Multidimensional gas chromatographic analysis of toxaphene - test of different column combinations

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Abstract
A technical toxaphene mixture and a number of biological samples were analysed by multidimensional gaschromatography (MDGC). Three different column combinations were used: Ultra 2/FFAP, Ultra/DX4 and Ultra 2/Rtx 2330. The major peaks present in the technical mixture and biological samples mostly consist of up to 10 different toxaphene congeners. The results show that single-column chromatography is in general not suitable for a congener-specific determination of the major toxaphene congeners.

1. Introduction

Toxaphene is a complex mixture primarily consisting of chlorinated bornanes (CHBs) with an average elemental composition of $C_{10}H_{10}Cl_8$[1,2]. Toxaphene has been detected as contaminant in various environmental compartments and has a widespread distribution[3-6]. Different chlorine substitution can theoretically lead to 32,768 possible congeners[7]. Technical toxaphene mainly consists of Cl_7 to Cl_8 congeners and therefore could contain 6,840 congeners. However, a number of these CHBs is unlikely to be present because of unfavourable substitution positions on ring and bridge carbons[8]. In environmental samples the total number of congeners will be smaller due to degradation and biotransformation.

Attempts made recently to improve the mutual analytical comparability of the determination of total toxaphene and individual CHBs have shown that there are major difficulties in separation when using single-column gas chromatography (GC)[9]. Nevertheless, a single-column GC/ECD (electron capture detection) method was recently introduced as a congener-specific method for the determination of individual CHB congeners[10]. The use of high resolution mass spectrometry

(HRMS) may be a way to obtain a better congener-specific method for the determination of CHBs. However, for reasons of sensitivity the use of negative chemical ionisation (NCI) is preferred, and with that technique mainly the molecular ions are formed which makes single congener identification more difficult.

A congener-specific method for the determination of CHBs using multidimensional gas chromatography (MDGC) was presented at the 15th International Dioxin Symposium in Edmonton, Canada[11]. In the present study the separation characteristics of three different column combinations were investigated. Indicative concentrations of four CHBs were measured in fish samples, dolphin and human milk.

2. Materials and methods

A technical toxaphene mixture was obtained from Polyscience (Warrington, PA, USA). Five individual toxaphene congeners were obtained from dr. Ehrenstorfer (Augsburg, Germany). Their codes (Parlar numbers)[12] and structures are:

CHB 26 (T_2) 2-exo, 4-endo,5-exo,6-endo-8b,8c,10a,10b-octachlorobornane
CHB 32 (ToxB) 2,2,5-endo, 6-exo, 8b,9c,10a-heptachlorobornane
CHB 50 (T_{12},ToxAc) 2-exo,3-endo,5-exo,6-endo,8b,8c,9c,10a,10b-nonachlorobornane
CHB 62 2,2,5,5,8b,8c,9c,10a,10b-nonachlorobornane
CHB 69 2,2,5,5,6-exo,8b,8c,9c,10a,10b-decachlorobornane

The CHBs were available as standard solutions, ampouled in cyclohexane, with a concentration of 1 mg/L ± 15%. Their purity (ECD) appeared to be >99% for the CHBs 26, 50 and 69, >98% for CHB 62 and >95% for CHB 32. The concentrations of the CHBs 26, 32, 50 and 62 were later verified by comparing them with another CHB mixture of Promochem (Wesel, Germany) (code USL 421), containing these CHBs in concentration of 5 mg/L.

Hake livers were obtained from the Atlantic Ocean, south-west and west of Ireland, and herring and dolphin blubber were obtained from the North Sea. A human milk sample was obtained from Managua, Nicaragua [5].

The extraction and clean-up method used were described by de Boer and de Geus[12]. This method included a Soxhlet extraction in n-pentane/dichloromethane (50:50, v/v) for the fish and dolphin samples and a cold n-hexane/acetone extraction for the human milk, followed by alumina and silica column chromatography for all samples. The silica gel fractionation was improved. Columns of 2.5 g SiO_2.2% H_2O were used for all other experiments. The bulk of the toxaphene compounds, including the most relevant congeners were now eluted in a second fraction of 12 ml diethyl ether/iso-octane (20:80, v/v) after a first fraction of 13 ml iso-octane, which contained most PCBs. PCBs were only present at 1-2% in the toxaphene fraction, which did not cause serious interference with toxaphene peaks with ECD. The recovery of total toxaphene of the entire clean-up procedure

