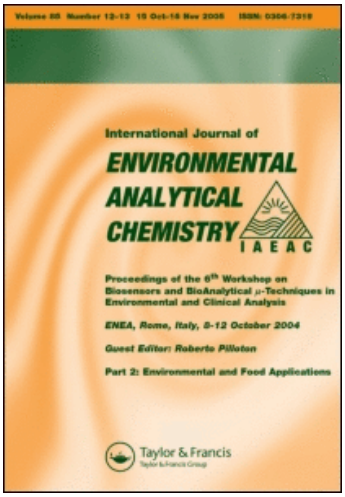

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Simultaneous Analysis of 32 Pcb-Congeners on Two Capillary Columns Operated in Parallel with a Glass *T*-Split

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SIMULTANEOUS ANALYSIS OF 32 PCB-CONGENERS ON TWO CAPILLARY COLUMNS OPERATED IN PARALLEL WITH A GLASS *T*-SPLIT

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The simultaneous analysis and confirmation of PCB-congeners by capillary GC–ECD on two parallel columns of different polarity is described. Compared to a normal single-column system, the stability of retention times and injection volume do not differ for the dual-column system operated with a glass *T*-split.

Analysis of a seal blubber extract yields evidence for differences in the identification of PCB-congeners in the sample by the two columns. This strongly indicates the usefulness of confirmation analysis on a column different from the normally used DB-5, in order to avoid false identification of components.

KEY WORDS: PCB-congeners, identification, dual-column, GC–ECD, analytical method.

INTRODUCTION

Analysis of specific PCB-congeners in environmental samples by capillary GC–ECD is a widely addressed topic.[1–3] The most commonly reported method uses one capillary column for separation and identification of the PCB-congeners. Although impressive chromatographic resolution can be achieved by use of 60 meter × 0.15 mm I.D. columns, complete separations of all congeners are not yet reported. Furthermore, identification based solely on retention time (t_R) on a single-column with EC detection may still leave interfering compounds to be falsely identified as PCBs. This complication is particularly pronounced in the analysis of congeners with low relative abundance.

The most frequent procedure for confirmation analysis is to perform the sample analysis on a second column of a different polarity. A number of relatively more polar columns have been used for this purpose, in a recent intercalibration study organized by the International Council for the Exploration of the Sea, ICES.[4]

Dual-oven chromatography, where the two columns of different polarity are connected serially, is reported for PCB mixtures and environmental samples.[5] This type of chromatography separates the difficult clusters successfully by cutting out a part of the chromatogram from the first column, and then separates this part further on the second column. Still this technique is not widely used as a tool for routine work.

For this type of work, a confirmation analysis may well be done in one step in a simpler way, and may thus enhance the quality of the analytical data in PCB analysis. This paper describes a chromatographic system, where a glass *T*-piece splits the carrier gas-stream before the chromatographic analysis. Analysis of technical PCB-mixtures (Aroclors) and organochlorine pesticides in various samples are reported in a similar dual-column system.[6] In this system, the two columns are connected to the injector via a Vespel ferrule with two holes. In both cases the variability due to repeated injections of the sample is eliminated in a parallel dual-column system.

The method is tested for the analysis of specific PCB-congeners in a test mixture containing 32 PCB-congeners, and in a seal blubber extract.

EXPERIMENTAL SECTION

Chemicals

In the following text, the PCB-congeners are referred to by their IUPAC numbers;[7] the relation between IUPAC number and molecular structure of the investigated congeners are given in Table 1 below. The PCB-congeners were selected as a basis for a study of chlorinated organic micropollutants in marine mammals. Two criteria of selection were applied, e.g. toxicity and persistency. Toxicity criteria resulted in selection of the co-planar non-ortho substituted PCB-congeners, and their mono- and di-ortho derivatives.[8,9] The persistency criteria yielded congeners with substitution in both para positions and without vicinal hydrogens.[9] Finally, the set of congeners recommended by ICES were included.[4] Some additional congeners were added as internal standards and recovery surrogate standards. The three most toxic non-ortho substituted co-planar congeners (PCB-77/126/169) were not included in the standard mixture, as these components are analyzed by a different technique (GC–MS) in this marine mammal study.

PCB standards were purchased from Ultra Scientific, Promochem and Cambridge Isotope Laboratories. PCB-15/44/136/70/95 were a generous gift from Dr. L. G. Hansen, University of Illinois at Urbana-Champaign.

