Characterisation of partially ethylated γ-cyclodextrins, a well suited alternative for the enantioselective separation of toxaphenes by HRGC

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Introduction

The enantioselective analysis of compounds of technical toxaphene (CTTs) has gained in interest in recent years since enantiomer ratios can be changed by enantioselective metabolisation in biota [1,2]. The first enantiomer separation of CTTs by high resolution gas chromatography (HRGC) was accomplished in 1994. Only a very limited number of phases is able to separate toxaphene congeners into enantiomers. For a long time, randomly derivatized heptakis-(2,3,6-*O*-*tert.*-butyldimethylsilyl)-β-cyclodextrin (TBDMS-CD) [3] was thought to be the only cyclodextrin derivative which is able to separate most CTTs into enantiomers [2].

Very recently, not completely derivatised heptakis-(2,3-*O*-dimethyl-6-*O*-*tert.*-butyldimethylsilyl)-β-cyclodextrin (2,3M6T-CD) was introduced as an alternative for the enantiomer separation of CTTs [4,5]. However, both TBDMS-CD and 2,3M6T-CD do not separate all important CTTs. In addition, these phases are not well defined and consist of a mixture of differently substituted isomers and congeners. Therefore, the availability of complementary chiral stationary phases of known and reproducible composition is highly desirable.

In order to elucidate the composition of cyclodextrins, a HPLC-method was developed with light scattering or mass spectrometric detection [6,7]. During the search for alternatives for TBDMS-CD incompletely derivatised batches of oktakis(2,3,6-tri-*O*-ethyl)-γ-cyclodextrin (TEG-CD) were found to be very suitable for the enantioselective analysis of CTTs and other polychlorinated pesticides [7].

Material and Methods

Reference compounds and solutions: Batches of ethylated γ-cyclodextrin with variable degree of ethylation were synthesised and fractionated further by silicagel column chromatography (Dr. Mark, Worms, Germany). HPLC water was obtained from an Elgastat maxima HPLC water purification unit (Elga Ltd., Bucks, England) and methanol (pesticide grade) from sds (Peypin, France). A toxaphene reference standard was used containing 400 pg each of 23 toxaphene congeners (Parlar no. 11, 12, 15, 21, 25, 26, 31, 32, 38, 39, 40, 41.1/42.2, 42, 44, 50, 51, 56, 58, 59, 62, 63, 69) in cyclohexane (Ehrenstorfer, Augsburg, Germany). Single congeners obtained from Ehrenstorfer (1 ng/µl in cyclohexane) and Promochem (Wesel, Germany, 5 ng/µl in iso-octane) were used to identify the elution order. All standards were diluted to 100 pg/µl with iso-octane of pesticide quality (Scharlau, Barcelona, Spain).

Purity control of cyclodextrin derivatives: The ethylated γ-cyclodextrins were characterised by reversed phase HPLC (Nucleosil 100-5, C_{18}, normal densitiy, particle size 5 µm, pore size 100 Å,

125 x 2 mm, Macherey-Nagel, Oensingen, Switzerland) using a gradient from methanol/water (90:10, v/v) to 100 % methanol within 7 min. Detection was performed with an evaporative light scattering detector (ELSD, Polymer laboratories, Shropshire, UK, 45 °C, air flow 3 l/min) or by a Finnigan LCQ LC-MS. Any fragmentation was suppressed by using atmospheric pressure chemical ionisation (APCI) in the positive ion mode without the corona discharge current switched on. Further analytical details are given in [7]. Compared to electrospray ionisation, the formation of undesired ion adducts is greatly suppressed.

HRGC columns: Capillaries (12 m length, 0.25 mm ID, 0.15 μm film thickness) were home-made as described in [7] diluting the ethylated γ-cyclodextrin in OV 1701-OH (Fluka, Buchs, Switzerland) in a ratio 1+4 (w/w). The columns were evaluated by the Grob-test and by a special test mixture containing polychlorinated pesticides [7].

HRGC separation: Separations were carried out on a Varian 3800 gas chromatograph (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector. The injector was kept at 160 °C and the detector at 280 °C. Nitrogen at a flow of 30 ml/min was used as detector make-up gas and He at a constant flow of 0.9 ml/min (30 cm/s) as carrier gas. The separation conditions were as follows: Splitless injection of 1 μl; splitless time 2 min; temperature programme, 2 min isothermal at 100 °C, then 2.5 °C/min to 230 °C (isothermal until the last compound eluted).

Results and discussion

Capillaries based on TBDMS-CD are frequently used for the enantioselective analysis of polychlorinated pesticides. Besides all chiral chlordane compounds, most toxaphene congeners are separated into enantiomers, too. This phase, however, is described as randomly derivatised [3], which leads to considerable batch to batch variations resulting in inconsistent separation properties [8]. Nevertheless, due to its unique separation properties, TBDMS-CD is of great importance for the enantioselective analysis of CTTs. The newly introduced alternative phase 2,3M6T-CD showed only a favourable enantioselectivity when not completely purified batches were employed. The pure product was far less suited for enantioselective toxaphene analysis [5].

During the investigation of alternative phases, differently alkylated cyclodextrins were characterised with HPLC-ELSD and LC-MS, and capillary columns were made from various batches. The type of cyclodextrin (α, β or γ) and substituent influenced the observed degree of alkylation [6,9]. Especially ethylated γ-cyclodextrin consisted of a complex mixture of under-ethylated products which could be fractionated further. The HPLC chromatogram of a polar fraction is shown in Figure 1. Its composition is quite complex and co-elutions of isomers are observed. Almost no fully derivatised product is present, the most abundant congeners lack 4-5 ethyl groups. Only underethylated γ-cyclodextrin was found to be able to separate numerous toxaphene congeners into enantiomers.

