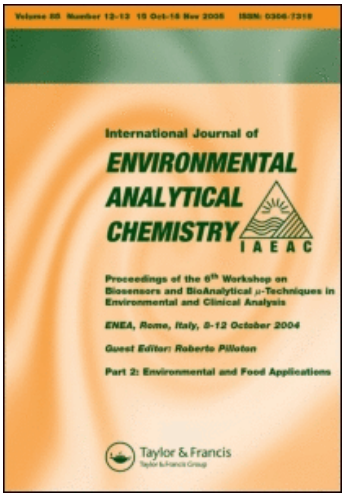

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Analysis of PCBs in Biotic Matrices by Two-Dimensional GC-ECD

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ANALYSIS OF PCBs IN BIOTIC MATRICES BY TWO-DIMENSIONAL GC-ECD

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The suitability of three GC-ECD systems for the separation of a model mixture containing various congeners of PCBs together with persistent chlorinated aromatics (pesticides and their metabolites, industrial chemicals) was tested. Analyses were performed on two parallel capillaries, the stationary phase of one of them was always in routine practice very common 5% phenyl-methylpolysiloxane, the second one was either 50% phenyl-methylpolysiloxane or 7% phenyl-7% cyanopropyl-methylpolysiloxane. The number of unresolved (coeluted) analytes was significantly reduced in these systems and thus unbiased quantitation of PCBs and other components used for regulation was possible. The utilization of this type of multidimensional chromatography for rapid and reliable analysis of real samples (fish oil, human fat) was documented.

KEY WORDS: PCBs, congeners, interferences, two-dimensional gas chromatography, parallel columns, separation, critical pair.

INTRODUCTION

To overcome a poor comparability of data on PCB content in environmental matrices expressed as "equivalent of technical mixture", quantitation based on 7 indicator PCBs was introduced[1] in 1982. However, potential interferences in these analytes occurring under routinely used conditions were proven several years later[2–4]. For both the regulatory purpose and for toxicological risk assessment, unbiased information on the levels of individual congeners is necessary and therefore there still exists the need to improve analytical methodology in this field.

The aim of our project was to demonstrate that multidimensional GC-ECD (simultaneous analysis of sample on two parallel columns with different selectivities) is a simple method rapidly providing precise and accurate results if suitable chromatographic conditions are selected. Several systems were tested in our study in order to characterise them with respect to the possible sources of bias caused by co-elution and/or poor separation of individual components typically contained in a sample extract. The test mixture, composed of 32 congeners of PCBs, 19 other chlorinated aromatics (pesticides and their metabolites, industrial contaminants) and 6 esters of phthalic acid was prepared for this purpose.

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EXPERIMENTAL

Analytical standards

PCBs (congeners No. 28, 31, 44, 52, 66, 70, 74, 77, 81, 84, 101, 105, 110, 114, 118, 123, 126, 128, 129, 138, 149, 153, 156, 157, 158, 163, 166, 167, 169, 170, 180, 189) and other chlorinated aromatics (α-HCH, HCB, β-HCH, γ-HCH, δ-HCH, Heptachlor, Heptachloroepoxide, Aldrin, Dieldrin, Endrin, Endosulphan I, Endosulphan II, o,p′-DDE, p,p′-DDE, o,p′-DDD, p,p′-DDD, o,p′-DDT, p,p′-DDT, Methoxychlor) were obtained from Dr. Ehrenstorfer (Germany), esters of phthalic acid: dimethyl- (DMP), diethyl- (DEP), di/n-butyl/- (DnBP), dibenzyl- (BBP), di/2-ethylhexyl/- (BEHP), di/n-octyl/- (DnOP) were supplied by Supelco Co. (U.S.A). All reference substances were dissolved in isooctane. The concentration of PCBs in the working solution (test mixture) was 0.02 μg/mL. Other chlorinated aromatics ranged from 0.005 to 0.05 μg/mL. The concentration of phthalates was approx. 250 μg/mL.

Materials, sample preparation

Fish (carp) and necroptic human tissue were used for the analyses. Acetone: n-hexane (1:2, v/v) were used for isolation of the analytes. Gel permeation chromatography on Bio-Beads SX-3 (mobile phase chloroform) was used for removing fat[5].

