electrospray ionisation MS [12–14] were reported. The latter was also combined with high-performance liquid chromatography (HPLC) [6] or capillary electrophoresis [15]. For this work, a reversed-phase HPLC method was developed and combined with either evaporative light scattering or MS detection based on atmospheric pressure chemical ionisation in the positive ion mode [APCI(+)]. This method has been successfully applied for the analysis of several cyclodextrin derivatives [16,17].

Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (PM-CD) is one of the most universally applicable chiral phases in HRGC [18,19] and capillaries coated with PM-CD are available from nearly all commercial manufacturers. Characterisation of this cyclodextrin (CD) derivative was already performed by electrospray MS [12] as well as UV matrix-assisted laser desorption MS [11] and no other compounds than completely derivatised PM-CD could be detected. During our study the composition of 10 PM-CD batches was investigated with HPLC–APCI(+)-MS. Nine of the batches were commercially available and one was synthesised in our laboratory. A detailed composition of the various batches is given, and the influence of composition differences on the achieved enantioselectivity is discussed for selected polychlorinated compounds. In addition, the structures of unknown cyclodextrin by-products are investigated.

2. Experimental

2.1. Reference compounds and solvents

Methanol (pesticide grade) was obtained from SDS (Peypin, France). *n*-Hexane, cyclohexane and *iso*-octane (for pesticide residue analysis) were purchased from Scharlau (Barcelona, Spain). HPLC-water from an Elgastat maxima HPLC water purification unit (Elga, Bucks., UK) was employed.

The chiral chlordane compounds MC5 (1-exo-2-endo-3-exo-4,5,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane), MC7 (1-exo-2-exo-3-endo-4, 5, 7, 8, 8 - octachloro - 3a, 4, 7, 7a - tetrahydro - 4, 7 - methanoindane) and MC8 (1-exo-2-exo-3-exo-4, 5, 7, 8, 8 - octachloro - 3a, 4, 7, 7a - tetrahydro - 4, 7-methanoindane) were isolated from technical chlordane as described in Ref. [20] and diluted with cyclohexane to concentrations of 100 pg/μl. *cis*-Heptachlor epoxide and oxychlordane (10 ng/μl in cyclohexane) were from Dr. Ehrenstorfer (Augsburg, Germany), crystalline heptachlor (99.7% purity) from Promochem (Wesel, Germany) and *o,p′*-DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane) (99.8-%) from Supelco (Bellefonte, PA, USA). All compounds were diluted with cyclohexane to concentrations of 100 pg/μl. The polychlorinated biphenyls (PCBs) 45, 91, 95, 136 (10 ng/μl in isooctane, 99.9% purity) were from Dr. Ehrenstorfer. Crystalline PCB 149 (purity>99%) was purchased from Promochem. All PCBs were diluted to a concentration of 250 pg/μl with isooctane. The ''Chirotest'', a mixture containing polychlorinated pesticides in mostly non-racemic ratios in *n*-hexane, is described in detail in Ref. [16]. However, the quantities of several enantiomers are not given correctly in this reference and are as follows: (+)-α-hexachlorocyclohexane (HCH), 35 pg/μl; (−)-α-HCH, 33 pg/μl; U82(1), 109 pg/μl; U82(2), 48 pg/μl; (+)-*trans*-heptachlor epoxide, 70 pg/μl; (−)-*trans*-heptachlor epoxide, 61 pg/μl; (+)-*trans*-chlordane, 50 pg/μl; (−)-*trans*-chlordane, 127 pg/μl; (+)-*cis*-chlordane, 208 pg/μl; (−)-*cis*-chlordane, 165 pg/μl; (±)-*o,p′*-1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT), 368 pg/μl; (±)-toxaphene 50, 143 pg/μl.

