Isomer and Enantiomer Specific Separation of 16 Toxaphene Congeners by HRGC on Different Stationary Phases

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Abstract

The elution order of 16 toxaphene congeners (Parlar no. 21, 25, 26, 31, 32, 38, 39, 40, 41, 42, 44, 50, 51, 56, 58, 62) was determined on the following stationary phases: 5%-diphenyl-95%-dimethyl-polysiloxane (Ultra 2), a smectic phase (N,N'-bis(p-butoxy-benzylidene)-α,α'-bis-p-toluidine), 10%-phenylcyanopropyl-90%-biscyanopropyl-polysiloxane (RTx-2330) and on heptakis(2,3,6-O-t-butyl-dimethylsilyl-β-cyclodextrin diluted in OV-1701-OH using two different temperature program rates. Substantial changes in the elution order were found for the different phases. This can be useful for additional confirmation of the congener identity or to avoid co-elution. The modified cyclodextrin phase separated all chiral congeners into enantiomers except Parlar no. 42 and 58. However, several co-elutions between enantiomers of different congeners were observed.

Introduction

The determination of toxaphene in environmental samples by high resolution gas chromatography (HRGC) is very challenging due to interferences between congeners. On the most frequently applied stationary phase for toxaphene separation, 5%-diphenyl-95%-dimethylpolysiloxane, some co-elutions are observed (see also Figure 1a,d). Different approaches have been used to improve the selectivity of the toxaphene analysis such as HRGC combined with mass spectrometry, multidimensional HRGC or pre-separation by high performance liquid chromatography[1-3]. However, little is known about the elution order of toxaphene congeners on more polar stationary phases. Due to different interactions a change of the elution order should be possible. In this way interferences by co-elutions can be eliminated, and the presence of a given congener in real samples can be confirmed on different phases. Furthermore, most toxaphene congeners are chiral, and, therefore, an additional enantioselective separation is requested.

In this work the elution order of 16 toxaphene congeners (Parlar no. 21, 25, 26, 31, 32, 38, 39, 40, 41, 42, 44, 50, 51, 56, 58, 62) is compared on the following stationary phases: Ultra 2 as reference phase (5%-diphenyl-95%-dimethylpolysiloxane), a smectic phase (N,N'-bis(p-butoxy-benzylidene)-α,α'-bis-p-toluidine), RTx-2330 (90%-biscyanopropyl-10%-phenylcyanopropyl-polysiloxane) and heptakis(2,3,6-O-t-butyldimethylsilyl)-β-cyclodextrin (TBDMS-CD) diluted in OV-1701-OH. Many of the selected toxaphene congeners are persistent in biota. The selected chiral phase TBDMS-CD has already been used successfully for the enantioselective separation of chiral toxaphenes[4-7]. To study possible influences of the temperature program on the separation and elution order, a slow and fast heating rate were applied. The results are presented and discussed.

Experimental Methods

Standards and chemicals: A toxaphene reference standard was used containing 400 pg of each congener given in Table 1 in cyclohexane as well as Parlar no. 11, 12, 15, 59, 63 and 69 (Ehrenstorfer, Germany). Single congeners obtained from Ehrenstorfer (1 ng/µl in cyclohexane) and Promochem (Germany, 5 ng/µl in iso-octane) were used to identify the elution order on the tested columns. Dilutions were made with iso-octane of pesticide quality (Scharlau, Germany) resulting in concentrations of about 100 pg/µl.

Instrumental: Separations were carried out on a Hewlett-Packard 6890 gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD). Helium (99.999 % purity) was used as carrier gas and nitrogen (99.999 % purity) as make-up gas. The temperature of the split/splitless injector was 150°C and 250 °C for the ECD. Splitless injections of 1µl were performed manually at 100°C. The isothermal period and splitless period were 2 minutes followed by a temperature programming with 1°C/min or 10°C/min to 240°C. This temperature was kept until the last congener eluted. The constant flow modus was used applying the following carrier gas flow rates: 0.8 ml/min (Ultra 2), 1.7 ml/min (smectic phase), 1.8 ml/min (RTx-2330) and 2 ml/min (TBDMS-CD capillary).