Gas chromatography (GC)

GC analyses were performed on a Hewlett Packard 5890 ser. II gas chromatograph equipped with split/splitles injector (electronically programmed pressure, EPP) and two ^{63}Ni electron capture detectors (ECDs). Hewlett Packard Chemstation software was used for processing data.

Three types of chromatographic systems, consisting of two parallel GC capillaries were tested. Their description is presented in Table 1.

RESULTS AND DISCUSSION

Until now, there is not a GC capillary column available, which could cope with the separation of all 209 existing congeners of PCBs. Moreover, clean-up procedures such as gel permeation chromatography that are nowadays currently being used[6] for removal of lipids from tissue extracts are not able to isolate a fraction free of chlorinated pesticides and similar lipophilic compounds (contrary to "classic" Florisil and/or alumina adsorption columns which can separate non polar PCBs from these chloroaromatics) commonly occurring in this type of matrice and that can interfere with GC peaks of individual PCBs.

In most of the comprehensive studies[7-9] concerned with retention time data of PCBs on various stationary phases, complex mixtures of available congeners and/or defined technical mixtures were used for such testing. Since our study was focused on problems related to the analyses of real samples, the following considerations were taken into account for selection of components of our model mixture:

Table 1 Tested chromatographic systems.

System	*A*	
Column	1st	2nd
Name	DB-5	DB-17
Supplier	J&W Scientific (USA)	J&W Scientific (USA)
Stationary phase	5% phenyl-methylpolysiloxane	50% phenyl-methylpolysiloxane
Length (m)	30	30
Int. diameter (mm)	0.25	0.25
Film thickness (μm)	0.10	0.11
System	*B*	
Column	1st	2nd
Name	DB-5	DB-17
Supplier	J&W Scientific (USA)	J&W Scientific (USA)
Stationary phase	5% phenyl-methylpolysiloxane	50% phenyl-methylpolysiloxane
Length (m)	60	60
Int. diameter (mm)	0.25	0.25
Film thickness (μm)	0.25	0.25
System	*C*	
Column	1st	2nd
Name	Ultra 2	NB-1701
Supplier	Hewlett Packard (USA)	Nordion (Finland)
Stationary phase	5% phenyl-methylpolysiloxane	7% phenyl-7% cyanopropyl-methylpolysiloxane
Length (m)	50	50
Int. diameter (mm)	0.20	0.20
Film thickness (μm)	0.11	0.10

Table 2 GC conditions applied for testing.

System	*A*	*B*	*C*
Injector temp (°C)	225	250	225
Detectors temp (°C)	300	300	300
Splitless time (min)	2.0	2.5	2.5
Oven temperature program	60°C–2.0 min, 30°C/min to 170°C, 1.2°C/min to 250°C	60°C–2.5 min, 30°C/min to 220°C, 1°C/min to 280°C 10 min	60°C–2.5 min, 30°C/min to 220°C. 1°C/min to 260°C
Carrier gas linear velocity (cm/s)	16.4	20.5	20.5
Pressure program:	200kPa-2min, 99kPa/min to 50kPa, then: constant flow: 0.4 (ml/min)	constant flow: 0.9 (ml/min)	constant flow: 0.7 (ml/min)
	50kPa at 120°C	130kPa at 60°C	170kPa at 60°C

PCBs. This group involved (i) "indicator" congeners used for regulation in many countries, (ii) other (if available) abundant PCBs typically occurring in the market basket, (iii) most toxic (planar) PCBs and (iv) congeners reported to interfere with some of the previous (important) PCBs on common stationary phases (5% phenyl-methyl polysiloxane or with similar selectivity). Characterisation of this group is summarised in Table 3.

Chlorinated pesticides. Ubiquitous persistent pesticides such as DDT, HCB and lindane including their metabolites and/or isomers possessing accumulation potential were used.

Esters of phthalic acid. From the practical point of view the most important representatives of this group of chemicals were added.

The priority of our effort was to find out conditions for unbiased determination of indicator PCBs for which maximum residue levels have been fixed. According to the reason stated above, i.e. due to the impossibility to achieve accurate determination of PCBs by a single GC capillary as well as taking into account the demand for favourable economic parameters of control and/or monitoring activities, analysis of samples simultaneously on two parallel columns of different selectivity—seemed to offer a good solution.

