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Liquid chromatographic profiles of individual compounds of technical toxaphene

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Abstract

The elution orders of 20 hexa- to nonachlorobornanes and five hexa- to octachlorocamphenes were studied with normal-phase silica and amino phase HPLC, reversed-phase HPLC, as well as gel-permeation chromatography (GPC). Twenty-one compounds of technical toxaphene (CTTs) are commercially available and four were isolated from environmental samples. Structure-activity relationships and chromatographic properties were deduced from the data sets derived on these LC systems. The retention on silica (low-resolution LC and HPLC) increased with the polarity of the CTTs. The elution order of CTTs on amino normal-phase HPLC was, for the most part, the same as on silica normal-phase HPLC. The degree of chlorination determined the elution order of CTTs on C_{18} RP-HPLC. CTTs eluted from medium-pressure GPC with decreasing molecular size. Chlorobornanes with dichloro substituents on the six-membered ring eluted after the chlorobornanes without geminal chlorine atoms on secondary carbons, indicating that these congeners are larger. Altogether, the results increase the knowledge of complex substance class and may serve as a tool in order to gain further standard components.

Keywords: Toxaphene; Organochlorine compounds; Pesticides; Polychlorinated biphenyls

1. Introduction

Toxaphene is a non-systemic organochlorine pesticide with a composition only partly known. The number of components identified in technical mixtures ranges from 177 to >670 [1–3]. The major substance classes are chlorobornanes, which theoretically exist in a variety of 16 640 congeners or 32 768 enantiomers and non-chiral congeners [4]. Compounds of technical toxaphene (CTTs) identified in environmental samples are almost exclusively chlorobornanes [5]. Chlorodihydrocamphenes, chlorocamphenes, and chlorobornenes are present as minor components in technical mixtures (Fig. 1) [5,6].

In environmental samples, many CTTs are susceptible to degradation and only a few are bioaccumulated in the top predators of diverse food webs [5,7]. A direct synthesis of polychlorobornanes has not been achieved [8], therefore, single standards are prepared by stepwise chlorination followed by liquid chromatographic separation and re-crystallization [9–

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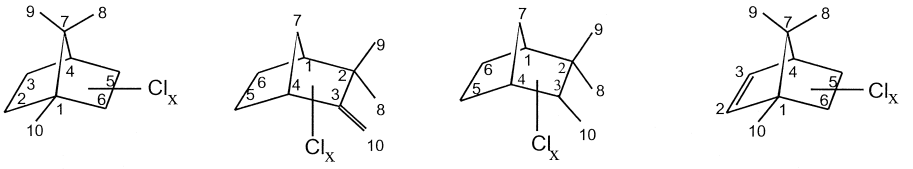


1 2 3 4

Fig. 1. Carbon backbone of (1) chlorobornanes, (2) chlorocamphenes, (3) chlorodihydrocamphenes, and (4) chlorobornenes.

11]. Only a few CTTs have been isolated from environmental samples [12–16]. During the last few years, the congener-specific determination of CTTs has become a promising quantitation method for toxaphene residues [5]. However, not all environmentally relevant CTTs are structurally known and there is a great interest to commercially produce relevant single CTT standards. Some progress in this field is hindered by the failure in the re-crystallization of each fraction, which can contain several components [17]. Such fractions may be purified with LC methods as well.

Congener-specific analysis enabled the development of quantitative structure–retention relationships. The physico-chemical behavior of the chlorobornanes that resist degradation follows certain rules; liquid chromatographic investigations may help to identify unknown CTTs and to provide information regarding the isolation of components from the complex mixture. For this reason, elution orders were studied using different liquid chromatographic methods for the separation of CTTs.

2. Experimental

2.1. Solvents, standards, and samples

Residue analysis-grade ethyl acetate (Fluka, Neu-Ulm, Germany) and *n*-hexane (Promochem), along with Pestanal-grade cyclohexane (Riedel-de Haen, Seelze, Germany) and acetonitrile (Baker, Deventer, Netherlands) were used throughout the study. All water was demineralized before use with the help of a Millipore-Q RG Ultra Pure Water System (Millipore, Milford, USA).