On a DB-5 capillary column, the elution pattern of congeners relative to octachloronaphthalene was found to be the same as reported by Mullin.[1] This was considered as a confirmation of the identity of the components.

Analytical System

HP 5890A(II) gas chromatograph. Two ^{63}Ni–ECDs, operated at 300 °C. Autosampler HP7376.

Splitless injection of 1 μl (single-column) or 2 μl (dual-column) sample; splitless time, 1 min; injector temperature, 250 °C.

Carrier gas He. Column head pressure, 170 kPa (25 psi), corresponding to a linear flow rate of approx. 25 cm/s at 150 °C. Make-up gas N_2, 50 ml/min.

Temperature programming: 90 °C (1 min); 90 °C→180 °C (25 °C/min); 180 °C (2 min); 180 °C→220 °C (1.50 °C/min); 220 °C (2 min); 220 °C→275 °C (3 °C/min); 275 °C (10 min).

Column 1: J&W DB-5, 60 meter, 0.25 mm I.D. and 0.11 μm d_f. Column 2: J&W DB-1701, 60 meter, 0.25 mm I.D. and 0.15 μm d_f. The film thickness constitutes only appprox. 0.06% of the internal diameter, and the difference between the two columns is therefore not considered important in terms of flow resistance.

Collection and processing of data was performed by a Vectra QS/20 PC, with HP Chemstation Software.

Test Procedure

A PCB test mixture containing approx. 50 ng/ml of all congeners was prepared in iso-octane. The t_R's on DB-5 and DB-1701 were determined by running the congeners one at a time. PCB-53 and PCB-155 were used as internal standards, as recommended by Wells *et al.*[10] As a late eluting internal standard, octachloronaphthalene was used. Internal standards (IS) are identified as follows: IS-1 (PCB-53), IS-2 (PCB-155) and IS-3 (octachloronaphthalene).

First, a single-column (DB-5) chromatographic analysis of the test solution was repeated five times. Then the two columns (DB-5/DB-1701) were installed in parallel, with the help of a Chrompack Quick-seal glass *T*-split. The *T*-split was connected to the injector with 1 meter of deactivated 530 μm pre-column. The columns and *T*-split were sealed by heating the GC-oven to 280 °C for 1 hour. Five repeated analyses of the test mixture were then performed by the dual-column system. The t_R's and peak heights of the PCBs were collected and analyzed.

To test the system's ability to identify PCB-congeners in a "real-life" sample, a seal blubber extract was analyzed by the dual-column system. The sample originated from an animal found dead in Limfjorden, Denmark. The blubber extract was prepared by clean-up by sulphuric acid on silica gel, followed by chromatography on basic aluminium oxide.

The stability of the t_R's from later batchwise analysis of samples is included in the present report.

RESULTS AND DISCUSSION

Elution Patterns of PCB-Congeners

Chromatograms of the PCB-congener test mixture analyzed on DB-5 and DB-1701 are shown in Figures 1 and 2. t_R's of the test mixture PCB-congeners on both columns are listed in Table 1.

Even though a baseline or near-baseline separation between two (or more) PCB-congeners were present in the standard mixture, they may not be separated in an environmental sample due to interferences or large differences in concentration. The separation of the following set of congeners, which are identified in environ-

