Liquid Chromatographic Profiles of Compounds of Technical Toxaphene (CTTs)

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Introduction

Due to the standard substances which have been prepared [1-2] or isolated from environmental samples [3-7], the congener specific determination of CTTs has become a promising method for the exact quantitation of toxaphene residues. Furthermore, there is some interest to isolate single CTTs from other organochlorines as well as other CTTs which makes easier an unambiguous quantitation of CTTs [8]. For this reason separation efficiencies were studied on silica gel and RP-HPLC methods together with CTT standards.

Starting point of the present study was the recently developed method on silica gel for the PCB/CTT group separation [8] and the RP-HPLC method that was often used in sample preparation [5].

Material and Methods

Standards

The commercially available "Parlar 22 component standard" (Dr. Ehrenstorfer, Augsburg, Germany). Additionally, a standard solution of 8 CTTs (100 pg/µL) was used (see **Table 1**).

Table 1: Systematic codes and structures of important CTTs in biological samples

Code [9]	Parlar-No.[10]	structure	source
B7-1453	-	2-*exo*,3-*endo*,5-*exo*,9,9,10,10-heptachlorobornane	[5]
B8-1413	#26	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-octachlorobornane	P
B8-1412	-	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane	[6]
B8-1414	#40	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10-octachlorobornane	E
B8-1945	#41	2-*exo*,3-*endo*,5-*exo*,8,9,9,10,10-octachlorobornane	E
B8-2229	#44	2-*exo*,5,5,8,9,10,10-octachlorobornane	E
B9-1679	#50	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-nonachlorobornane	P
B9-1025	#62	2,2,5,5,8,9,9,10,10-nonachlorobornane	P

P = Promochem (Wesel, Germany); E = Dr. Ehrenstorfer (Augsburg, Germany)

Gas chromatography/electron capture detector (GC/ECD)

GC measurements were performed on an HP 5890 II gas Chromatograph (Hewlett-Packard) equipped with an HP 7673 autosampler and two ^{63}Ni electron capture detectors (ECD). CP-Sil 8/C18 20% and CP-Sil 2 stationary phases were obtained from Chrompack (Middelburg, The Netherlands). Both columns were 50 m × 0.25 mm, film thickness 0.25 µm. Injector and

detector temperatures were 250 °C and 300 °C, respectively. The column head pressure was set at 1.2 bar (helium). Oven program: 60 °C (1.5 min), 40 °C/min to 180 °C (2 min), 2 °C/min to 230 °C (25 min), 10 °C/min to 270 °C (15 min).

Reversed phase high performance liquid chromatography (RP-HPLC)
An HPLC pump 64 (Knauer, Germany) was equipped with an injection loop of 100 µL. Separations were performed on a Supelcosil LC-18-DB 250 × 4.6 mm column, mash size: 5 µm. A SPD 7A UV spectrophotometric detector (Shimadzu, Germany) was operated at 220 nm. Chromatograms were recorded with an HP 3395 integrator. The mobile phase was acetonitrile/water 86/14 (v/v) at a flow rate of 0.9 mL/min.

Ambient liquid chromatography (LC)
Separation on 8.0 g activated (16 h at 130 °C) silica gel (70 - 230 mesh ASTM) in a 200 mm × 10 mm i.d. glass column were performed with 48 mL n-hexane to elute PCBs followed by either 50 mL n-hexane/toluene 65/35 (v/v) or 50 mL n-hexane/ethyl acetate 90/10 (v/v) for the elution of the CTTs.

Separation of 8 environmentally relevant CTTs on silica gel
0.5 mL CTT-8 component standard (see Table 1) were eluted and distributed in 10 fractions à 5 mL. Each of the fractions was concentrated to 0.5 mL and 1 µL was injected into the GC.

Separation of four CTT isolates and the "Parlar 22 component standard" on RP-HPLC
30 µL of the standard solution were evaporated in a nitrogen flow and CTTs were dissolved in 80 µL eluent of the HPLC. During the elution fractions of 1 min were collected, then 1 mL n-hexane was added to each fraction and shaken 4-5 times to extract the CTT content. 0.75 mL n-hexane was removed from each fraction and the final volume was set at 0.2 mL. 1 µL was injected into the GC-system in each case.