The following CTTs were investigated in this study which will be abbreviated with systematic codes according to Andrews and Vetter [18] (''AV'' codes) and Parlar numbers — if available — in parenthesis [19]. B6-923 and B7-1001 were identified on the basis of the isolation of Stern et al. [16]. Fractions containing significant amounts of the CTTs were produced in our laboratory by microbial degradation of toxaphene. B7-1453 was isolated by Krock et al. [14] and B8-1412 by Vetter et al. [15]. All further CTTs were present at 400 pg/μl in the Parlar 22 components standard (Dr. Ehrenstorfer, Augsburg, Germany). The production and characterization has been described by Hainzl et al. [10]. Several components in the ''Parlar 22 component'' standard were also available as single standards. Different mixtures of CTTs were investigated (see Table 1) and all contained B8-2229 (P-44), which was used to calibrate the LC systems.

Originally packed Melipax powder (technical toxaphene, produced in the former GDR) was obtained from a garden shed in Jena, Germany, in 1998. An emulsion of Melipax was diluted in *n*-hexane, and extracted in an ultrasonic bath and filtered. Stock solutions were diluted to a final concentration of 1.46 μg/μl. Additionally, a solution of 10 ng/μl of the polychlorobiphenyl (PCB) congeners PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, and PCB 180 (PCB Mix 1, Dr. Ehrenstorfer), and 1,2,3,4-tetrachloronaphthalene (TCN) (Promochem, Wesel, Germany) were used as internal HPLC standards.

2.2. Gas chromatography–electron-capture detection (GC–ECD) parameters

GC measurements were performed on a Hewlett–Packard 5890 II gas chromatograph equipped with an

autosampler (HP 7673) and two ^{63}Ni electron-capture detectors using nitrogen as the make-up gas. Columns of 50 m×0.25 mm I.D., coated with 0.25 μm CP-Sil 2 and CP-Sil 8/20% C_{18} were obtained from Chrompack (Middelburg, Netherlands). Injector (splitless injection mode; splitless time, 1.5 min) and detector temperatures were 250°C and 300°C, respectively. The column head pressure was set at 1.2 bar (helium). The GC oven temperature was programmed as follows: 60°C (hold for 1.5 min), at 40°C/min to 180°C (hold for 2 min), at 2°C/min to 230°C (hold for 25 min), and at 10°C/min to 270°C (hold for 15 min).

2.3. High-performance liquid chromatographic (HPLC) conditions

An HPLC pump 64 (Knauer, Berlin, Germany) was equipped with a 100 μl injection loop. An SPD 7A UV (Shimadzu, Germany) or a Gilson Model 116 (Middleton, USA) UV detector was used at 220 nm. HPLC chromatograms were recorded with a Hewlett–Packard 3395 integrator.

In the normal-phase mode a 250 mm×3.2 mm Waters Spherisorb Silica 5 μm column (Sigma–Aldrich, Deisenhofen, Germany) was used with *n*-hexane at a flow of 0.9 ml/min. A 250 mm×4 mm Nucleosil 10 NH_2 column (Macherey–Nagel, Düren, Germany was used for amino phase with a flow of 0.9 ml (*n*-hexane).

In the reversed-phase mode, separations were performed on a Supelcosil LC-18-DB 250 mm×4.6 mm column with 5 μm size. The mobile phase was acetonitrile–water (86:14, v/v) at a flow-rate of 0.9 ml/min. PCBs and TCN were used as internal standards. CTTs were injected after establishing reproducible conditions for the PCBs.

Solution which contained approximately 15 ng of each CTT in the respective standards were evaporated under a nitrogen flow and re-dissolved in 80 μl HPLC eluent, which was then injected into the 100 μl sample loop.

Table 1
AV codes, Parlar numbers, chemical names and structural relationship between chlorobornanes[a], as well as their relevance in the environment