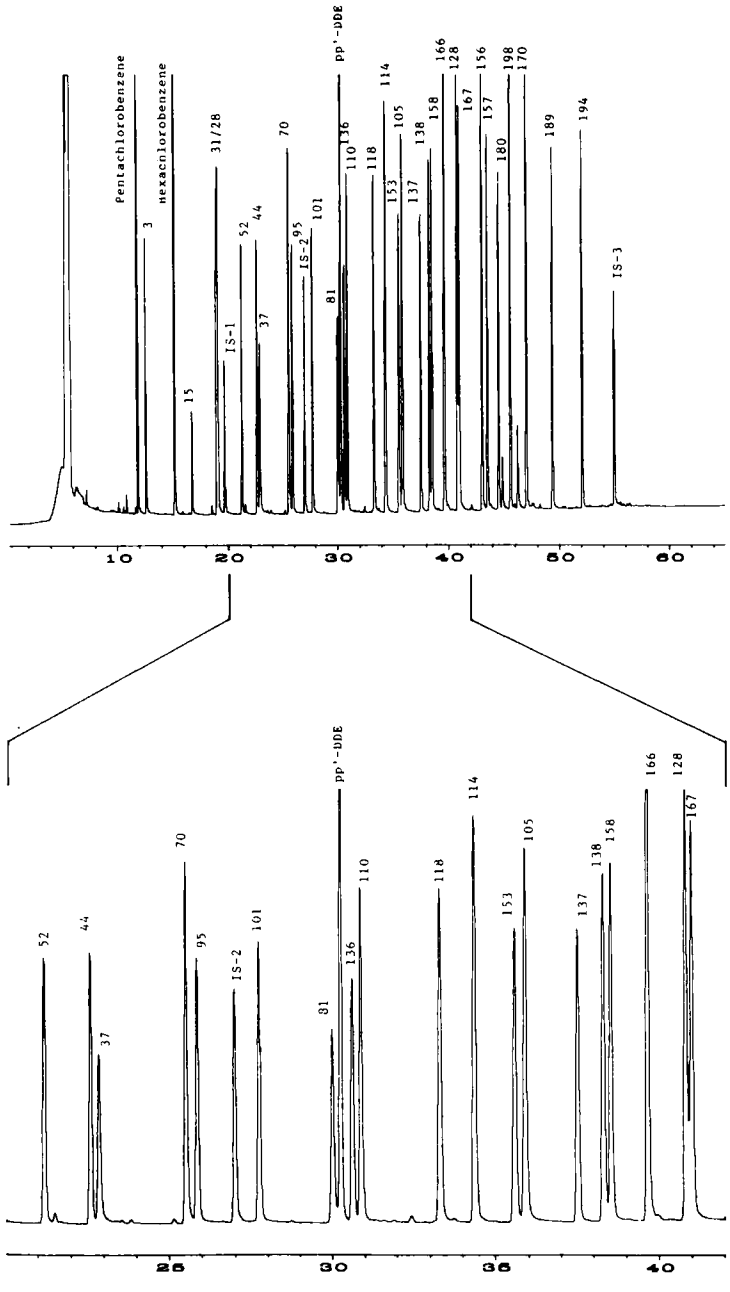

Figure 1 GC–ECD of the PCB-congener mixture analyzed on DB-5 in a dual-column system. Lower part of the figure is an expansion of the 20–42 min range.