Results and Discussion

Elution order of CTTs on silica gel (see Table 2)
1. The elution order of the 8 CTTs is partly determined by the polarity of the compounds. The elution volume of the CTTs is depending on the polarity of the mobile phase.
2. The time of the analysis after the PCB/CTT separation with n-hexane/ethyl-acetate is much faster due to the high volatility of ethyl acetate.
3. Separation of single CTTs can not be effectively done.

RP-HPLC (see Table 3)
1. B10-1110 (#69) was not found and Parlar #31 could not be unambiguous identified.
2. Chlorination level is an important factor in the elution order of bornanes.
3. Hexa- and heptachlorocamphenes (Parlar # 31 was not identified), are easily separated from the higher chlorinated bornanes, but this may be due to the different chlorination levels and not to the different polarity.
4. RP-HPLC is an effective method for the separation of single CTTs.

The knowledge of the elution orders of single CTTs with known structure allows the systematic separation of unknown CTTs from complex mixtures. The major separation factor on silica gel is the polarity of the CTTs, while on reverse-phase (C_{18}) it seems to be the degree of chlorination of the CTTs.

Table 2: The qualitative and quantitative elution order of the CTT-8 component standard and B6-923 (Hx-Sed), B7-1001 (Hp-Sed) on silica gel. Eluent: 48 mL of n-hexane followed by 50 mL of a more polar eluent

Volume of Eluent	Eluent: 48 mL n-hexane + 50 mL n-hexane/toluene 65/35 (v/v)	Eluent: 48 mL n-hexane + 50 mL n-hexane/ethyl acetate 90/10 (v/v)
mL	CTTs	CTTs
0-48 (n-hexane)	-	-
0-5	B8-1413 (9%)*	B8-1413 (5%)
5-10	B8-1413 (17%)	B8-1413 (18%)
10-15	B8-1413 (18%), B7-1453 (4%)	B8-1413 (19%), B7-1453 (4%)
15-20	B8-1413 (52%), B7-1453 (81%), B8-1412 (74%), B9-1679 (76%), B9-1025 (68%), B8-2229 (62%), B8-1945 (46%), B8-1414 (51%), B7-515 (17%)	B8-1413 (19%), B7-1453 (11%)
20-25	B8-1413 (5%), B7-1453 (15%), B8-1412 (26%), B9-1679 (24%), B9-1025 (32%), B8-2229 (38%), B8-1945 (54%), B8-1414 (49%), B7-515 (66%)	B8-1413 (33%), B7-1453 (36%), B8-1412 (13%), B9-1679 (9%)
25-30	B7-515 (17%)	B8-1413 (6%), B7-1453 (47%), B8-1412 (84%), B9-1679 (87%), B9-1025 (100%), B8-2229 (95%), B8-1414 (92%), B8-1945 (94%), B7-515 (90%) *B6-923 (100%), B7-1001 (100%)*
30-35	-	B7-1453 (3%), B8-1412 (3%), B9-1679 (4%), B8-2229 (5%), B8-1414 (8%), B8-1945 (6%), B7-515 (10%)
35-50	-	-

* Percentage of the eluted CTT - based on peak heights - in the given fraction. 100 % is the quantity of all the eluted CTTs.

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Table 3: The elution order of the "Parlar 22 component standard" on RP-HFLC

Elution time (min)	Eluted CTTs (Codes, Parlar Numbers in bracket)	
0-5	-	
5-6	(#15)	
6-7	(#11), (#12)	B6-923 (Hx-Sed)
7-8	(#25)	
8-9	B7-515 (#32)	B7-1001 (Hp-Sed)
9-10	B8-1414 (#40), B8-1945 (#41)	
10-11	B7-499 (#21), B8-531 (#39), B8-1414 (#40) B8-806/809 (#42), B8-786 (#51)	B7-1453 (TOX 7) B8-1412
11-12	B8-2229 (#44), B8-786 (#51)	
12-13	B9-1679 (#50), B9-1025 (#62), B9-2206 (#63)	
13-14	B8-1413 (#26), B9-1025 (#62), B9-2206 (#63)	
14-15	B8-789 (#38), B9-1046 (#56), B9-1049 (#59)	
15-16	-	
16-17	B9-715 (#58)	
18-30	-	

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