System (A) consisted of two 30 m, 0.25 mm (i.d.) capillaries: DB-5, for analysis of PCBs, was used in combination with a DB-17 (50% phenyl-methylpolysiloxane) capillary. The results obtained in this experiment are summarised in Table 4. Under the chromatographic conditions described in Tables 1 and 2, it was not possible to separate "indicator" PCB 28 from PCB 31 that represents an abundant component*) of the lower chlorinated technical mixtures. Consequently, in biota with the limited extent of PCB biotransformation (e.g. fish), this lack of separation can lead to an overestimation of target analyte. On the other hand, a problem related to the correct regulation based on

Table 3 Characterization of PCBs selected for GC separation study.

Type of PCB congener					
Ind	*Rec*	*Int (with PCB No)*	*Non*	*Mono*	*Di*
28	44	31 (28)	77	105	128
52	66	84 (101)	81	114	138
101	70	105 (153)	126	118	153
118	74	110 (77)	169	123	158
138	110	129 (126)	–	156	166
153	128	149 (118)	–	157	170
180	170	163 (138)	–	167	180
–	–	166 (126)	–	189	–
–	–	170 (169)	–	–	–

(ind = "indiactor" PCBs, rec = abundant PCbs recommended for monitoring, int = PCBs reported to interfere with indicator or planar congeners on conventional 5% phenyl-methylsiloxane stationary phases, non′ = non-ortho PCBs, mon = mono-ortho PCBs, di = di-ortho PCBs.

*) the main source of pollution in our country are Delor 103 and Delor 106, technical mixtures (similar to Aroclor 1242 and Aroclor 1254, resp.), produced by Chemco in the former Czechoslovakia. Delor 103 contains 9.1% (w/w) of PCB 31 and 13.0% (w/w) of PCB 28.[10]

Table 4 Characterisation of separation problems in system A (shaded areas indicate groups of analytes that are co-eluted on both columns).

DB-5		*DB-17*	
Coelution	*Poorly resolved*)*	*Coelution*	*Poorly resolved*)*
28+ 31	123+ 149, Endrin	28+ 31	BBP + 129, 166, BEHP
70+ Heptachloroepoxide	p,p′-DDD, o,p′-DDT	81+ p,p′-DDE	o,p′-DDT, 114
101 + 84+ Endosulphan I	163 + 138, 158 BBP	77+ Dieldrin	128, 156
81+ p,p′-DDE	– –	123 + 118 o,p′-DDD	138 + 126+ p,p′-DDT, 158
77+ Dieldrin	– –	114+ Endrin	– –
123+ 149	– –	o,p′-DDT+ Endosulphan II	– –
118+ Endosulphan II	– –	138 + 126+ p,p′-DDT	– –
114+ o,p′-DDT	– –	129+ BBP	– –
163 + 138 158	– –	170+ DnOP	– –
157+ Methoxychlor	– –	– –	– –

*)resolution Rs < 1

accurate determination of indicator PCB 138 was solved in this system thanks to its good separation from relatively abundant and quite persistent PCB 163 on the 30 m DB-17 capillary (contrary to the conventional DB-5 where co-elution occurred). Although PCB 158 was not separated from PCB 138 on any of the two columns, this fact in practice does not pose a serious problem because only low levels of the later compound are contained in biotic matrices. The remaining two"critical" pairs (PCB 77 and dieldrin, PCB 81 and p,p′-DDE) could be theoretically a problem in the case of analysis of fractions containing (minor) toxic non-ortho PCBs from which respective chlorinated pesticides were not completely removed.