Source[b]	AV code [18]	IUPAC name		Abundant
		Enantiomer a	Enantiomer b	
1	B6-923 (–)	2*x*,3*n*,6*x*,8,9,10[c]	2*x*,5*n*,6*x*,8,9,10	Major CTT in sediment
4	B7-499 (P-21)	2,2,5,5,9,10,10	3,3,6,6,8,10,10	
3,4	B7-515 (P-32)	2,2,5*n*,6*x*,8,9,10	2*x*,3*n*,6,6,8,9,10	Major CTT in technical toxaphene
1	B7-1001 (–)	2*n*,3*x*,5*n*,6*x*,8,9,10	2*x*,3*n*,5*x*,6*n*,8,9,10	Major CTT in sediment
2,3	B7-1453 (–)	2*x*,3*n*,5*x*,9,9,10,10	3*x*,5*n*,6*x*,8,8,10,10	Present in marine biota
4	B8-531 (P-39)	2,2,3*x*,5*n*,6*x*,8,9,10	2*x*,3*n*,5*x*,6,6,8,9,10	
4	B8-786 (P-51)	2,2,5,5,8,9,10,10	3,3,6,6,8,9,10,10	
4	B8-789 (P-38)	2,2,5,5,9,9,10,10	3,3,6,6,8,8,10,10	
4	B8-806 (P-42a)	2,2,5*n*,6*x*,8,8,9,10	2*x*,3*n*,6,6,8,9,9,10	Major CTT in technical toxaphene
4	B8-809 (P-42b)	2,2,5*n*,6*x*,8,9,9,10	2*x*,3*n*,6,6,8,8,9,10	Major CTT in technical toxaphene
2,3	B8-1412 (–)	2*n*,3*x*,5*n*,6*x*,8,8,9,10	2*x*,3*n*,5*x*,6*n*,8,9,9,10	Present in marine biota
2,3,4	B8-1413 (P-26)	2*n*,3*x*,5*n*,6*x*,8,8,10,10	2*x*,3*n*,5*x*,6*n*,9,9,10,10	Major CTT in marine biota
2,3,4	B8-1414 (P-40)	2*n*,3*x*,5*n*,6*x*,8,9,10,10	2*x*,3*n*,5*x*,6*n*,8,9,10,10	Present in marine biota
2,3,4	B8-1945 (P-41)	2*x*,3*n*,5*x*,8,9,9,10,10	3*x*,5*n*,6*x*,8,8,9,10,10	Present in marine biota
1,2,3,4	B8-2229 (P-44)	2*x*,5,5,8,9,9,10,10	3,3,6*x*,8,8,9,10,10	Present in marine biota
4	B9-715 (P-58)	2,2,3*x*,5,5,8,9,10,10	3,3,5*x*,6,6,8,9,10,10	
2,3,4	B9-1025 (P-62)	2,2,5,5,8,9,9,10,10	3,3,6,6,8,8,9,10,10	Major CTT in fish
4	B9-1046 (P-56)	2,2,5*n*,6*x*,8,8,9,10,10	2*x*,3*n*,6,6,8,9,9,10,10	
4	B9-1049 (P-59)	2,2,5*n*,6*x*,8,9,9,10,10	2*x*,3*n*,6,6,8,8,9,10,10	
2,3,4	B9-1679 (P-50)	2*n*,3*x*,5*n*,6*x*,8,8,9,10,10	2*x*,3*n*,5*x*,6*n*,8,9,9,10,10	Major CTT in marine biota
4	B9-2206 (P-63)	2*x*,3*n*,5*x*,6*x*,8,8,9,10,10	2*x*,3*x*,5*n*,6*x*,8,9,9,10,10	

[a] Structures of chlorocamphenes are not presented or discussed in this work due to the low number (n=5).
[b] Component present in a (1) five, (2) eight, (3) nine or (4) 22 component standard, underlined: also available as single standard.
[c] n=−*endo*; x=−*exo*.

In NP-HPLC, 0.5 ml fractions were collected, and the aliquots were analyzed by GC–ECD. The percentage distribution was calculated and retention times were assigned to a CTT. For example, if 100% eluted within one fraction then the retention times were defined as starting time of the fraction plus 0.2 min. Retention times of CTTs that were found in two fractions were calculated from the percent distribution plus 0.2 min. The elution order was established in different runs and the accuracy of the retention times was ±0.1 min. In the amino phase tailing was observed for some compounds yielding <10% in a third fraction. These small amounts were disregarded.

In RP-HPLC, the CTT standards were spiked with 15 ng PCB Mix 1 in 15 μl of the mobile phase. The combined solution (95 μl) was injected into the HPLC system. Thirty fractions at 1 min intervals were collected in 4 ml screw cap glass vials. 1 ml of *n*-hexane was added to each HPLC fraction and the closed vials were shaken 4–5 times to extract the CTTs into the non-polar phase. 0.75 ml of *n*-hexane were removed from each fraction and the final volume was set at 0.2 ml. 1 μl was injected into the GC–ECD system in each case. Only the percent distribution was reported.

