Enantioselective separation of toxaphene congeners. Results, problems and challenges

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1. Introduction

Several studies in the past have shown that the enantiomer ratio of pesticides applied as racemates is changed during bioaccumulation/metabolization. Examples are α-hexachlorocyclohexane and chlordanes[1,2]. Another important pesticide which has been used in huge quantities is toxaphene. Most of the large number of toxaphene congeners present in the technical mixture are chiral[3]. The enantiomers of a congener might have different properties concerning accumulation and toxicology. Therefore, an enantioselective separation and quantification is important. It could be shown that the two most abundant toxaphene congeners in seal blubber still are racemic[4]). However, nothing is known about changes in the enantiomer ratios of toxaphenes in marine biota such as fish. In cod liver and cod liver oil a larger number of congeners are present than in seal blubber.

Own preliminary studies have shown, that even small changes in the steric structure of toxaphenes change drastically the enantioselective interaction with the stationary phase. As part of the work to find a stationary phase which is able to separate most toxaphenes into enantiomers, some factors were studied which influence the enantioselectivity of stationary phases based on dimethyl-t-butylsilylated ß-cyclodextrin. This chiral compound has given the best enantioselectivity for toxaphenes so far.

2. Experimental

Standards: Single toxaphene congeners were obtained from Ehrensdorfer (Augsburg, Germany) or the group of Nikiforov and co-workers[5] via Promochem (Wesel, Germany). The concentrations were 1 or 5 ng/µl in iso-octane. The origin of the standards is marked in Table 1. The applied nomenclature is according to references [6,7].

Separation capillaries:
The surface of fused silica capillaries with 0,25 mm i.d. was either completely untreated or leached with 20% HCl and afterwards persilylated according to the procedure described by Blum[8]. The surface was statically coated with the following stationary phases:
Capillary Type 1: Capillary of 25 m using a solution of 2,5‰ PS086 (a 85% methyl 15% phenyl OH-terminated polysiloxane) and 0,25‰ dimethyl-t-butylsilylated ß-cyclodextrin (DBSCD), prepared according to Blum and Aicholz[9] 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 0,14 µm.

Capillary type 2: Same as no. 1 except that 2,5‰ of OV-1701-OH ((14% phenyl cyanopropyl-, 86% dimethyl polysiloxane) was used.
Capillary type 3: Capillary of 16 m, untreated surface, coated with a solution of 2,5‰ of OV-1701-OH and 1,4 ‰ of BSCD (56 % of stationary phase content).

Instrumentation: A part of the separations on the type 1 capillaries were carried out on a Hewlett-Packard (HP) 5890II gas chromatograph and the compounds detected with electron capture negative ion (ECNI) mass spectrometry on a HP 5987 GC/MS using CH_4 at a pressure of 0,45 torr and an ion source temperature of 200°C. For quantification the $(M\text{-}Cl)^-$ and $(M\text{-}Cl+2)^-$ ions were employed. For all other capillaries a HP 6890 gas chromatograph with an electron capture detector (ECD) was used.

Separation conditions: The separation conditions were as follows: Injector temperature, 220°C; transfer line temperature, 260°C (if adequate); ECD temperature 300°C; splitless injection of 1 µl at the start temperature given below, 2 min splitless time. Temperature program type 1: 90-160°C at 30°C/min, 160-190°C at 1°/min, 190-210°C, 0,5°C/min, 10 min isothermal, and 210-240°C at 5°C/min; type 2: 90-160°C at 30°C/min, 160-200°C at 3°C/min, 200-240°C at 1°/min, 10 min isothermal, type 3: 90-160°C at 30°C/min, 160-180°C at 2°C/min, 30 min isothermal, 180-220°C at 4°/min, 5 min isothermal.

3. Results and discussion

The first reported enantioselective separation of Tox 26 and 50 was carried out on a glass capillary of 16 m length and 0,32 mm i.d. coated with 2,5‰ PS086 and 0,25‰ DBSCD (capillary type 1) and a fused silica capillary of 20 m length and 0,25 mm i.d. coated with 2,5‰ OV-1701-OH and 0,25‰ DBSCD (capillary type 2)[4]. It could be shown that the dilution of the cyclodextrin in a medium-polar phase such as OV-1701 allowed to separate more congeners into enantiomers than in the non-polar polymethylsiloxane PS086[4]. However, OV-1701 is less favorable for the separation of toxaphene isomers and gives more co-elutions between pairs of enantiomers[10]. Since there is a larger number of major toxaphenes present in cod liver and cod liver oils, the enantioselective separation of most of the single congeners available at present was studied on different capillaries (see experimental). The best results obtained so far are summarized in Table 1. Figure 2 shows some selected separations. Based on these results the following conclusions can be drawn:

- A content of 10% of the chiral modificator is sufficient for the enantioselective separation (type 1 and 2 capillaries). Higher amounts influence too much the polarity of the basic stationary phase.
- On non-deactivated fused capillaries with a low surface activity[11] mixtures of more than 50% of DBSCD in OV-1701 still give films which are stable up to 240°C. Though these columns are excellent for the separation of chiral chlordanes, they do not show any longer an enantioselectivity for toxaphenes. A similar capillary with only 10% DBSCD was able to separate Tox 26 and 50 into enantiomers[4].
- Capillaries prepared with the same batch of DBSCD but at different times showed a deviating enantioselectivity. Freshly synthesized DBSCD gave better columns than the same batch after a storage period of some months. However, after preparation the capillaries were stable and did not show any change of the enantioselectivity.
- Capillaries coated with different batches of DBSCD showed also different enantioselectivities. A similar problem was also observed earlier for chlordanes.

DBSCD prepared after the standard procedure of Blum and Aicholz[9] is obviously not pure enough and degrades slowly. Furthermore, the introduction of the rather bulky t-butyl groups leads to a not reproducible derivatisation reaction which stops at random due to steric hindrance. Another problem might be the blocking of the DBSCD cavity by chiral by-products. Therefore, work is now in progress to improve the purity of DBSCD and to determine its exact composition and steric structure.

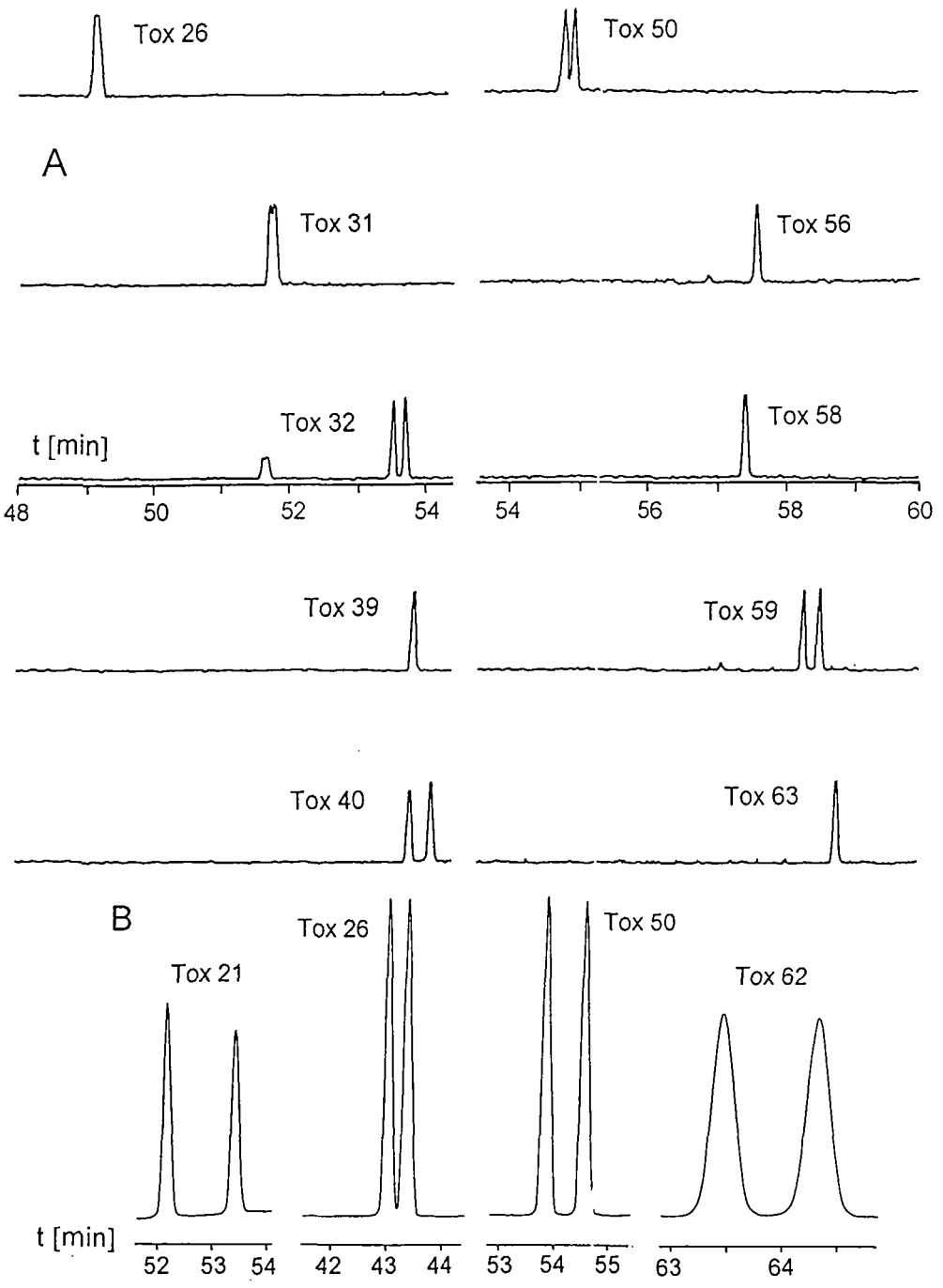
Table 1. Enantioselective resolution of selected toxaphene congeners on capillaries coated with 10% DBSCD in different polysiloxanes. An abbreviated nomenclature is added in parentheses. Primed positions are endo. E: obtained from Ehrendorfer, P: obtained via Promochem.

