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High-resolution gas chromatographic test for the characterisation of enantioselective separation of organochlorine compounds Application to *tert.*-butyldimethylsilyl β-cyclodextrin

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Abstract

Capillaries based on *tert.*-butyldimethylsilylated β-cyclodextrin (TBDMS-CD) diluted in OV-1701 have gained increasing interest for the enantioselective separation of lipophilic polychlorinated pesticides. A modified test according to Schurig for chiral stationary phases was not suitable for testing TBDMS-CD due the lack of sufficiently lipophilic compounds. A test mixture was developed containing the chiral polychlorinated pesticides α-hexachlorocyclohexane, *cis*- and *trans*-chlordane, heptachlor-exo-epoxide and 2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,8,9,10,10-nonachloro-bornane. They are especially sensitive to changes in the enantioselective properties of TBDMS-CD which is also frequently used for these compounds. *Cis*- and *trans*-chlordane with different enantiomer ratios facilitate the detection of changes of the enantiomer elution order. Test results of laboratory-made and commercial capillaries are presented showing the usefulness of the test to detect differences in the enantioselective behaviour such as changes in the elution order of enantiomers. Electrospray MS was used for the characterisation of TBDMS-CD. The analysed batches contained homologues with 7–10 TBDMS groups in a mixture of variable composition. This is assumed to be the reason for the observed differences. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Chiral stationary phases, GC; Cyclodextrin-based stationary phases; Organochlorine compounds; Pesticides; Chlordane

1. Introduction

Recently published literature shows that enantioselective separations on modified cyclodextrins by high-resolution gas chromatography (HRGC) have increased in importance though the application range is restricted to relatively lipophilic compounds of low to medium polarity [1–4]. Differently substituted cyclodextrins are important to achieve the desired selectivity of the enantioselective separation. A large number of commercial columns coated with mixtures of polysiloxanes and cyclodextrin derivatives or the pure modified cyclodextrin alone are now available.

When using different cyclodextrins as stationary phases, changes in the elution order of enantiomers can be observed. If not properly documented, they might cause problems such as wrongly assigned enantiomer ratios. One well-known example is the inversion of the elution order of α-(+)- and α-(−)-hexachlorocyclohexane (HCH). On β-cyclodextrins α-(+)- elutes before α-(−)-HCH but vice versa on

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γ-cyclodextrins which have one more sugar unit [5]. Müller and Buser reported differences in the elution order of chiral chlordanes and derivatives between HRGC on permethylated β-cyclodextrin and HRGC on permethylated, perethylated and *tert.*-butyl dimethylsilylated β-cyclodextrins [6].

Recently, increasing interest has been focused on the technical pesticides chlordane and toxaphene due to their strong synergistic amplification of oestrogenic effects when added to weak artificial oestrogens such as dieldrin or other polychlorinated pesticides [7]. Toxaphene consists of a large number of chiral polychlorinated bornanes and related compounds [8]. Due its ubiquitous presence in any food chain of the world and the decrease of the maximum allowable concentration in food in Germany and other countries, the analysis of this compound group has gained increased interest. Recent publications have shown that capillaries based on *tert.*-butyldimethylsilylated β-cyclodextrin (TBDMS-CD) allow a simultaneous separation of a large number of chiral polychlorinated pesticides including chlordane and toxaphene enantiomers [9–15]. At present there are no alternatives with a similar enantioselectivity for toxaphene congeners. Unfortunately, this phase originally introduced by Blum and Aicholz [16] is not well defined and was described as randomly derivatised by these authors. Nevertheless, capillaries based on TBDMS-CD are now commercially available.

Species and sex related differences in the bioaccumulation and degradation of chlordane isomers and enantiomers have been studied by our group [17] using both laboratory-made and commercial capillaries. During this work a reversion of the elution order of only the enantiomers of *cis*-chlordane was observed between two columns made from the same stationary phase mixture containing OV-1701 and TBDMS-CD [17]. The enantiomer elution sequence of all other chiral octachloro congeners remained unchanged. This important difference could only be recognised by comparing the separation of well characterised samples and of pure enantiomers on both capillaries. Later, similar differences in the separation properties were also observed between two columns obtained from the same commercial supplier. Interestingly, the test mixtures normally applied for the characterisation of chiral separations looked to be unsuitable for capillaries based on TBDMS-CD. Such chiral test solutions contain racemates of a wide range of structures of different polarity [18,19]. Most of them were not even separated into enantiomers. Furthermore, the elution order differed between similar capillaries indicating changes in the polarity of the stationary phase.