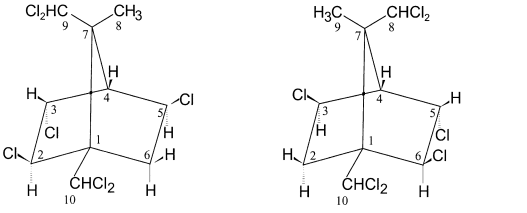
2.4. Gel-permeation chromatography (GPC)

An HPLC pump 64 (Knauer), equipped with a 100 μl injection loop, was connected to a GPC 330×25 mm I.D. glass column filled with 50 g Bio-Beads S-X3 (ABC, Analytical Biochemistry, USA). The mobile phase was ethyl acetate–cyclohexane (1:1, v/v) at a flow-rate of 4.9 ml/min. Separation efficiencies were tested with the ''Parlar 22 component standard'' as well as the CTT 8 standard. Solutions containing 6–15 ng/CTT were evaporated under nitrogen and re-dissolved in 80 μl of the mobile phase. Before injection of the CTTs, the dump and collection volumes were optimized using *trans*-chlordane and hexachlorobenzene (HCB), which are among the first and last eluted organochlorine compounds [20]. After 90 ml of waste (dump volume), 15 fractions were collected from 5 to 165 ml. The volume of each fraction was adjusted to 150 μl and 1 μl was injected into the GC system.

3. Results and discussion

3.1. General remarks

Fig. 2 shows the structure of enantiomers of two chlorobornanes frequently identified in environmental samples. The mirror image of a chlorobornane is obtained by the exchange of substituents at C2, C3, and C9 with those at C6, C5, and C8, respectively [4]. Therefore, enantiomers of chlorobornanes have different chemical names (Table 1 and Fig. 2). The two examples shown in Fig. 2 differ only by one Cl, however, the IUPAC names of the two components (B8-1413a and B7-1453a) are very different. The similarity is visible only when the left and the right enantiomers are compared (B8-1413a and B7-1453b or B8-1413b and B7-1453a). For a better understanding, the chemical names of both enantiomers are listed in Table 1, along with information on the relevance of single CTTs in technical products and environmental samples.



IUPAC name (Enantiomer 1)
B7-1453a: 2-exo,3-endo,5-exo,9,9,10,10-heptachlorobornane

Enantiomer 2
B7-1453b: 3-exo,5-endo,6-exo,8,8,10,10-heptachlorobornane

IUPAC name (Enantiomer 1)
B8-1413a: 2-endo,3-exo,5-endo,6-exo,8,8,10,10-octachlorobornane

Enantiomer 2
B8-1413b: 2-exo,3-endo,5-exo,6-endo,9,9,10,10-octachlorobornane

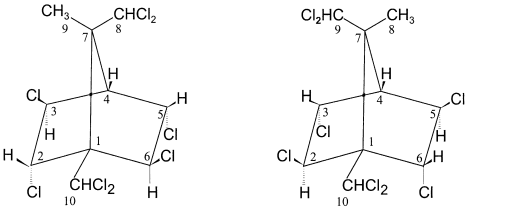


Fig. 2. Structure of the enantiomers of B7-1453 and B8-1413 (P-26). The carbon backbone of bornane can be distributed in the six-membered ring (C1-C6), the bridge (C7-C9), and the bridgehead (C10). Enantiomers are formed by mirroring the substituents at a plane of symmetry though C1, C4, and C7.

A drawback in HPLC analysis of CTTs is the low sensitivity of classical LC detectors for this substance class. Chlorobornanes have a saturated alicyclic hydrocarbon backbone, which provides no significant response in UV–Vis detectors. Refractive index detectors do not exhibit appropriate response for CTTs either, and most of the separations had to be carried out ''blindly''. For this reason, LC fractions were collected and analyzed by GC–ECD after solvent adjustment.