Compound[a]	Origin	Enantiomer Resolution R	
		PS086/DBSCD	OV-1701/DBSCD
Tox 21 (2,2',5,5',8,10,10)	E	8.2	4.6
Tox 26 (2,3',5,6'-8,8,10,10)	P	n.s.	1.45
Tox 31 (2,2',3'-8,8,9,9,10)	E	0.34	n.s.
Tox 32 (2,2',5',6-8,9,10)	P	1.3	0.87
Tox 38 (2,2',5,5'-8,8,10,10)	E	5.9	1.5
Tox 39 (2,2',3,5',6-8,9,10)	E	n.s.	n.d.
Tox 40 (2,3',5,6'-8,9,10,10)	E	2.6	1.2
Tox 41 (2,3',5-8,8,9c,10,10)	E	2.9	6.2
Tox 42A (2,2',5',6-8,9,9,10)	E	n.s.	n.s.
Tox 42B (2,2',5',6-8,8,9,10)	E	1.06	2.2
Tox 44 (2,5,5'-8,8,9,10,10)	E	4.2	n.d.
Tox 50 (2,3',5,6'-8,8,9,10,10)	P	1.4	2.9
Tox 51 (2,2',5,5'-8,9,10,10)	E	n.s.	0.7
Tox 56 (2,2',5',6-8,9,9,10,10)	E	n.s.	3.0
Tox 58 (2,2',3,5,5'-8,9,10,10)	E	n.s.	n.s.
Tox 59 (2,2',5',6-8,8,9,10,10)	E	1.9	1.2
Tox 62 (2,2',5,5'-8,8,9,10,10)	P	2.2	2.3
Tox 63 (2,3,5',6-8,8,9,10,10)	E	n.s.	2.1
Tox 2439 (2,5,5',9,9,10,10)	P	2.2/4.3	1.3[b]
Tox 3157 (2,3',6'-8,9,10,10)	P	2.9	2.5
Tox 6551 (2,2',5,5'8,9,9,10,10)	P	2.0	2.4
Tox 6535 (2,2',5,5',9,9,10,10)	P	2.8[c]	1.5[c]
Tox 7047 (2,2',3,5,5'-9,9,10,10)	P	4.0/0.65[d]	3.0/1.3[d]

[a] Two digit number, nomenclature according to Parlar et al.[6], four digit numbers, nomenclature according to Nikiforov et al.[7], [b] consists of two isomers, only the second separated on type 2, [c] enantiomer ratio not racemic, [d] consists of two isomers. n.s.: not separated, n.d.: not determined at the moment.

Recently, Coelhan and Parlar published a different numbering scheme for polychlorinated bornanes by claiming that the nomenclature used so far is not in accordance with IUPAC nomenclature[3]. Until 1995 Parlar has applied another numbering system which has been generally accepted and used. If the now proposed numbering system is the correct one, it is very important to avoid a confusing use of the old and new nomenclature in parallel.

TOXA II



Figur 1. Enantioselective separation of selected toxaphene congeners on (A) PS086/DBSCD by EC-NI detection and (B) on OV-1701/DBSCD by ECD.

Of the 23 toxaphenes studied so far, 7 congeners could not be separated on PS086 with 10% DBSCD. Tox 26 was separated on a similar glass capillary but not on this fused silica capillary. It can occasionally be separated with this capillary type. As mentioned before, small changes in the compostion and the content of impurities as well as a slow degradation over time seem to be responsible for the observed differences. They are much more pronounced for toxaphenes than for chiral chlordanes. By exchanging the non-chiral polysiloxane PS086 with OV-1701, more congeners could be separated into enantiomers. However, on both column types overlaps between enantiomer pairs of different isomers were observed. They are more severe than on the pure polysiloxane phases without DBSCD (see also figure 3 in [4] as example and [10]). Therefore, a better isomer selectivity has also to be achieved before real samples can be analysed.

5. References

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