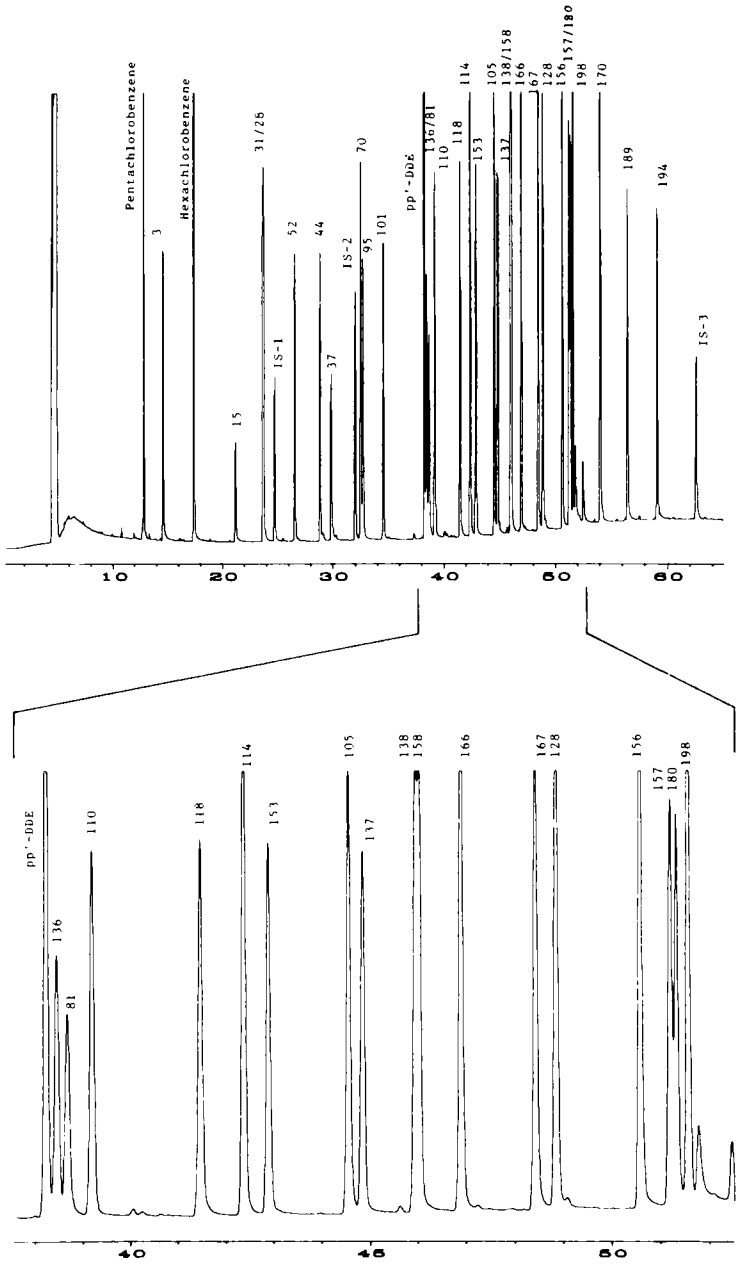

Figure 2 GC–ECD of the PCB-congener mixture analyzed on DB-1701 in a dual-column system. Lower part of the figure is an expansion of the 38–52 min range.

Table 1 Retention times (t_R) of PCBs on A = DB-5 and B = DB-1701 columns[a]

Structure	*IUPAC*	t_R-A	$RRT-A$	t_R-B	$RRT-B$
4	CB-3	12.563	0.2285	14.588	0.2333
4,4′	CB-15	16.737	0.3044	21.141	0.3380
2,4,4′	CB-28	19.079	0.3470	23.722	0.3793
2,4′,5	CB-31	18.989	0.3453	23.650	0.3782
3,4,4′	CB-37	22.854	0.4156	29.852	0.4773
2.2′,3′,5	CB-44	22.597	0.4110	28.828	0.4610
2,2′,5,5′	CB-52	21.186	0.3853	26.546	0.4245
2,2′,5,6′	CB-53	19.697	0.3582	24.726	0.3954
2,3′,4′,5	CB-70	25.507	0.4639	32.445	0.5188
3,4,4′,5	CB-81	30.014	0.5459	38.654	0.6181
2,2′,3,5′,6	CB-95	25.857	0.4703	32.656	0.5222
2,2′,4,5,5′	CB-101	27.733	0.5044	34.493	0.5515
2,3,3′,4,4′	CB-105	35.903	0.6530	44.538	0.7122
2,3,3′,4′,6	CB-110	30.893	0.5618	39.168	0.6263
2,3,4,4′,5	CB-114	34.367	0.6250	42.355	0.6772
2,3′,4,4′,5	CB-118	33.306	0.6057	41.446	0.6627
2,2′,3,3′,4,4′	CB-128	40.938	0.7445	48.843	0.7810
2,2′,3,3′,6,6′	CB-136	30.633	0.5571	38.434	0.6146
2,2′,3,4,4′,5	CB-137	37.512	0.6822	44.834	0.7169
2,2′,3,4,4′,5′	CB-138	38.295	0.6965	45.946	0.7347
2,2′,4,4′,5,5′	CB-153	35.588	0.6472	42.876	0.6856
2,2′,4,4′,6,6′	CB-155	26.979	0.4907	31.951	0.5109
2,3,3′,4,4′,5	CB-156	43.044	0.7828	50.590	0.8089
2,3,3′,4,4′,5′	CB-157	43.540	0.7919	51.187	0.8185
2,3,3′,4,4′,6	CB-158	38.531	0.7008	46.015	0.7358
2,3,4,4′,5,6	CB-166	39.657	0.7212	46.888	0.7497
2,3′,4,4′,5,5′	CB-167	41.009	0.7458	48.419	0.7742
2,3,3′,4,4′,5	CB-170	47.017	0.8551	54.001	0.8635
2,2′,3,4,4′,5,5′	CB-180	44.569	0.8106	51.313	0.8205
2,3,3′,4,4′,5,5′	CB-189	49.362	0.8977	56.840	0.9089
2,2′,3,3′,4,4′,5,5′	CB-194	52.080	0.9472	59.101	0.9450
2,2′,3,3′,4,5,5′,6	CB-198	45.570	0.8288	51.552	0.8243
	OCN	54.985	1.0000	62.540	1.0000
	pp′-DDE[b]	30.268	0.5505	38.216	0.6111

[a]RRT is retention time relative to octachloronaphthalene.
[b]pp′-DDE is included, as it appears in the PCB-fraction from clean-up of samples.

mental samples,[5,11,12] are critical on the non-polar DB-5 column: PCB-31/28; PCB-77/110; PCB-52/49/47/73; PCB-101/90; PCB-153/132/105; PCB-118/149; PCB-126/129; PCB-138/158/160/163/164/186; PCB-128/167 and PCB-187/182. The present study does not concern all of the above-mentioned congeners. With regard to the congeners included in the test, the relatively polar column DB-1701 separates PCB-110/77, PCB-153/105 and PCB-128/167 better than does DB-5. On the other hand, separation of the congener pairs PCB-138/158 and PCB-157/180 is less good on DB-1701 than on DB-5. The separations of these congener pairs on a column similar to DB-1701 in polarity, CP-Sil 19CB[11] (Chrompack), seems better for the pair PCB-110/77, and comparable for PCB-153/105.