2.2. Cyclodextrin derivatives

The PM-CD batches 1–9 were from six commercial suppliers. PM-CD batches 1–3 were synthesised by C. Mark (Chemical Labs. Mark, Worms, Germany) using a modified standard procedure [21] based on sodium hydride and methyl iodide in

dimethylsulfoxide. PM-CDs 4–8 were batches obtained from a commercial column manufacturer. Batches 4 and 5 were synthesised by the same laboratory. PM-CD batch 9 was from a commercial CD synthesis laboratory and batch 10 was synthesised by L. Müller (Organic Analytical Chemistry, University of Basel, Basel, Switzerland) with sodium hydroxide and methyl iodide in dimethylsulfoxide according to Schomburg et al. [22]. It was purified by several re-crystallisations until a colourless product was obtained.

Solutions containing 20 ng/μl PM-CD were prepared in methanol–water (4:1, v/v). Aliquots of 5 μl were injected and characterised by reversed-phase HPLC combined with APCI-MS in the positive ion mode (LCQ, Finnigan MAT, San Jose, CA, USA). A column containing a C_{18} phase and an isocratic eluent of methanol–water (85:15, v/v) were used. Further details of the method and the instrument set-up are given in Ref. [16].

The APCI-MS conditions were further optimised for optimum peak shape and sensitivity: The sheath gas flow was increased from 30 to 50 arbitrary units, the vaporiser was heated to 350°C, the transfer capillary to 250°C and the corona discharge current was set to zero. Under these conditions only the formation of sodium adducts was observed and no fragmentation occurred. Quantities exceeding 2% were included into the quantification. Each batch was analysed three times. The differences among measurements were maximum 0.6% absolute for amounts present at quantities <20% and within 2% for larger amounts. MS–MS was performed with a collision energy of 70% and a mass isolation width of 0.5 u.

^{1}H nuclear magnetic resonance (NMR) spectroscopy was performed on a Bruker DRX 600 spectrometer operating at 600.13 MHz. All samples were dissolved in $C^{2}HCl_3$ (Dr. Glaser, Basel, Switzerland). Chemical shifts were detected relative to the solvent signal at 7.26 ppm. $^{2}H_2O$ was from Dr. Glaser. Between 512 and 1024 data points were recorded for all experiments. The spectral width was 2800 Hz; 384 or 512 t_1 increments were recorded with 16–56 scans per increment. The 90° pulse length was 13 μs. The spinlock time for the rotating frame homonuclear Overhauser spectroscopy (ROESY) experiments was 300 ms. A mixing time of 100 μs was used for the total correlation spectroscopy (TOCSY) experiments.

The three PM-CD batches with the most differing composition were selected to prepare HRGC capillaries: PM-CD 10 was the purest batch and no other compound than completely methylated PM-CD could be detected at levels exceeding 2%. PM-CD 2 was the most undermethylated product. PM-CD 5 did not contain undermethylated compounds but a considerable amount (>35%) of unknown by-products was present (see below).

2.3. HRGC columns

Capillaries (12 m×0.25 mm I.D., 0.15 μm film thickness) were made from raw fused-silica (Microquartz, Munich, Germany). The columns were manufactured as described in Ref. [16] diluting the PM-CD in OV 1701-OH (Fluka, Buchs, Switzerland) in a ratio of 1:4 (w/w). Dilution of PM-CD with polysiloxanes was introduced by Schurig and Nowotny in 1988 [23]. Furthermore, 10-m columns were coated with PM-CD 10 in PS 086 (Fluka) 1:4 (w/w) or OV 35 (BGB-Analytik, Anwil, Switzerland) 1:10 (w/w). The Grob-test [24] was modified by adding the methylesters of tetra-, hexa- and octadecanoic acid (E_{14}, E_{16} and E_{18}, all purissimum; Fluka). This allowed one to determine column performance in terms of separation numbers (Trennzahl, TZ) also at higher temperatures. After conditioning, a first capillary evaluation was carried out with this modified Grob-test under standardised conditions [24].