Two capillary columns of different polarity were installed in the GC oven. The main column was a non-polar CP-Sil 2 phase. Separation characteristics of this stationary phase have been reported earlier [21]. However, due to the availability of more single standards we found the following elution order: B9-1046 (P-56) < B9-1049 (P-59) < B9-715 (P-58); the latter two had been switched in Ref. [21]. The elution order of the CTTs on the second capillary column (CP-Sil 8/20% C_{18}) was identical with that reported on certain DB-5 columns. The contribution of each congener was established by adding the peak heights in the GC fractions. Results on both columns correlated with typical and maximum deviations between the two columns, 2% and 10%, respectively. The GC data was transformed to LC retention times or distribution in percentages as described in Section 2.3.

3.2. Elution order of CTTs on normal-phase (silica) HPLC

Liquid chromatographic separation on silica with *n*-hexane as the mobile phase has been used extensively to isolate CTTs and separate PCBs from CTTs, since these lipophilic components are readily soluble in organic solvents. Beginning in the 1970s, adsorption chromatography in columns packed with up to several kilograms of silica has been used for the isolation of large amounts of single standards [22–25]. This technique enabled isolation of the abundant CTTs in technical mixtures, i.e. B7-515 (P-32) and B8-806/809 (P-42), but was unable to produce environmentally relevant single standards. There are several reasons that can account for this inadequacy. (1) HPLC resolution was not suitable for separating the complex technical mixture (see below). (2) Each CTT was found in several fractions and each fraction contained many CTTs. (3) The elution order of the chlorobornanes increased with the CTT polarity [26]. The early eluting non-polar CTTs comprise the persistent and thus environmentally more relevant congeners [27]. (4) *n*-Hexane, a relatively non-polar solvent, has to be used in order to obtain sufficient retention for the CTTs of interest. However, even with *n*-hexane, LC retention times for the CTTs were relatively short, which limits the chances of isolating pure environmentally relevant components. In analytical laboratories, silica is widely used to separate PCBs from CTTs. Silica (1–8 g) is used to separate PCBs and other aromatic organochlorine compounds from the later eluting alicyclic chloropesticides [20,26,28]. An 8-g amount of silica yields a quantitative PCB/CTT separation [26]. However, elution of polar CTTs requires large amounts of solvent, therefore, a more polar solvent is used. For example, the addition of toluene or ethyl acetate to the mobile phase increases the polarity and thereby increasing the elution of chlorobornanes, however, decreasing the separation efficiency. Despite this drawback, the study of the elution order of CTTs on silica is important and helps to explain the persistence of known and unknown CTTs (see above).

NP-HPLC separation on a silica phase yields better resolution than column chromatography (low-resolution LC). Nevertheless, the HPLC elution order of CTTs (Table 2) was comparable with the results of Hainzl et al. who reported retention volumes of approximately 25 CTTs on a column packed with 1.8 kg of silica. Zhu et al. used HPLC with a silica phase for studying the composition of technical products and identified many co-elutions [29]. Several rules can be derived from the data sets. CTTs with the same pattern except for a non-symmetric 9,9-/8,8- or a symmetric 8,9-substitution on the methyl groups of the bridge had very different retention times. The non-symmetric substituted B8-789 (P-38) and B8-1413 (P-26) eluted much later than the symmetric-substituted B8-786 (P-51) and B8-1414 (P-40). This rule is valid for non-polar GC columns as well [21]. An exception was discovered for the first eluting CTT under investigation, B9-715 (P-58), which has an 8,9-pattern on the methyl groups of the bridge. No explanation can be made