A test was developed to evaluate the reasons for such deviations and to obtain a better characterisation of the phase properties for the enantioselective separations of lipophilic polychlorinated compounds. It consists of a set of polychlorinated pesticides which are especially sensitive to small changes in the enantioselective behaviour of the stationary phase. The preparation and composition of the test mixture is described. Test results for capillaries containing mixtures of OV-1701 and TBDMS-CD are presented. The differences in the enantiomer separation behaviour detected by this test demonstrates its usefulness. In addition, first results are presented about the composition of TBDMS-CD by electrospray mass spectrometry. It is assumed that the observed differences are the reason for the observed deviations in the enantioselective behaviour of apparently similar stationary phase compositions.

2. Experimental

2.1. Reference compounds and solvents

All solvents were of pesticide-grade. The compounds for the modified Schurig test were obtained from Fluka (≥95% purity, Buchs, Switzerland) and had a purity of 99% or better. Pure racemic reference pesticides as well as technical chlordane (Velsicol, Chicago, IL, USA) were obtained from Dr. Ehrenstorfer, Augsburg, Germany. The elution order of the enantiomers on the enantioselective HPLC column was controlled by enantiopure standards (purity better than 95% controlled by HRGC) obtained from Dr. Markus Müller at the Federal Research Station [6]. Similar enantiopure reference compounds were also prepared by König et al. [9] and are commercially available from Dr. Ehrenstorfer. Solutions containing single reference compounds or mixtures were prepared in hexane having concentrations of about 50–600 pg/μl. The enantiomer ratios of *cis*-

and *trans*-chlordane in the test solution were obtained by mixing pure enantiomers.

2.2. Preparation of enantiopure standards

A 2.5% solution of the racemic isomer was prepared in tetrahydrofuran. A volume of 4 μl was injected via a loop valve into a Hewlett-Packard 1050 liquid chromatograph equipped with a ternary low pressure gradient system and a diode array detector. The separation was carried out isocratically on permethyl-β-cyclodextrin modified silicagel (200 mm×4 mm I.D., particle size 5 μm, porosity 100 nm, ET200/4 Nucleodex, Macherey–Nagel, Switzerland). As mobile phase methanol–water (80:20, v/v) was used at a flow-rate of 0.5 to 0.8 ml/min. Detection was carried out at 220 nm. Designation of the enantiomer elution order was made as described in Ref. [6] using the same enantioselective HPLC column and enantiopure standards supplied by these authors.

2.3. Enantioselective test mixtures

The polychlorinated pesticide test mixture contained the following concentrations in hexane: α-hexachlorocyclohexane (α-HCH, racemic), 350 pg/μl; heptachlor-exo-epoxide (HEP), 300 pg/μl; *cis*-(+)-chlordane, 250 pg/μl; *cis*-(−)-chlordane 100 pg/μl; *trans*-(+)-chlordane, 510 pg/μl; *trans*-(−)-chlordane 420 pg/μl; 2-*exo*,3-*endo*,5-*exo*,6-*endo*-,8,8,9,10,10-nonachlorobornane (Tox 50), 300 pg/μl. Alternatively, α-HCH with a given α-(+)-/α-(−) ratio can be used.

The enantioselective properties of the columns were also tested by a modified test mixture according to Schurig. It consisted of the compounds α-pinene, γ-valero-lactone, α-phenylethylamine and 2-ethylhexanoic acid, being also part of the original Schurig test mixture, as well as additional substances such as linanool, 2-ethyl-hexanoic acid methylester and phenyl ethylene glycol. For split injections concentrations of 0.05% per compound were prepared with chloroform as solvent. This test is also used by Macherey–Nagel to test enantioselective capillaries.

2.4. Herring oil sample

The origin and clean-up of the herring oil sample is described in Ref. [20].

2.5. Chiral capillary columns

(2,3,6-Tri-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin (TBDMS-CD) was prepared following precisely the procedure published by Blum and Aicholz [16]. A simple composition control was carried out by thin-layer chromatography on silica gel 60 (E. Merck, No. 1.15327) using toluene–ethanol (15:1, v/v) as eluent and by ^{1}H NMR (Varian Gemini 300 MHz). TLC showed one spot at an R_F value of 0.75 and NMR confirmed the introduction of *tert.*-butyldimethyl silyl groups but no further detailed information could be obtained. Further characterisation was carried out by HPLC combined with electrospray mass spectrometry as outlined in Section 2.7.

Fused-silica capillaries of 15–25 m×0.25 mm I.D. (Siemens, Germany) were statically coated with a mixture of 2.5% OV-1701 (a 7% phenylmethyl 7% cyanopropylmethyl 86% dimethyl OH-terminated polysiloxane, Ohio Valley, Marietta, OH, USA) and a variable amount (see below) of TBDMS-CD in dichloromethane–pentane (1:1, v/v). Methyltriethoxysilane was added as a crosslinking reagent corresponding to 0.1% of the stationary phase amount. The resulting film thickness was about 0.15 μm.