2.4. HRGC separation

The enantioselective separations of polychlorinated compounds were carried out on a Varian 3800 gas chromatograph (Varian, Palo Alto, CA, USA) equipped with a ^{63}Ni electron-capture detection (ECD) system and a split/splitless injector. The injector was kept at 250°C and the detector at 280°C. Nitrogen (purity 99.995%) at a flow-rate of 30 ml/min was used as detector make-up gas and helium (purity 99.999%) at a flow velocity of 36 cm/s as carrier gas. The separation conditions for the ''Chirotest'' and other chlordane-related compounds

were as follows: splitless injection of 1 μl; splitless time, 2 min; 60°C for 2 min, then 20°C/min to 120°C and 1°C/min to 220°C, isothermal until the last compound eluted.

For enantioselective analysis of PCBs, the flow velocity of the carrier gas flow was 52 cm/s and the temperature program: 2 min isothermal at 100°C, then 2°C/min to 220°C, isothermal.

3. Results and discussion

3.1. Cyclodextrin characterisation

The isocratic or gradient separation of the PM-CD compounds [molecular mass of PM-CD 1428.7 u, $(C_9H_{16}O_5)_7$] was not significantly different for methanol–water eluents within the range 70:30 to 90:10. Therefore, an isocratic eluent with 85% methanol and 15% water was chosen. Important composition differences were found among the investigated PM-CDs (see Fig. 1), and the batches could be divided into three groups: batches of high purity (PM-CDs 6, 8 and 10), those with an important fraction of undermethylated products (PM-CDs 1, 2, 3 and 9) and batches containing 30% or more of unknown cyclodextrin derivatives with molecular masses of 30 u, 60 u or 90 u higher than those of completely methylated β-cyclodextrins (PM-CDs 4, 5 and 7). A HPLC–MS chromatogram of batch 5 is given in Fig. 2. As shown in this figure, at least two isomers each of the [M+30/60/90] impurities were present which eluted all close to the retention time of the fully derivatised PM-CD. Investigations to elucidate the structure of these compounds are described below. Furthermore, small amounts of unknown compounds with the sodium adduct masses 1460 u, 1474 u and 1514 u were detected in various batches. Due to their low abundance (maximum 4%) no further investigations could be performed.

For batches originating from the same manufacturer (batches 1–3, 4 and 5) typical composition patterns were observed such as undermethylation or presence of [M+30/60/90] by-products. Therefore these batches were presented within one graph (see Fig. 1).

MS–MS and ^{1}H-NMR were used to obtain additional structural information about the [M+30/60/90] impurities present in batches 4, 5 and 7. MS–MS of PM-CD sodium adducts (exact mass of parent ion 1451.7 u) resulted mainly in the following ions with the assigned tentative compositions: m/z 1247.6 u (base peak, [6 methylated glucose units+Na]$^+$), 1043.5 u (25% relative abundance, [5 methylated glucose units+Na]$^+$), 839.4 u (15% relative abundance, [4 methylated glucose units+Na]$^+$). Moreover, for each [4–6 methylated glucose units+Na]$^+$ fragment further ions with a m/z ratio being 18 u higher (m/z 1265.7 u, 1061.6 u, 857.4 u) were observed at an abundance of 10–40%.

All fragments listed above were also found in the MS–MS spectra of the [M+30] products (exact mass of parent ion 1481.5 u) with a relative abundance of 10–30%. In addition, the main fragments m/z 1277.4 u (base peak), 1073.3 u (relative abundance 40%) and 869.3 u (relative abundance 20%) with 30 u higher m/z ratios compared to the fragments resulting from the PM-CD molecule were present. Also for all these +30-u compounds further fragments with a m/z ratio being 18 u higher each were observed [m/z 1295.4 u (relative abundance 95%), 1091.3 u (20%), 887.3 u (15%)]. For the [M+60] by-products (mass of parent ion 1511.7 u), correspondingly, mass differences of +30 and +60 u could be found as well as the original PM-CD fragments. Analogous results were also obtained for the [M+90] compounds (mass of parent ion 1541.7 u). Therefore, it can be assumed that the [M+60] and [M+90] by-products contain additional structures of 30 u, which can be cleaved off separately. No direct loss of 30 u from the sodium adduct of the molecule was observed for any compound. Further fragment assignment to elucidate the structure of the by-products was not possible.