Table 1: Numbering, structure and suppliers of the studied toxaphene congeners

Parlar no.	Structure[8)]	Supplier
21	(±)-2,2,5,5,9,10,10-heptachlorbornane	E
25	(±)-2,2,3-exo-trichloro,5-endo-chloromethyl,6-(E)-chloromethylen,5-dichloromethyl,8,9,10-trinorbornane	E
26	(±)-2-endo,3-exo,5-endo,6-exo,8,8,10,10-octachlorobornane	E
31	(±)-2,2,3-exo-trichloro,6-(E)-chloromethylen,5,5-bis(dichloromethyl),8,9,10-trinorbornane	E
32	(±)-2,2,5-endo,6-exo,8,9,10-heptachlorobornane	P
38	(±)-2,2,5,5,9,9,10,10-octachlorobornane	E
39	(±)-2,2,3-exo,5-endo,6-exo,8,9,10-octachlorobornane	E
40	(±)-2-endo,3-exo,5-endo,6-exo,8,9,10,10-octachlorobornane	E
41	(±)-2exo,3-endo,5-exo,8,9,9,10,10-octachlorbornane	E
42	(±)-2,2,5-endo,6-exo,8,8,9,10-octachlorobornane (±)-2,2,5-endo,6-exo,8,9,9,10-octachlorobornane	E
44	(±)-2-exo,5,5,8,9,9,10,10-octachlorobornane	E
50	(±)-2-endo,3-exo,5-endo,6-exo,8,8,9,10,10nonachlorobornane	P
51	(±)-2,2,5,5,8,9,10,10-octachlorobornane	E
56	(±)-2,2,5-endo,6-exo,8,8,9,10,10-nonachlorobornane	E
58	(±)-2,2,3-exo,5,5,8,9,10,10-nonachlorobornane	E
62	(±)-2,2,5,5,8,9,9,10,10-nonachlorobornane	E

E: Ehrenstorfer; P: Promochem

Capillary columns: The following columns were employed: 1) *Ultra 2:* 25 m length x 0.2 mm i.d., coated with 0.11 µm 5%-diphenyl-95%-dimethylpolysiloxane (Hewlett Packard). 2) *Smectic phase:* 12 m length x 0.25 mm i.d., coated with 0.15 µm OV-1701 containing 15 % BBBT (N,N`-bis(p-butoxy-benzylidene)-α,α`-bis-p-toluidine). 3) *Rtx-2330:* 30 m length x 0.25 mm i.d., coated with 0.2 µm 10%-phenylcyanopropyl-90%-biscyanopropyl polysiloxane (Restek). 4) *Enantioselective phase:* home-made, 25 m length x 0.25 mm i.d., coated with 0.15 µm of OV-1701-OH with 10% heptakis(2,3,6-O-t-butyldimethylsilyl)-β-cyclodextrin (TBDMS-CD).

For a better visibility, the chromatograms were plotted by computer using the optimization program Pro ezGC for Windows (Analytical Innovations, Inc., USA). Signal overlap is indicated by a corresponding increase of the intensity and also the correct signal width is given. The enantiomer resolution R_s was calculated using the equation: $R_s = 1.177 \cdot \Delta t \cdot (w_{h1} + w_{h2})$, where Δt is the retention time difference between the second and first eluting enantiomer and w_h the signal width at half height.

Results and discussion

Isomer selective separation: Figure 1 shows the elution sequences obtained with a temperature rate of 1 °C/min and 10 °C/min on the achiral stationary phases. Compared to Ultra 2, a very substantial change of the elution order of the toxaphene congeners is observed for the smectic phase BBBT and RTx-2330. The information available at present did not allow to find any relation between the elution order and the structure for any of the phases. The increase of the temperature rate to 10 °C/min had no influence on the separation sequence on Ultra 2 and RTx-2330 indicating that the influence of temperature is weak on the interactions between compound and stationary phase. However, on the smectic phase changes in the elution order were observed leading to two more signal overlaps (Parlar no. 44 with 41,42, Parlar no. 56 with 58). Compared to Ultra 2, Parlar no. 40 is easily separated from other congeners both on RTx-2330 and BBBT. Furthermore, there is no risk of interference of Parlar no. 32 by 31 on BBBT. On RTx-2330 no. 42 could not be assigned since the single congener standard showed five signals.