The fused-silica capillaries compared in this work had the following properties. Capillary (a): 25 m×0.25 mm I.D., 0.15 μm film thickness of OV-1701 with 10% TBDMS-CD (batch a); capillary (b): 20 m×0.25 mm I.D., 0.15 μm film thickness of OV-1701 with 10% TBDMS-CD (batch b), capillary (c): 20 m×0.25 mm I.D., 0.15 μm film thickness of OV1701 with 10% TBDMS-CD (BGB Analytik, Rothenfluh, Switzerland, catalogue No. 27220-015) capillary (d): as capillary (c) but a different order; capillary (e): 15 m×0.25 mm I.D., 0.15 μm film thickness of OV-1701 with 50% TBDMS-CD (batch b); capillary (f): tandem capillary as described in Ref. [20] made from a fused-silica capillary of 24 m×0.25 mm I.D., 0.15 μm RTx2330 (Restek, Bellefonte, PA, USA) coupled to a glass capillary of 18 m×0.3 mm I.D., 0.14 μm of PS-086 with 10%

TBDMS-CD; capillary (g): tandem capillary with the same first column as (f) coupled to a similar column as capillary (b).

2.6. Gas chromatographic separation

A 1-μl volume of the test solution with polychlorinated pesticides or of the sample extract was injected applying a splitless time of 2 min on all systems. A Hewlett-Packard 6890 gas chromatograph equipped with an electron-capture detector kept at 300°C and an injector temperature of 250°C was used for the chiral pesticide test mixture. The separation conditions were as follows: initial temperature 60°C, 2 min isothermal, then with 20°C/min to 120°C and with 2°C/min to 240°C, isothermal for 15 min.

The modified Schurig test was separated on a Carlo Erba 2150 gas chromatograph equipped with a flame ionisation detector. H_2 was used as carrier gas at a flow velocity of 50 cm/s. The injector and detector temperature was 250°C. A 1-μl volume was injected with a split rate of 30:1 and separated with the following temperature programme: 40°C for 2 min, then with 7°C/min to 200°C.

For the analysis of the herring oil a Hewlett-Packard 5989B mass spectrometer equipped with a HP5890 gas chromatograph was employed in the negative ion chemical ionisation (NICI) mode. The injector temperature was 230°C and the transfer line was kept at 250°C. The separation was carried out at an initial temperature of 90°C for 2 min, then with 15°C/min to 180°C/min, isothermal for 44 min, and then with 1–1.5°C/min to 230°C, isothermal for 2 min.

2.7. Characterisation of TBDMS-CD by HPLC–MS

TBDMS-CD batches synthesised according to [16] were analysed on a Finnigan LCQ HPLC–MS^n system. A 5-μl aliquot of a saturated solution consisting of 300 μl methanol, 100 μl tetrahydrofuran and 400 μl chloroform was injected into a flow of methanol–tetrahydrofuran (80:20, v/v) which was transferred into the MS system at a flow-rate of 100 μl/min. The mass spectrum of the double charged ions were recorded in the electrospray positive ion and negative ion mode.

3. Results and discussion

During the past years the enantioselective separation of chiral polychlorinated pesticides such as α-HCH, heptachlor epoxide, chlordane congeners and *o,p'*-DDT has found an increased interest. Many of them have been separated for the first time by König et al. on different stationary phases [9].

As already mentioned in Section 1, chiral stationary phases which are mixtures of OV-1701 with TBDMS-CD were found to be very suitable for the isomer and enantiomer specific separation of complex mixtures of technical pesticides such as chlordane and toxaphene. However, the enantioselective separation of chlordane and toxaphene congeners varied considerably on both different commercial and laboratory-made capillaries making their application for studies of enantiomer ratios in environmental samples rather troublesome [14,17]. Therefore it was decided to study the reproducibility of the enantioselective separations of polychlorinated compounds on TBDMS-CD in more detail.

A first complication was that one of the test solutions frequently used for modified cyclodextrins was were not suitable for TBDMS-CD. This test mixture containing four compounds of the Schurig test and some more chiral substances (see Section 2.3) was very well separated into enantiomers on for example heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin or heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin which is in accordance with [19]. However, as can be seen from the examples in Fig. 1, the results for the tested capillaries based on TBDMS-CD were quite puzzling. Different elution orders were observed and only some very few compounds were separated into enantiomers. Blum and Aicholz [16] also reported about a special behaviour of TBDMS-CD being unable to separate compounds into enantiomers containing free hydroxyl groups. In addition, some of the compounds such as γ-valerolactone, 2-ethylhexanoic acid and phenyl ethylene glycol showed signal broadening and strong adsorption effects and even losses. This is also in accordance with the experience of Blum and Aicholz observing similar effects for some more polar compounds of the Grob test [16]. According to these test results, the TBDMS-CD columns would have been considered as unusable. Nevertheless, some columns