The main signals in the ^{1}H-NMR spectra of the batches containing [M+30/60/90] compounds did not show any differences or shifts compared to the spectra of pure PM-CD (batch 10). The latter were in accordance with published ^{1}H-NMR data for PM-CD [19,25]. However, sets of additional signals with a height of 0.5–5% relative to the main signal were present for the protons at all carbon positions. Therefore, it was assumed that the by-products have varying substituents at the O-atoms. None of the extra signals could be assigned to free OH groups

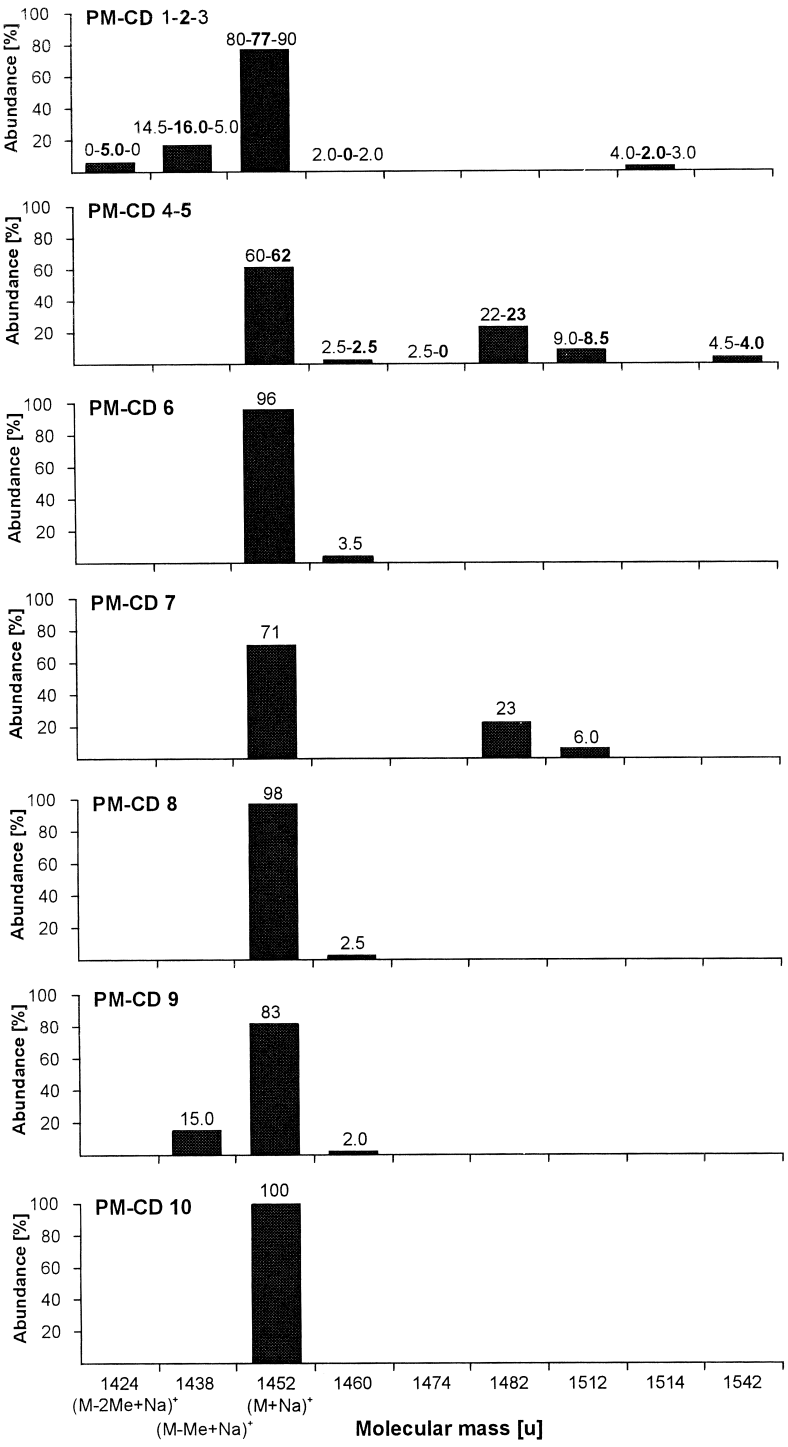

Fig. 1. Composition of 10 batches of PM-CD as determined by LC–MS. Quantities ≥2% are presented. The content was calculated assuming the same response factor for all compounds. The molecular masses of the sodium adducts are given. For batches 1 to 3 as well as 4 and 5 the graphs give the composition of batches 2 and 5, respectively, which were applied to manufacture HRGC capillaries.

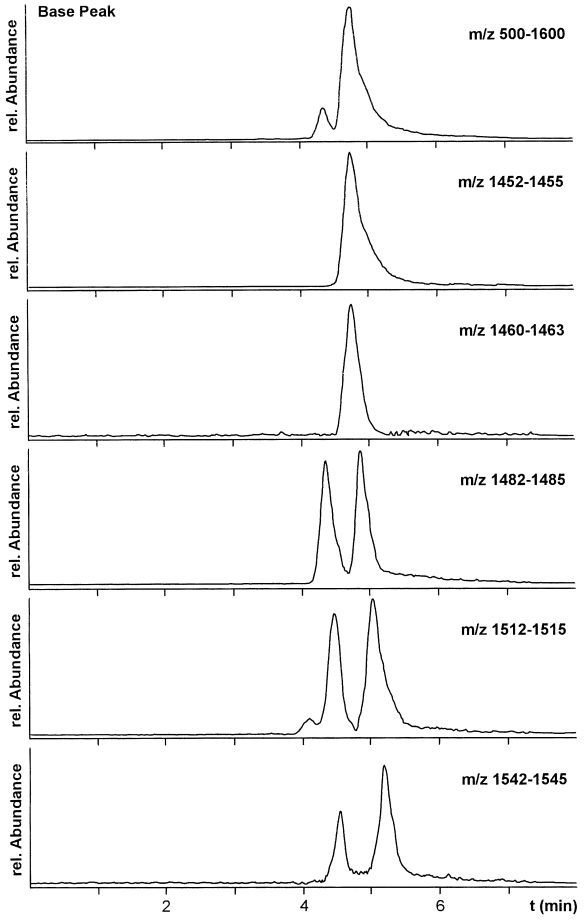

Fig. 2. HPLC–APCI(+)-MS chromatograms of PM-CD batch 5. Mass chromatograms of all compounds present ≥2% are given. For details, see text.

according to chemical shift, signal shape and change of signal form after addition of $^{2}H_2O$. Consequently, the mass differences of 30, 60 and 90 u cannot be explained by substituents such as –O-CH_2CH_2-OH instead of –O-CH_3. Due to the complexity of the ^{1}H-NMR spectra, a further assessment was not possible. This leaves the hypothesis that –O-CH_2-O-CH_3 groups might be present instead of –O-CH_3 which would also lead to different isomers. However, these compounds cannot be formed as by-products during standard synthesis conditions [19,22,26,27] from the reagents described in these references. Therefore, such substituents might origin from an impurity in the native β-cyclodextrin or the derivatisation reagent. Due to the low intensity of the additional signals, no further structural elucidation was possible by various two-dimensional ^{1}H-NMR experiments such as correlation spectroscopy (COSY), ROESY or total correlation spectroscopy (TOCSY).