Table 1 MDGC/ECD conditions

Parameter	DB5/FFAP	Ultra 2/DX4	Ultra 2/Rtx 2330
Injection volume (µl)	1-2	1-2	1-2
Splitless time (min)	1	1	1
Injector temperature (°C)	270	270	270
Septum purge (ml/min)	2	2	2
First dimension			
Stationary phase	DB5	Ultra 2	Ultra 2
Column length (m)	30	25	25
Internal diameter (mm)	0.25	0.20	0.20
Film thickness (µm)	0.25	0.30	0.30
Carrier gas pressure (kPa)	108	150	150
Linear gas velocity (cm/s)	10	18	18
Make-up gas flow (ml/min)	36	36	36
Initial oven temperature (°C)	90	90	90
Initial isothermal period (min)	1	1	1
Initial programming rate (°C/min)	20	30	30
Second isothermal temperature (°C)	220	220	220
Second isothermal period (min)	1	20	20
Second programming rate (°C/min)	5	3	3
Third isothermal temperature (°C)	260		
Third isothermal period (min)	1		
Tird programming rate (°C/min)	3		
Final isothermal temperarure (°C)	280	270	270
Final isothermal period (min)	30	40	40
Second dimension			
Stationary phase	FFAP	DX4	Rtx 2330
Column length (m)	15	15	15
Internal diameter (mm)	0.20	0.25	0.25
Film thickness (µm)	0.30	0.25	0.20
Carrier gas pressure (kPa)	110	70	70
Linear gas velocity (cm/s)	10	22	26
Make-up gas flow (ml/min)	38	38	38
Isothermal oven temperature (°C)	200	210	215

Manual injection; Detection ^{63}Ni ECD, 300 °C; Carrier gas and make-up gas N_2

including the improved fractionation varied from 80 to 96%. The recoveries of the CHBs 32, 50 and 62 varied from 84 to 100%. CHB 26 was divided over the two fractions with 41% in the first fraction and 59% in the second fraction. The overall recovery of CHB 26 varied from 84 to 92%. The occurrence of CHB 26 in two fractions urged an analysis of both fractions on this CHB.

All MDGC analyses were performed on a Sichromat 2-8 GC with two independently controlled ovens and two ECDs (Siemens AG, Karlsruhe, Germany). All column dimensions and operating conditions are given in Table 1. Heart-cuts from the first column were transferred to the second column by use of a valveless switching technique, in which instead of the gas flow, the pressure drop over the coupling piece - a glass capillary, i.d. 0.17 mm ("live-T-piece") - is used[13]. The temperature of the first oven was optimised to obtain a maximum resolution. The second columns were kept isothermally, close to their maximum allowable operating temperatures to prevent unnecessary lengthening of the total run time. Only the maximum temperature of the Rtx 2330 column was higher (275°C) than the working temperature (215°C). A higher working temperature for this column resulted, however, in less resolution.

Heart-cuts of the five CHBs were made from first-dimension chromatogram of the technical toxaphene mixture and the five biota samples, using the three column combinations. The concentrations (indicative because only single point calibration was used) of the five CHBs were measured and the composition of the heart-cuts made was studied.

3. Results and discussion

All heart-cuts, except those of CHB 69, made from technical toxaphene consisted of 4-12 peaks. The results of the heart-cuts of CHBs 26, 32, 50, 62 and 69 made from first dimension chromatograms of five biological samples are given in Table 2.

Most biota heart-cuts contained several peaks, which shows that it is very difficult to determine the congeners studied by single-column GC. CHB 32 was not detected at all in most biota samples, but several other peaks were present it its heart-cuts. Although differences between the column combinations studied are small, Ultra 2/Rtx 2330 is the most suitable column combination for this type of analysis, because generally the best separation is obtained and its stability at higher temperatures is good, which avoids excessive column bleeding. Also, only by using this combination CHB 69 could be analysed.