Table 2
Elution order of 20 chlorobornanes and 5 chlorocamphenes on NP-HPLC

	NP silica			Amino phase		
	CTT	t_R (min)	RRT[a]	CTT	t_R (min)	RRT[a]
1	B9-715 (P-58)	3.6	0.409	B9-715 (P-58)	4.8	
2	B8-1413 (P-26)	3.7	0.420	B8-1413 (P-26)	5.3	0.368
3	B7-1453	4.0	0.455	B7-1453	5.75	0.399
4	B8-1412	4.1	0.466	B8-789 (P-38)	5.85	0.406
5	B8-789 (P-38)	4.15	0.472	B9-1046 (P-56)	5.95	0.413
6	B9-1046 (P-56)	4.2	0.477	B8-1412	6.0	0.417
7	B9-1679 (P-50)	4.55	0.517	B7-499 (P-21)	6.35	0.441
8	B9-1049 (P-59)	4.7	0.534	B9-1679 (P-50)	6.45	0.448
8	C8-??? (P-31)	4.7	0.534	B9-1025 (P-62)	6.8	0.472
8	B7-499 (P-21)	4.7	0.534	B8-2229 (P-44)	7.05	0.490
11	B9-1025 (P-62)	4.9	0.557	B9-1049 (P-59)	7.4	0.514
12	B8-2229 (P-44)	5.2	0.591	B8-531 (P-39)	7.8	0.542
13	B8-531 (P-39)	5.3	0.602	C8-??? (P-31)	8.45	0.587
14	B8-806/9 (P-42)	6.0	0.682	B8-1945 (P-41)	9.0	0.625
15	B8-1945 (P-41)	6.2	0.705	B8-806/9 (P-42)	9.3	0.646
16	B7-1001	6.25	0.710	C6-??? (P-11)	10.6	0.736
17	B9-2206 (P-63)	6.3	0.716	B7-1001	10.85	0.753
18	B8-786 (P-51)	7.05	0.801	B6-923	11.1	0.771
19	B8-1414 (P-40)	7.2	0.818	B8-786 (P-51)	12.45	0.865
20	C6-??? (P-11)	7.25	0.824	B8-1414 (P-40)	13.6	0.944
21	C7-??? (P-25)	7.3	0.830	C7-??? (P-25)	14.3	0.993
22	C6-??? (P-12)	7.8	0.886	B7-515 (P-32)	14.4	1.000
23	B6-923	7.9	0.898	C6-??? (P-12)	14.45	1.003
24	B7-515 (P-32)	8.8	1.000	B9-2206 (P-63)	14.55	1.010
25	C6-??? (P-15)	11.1	1.261	C6-??? (P-15)	17.7	1.229

[a] RRT=relative retention time, retention time relative to the t_R of B7-515 (P-32)=1.000.

for the early elution of this CTT, which was not found on non-polar GC columns.

3.3. Elution order of CTTs on normal-phase (amino) HPLC

The amino phase was tested in the normal- and reversed-phase modes. However, in the RP-mode no retention of CTTs was obtained, and only results from experiments in the normal-phase mode were presented. The elution order on the amino phase was roughly the same as on silica except that CTTs eluted 1.2–6.6 min later than on silica. Consequently, CTTs were distributed over a larger retention range on the amino phase (Table 2). On the other hand, some tailing was observed and relatively small amounts of some CTTs were found in more than two fractions. CTTs with different substitution patterns on the methyl groups of the bridge (and otherwise identical substitution) showed larger Δt_R than on silica. B8-1046 (P-56) and B9-1049 (P-59) (8,8,9 versus 8,9,9 substitution) were better resolved on the amino than on the silica phase. Unfortunately, B8-806 and B8-809 (P-42a and b) which have the same substitution difference, were not separated on the silica or on the amino phase and are not presently available as single standards. Here it is important to note that only one of the two isomers was detected in sediments [27]. B9-2206 (P-63) eluted late on the amino phase, and the elution order of B8-1945 (P-41) and B8-806/9 (P-42) were switched relative to silica NP-HPLC.

Fig. 3 shows the HPLC–UV chromatogram of the technical product Melipax. The retention times of the major components in technical products, B7-515 (P-32) and B8-806/9 (P-42), and biological samples, B8-1413 (P-26) and B9-1679 (P-50), are labeled in Fig. 3. The unresolved signals support the evidence that normal-phase HPLC alone is not suitable for the isolation of relevant CTTs from technical products.

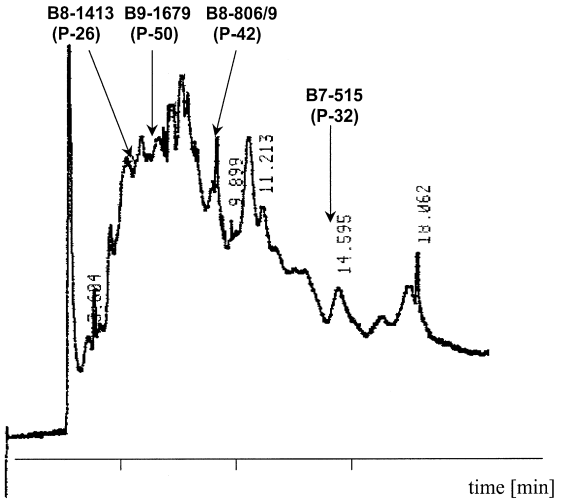


Fig. 3. HPLC–UV chromatogram (amino phase with mobile phase *n*-hexane) of the technical mixture Melipax. Numbers at the peaks are retention times in min.