Since no crude product of the self-synthesised batch 10 was any longer available when this study was performed, it was not possible to check the presence of any of the impurities found in the other products.

3.2. Manufacturing of HRGC capillaries

Columns were coated with the three batches of most differing composition (PM-CDs 2, 5 and 10) to investigate the influence of cyclodextrin composition on the enantioselective separation properties. The polysiloxane OV 1701-OH, which has very favourable properties in terms of coatability and cyclodextrin solubility [23,28], was applied to dilute the PM-CD. Compared to other studies usually adding 10% of PM-CD to the polysiloxane [18,19,23,29], the higher ratio of 1:4 was chosen to amplify separation differences caused by varying composition of the PM-CD. As reported by Bicchi and co-workers [25,30] stable films can be obtained for at least up to 30% PM-CD in OV 1701-OH. Moreover, they often observed improved enantioselectivity on 30% PM-CD compared to 10%.

The reproducibility of the manufacturing process was controlled by preparing a second column from PM-CDs 2 and 10. The separations of the Grob-test and the ‘‘Chirotest’’ were very similar for both sets of capillaries, and enantiomer resolutions (R_s) deviated less than 10%. Therefore, the observable differences among capillaries prepared from various batches can be assigned to changes in the cyclodextrin composition.

3.3. Grob-test

The separations of the modified Grob-test were very different. PM-CD 10 showed symmetrical peak shapes and a sufficient column performance ($TZ_{E10/E12}$=18, $TZ_{E16/E18}$=25). The Grob-test results observed on this capillary were very similar to that reported for PM-CD in Ref. [31]. For PM-CD 2 broad and tailing peaks were observed up to E_{14}.

Only the two last eluting esters E_{16} and E_{18} had an acceptable signal form resulting in a $TZ_{E16/E18}$ of 17. PM-CD 5 showed broad signals for the first compounds up to E_{10}, whereas the late eluting ester peaks were of excellent shape ($TZ_{E16/E18}$=21 compared to $TZ_{E10/E12}$=12). The poor separations on PM-CDs 2 and 5 at low temperatures are probably caused by limited solubility of the cyclodextrin in the polysiloxane [32]. However, a reasonable TZ at higher temperature confirmed successful phase coating on the capillary surface. This comparison clearly shows that an incomplete methylation and/or the presence of impurities has a substantial influence on the lower applicable temperature limit.

3.4. Enantioselectivity for polychlorinated compounds

Differences in the separation properties of the investigated columns were also observed for polychlorinated compounds. The capillary coated with the pure PM-CD 10 showed by far the best enantioselectivity. It was able to separate the ''Chirotest'' compounds α-HCH, *trans*-heptachlor epoxide, *cis*- and *trans*-chlordane into enantiomers, and even for *o,p′*-DDT a peak splitting could be observed (see Fig. 3). Moreover, this column was able to resolve MC5, MC7, MC8 and oxychlordane as well as PCBs 95 and 149. Table 1 summarises the achieved resolutions. No changes in the separation properties were observed when the capillary was kept at room temperature for several days and then re-installed in the GC system.

In contrast, the column coated with the most undermethylated batch PM-CD 2 was not applicable at all. Broad signals were observed and, in addition, the separation properties changed within the first few hours: at the beginning α-HCH partially split and enantiomer resolutions for *cis*- and *trans*-chlordane were >0.7. However, the separation properties deteriorated very quickly and Fig. 3 shows the poor separation of the ''Chirotest'' after several days. Furthermore, only the chlordane constituents MC5 and MC7 could be resolved into enantiomers (see Table 1 for resolutions). Obviously, incompletely derivatised PM-CD does not form stable films over time and, moreover, shows only unsatisfactory separation properties.