Just as important as positive confirmation of identity is the exclusion of false identifications. The combination of DB-5 and DB-1701 columns enables the separation and confirmed identification for all congeners present in the test mixture, except the congener pairs PCB-138/158 and PCB-157/180. Application of another type of column may be useful for separation of PCB-138/158. In real samples, additional congeners present may complicate the picture, as illustrated by the analysis of the blubber extract.

Stability of Retention Times

The stability of t_R's in the single-column (DB-5) system is indicated by the relative standard deviation (RSD) of the five analyses of each compound. During the five injections, the RSD was shown to be 0.03–0.06% for the single-column system. The corresponding stability of t_R's for each column in the dual-column system were calculated similarly.

Data from both single- and dual-column system are shown in Figure 3A, as a function of IUPAC number. RSD by the dual-column system indicated no discrimination between the two columns with respect to stability of t_R's of the compounds studied. It was in the range 0.004–0.014% and 0.004–0.011% for the DB-5 and DB-1701 columns, respectively. The stability of t_R's was better for the dual-column system than for the single-column analogue, which may be due to the 530 μm pre-column.

In a daily batch run with 16 samples, the RSD of the t_R's was 0.01–0.03% ($N=4$). During 8 weeks, in which four batches were analyzed, the corresponding RSD was found to be 0.03–0.08% ($N=4$). The daily and weekly variation in t_R's are illustrated in Figure 3B. Daily RSD was smaller than the single-column RSD, while weekly RSD exceeds the single-column variation.

The RSDs were used to determine the calibration window tolerances in the Chemstation Software. With environmental samples analyzed, larger variance was tolerated, because major peaks may influence the t_R of less abundant congeners. The tolerances were assigned the value 0.2% of the t_R for PCB-congeners, and 1% for internal standards.

The injected amount was estimated by coefficient of variation for area counts of the internal standard IS2. In the single-column system, this was 2.3% ($N=7$). The corresponding RSD was 1.7% ($N=5$) for the DB-5 column, and 4.3% for the DB-1701 column ($N=5$) in the dual-column system. A one-tailed F-test ($P=0.05$) revealed that the RSD of the injection volume on DB-1701 was not significantly different from the RSD of the injection volume on DB-5 ($F_{4,4}=6.388$). The same was the case for the difference in RSD between the single-column system DB-5 and each of the columns in the dual-column system ($F_{4,6}=3.495$).

Distribution of the injected amount between columns was estimated from the response ratio $(H/His)_{\text{DB-1701}}/(H/His)_{\text{DB-5}}$, and was calculated for four selected congeners (PCB-101/118/153/194) in each injection. The results are shown in Table 2. The data indicated a minor discrimination between columns 1 and 2. Some discrimination of PCB-194 seemed to occur in the glass-split, but this is an effect of different t_R's on the two columns. PCB-194 eluted on a programmed part of the