3.4. Elution order of CTTs on RP-HPLC with C_{18} material

A drawback of RP-HPLC methods is the relatively low solubility of CTTs in binary mixtures of acetonitrile or methanol with water, in which the typical solvents may be organic phase–water, (75:25) up to (90:10, v/v). In this presentation we used the azeotropic mixture of acetonitrile–water (86:14, v:v) which has been suggested earlier for the isolation of CTTs from environmental samples [13]. Using this eluent, the chlorobornanes exhibited a better distribution than silica based HPLC (Table 3). However, RP-HPLC does not separate alicyclic (CTTs) and aromatic (PCBs) organochlorine compounds. For this reason, PCB congeners, recorded at 220 nm, were used as internal standards in order to control the reproducibility of the system. In RP-HPLC, the dominating factor for the elution of CTTs was the degree of chlorination (Table 3). Chlorocamphenes eluted within the same degree of chlorination as chlorobornanes. Retention of PCBs also correlated with their degree of chlorination. However, PCBs and CTTs found in the same retention range deviated by 4 chlorine atoms on the respective backbones, for example, the tetrachloro-PCB 52 eluted with octachloro-CTTs, and the penta-PCB 101 with nonachloro-CTTs.

Only a few exceptions from this ''degree of chlorination rule'' were found; B7-1453, B7-499 (P-21), B8-1413 (P-26) and B8-789 (P-38) eluted late in relation to their degrees of chlorination. Note that these four chlorobornanes have chlorine substitution on either the C8 or C9 of the bridge (non-symmetric distribution). Since these CTTs eluted early on silica, the polarity was the second factor that determined the retention of chlorobornanes on RP-HPLC, i.e non-polar CTTs eluted late.

Table 3
Elution order of CTTs and internal standards (PCBs and TCN) on RP-HPLC (C_{18}) with acetonitrile–water (86:14, v/v) as mobile phase

Time (min)	CTTs (AV codes, Parlar numbers in parentheses)	IS (t_R in parentheses)
0–5	–	–
5–6	C6-? (P-15, 100%)	–
6–7	C6-? (P-12, 100%), C6-? (P-11, 79%), B6-923 (Hx-Sed, 29%)	–
7–8	C6-? (P-11, 21%), B6-923 (Hx-Sed, 71%), C7-? (P-25, 100%), B7-515 (P-32, 46%)	–
8–9	B7-515 (P-32, 54%), B7-1001 (Hp-Sed, 44%)	–
9–10	B7-1001 (Hp-Sed, 66%), B8-1945 (P-41, 100%), B8-1414 (P-40, 85%), C8-? (P-31, 35%), B7-499 (P-21, 25%)	PCB 28 (9. 65 min)
10–11	B8-1414 (P-40, 15%), C8-? (P-31, 65%), B7-499 (P-21, 75%), B8-531 (P-39, 100%), B8-806/809 (P-42 a/b, 100%), B8-786 (P-51, 56%)	PCB 52 (10.16 min)
11–12	B8-786 (P-51, 44%), B7-1453 (100%), B8-1412 (100%), B8-2229 (P-44, 100%)	–
12–13	B9-1679 (P-50, 100%), B9-1025 (P-62, 77%), B9-2206 (P-63, 28%)	–
13–14	B9-1025 (P-62, 23%), B9-2206 (P-63, 72%), B8-1413 (P-26, 100%), B9-1049 (P-59, 24%)	PCB 101 (13.96 min)
14–15	B9-1049 (P-59, 56%), B8-789 (P-38, 100%), B9-1046 (P-56, 100%)	PCB 101 (13.96 min)
15–16	–	–
16–17	B9-715 (P-58, 100%)	–
17–30	–	TCN (17. 46 min)

Similar to other organochlorine compounds, the bioaccumulation of CTTs increases with the degree of chlorination. Together with the information observed on silica, CTTs eluting late within a degree of chlorination are environmentally relevant (Table 1), and it can be concluded that most of the investigated CTTs follow this rule. Some deviations were found for nonachlorobornanes, particularly B9-715 (P-58). The unique LC profile of this CTT observed on silica was also found on RP-HPLC.