System (B) employed columns with identical stationary phases but the lengths were doubled with the aim to get more theoretical plates for resolution of "difficult" groups of analytes. As can be seen from Figure 1, sufficient resolution of PCB 28 from PCB 31 was obtained on the DB-17 column. Nevertheless, (see Table 5) problems may be encountered with quantitation of PCB 138 in the presence of higher concentrations p,p′-DDT in the analysed sample. Only partial separation of this important congener from the preceeding peak containing (co-eluted) p,p′-DDT and PCB 163 and from the later closely eluting peak of PCB 158 was recorded on the DB-5 column. On the DB-17 capillary PCB 163 is totally (and PCB 158 partially) separated from congener 138. However, the last analyte is eluted together with PCB 126 and p,p′-DDT. As it will be

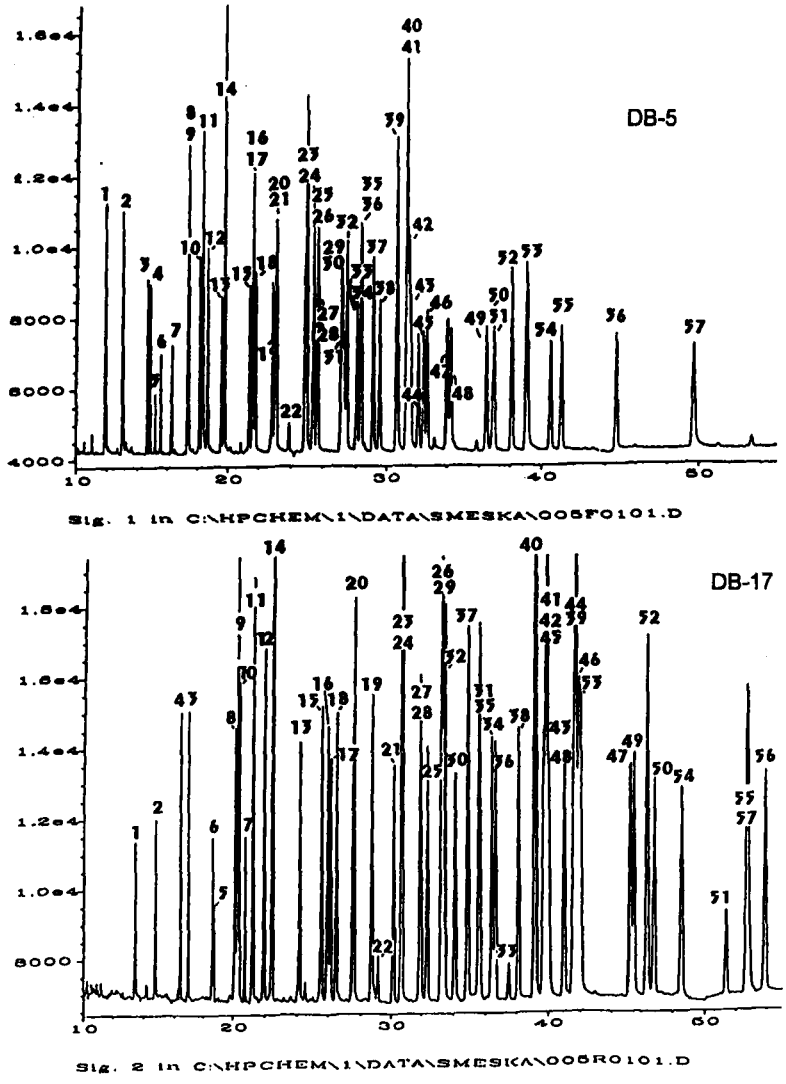

Figure 1 Chromatograms of the test mixture under conditions corresponding to system B.

Number of analytes:

1	DMP	16	PCB 70	31	Endrin	46	PCB 166
2	DEP	17	Heptachloroepoxide	32	PCB 118	47	PCB 128
3	α-HCH	18	PCB 66	33	Endosulphan II	48	PCB 167
4	HCB	19	o,p′-DDE	34	p,p′-DDD	49	PCB 156
5	β-HCH	20	PCB 101	35	PCB 114	50	PCB 157
6	γ-CH	21	PCB 84	36	o,p′-DDT	51	Methoxychlor
7	δ-CH	22	Endosulphan I	37	PCB 153	52	PCB 180
8	PCB 31	23	p,p′-DDE	38	PCB 105	53	BEHP
9	PCB 28	24	PCB 81	39	BBP	54	PCB 169
10	Heptachlor	25	PCB 110	40	PCB 163	55	PCB 170
11	DnBP	26	o,p′-DDD	41	p,p′-DDT	56	PCB 189
12	PCB 52	27	Dieldrin	42	PCB 138	57	DnOF
13	PCB 44	28	PCB 77	43	PCB 158		
14	Aldrin	29	PCB 123	44	PCB 129		
15	PCB 74	30	PCB 149	45	PCB 126		

Table 5 Characterisation of separation problems in system B (shaded areas indicate groups of analytes that are co-eluted on both columns).