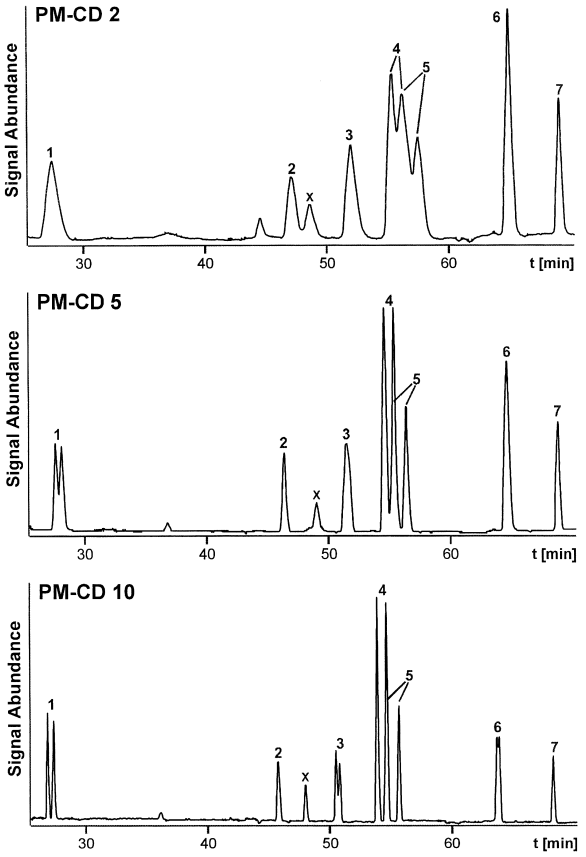

Fig. 3. HRGC–ECD chromatograms of the ''Chirotest'' containing chlorinated pesticides on PM-CD 2, 5, 10 in OV 1701-OH. For column details, see Experimental. 1=α-HCH, 2=U82, 3=*trans*-heptachlor epoxide, 4=*trans*-chlordane, 5=*cis*-chlordane, 6=*o,p′*-DDT, 7=toxaphene Parlar No. 50, x=impurity.

The enantioselective separation properties of a capillary prepared from PM-CD 5 containing the [M+30/60/90] by-products were stable but inferior compared to batch 10 (refer to Fig. 3 and Table 1).

On PM-CDs 5 and 10 the same elution order of (+)- before (−)-α-HCH was observed as reported for this chiral phase in the literature [8,29,33]. The (+)-enantiomer of *cis*- and *trans*-chlordane eluted first on all capillaries, however, (−)-*cis*-chlordane and (+)-*trans*-chlordane co-eluted. The enantiomer elution orders are in accordance with Refs. [29] and [34]. Interference of *cis*- and *trans*-chlordane on PM-CD was already observed in Refs. [35] and [36]. Buser et al. [35] suggested the use of a 20-m column with 10% PM-CD in PS 086 to separate all *cis*- and

Table 1
Enantiomer or atropisomer resolution R_s of selected PM-CD batches for polychlorinated compounds[a]