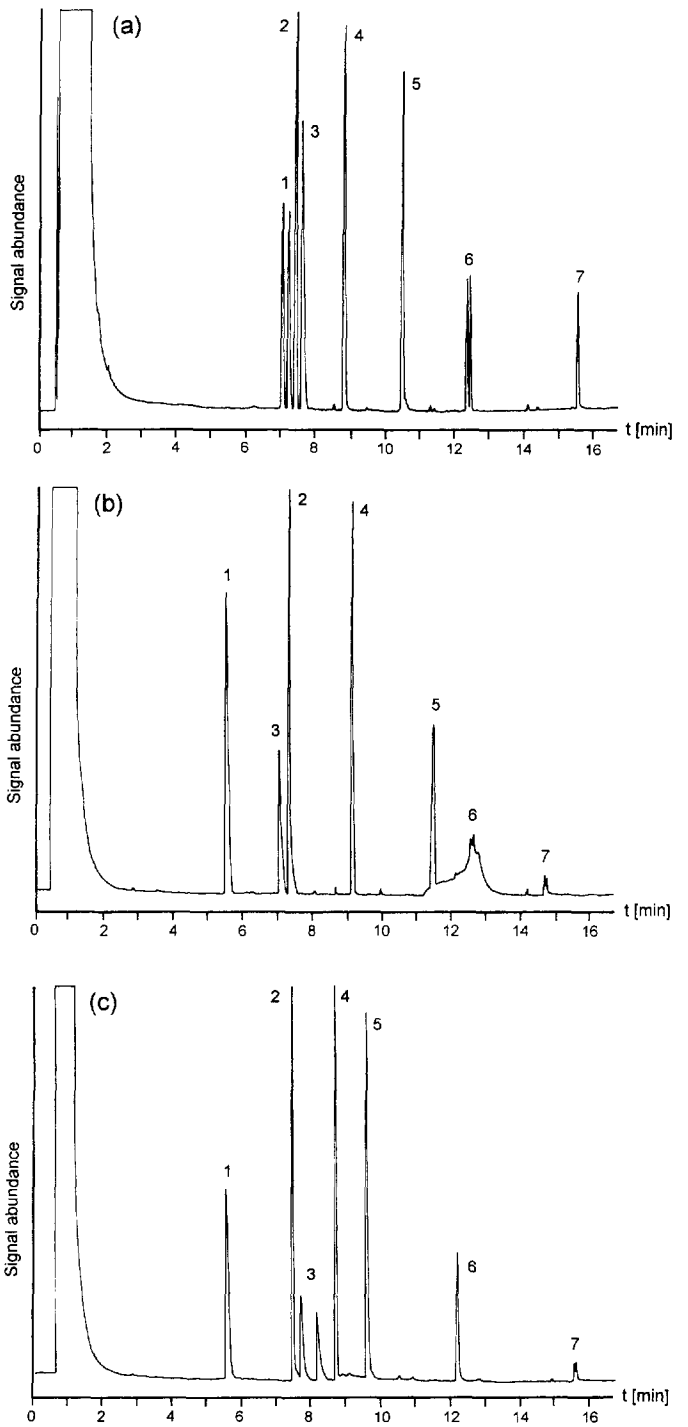

Fig. 1. Enantiomeric separation of the modified Schurig test on OV-1701 + TBDMS-CD on the capillaries (a), (b) and (c) from different batches. For column details see Section 2.5. Peaks: 1 = α-Pinene, 2 = 2-ethyl hexanoic acid methyl ester, 3 = γ-valero-lactone, 4 = α-phenyl ethyl amine, 5 = linanool, 6 = 2-ethylhexanoic acid, 7 = phenyl ethylene glycol.

showed a good enantiomer separation of *cis*- and *trans*-chlordane, of some toxaphene congeners and of other polychlorinated pesticides. However, some variations in the enantiomer and isomer elution order were also observed. To study such deviations in a more systematic way, a test mixture was developed which fulfilled the following points:

(i) Polychlorinated test compounds were selected which are very sensitive to minute differences in the chiral interactions.

(ii) Pairs of critical enantiomers with specific non-racemic enantiomer ratios are part of the mixture to facilitate the control of enantiomer elution orders. In addition, the preparation of the pure enantiomers by HPLC had to be simple and the elution order of the (+)- and (−)-enantiomers known for a reference column.

(iii) Compounds were chosen which are of interest in real samples and which also can be separated (at least partly) on other modified cyclodextrins to extend later on the applicability of the test to other phases than TBDMS-CD.