Isomer and enantioselective separation: As can be seen from Figure 2 and Table 2, the isomer elution order on TBDMS-CD diluted in OV-1701-OH is different once more. The addition of 10 % TBDMS-CD changes the polarity considerably compared to pure OV-1701-OH[9]. Furthermore, the picture is now complicated by the additional separation into enantiomers. The increased number of signals leads to several overlaps preventing the determination of enantiomer ratios. Except for Parlar no. 39 and 40 at least one enantiomer is completely free of interferences by other congeners. Of the most abundant congeners in marine biota, Parlar no. 26, 50 and 62, no co-elutions are observed for no. 26 and 62. The enantiomers of no. 50 are interfered by no. 21 or 32. The latter is easily metabolized in biota and therefore often not present in real samples. The abundance of no. 21 is not known yet.

Of all 16 congeners, only Parlar no. 58 could not be separated into enantiomers. Some of the observed enantiomer resolutions R_s belong to the largest observed so far for HRGC (see e.g. no. 25 and 31) indicating a very strong interaction of the stationary phase with the trinorbornane structure. Compared to earlier achieved results on the same stationary phase[6,10], a substantial improvement was observed with the capillary applied here. An increased temperature program rate of 10 °C/min led to shorter retention times but higher elution temperatures resulting in weaker interactions between the chiral phase and the enantiomers giving decreased R_s values and a different elution order.