DB-5		*DB-17*	
Coelution	*Poorly resolved*)*	*Coelution*	*Poorly resolved*)*
28+ 31	123+ 149, Endrin	81+ p,p′-DDE	123+ o,p′-DDD, 118
70+ Heptachloroepoxide	138, 158 163+ p,p′-DDT	77+ Dieldrin	138+ 126+ p,p′-DDT, 158
101+ 84		123+ o,p′-DDD	BBP+ 129, 166, BEHP
81+ p,p′-DDE		114+ Endrin	128, 156
77+ Dieldrin		138+ 126+ p,p′-DDT	
123+ 149		129+ BBP	
p,p′-DDD+ Endosulphan II		170+ DnOP	
114+ o,p′-DDT			
163+ p,p′-DDT			
157+ Methoxychlor			
110+ o,p′-DDD			

*)resolution Rs < 1

demonstrated in our next study, fine tuning of temperature program may result in some improvement of resolution of this problematic cluster. Nevertheless, the analysis time would exceed one and half hours even by maintaining constant flow by electronic pressure programming.

System C consisted of a 5% phenyl-methylpolysiloxane capillary, Ultra 2, and an NB-1701 capillary characterised by lower McReynolds constants than DB-17. In Figure 2 there are presented chromatograms obtained in this system which was operated under identical GC conditions as those used in previous experiment (system B). As can be seen in Table 6, only two pairs of analytes could not be separated. Unfortunately in both cases, indicator congeners were obscured. Complete co-elution of congeners 28 + 31 and 138 + 163 occurred. Contrary to the DB-5 column involved in system B, p,p′-DDT did not interfere with the later pair on the Ultra 2 column. A comparison of Tables 5 and 6 reveals some further differences in separation effects of the model mixture on these two columns with—as to the declaration of producers—identical stationary phases. Besides slightly different selectivity, a higher number of theoretical plates per meter was recorded for Ultra 2 capillary. System C was proved to be suitable