The following compounds were selected taken into account additional reasons given below: α-HCH, *cis*-chlordane with a (+)/(−) enantiomer ratio of 3:1 (concentration about 50% of that of *trans*-chlordane), *trans*-chlordane with a (+)/(−) enantiomer ratio of 5:4, heptachlor-exo-epoxide and the toxaphene congener 2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,8,9,10,10-nonachlorobornane (Tox 50).

The enantiomer selective determination of chlordane isomers is of particular importance. As shown earlier, the enantiomer ratios of the octachloro isomers are different between animals at different trophic levels such as fish and seals [17,21]. In addition, significant changes were also observed between different seal species [21]. Finally, the enantiomer ratio for *cis*- and *trans*-chlordane is inverted between male and female cod [22]. This is of special importance since chlordane has shown a high degree of synergism which when combined with other compounds acting as endocrine disrupters [7].

α-HCH was chosen as an important test compound due to the following reasons. Its the separation into enantiomers is possible on most derivatised β-cyclodextrins. However, the enantioselective separation of this compound looks to be quite sensitive to changes in the stationary phase composition of TBDMS-CD capillaries as can also be seen from Table 1. A reversion of the enantiomer elution on modified γ-cyclodextrins compared to substituted β-cyclodextrins [here α-(+)- comes before α-(−)-HCH] has also been reported [5] and indicates that this compounds also might be susceptible to impurities in the chiral stationary phase leading to a change in the elution order. Furthermore, a change of the 1:1 enantiomer ratio in racemic α-HCH by an enantiomer enriched α-HCH would offer the advantage of detecting such changes in the elution order.

3.1. Enantioselectivity of tested capillaries

Fig. 2 shows the separation of the test solution containing polychlorinated pesticides (chirapest test) on three capillaries coated with the same stationary

Table 1
Enantiomer resolution and elution order of *cis*- and *trans*-chlordane on the tested TBDMS-CD capillaries (see Section 2.5 for column description)

Capillary	Enantiomer resolution					Elution order
	α-HCH	HEP	*cis*-Chlordane	*trans*-Chlordane	Tox 50	*cis*(cs)- and *trans*(tr)-chlordane
a	1.5	1.8	2.1	1.7	1.4	cs(−), tr(+), cs(+), tr(−)
b	1.7	2.7	0.95	n.s.	1.0	cs(−), cs(+), tr(±)
c	1.5	2.0	1.2	n.s.	0.8	cs(−), cs(+), tr(±)
d	1.7	2.0	1.5	4.6	2.0	tr(+), cs(+), cs(−), tr(−)
e	4.0	1.6	3.3	1.8	n.s.	cs(+), cs(−), tr(−), tr(+)
f	0.7	1.1	0.7	2.1	0.9	tr(+), tr(−), cs(+), cs(−)
g	n.s	2.3	0.7	2.3	0.8	tr(+), tr(−), cs(−), cs(+)

n.s.: not separated; α-HCH: α-hexachlorocyclohexane; HEP: heptachlor-exo-epoxide; Tox 50: 2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,8,9,10,10-nonachlorobornane.