One of the main questions with regard to this work will relate to the essence of the use of MDGC compared to single-column GC. Because toxaphene is more easily biotransformed than other complex mixtures such as PCBs[2], it could be anticipated that a congener-specific analysis would be possible for CHBs in biological samples. However, the results of Table 2 show that the use of MDGC is essential for a congener-specifc analysis of CHBs in biota. There are some indications that for CHB 50 a single-column GC analysis would be possible. The problem is that different samples, even of one species, may have different toxaphene patterns. Because also quadrupole MS does not offer the selectivity required for a reliable congener-specific analysis, HRMS and MDGC are at the moment the only suitable methods for a congener-specific analysis of toxaphene. This observation is

Table 2 Number of peaks, peak area ratios and indicative concentrations of the CHBs 26, 32, 50 and 62 in heart-cuts of biological samples made with three column combinations[a]

CHB	Sample	n[b]			R[c]			Concentration (µg/kg)[d]		
		I	II	III	I	II	III	I	II	III
26[e]	Hake liver '91	-	3(4)	13(4)	-	72(20)	40(15)	na[f]	100	70
	Hake liver '89	3(4)	4(4)	10(4)	45(3)	39(29)	38(25)	50	30	90
	Herring muscle	-	5(2)	5(2)	-	39(37)	29(70)	<1	2	<1
	Dolphin blubber	-	9(4)	8(4)	-	85(37)	77(30)	na	3300	2600
	Human milk	3(1)	5(2)	9(2)	21(100)	21(92)	22(89)	1	1	1
32	Hake liver '91	2	4	8	-	-	-	<1	<1	<1
	Hake liver '89	1	2	11	-	-	-	<1	<1	<1
	Herring muscle	-	4	5	-	13	-	<1	0.4	<1
	Dolphin blubber	7	9	13	-	-	-	<1	<1	<1
	Human milk	-	-	6	-	-	-	<1	<1	<1
50	Hake liver '91	1	3	3	100	95	92	20	30	10
	Hake liver '89	2	4	5	92	95	85	10	30	7
	Herring muscle	1	2	3	100	84	40	2	3	1
	Dolphin blubber	-	10	6	-	95	84	4400	3500	1900
	Human milk	1	3	3	100	92	95	3	6	3
62	Hake liver '91	-	-	-	-	-	-	<1	<1	<1
	Hake liver '89	-	2	3	-	83	32	<1	1	1
	Herring muscle	-	2	2	-	57	51	<1	2	1
	Dolphin blubber	4	6	3	75	83	28	100	190	40
	Human milk	-	4	3	-	29	14	<1	0.6	0.4

[a] I: DB5/FFAP, II: Ultra 2/DX4, III: Ultra 2/Rtx 2330; [b] n, number of peaks present in heart-cut; [c] R, peak area ratio (%): peak area of target CHB divided by sum of peak areas of other peaks in same heart-cut; [d] Concentrations in µg/kg wet weight; [e] CHB 26 was present in two fractions, which were both analysed. The concentration is sum of CHB 26 concentrations in both fractions. Under n and R the number of peaks and peak area ratios in the first fraction are given in brackets; [f] na, not analysed. Total toxaphene concentrations measured by GC/NCI-MS (8) in µg/kg wet weight: hake liver '91: 1300, hake liver '89: 690, herring: 60, dolphin: 19,000, human milk: not determined.

important with regard to discussions on tolerance levels for toxaphene. In case these discussions would result in congener-specific tolerance levels, because of the toxicity or persistence of some CHBs, one should be aware of the limitations of using single-column GC for this purpose. MDGC may well serve as a suitable alternative, also in routine control and monitoring programmes.

4. Conclusions

Concentrations of the most persistent CHB congeners determined by MDGC/ECD can be up to 10-fold lower than those determined by single-column GC/ECD or GC/MS (quadrupole) and are, therefore, regarded to be more accurate. Heart-cuts made by MDGC show that in single-column chromatograms peaks of several CHBs in biota samples consist of up to 10 different compounds. Therefore, the use of MDGC is recommended for a congener-specific determination of CHBs. MDGC/ECD is suitable for use in routine control work and monitoring programmes, and is cheaper than than GC/HRMS.

There were only small differences between the column combinations tested, and only five congeners were studied until now. Based on these preliminary results there is a slight preference for the Ultra 2/Rtx2330 column combination.

The relatively high concentrations of toxaphene in European fish and marine mammals samples demand a proper determination of total toxaphene and individual CHBs in samples from European aquatic ecosystems.

5. References

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