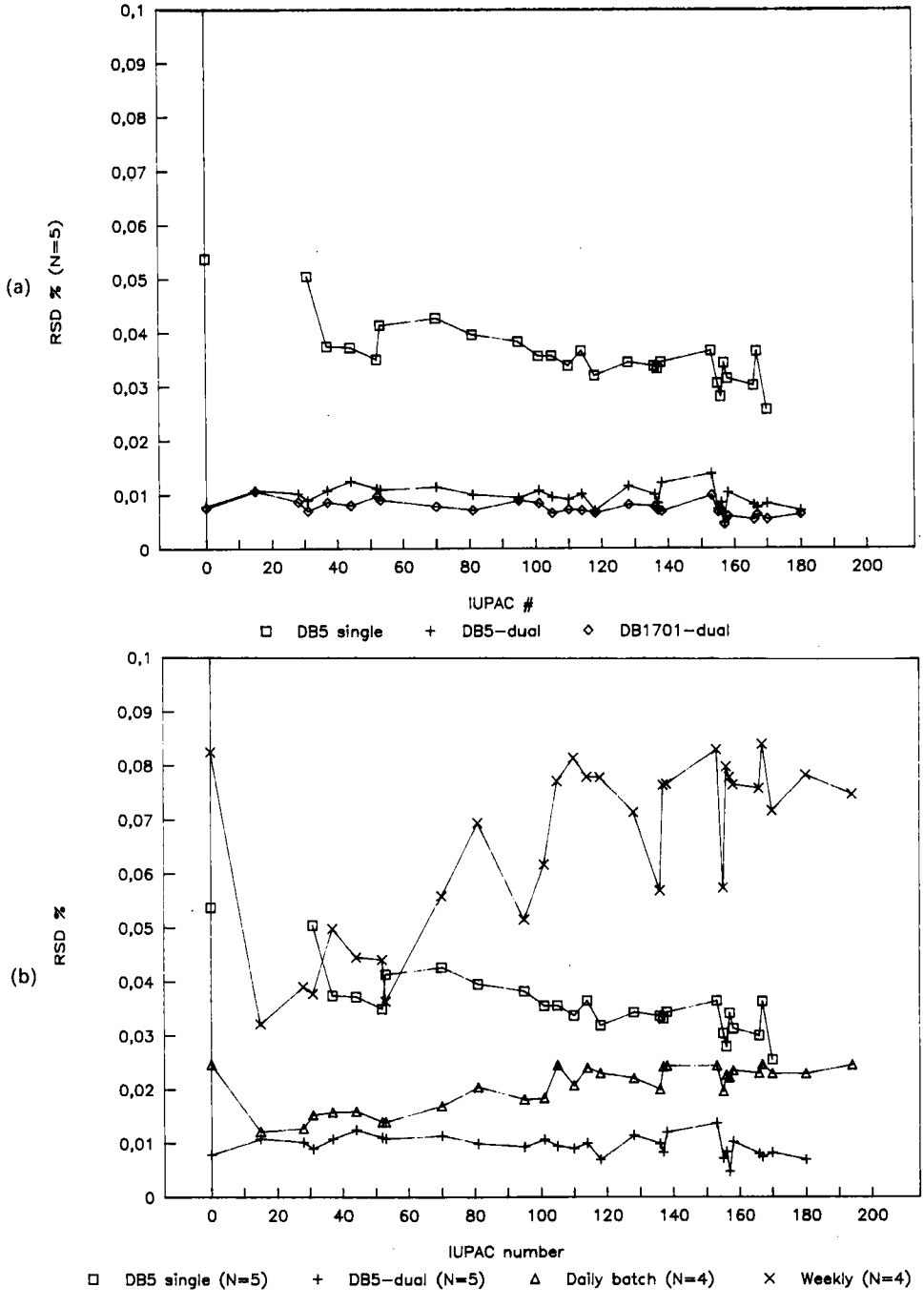

Figure 3 (A) Stability of the single- (DB-5) and dual-column (DB-5/DB-1701) system with respect to retention times. The RSD (%) of the retention times for each compound (five injections) is plotted against IUPAC number. (B) Long-term stability of retention times on DB-5 compared to daily single- and dual-column system. For the daily batch, four samples are injected between standards. "Weekly" standards are analyzed about 2 weeks apart. The RSD (%) of the retention times for each compound is plotted against IUPAC number.

Table 2 Distribution of injected volume between columns, estimated from the response ratio $(H/His)_{DB\text{-}1701}/(H/His)_{DB\text{-}5}$

CB[a]	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Inj. 4*	*Inj. 5*	$\langle X \rangle + -s$	*RSD* (%)
101	0.950	0.951	0.957	0.963	0.942	$0.953 + -0.007$	0.74
118	0.896	0.887	0.903	0.918	0.881	$0.897 + -0.013$	1.45
153	0.908	0.859	0.890	0.891	0.781	$0.866 + -0.045$	5.24
194	0.677	0.674	0.712	0.720	0.697	$0.696 + -0.018$	2.63
$\langle X \rangle$	0.858	0.843	0.865	0.873	0.825		
s	0.106	0.103	0.092	0.092	0.094		
RSD (%)	12.4	12.2	10.7	10.5	11.4		

[a]IUPAC number of congener.

temperature program on DB-5, but on the final isothermal part on DB-1701. As the response ratio is calculated with peak heights, the ratio for PCB-194 was thus lower on DB-1701 than on DB-5. During a normal run this problem is eliminated, as separate calibration tables exist for each column. The data indicated a strong injection-to-injection stability of the distribution between columns, and thus a stable distribution ratio during batch-analysis of samples. This is further confirmed by quantitative results from analysis of the PCB-congener test mixture.