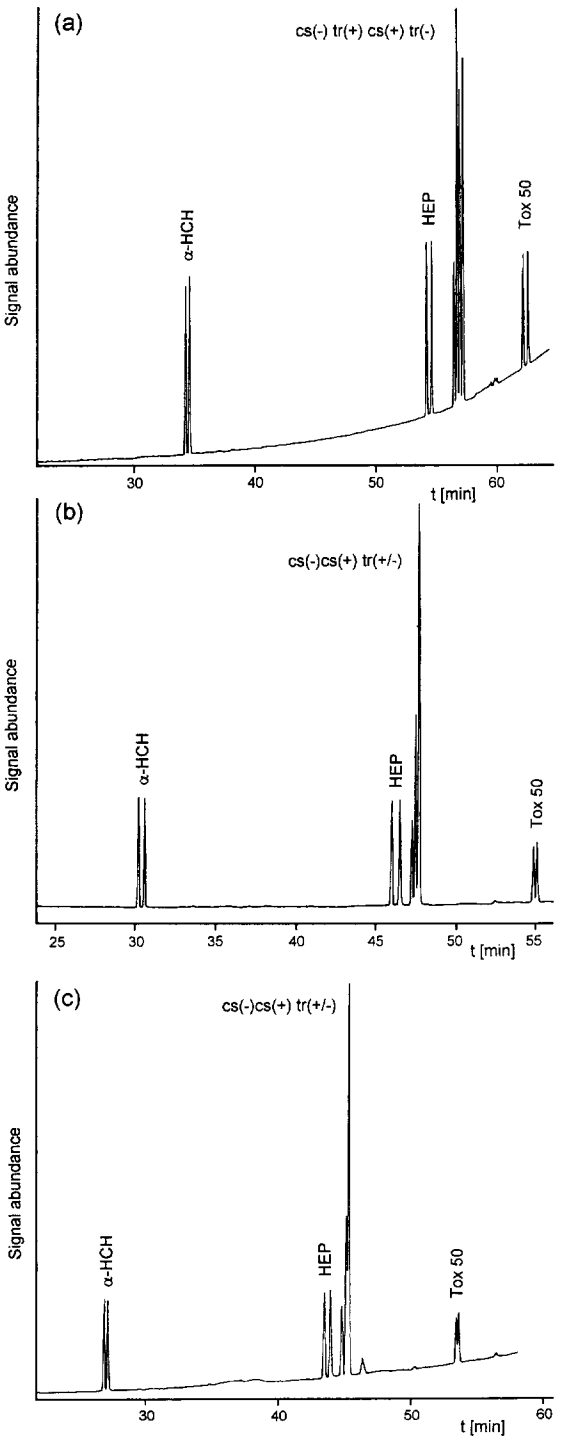

Fig. 2. Enantiomer selective separation of the polychlorinated pesticides present in the proposed test mixture on capillary (a), (b) and (c). For compound abbreviation, see Table 1.

phase composition (OV-1701-OH+TBDMS-CD). Table 1 summarises the achieved enantiomer separation and the elution order of the enantiomers of *cis*- and *trans*-chlordane on these three columns and the results for four more capillaries. The last three are the capillaries used for the separation of the herring oil shown in Fig. 3 and include two tandem capillaries. As can be seen from Fig. 2, the elution order of the chlordane isomers and enantiomers for capillary (a) and (b) is quite different. Besides a minor deviation in the length, the main difference is the use of two different batches of TBDMS-CD. The commercial capillary (c) is quite similar to (b) but completely different compared to the other commercial column (d) obtained from the same supplier.

Both capillaries (a) and (d) allow a separation of the enantiomers of the stereoisomers *cis*- and *trans*-chlordane. Both pairs of enantiomers were just separated from each other. However, the risk is high that small changes in the column properties or the temperature programme will cause a partial overlap preventing the determination of the *cis*- and *trans*-chlordane enantiomer ratio. The tandem columns (f) and (g) as well as capillary (e) with 50% TBDMS-CD separated the pairs of enantiomers of *cis*- and *trans*-chlordane far enough from each other to avoid such problems. However, capillary (e) showed no enantioselectivity for Tox 50 or any other toxaphene congener. The tandem capillaries are considered as the best compromise for the enantioselective separation of the largest possible number of polychlorinated pesticides and their isomers in routine analysis [20]. Furthermore, the capillary in front acts as a precolumn preventing a deposition of matrix residues on the chiral stationary phase and as a consequence its quick deterioration. Columns with high amounts of TBDMS-CD such as capillary (e) are quite sensitive against traces of matrix residues in the sample extracts. They are only to a limited extent suitable for real samples.

As can be seen from Fig. 3, not only the elution order of the enantiomers of *cis*- and *trans*-chlordane can be different on columns based on TBDMS-CD, but also of other octachloro isomers such as MC5 and MC7 (for their structures, see Fig. 3). Their relative tentative enantiomer elution order was determined by extracts which were isolated from different herring oils and which contained the en-