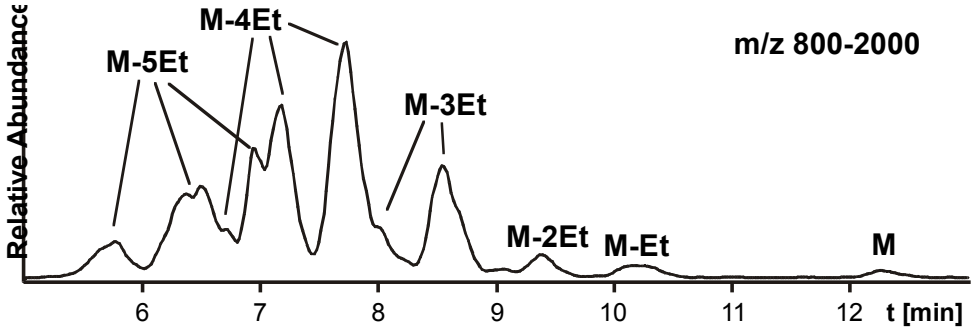

Figure 1: HPLC-MS base peak chromatogram of a partially ethylated γ-CD; M: perethylated TEG-CD, Et: ethyl group. Only the most abundant congeners are assigned.

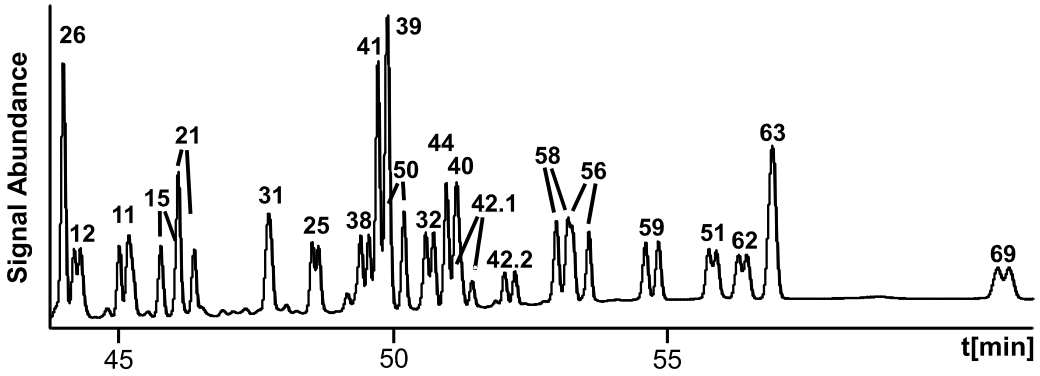

Figure 2: HRGC-ECD chromatogram of a mixture consisting of 22 toxaphene congeners. Column: 25 % partially ethylated γ-cyclodextrin in OV 1701-OH, for details see experimental.

Completely ethylated γ-cyclodextrin lacked any enantioselectivity for CTTs. On mixed phases containing about 60 % fully derivatised TEG-CD (the rest was mainly mono-underethylated), still no enantiomer separation could be observed. A further reduction of the degree of ethylation (20 % perethylated, 20 % mono- and tri-underethylated, 35 % di-underethylated product) led to the enantiomer separation of Parlar no. 15, 42.2, 58, 56 and peak splitting for Parlar no. 32 and 50. The gas chromatogram of an even less ethylated fraction (for exact composition, see Figure 1) is shown in Figure 2. Besides the above mentioned CTTs, this column separated also Parlar no. 11, 15, 21, 38, 42 (42 is separated into 4 peaks without overlapping!) and 59. The obtained resolutions are presented in Table 1. Moreover, for Parlar no. 12, 25, 51, 62 and 69 peak splitting could be observed.

Obviously, a mixture containing mainly 4-5 free hydroxyl groups offers the best separation properties for CTTs as well as for chlordanes [7]. It is noteworthy that a considerably improved enantiomer separation for CTTs was also observed for not purified 2,3M6T-CD containing residual OH-groups [5].

Table 1: Enantiomer resolution Rs of CTTs on partially ethylated γ-cyclodextrin. R_S values were calculated as $R_S = 1.18\Delta t\ (w_{b1} + w_{b2})^{-1}$, where Δt = time difference between enantiomer peaks, w_{b1},w_{b2} = peak width at half hight

Parlar no.	11	15	21	32	38	42.1	42.2	50	56	58	59
R_S	0.85	1.81	1.84	0.82	0.89	1.33	1.22	1.74	1.75	1.24	1.31

Furthermore, batches with a comparable HPLC elution pattern showed similar separation properties, thus indicating that the reproducibility of the exact composition is not too critical.

This is the first time that an alkylated cyclodextrin without a bulky substituent in position 6 shows a good enantiomer separation of CTTs. Compared to TBDMS-CD, the elution order of the CTTs on TEG-CD is quite different. Moreover, the resolution R_S does not exceed about 1.5 in opposite to TBDMS-CD where extreme R_S >5 were observed [10]. Unfortunately, Parlar no. 26 which is abundant in biota cannot be split into enantiomers. On the other hand, the TEG-CD column shows complementary properties and is able to separate Parlar no. 58 and 42.1 which is not possible with TBDMS-CD.

The enantioselectivity of TBDMS-CD deteriorated a few weeks after synthesis due to decomposition [8] (not valid for prepared columns), which impeded composition analysis. In contrast, columns with identical properties can be made from stored TEG-CD coating solutions over weeks or from the pure cyclodextrin over months.

Acknowledgement

The financial support by the Swiss Science Foundation under the project no. 20-50474.97 is gratefully acknowledged. In addition, we like to thank Ruth Baycan-Keller from our group for preparing the toxaphene standards.

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