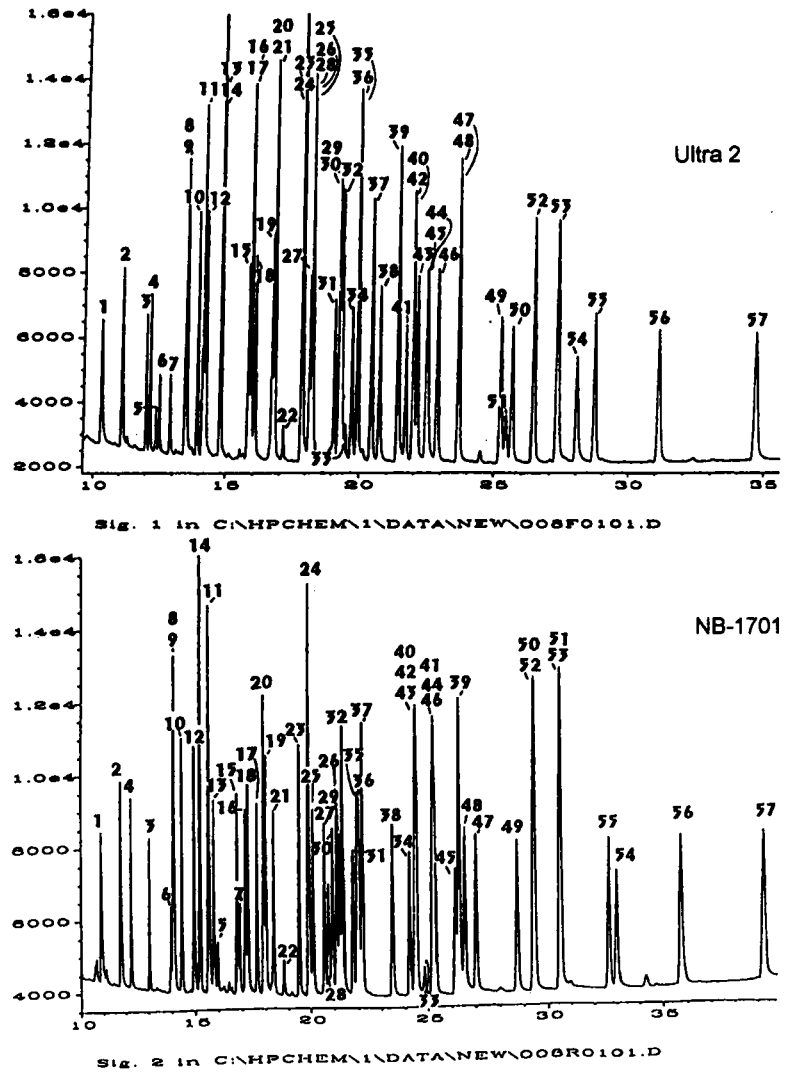

Figure 2 Chromatograms of the test mixture under conditions corresponding to system C (for analytes corresponding to individual numbers see Figure 1).

for determination of the most toxic (planar) PCBs. Fractionation aimed at preconcentration of these analytes is commonly carried out prior to their GC analysis. Nevertheless, traces of major components of original extract could negatively influence the results on a 5% phenyl methylsiloxane stationary phase (for details see Table 3). The NB-1701 column gave reliable separation.

The last set of experiments demonstrated application possibilities and limitations of the individual systems of analysis of real samples. Figure 3 illustrates the analysis of fish oil carried out under conditions corresponding to system B. As it follows from the previous discussion, overestimation of "indicator" congeners can occur on the conventional 5% phenyl-methylpoly-siloxane stationary phase. The extent of this bias

Table 6 Characterisation of separation problems in system C.

Ultra 2		*NB-1701*	
Coelution	*Poorly resolved*)*	*Coelution*	*Poorly resolved*)*
28 + 31	52, DnBP	28 + 31	101, o,p'-DDE
44+ Aldrin	74, 70+Heptachloro-epoxide	138+ 158+ 163	123, o,p'-DDD
70+ Heptachloroepoxide	o,p'-DDE, 84 + 101	p,p'-DDT 129 + 166	118, o,p'-DDD
101 + 84	Dieldrin, 77 + 110+ o,p'-DDD	180+ 157	o,p'-DDT, 114
81+ p,p'-DDE	123 + 149, 118	Methoxychlor+ BEHP	
77 + 110+ o,p'-DDD			
123+ 149			
114+ o,p'-DDT			
138+ 163			
126+ 129			
128+ 167			

*)resolution Rs < 1

for two types of biotic matrices differing in the degree of biotransformation of xenobiotics is documented in Table 7. Results presented here were calculated by a combination of data obtained in systems B and C (peaks recorded without any interference were considered to be 100%). The Ultra 2 capillary column (with the highest performance) was considered representative of a "good" column and used routinely. As anticipated, the major problem was encountered for congeners 28 and 138. Incorrect integration caused by close elution of other compounds could contribute to apparent "overestimation" of other analytes.

CONCLUSIONS

Simultaneous GC/ECD analysis of samples (extracts from biotic matrices) containing PCBs, chlorinated aromatics and phthalates on two parallel capillaries provides improved identification and quantitation of analytes. Rapid and unbiased determination of all indicator PCBs and many other abundant PCBs is possible in a system consisting of 5%