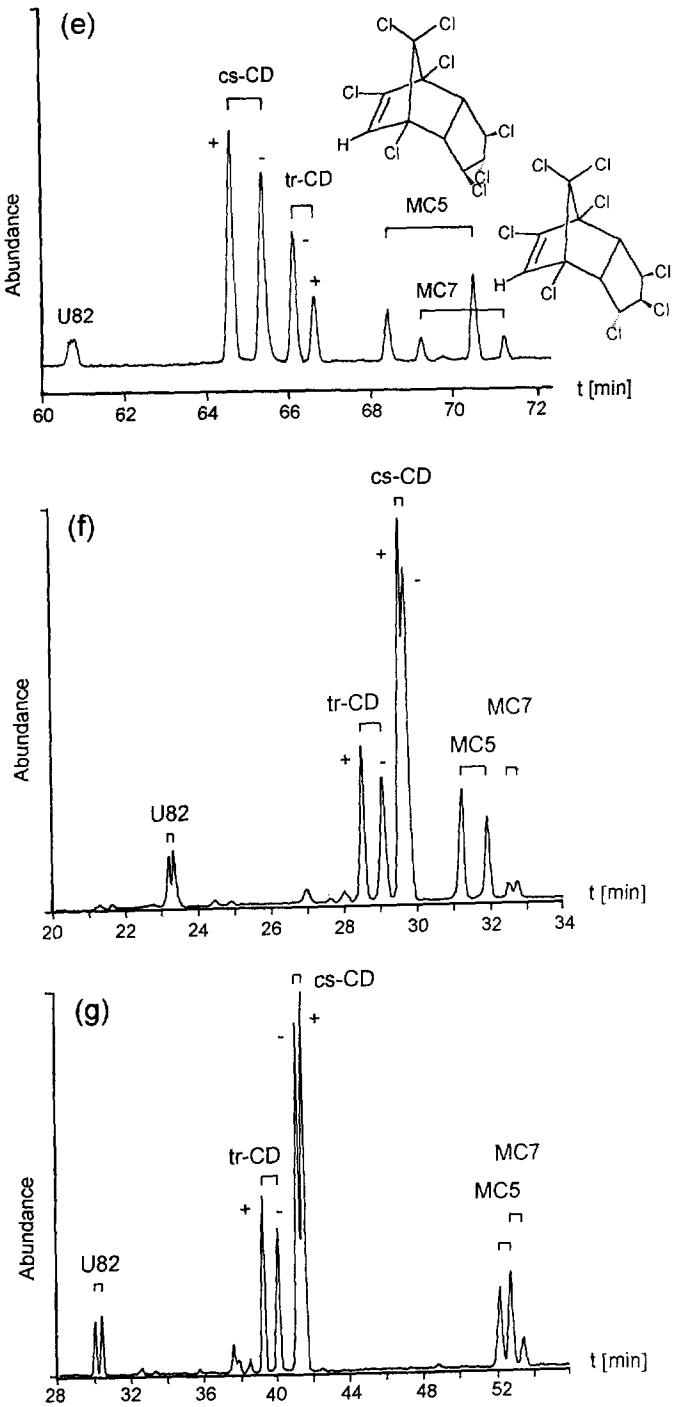

Fig. 3. Separation of the chlordane isomers U82, *cis*-, *trans*-chlordane, MC5 and MC7 in a herring oil sample [20] on the capillaries (e), (f) and (g) based on TBDMS-CD. The GC–NICI-MS mass chromatograms of *m/z* 408 are shown. For column details see Section 2.5. The temperature program was optimised for the best separation of MC5 and MC7. U82: Chlordane isomer of unknown structure, cs-CD, tr-CD: *cis*-, *trans*-chlordane.

antiomers in different concentration ratios (see also [20]). Work is in progress to prepare pure enantiomers of MC5 and MC7 from technical chlordane. The same herring oil as separated earlier on a tandem column (Fig. 2B in Ref. [20]) was injected into the columns (e) to (g). The enantiomers of MC5 and MC7 were completely separated on (e) and the tandem column (f) [20] while a co-elution was observed on tandem column (g) which did not allow to calculate any enantiomer ratio (see Fig. 3). Furthermore, the elution order of the *cis*- and *trans*-chlordane enantiomers changed as well.

3.2. Electrospray mass spectrometry

It was assumed that incomplete derivatisation of the β-cyclodextrin and/or by-products could be the reasons for the observed changes in the isomer and enantiomer selectivity. Using HPLC and high-temperature gas chromatography after trimethylsilylation, Deege et al. [23] found that partially alkylated β-cyclodextrins were present as by-products in peralkylated cyclodextrins. Blum and Aicholz [16] tried to determine the composition of TBDMS-CD by ^{252}Cf plasma desorption mass spectrometry. Only masses above m/z 4500 could be detected which is far beyond the mass of the completely derivatised molecule. They assumed that higher oligomers were present or formed during plasma desorption. They also reported signal broadening in the ^{1}H NMR spectra due to the presence of the homologues and isomers. An improved purity control was obtained by electrospray ionisation (ESI) mass spectrometry in the positive or negative ion mode. The latter is preferable since only the $[M-H]^-$ ion is formed. No further fragmentation of the cyclodextrins was observed with this ionisation technique allowing a direct characterisation of a mixture without separation. In the positive ion mode the ion adduct formation with different cations gives a more complex picture which complicates the interpretation of the mass spectra.