Analysis of a Seal Blubber Extract

Chromatograms of the seal blubber extract analyzed on the dual-column system are shown in Figures 4 and 5. Identifications of the PCB-congeners selected for the marine mammal study in the blubber sample are listed in Table 3, and they are marked on the chromatograms. As for the analysis of the test PCB-mixture, the t_R tolerance used by the Chemstation software was set to 0.2%. Co-elutants known from the analysis of the test mixture of PCB-congeners are listed in Table 3 under "Remarks". As discussed earlier, the separation in a real sample may be much less than in the standard mixture, when major differences in concentration of two (or more) compounds are present. Unidentified co-eluting congeners may also deter separation and thus identification. For congeners that are close eluting in the standard mixture, special criteria should be applied for the identification and quantitation in a "real life" sample.

PCB-114 had a major unidentified co-elutant on the DB-1701 column, which indicates that DB-5 may be preferred for the quantitation of this component. A small peak showed up on the PCB-189 position on DB-5, but no corresponding peak was seen on DB-1701. In a higher concentration of the seal blubber extract (dilution 1:20), this was also the case. Thus PCB-189 would have been false-positive identified on DB-5 if no confirmation column was used. The same was the case for PCB-156, where a peak was present on DB-1701, but not on DB-5. These congeners should then be registered as not present in this sample (n.d.).

PCB-105, PCB-158 and PCB-157 were all masked by a major close eluting congener (PCB-153, PCB-138 and PCB-180, respectively). In the case of PCB-105,

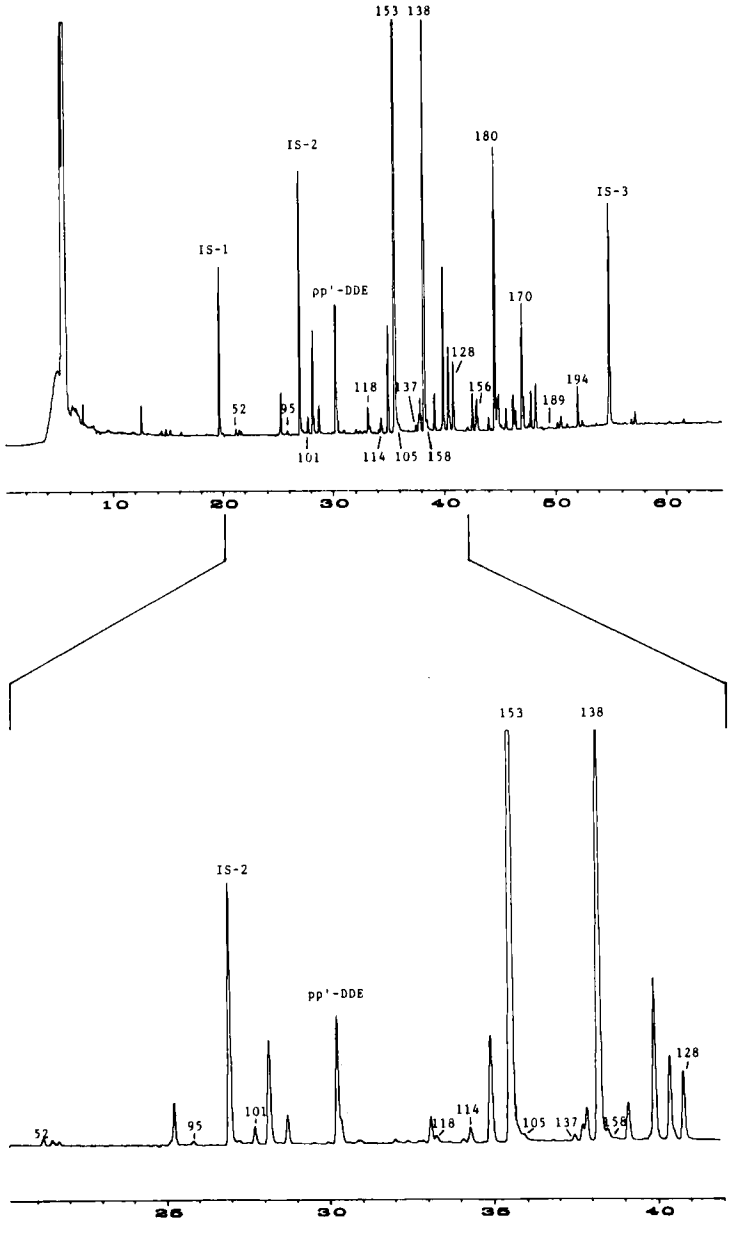

Figure 4 GC–ECD of seal blubber extract analyzed on the dual-column system; signal from DB-5 column. Extract diluted 250 times. Lower part of the figure is an expansion of the 20–42 min range.