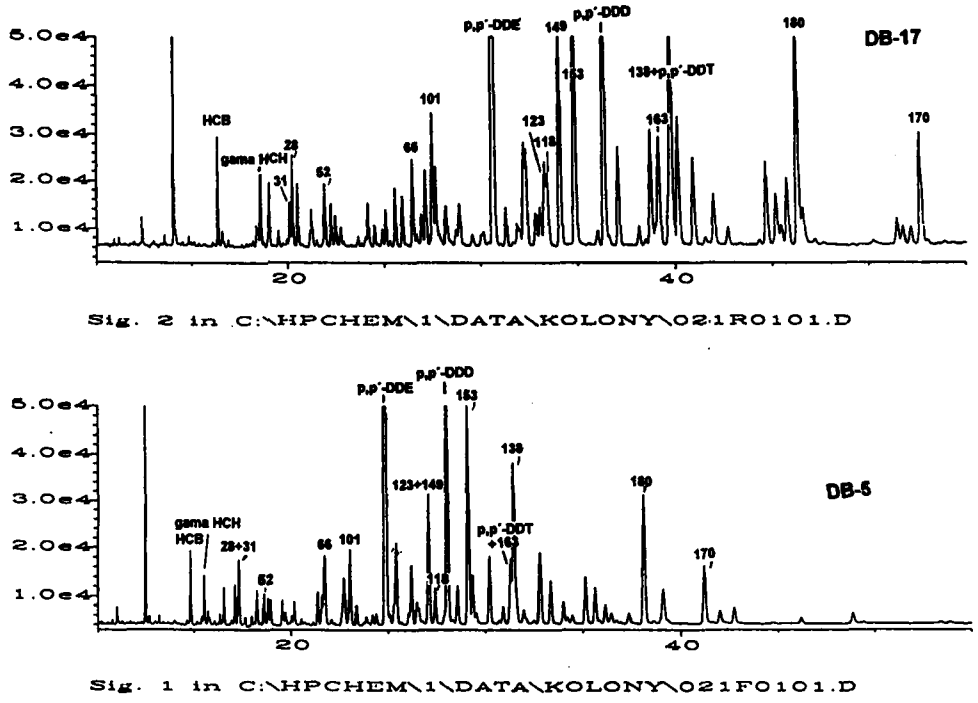

Figure 3 Real sample of fish oil analysed under conditions corresponding to system B.

Table 7 Overestimation (% of content of indicator PCBs by analysis carried out on Ultra 2 column (for GC conditions see experiment B).

Congener No.	*Fish oil*	*Human fat*	*Potential interference*
28	30	11	PCB 31
52	2	–	
101	8	6	PCB 84
118	5	2	PCBs 149, 123
138	32	25	PCB 163
153	7	–	
180	–	–	

phenyl-methylpolysiloxane and 50% phenyl-methylpolysiloxane. The first column of this pair must be characterised by a high number of theoretical plates to avoid with a "fast" temperature program, co-elution of p,p'-DDT with the peaks corresponding to PCB 163 and 138.

A capillary coated with 5% phenyl-methypolysiloxane stationary phase in combination with NB-1701 (or other relevant) column represents a system that can be

conveniently used for analysis of toxic planar PCBs. The high sensitivity of detection by ECD together with confirmation based on the agreement of retention times on both columns makes this method comparable with GC-MS procedures often used for this purpose.

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References

1. D. E. Wells, J. de Boer, L. G. M. T. Tuinstra, L. Reutergardh and B. Griepink, *Fresenius Z. Anal. Chem.*, **322**, 591–597 (1988).
2. A. H. Roos, P. G. M. Kienhuis, W. A. Traag and L. G. M. T. Tuinstra, *Intern. J. Environ. Anal. Chem.*, **36**, 155–161 (1989).
3. B. Larsen and J. Riego, *Intern. J. Environ. Anal. Chem.*, **40**, 59–68 (1990).
4. J. de Boer and Q. T. Dao, *Intern. J. Environ. Anal. Chem.*, **43**, 245–251 (1992).
5. J. Hajšlová, K. Holadová, V. Kocourek, J. Poustka, P. Cuhra and V. Raverdino, *Z. Lebensm. Unters. Forsch.*, **197**, 562–569 (1993).
6. D. E. Wells in: *Environmental Analysis: Techniques, Applications and Quality Assurance* (D. Barceló, ed. Elsevier Science Publishers, Amsterdam, 1993).
7. M. H. Mullin, C. M. Pochini, S. McCrindle, M. Romkes, S. H. Safe and L. M. Safe, *Environ. Sci. Technol.*, **18**, 468–476 (1984).
8. J. de Boer, Q. T. Dao and R. van Dortmund, *J. High Resolut. Chromatogr.*, **15**, 249 (1992).
9. S. Bowadt and B. Larsen, *J. High Resolut. Chromatogr.*, **15**, 377 (1992).
10. P. De Voogt, D. E. Wells, L. Rautergardh and U. A. T. Brinkman, *Intern. J. Environ. Anal. Chem.*, **40**, 1–46 (1990).