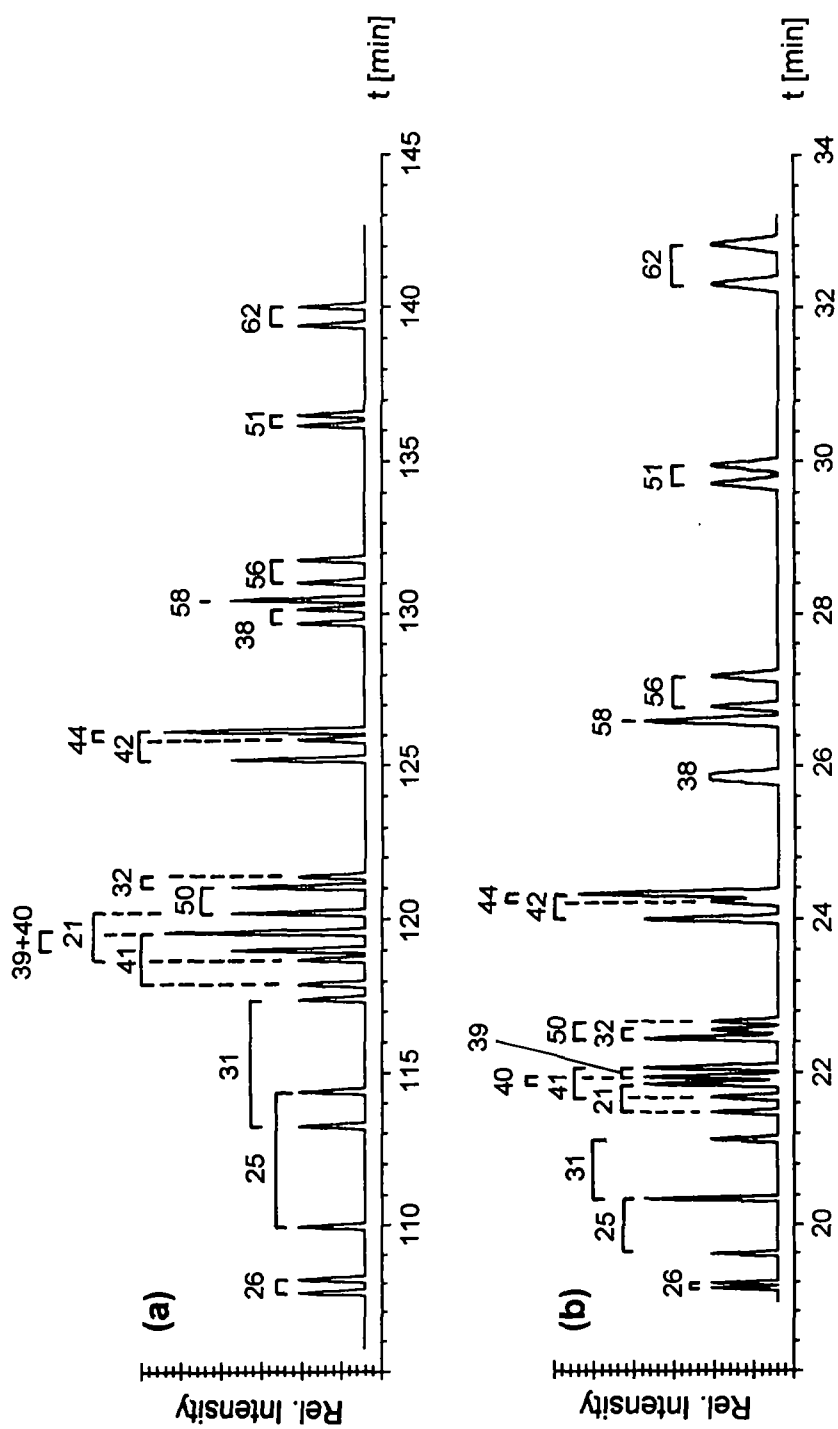

Figure 2: Isomer and enantiomer selective separation of 16 toxaphene congeners on the chiral phase TBDMS-CD using a temperature program rate of 1 °C/min (a) and 10 °C/min (b). Co-elution of one or more congener/enantiomer are indicated by a corresponding change of the signal height.