Compound	PM-CD 10 (retention time, min)	PM-CD 2 (retention time, min)	PM-CD 5 (retention time, min)
trans-Chlordane	2.84 (54.612/55.672)	p.s. (56.795/57.410)	1.43 (55.285/56.352)
cis-Chlordane	1.98 (53.840/54.614)	0.69 (55.194/56.067)	2.00 (54.565/55.373)
α-HCH	1.92 (26.929/27.439)	n.s. (27.441)	0.84 (27.591/28.090)
MC5	2.34 (59.430/60.187)	1.35 (61.123/62.300)	1.73 (60.315/61.128)
MC7	4.21 (60.839/62.391)	1.80 (62.563/64.195)	3.23 (61.579/63.160)
MC8	0.89 (64.808/65.148)	n.s. (67.555)	p.s. (65.646/65.976)
Oxychlordane	0.85 (45.738/46.087)	n.s. (47.242)	n.s. (46.831)
Heptachlor	n.s.[b](34.340)	n.s. (35.315)	n.s. (35.198)
U82	n.s. (45.870)	n.s. (47.095)	n.s. (46.348)
o,p′-DDT	p.s.[c](63.691/63.833)	n.s. (64.969)	n.s. (64.562)
o,p′-DDD	n.s. (62.655)	n.s. (63.640)	n.s. (63.343)
Toxaphene Parlar No. 50	n.s. (68.330)	n.s. (69.124)	n.s. (68.757)
trans-Heptachlor epoxide	0.83 (50.452/50.747)	n.s. (52.047)	n.s. (51.464)
cis-Heptachlor epoxide	n.s. (48.875)	n.s. (50.447)	n.s. (49.556)
PCB 45	n.s. (30.371)	n.s. (30.405)	n.s. (30.858)
PCB 91	p.s. (37.083/37.189)	n.s. (37.335)	n.s. (37.598)
PCB 95	0.78 (36.300/36.478)	n.s. (36.574)	n.s. (36.892)
PCB 136	n.s. (40.739)	n.s. (40.906)	n.s. (41.183)
PCB 149	0.76 (42.354/42.514)	n.s. (42.636)	n.s. (42.885)

[a] R_s values were calculated as $R_s = 1.18\Delta t(w_{b1}+w_{b2})^{-1}$, where Δt is the time difference between the peaks and w_{b1}, w_{b2} the peak width at half height. Retention times are given in parentheses.
[b] n.s.=Not separated.
[c] p.s.=Peak split.

trans-chlordane enantiomers without overlap. However, Vetter et al. [36] could not reproduce these separations on an identical capillary. A complete resolution was also achieved on the commercially available Betadex column [34]. Own results showed that an interference-free isomer and enantiomer separation of *cis*- and *trans*-chlordane could be achieved on a 10-m capillary coated with pure PM-CD/PS 086, 1:4 or PM-CD/OV 35, 1:10 (data not shown). The second phase required a change of the heating rate to 2°C/min from 120 to 220°C for optimum separations. Since even minute changes of the experimental conditions have a great influence on the achieved separations, it is still very demanding to avoid co-elutions.

The enantiomer resolution of *trans*-heptachlor epoxide [the (+)-enantiomer eluted first] and oxychlordane was only possible on completely derivatised PM-CD 10. These separations are especially remarkable since so far both compounds could not be resolved on PM-CD [8,36,37]. The broader enantioselectivity observed here may be owing to a higher portion of PM-CD in the stationary phase than described in the literature. This has a significant influence on the enantioselectivity as reported by Bicchi and co-workers [25,30]. Moreover, not pure and completely methylated PM-CD might have been used previously leading to a deteriorated enantioselectivity as shown in this study for batches 2 and 5.

So far, the suitability of PM-CD for the separation of atropisomeric PCBs was only investigated with an immobilised phase, which resolved nine congeners (among others PCBs 91, 95, 136 and 149) [38–40]. Normally, the enantioselectivity of immobilised and non-bonded phases is different [41]. Therefore, no direct comparison with the separations obtained here (resolution of PCBs 95 and 149, but not of PCBs 45, 91, 136) is possible.

The behaviour of PM-CD is in contrast to the previously studied octakis(2,3,6-tri-*O*-ethyl)-γ-cyclodextrin. On the latter phase far better separations of polychlorinated compounds could be achieved on incompletely derivatised batches and the most completely derivatised product showed the poorest enan-

tioselectivity [16,17]. It obviously strongly depends on the cyclodextrin derivative whether a complete derivatisation is favourable or not.

In conclusion, the presented results demonstrate that the composition of PM-CD may vary considerably despite its relative simple synthesis. The content and nature of these impurities have an enormous influence on the enantioselectivity for the investigated polychlorinated compounds. Therefore, an adequate purity control is indispensable for the reproducible manufacturing of HRGC capillaries.

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