Despite the good distribution of CTTs, several important congeners such as B7-1453 (-), B8-1412 (-), and B8-2229 (P-44) co-eluted. In order to isolate pure compounds, the collection of smaller fractions may yield better separation of unresolved CTTs [13]. However, a complete separation of these components was not achieved [15]. Nevertheless, RP-HPLC is more effective for the isolation of pure CTTs than NP-HPLC.

3.5. Elution order of CTTs on GPC

GPC separation of the CTTs was performed with Bio-Beads SX-3. No high-resolution technique was available to us. The system applied is usually used to separate lipids and organochlorines, therefore, we did not expect an above-average CTT separation. From the literature it has been stated that hexachlorocyclohexanes are distributed over a wide range in this system [20]. Therefore, we found it worthwhile to study the distribution of CTTs according to the size of the molecules. After the dump volume was collected (usually for the separation of the lipids), the CTTs were distributed between 15 and 60 ml (Table 4) and the CTTs could be found in several fractions, as expected. However, an interesting trend was observed. Chlorobornanes with 2,2 and 2,2,5,5 substitution eluted after CTTs without this structural feature. On the other hand, the degree of chlorination did not play the expected role. Congeners without geminal chlorine atoms on the secondary carbons are obviously larger than the respective components with 2,2 or 5,5 substructure. Only B8-1945 (P-41) eluted together with three CTTs with $-CCl_2$ substructure, i.e. B8-531 (P-39), B7-515 (P-32) and B8-806/9 (P-42), which may be due to the low resolution of the GPC system. Chlorobornanes with a geminal Cl on the six-membered ring seem to be less stable than those without, therefore, molecular size might be another factor playing a role in this phenomenon.

Table 4
Elution order of CTTs from Bio-Beads SX-3

GPC fractions of CTTs[a]		
15–35 ml	C6-? (P-15)	C7-? (P-25)
20–35 ml	B8-1413 (P-26)	B8-1414 (P-40)
20–40 ml	C6-? (P-12)	C8-? (P-31)
	C6-? (P-11)	B9-2206 (P-63)
25–40 ml	B7-1453	B8-1412
	B9-1679 (P-50)	
25–45 ml	B7-515 (P-32)	B8-531 (P-39)
	B8-1945 (P-41)	B8-806/9 (P-42)
30–45 ml	B7-499 (P-21)	
30–50 ml	B9-1046 (P-56)	B8-789 (P-38)
	B8-786 (P-51)	B9-1049 (P-59)
35–50 ml	B8-2229 (P-44)	
35–55 ml	B9-715 (P-58)	
40–60 ml	B9-1025 (P-62)	

[a] Fractions containing<5% of the total quantity are not included.

Note that chlorocamphenes eluted fast, and a selective enrichment of these compounds seems to be possible with GPC. Such investigations should, however, be performed with a high-resolution GPC system.

4. Conclusion

Congener-specific LC separation was suited to develop structure-specific elution orders of single CTTs which may be helpful in order to isolate unknown CTTs from complex mixtures. With NP-HPLC fractions of non-polar and more polar CTTs may be obtained. RP-HPLC is the method of choice for fractionating according to the degree of chlorination. A combination of both techniques is suitable for the isolation of single CTTs, particularly those associated with biological samples [14,15], since RP-HPLC does not separate aromatic from alicyclic organochlorines. The use of silica enables the separation of PCBs and other prominent aromatic organochlorine compounds from CTTs (and further alicyclic organochlorines), and should be performed prior to RP-HPLC. This also takes advantage of using *n*-hexane as the solvent which has been known to separate extracts containing high sample masses.

Furthermore, environmentally relevant CTTs are found in the early silica fractions [27].

With the help of GPC fractionation, chlorocamphenes and chlorobornanes with or without a geminal Cl on the six-membered ring may become enriched.

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