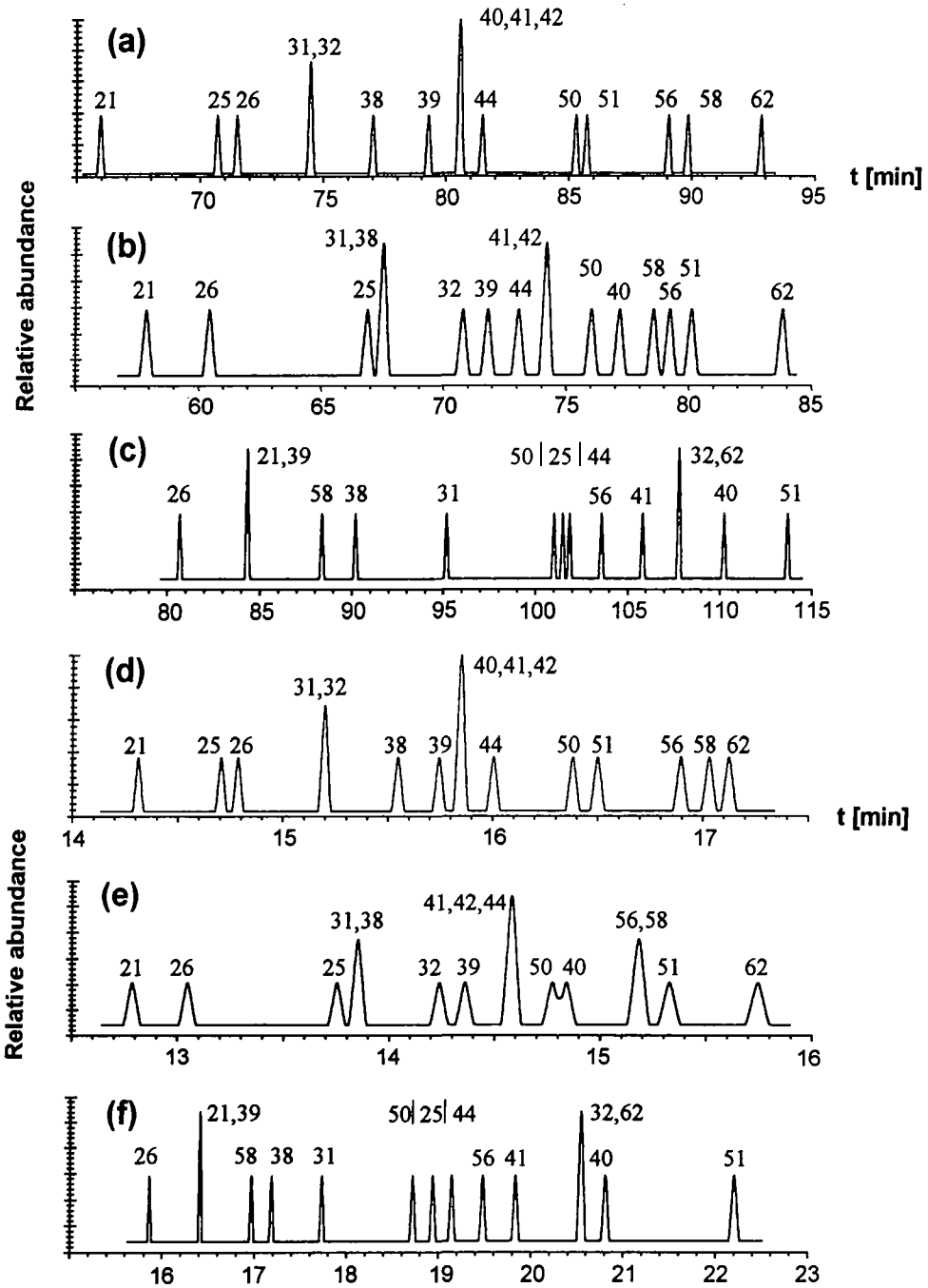

Figure 1: Elution order of 16 toxaphene congeners on three different achiral stationary phases at a temperature program rate of 1 °C/min (a-c) and 10 °C/min (d-f). Co-elutions of congeners are indicated by a change of the signal height. (a,d): Ultra 2; (b,e): smectic phase, (c,f): RTx-2330

Table 2: Retention times R_T of toxaphene enantiomers separated on TBDMS-CD as well as enantiomer resolution values R_s for a temperature program rate of 1 and 10 °C/min.

Congener Parlar no.	R_T [min] (1 °C/min)	R_T [min] (10 °C/min)	R_s (1 °C/min)	R_s (10 °C/min)
21	118.6/120.1	21.4/21.8	4.95	3.87c
25	109.8/114.3	19.5/20.3	14.09	5.99c
26	107.6/108.0	19.0/19.1	1.63c	1.21
31	113.1/117.3	20.3/21.0	13.27	5.95c
32	120.9/121.3	22.4/22.5	1.14c	0.81c
38	129.6/130.1	25.6/25.8	1.61	1.11
39	118.9/119.5	21.9/22.0	1.85c	0.89c
40	118.9/119.5	21.8/21.9	1.85c	0.66c
41	117.8/119.5	21.6/22.0	5.38c	2.91c
42A	125.1	23.9	c[a])	c[a])
42B	126.1	24.2	c[a])	c[a])
44	125.8/126.1	24.1/24.2	0.84c	0.62c
50	120.1/120.9	22.4/22.6	2.67c	1.59c
51	136.2/136.5	29.6/29.8	0.96	0.73
56	130.9/131.7	26.7/27.0	2.45	1.95
58	130.4	26.5	0	0
62	139.4/140.0	32.0/32.5	2.37	1.64

c: co-elution with enantiomers of other congeners. This might affect R_s; [a]) Parlar no. 42 consists of two isomers (toxicant A_1 and A_2), an assignment was not possible.

Acknowledgment

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