Fig. 4 shows the ESI mass spectrum of a batch of TBDMS-CD recorded in the negative ion mode. The range of double charged ions is presented. The mass differences of m/z 57 ($z=2$) correspond to m/z 114 ($z=1$) which is the mass of the *tert.*-butyldimethylsilyl rest. The most abundant ion is from the octa(2,3,6-tri-O-*tert.*-butyldimethylsilyl)β-cyclodextrin with a mass of 2046 giving for $z=2$ m/z 1023. A mixture containing 7 to 10 *tert.*-butyldimethylsilyl groups is present instead of 21 substituents for the persilylated structure. Other batches showed the same ions but with different abundance distributions. This confirms the assumption of Blum and Aicholz [16] about an incomplete derivatisation. The low number of introduced groups will give large batch-to-batch variations due a random distribution of the derivatised sites and a different yield of the formed homologues. It is assumed that this is the most likely reason for the observed changes in the isomer and enantiomer elution patterns shown in Figs. 2 and 3. Furthermore, the free OH groups will increase the adsorption of more polar compounds as also confirmed by the modified Schurig test. However, some of the capillaries made so far, had very unique separation properties such as capillary (a) which allowed the enantioselective separation of all chlordane congeners and most toxaphenes. At present, this cannot be achieved on any well-defined modified β-cyclodextrin. Therefore, TBDMS-CD has to be considered as an important phase for chiral separations of lipophilic polychlorinated pesticides despite the obvious drawback of a variable enantioselectivity. Work is in progress to produce better defined TBDMS-CD mixtures and/or to substitute them by other β-cyclodextrins.

A further problem is the stability of the randomly derivatised TBDMS-CD. The large number of remaining unreacted OH groups leads to a slow degradation over several weeks resulting in a loss of any enantioselectivity. However, once a capillary is coated with the stationary phase mixture, its properties do not change any longer, and capillaries of excellent thermal stability (up to 260°C) are obtained. It is assumed that the reaction of the free OH groups with the added crosslinking agent (see Section 2.5) is responsible for this stabilisation. This indicates that a partial derivatisation of β-cyclodextrins might be even advantageous in the preparation of stable stationary phases.

Acknowledgments

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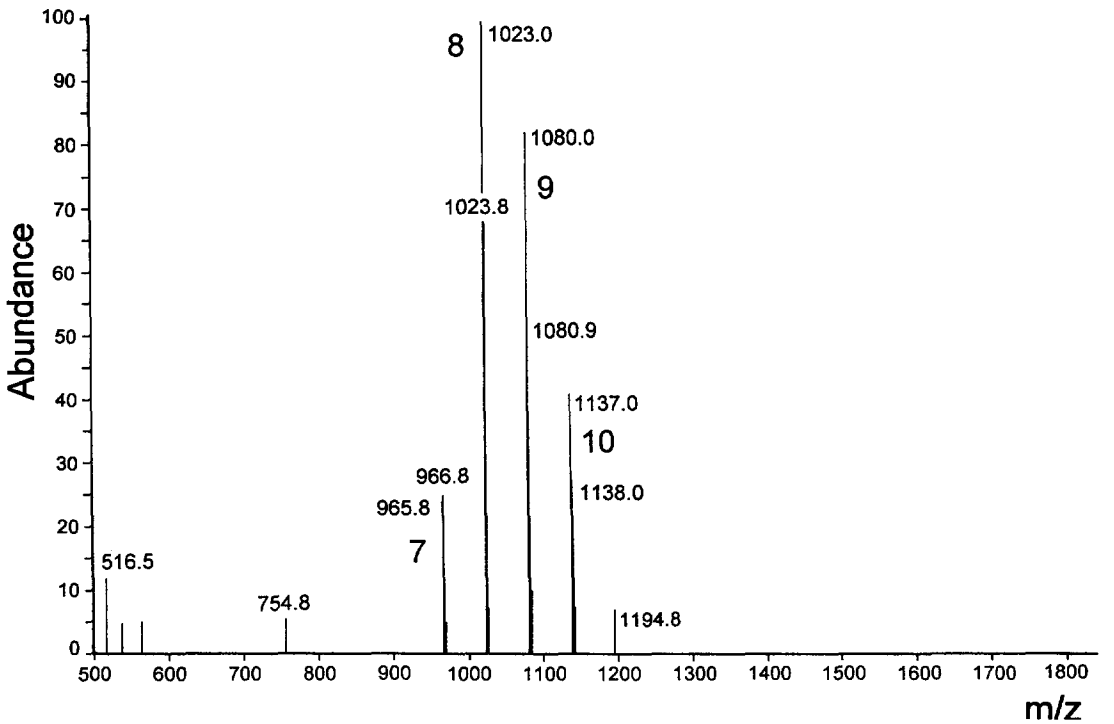

Fig. 4. Mass spectrum of a TBDMS-CD batch obtained by ESI-MS in the negative ion mode. The range of double charged ions is shown. Only relative abundances over 5% are presented. TBDMS-CD homologues with 7 to 10 *tert.*-butyldimethylsilyl rests are marked. The mass difference $m/z = 57$ ($z = 2$) corresponds to $m/z = 114$ ($z = 1$). The mass m/z 1080 ($z = 2$) is from nona(2,3,6-tri-O-*tert.*-butyldimethylsilyl)β-cyclodextrin [m/z 2160 ($z = 1$)].

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