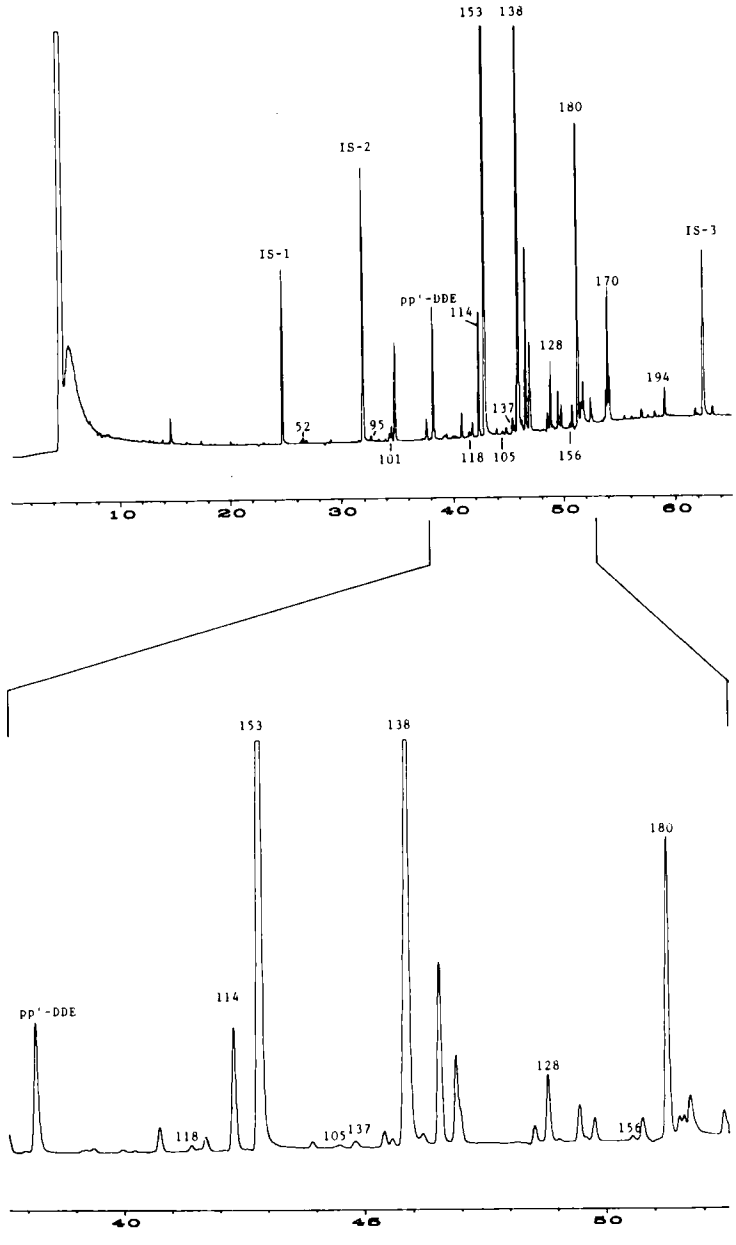

Figure 5 GC–ECD of seal blubber extract analyzed on the dual-column system; signal from DB-1701 column. Extract diluted 250 times. Lower part of the figure is an extension of the 38–52 min.

Table 3 Identified PCB-congeners in seal blubber extract, analyzed in dilution 1:250[a]

Congener	*C1*	*C2*	*Remarks*
CB-15	n.d.	n.d.	
CB-31	n.d.	n.d.	
CB-28	n.d.	n.d.	
CB-52	25.5	19.2	
CB-44	n.d.	n.d.	
CB-37	n.d.	n.d.	
CB-70	n.d.	n.d.	
CB-95	13.6	15.4	
CB-101	56.1	45.8	
CB-81	n.d.	n.d.	
CB-136	n.d.	n.d.	
CB-110	n.d.	n.d.	
CB-118	23.4	19.2	
CB-114	46.8	326	unidentified co-elutant on C2
CB-153	2477	2516	
CB-105	23.6	9.6	masked by CB-153 on C1
CB-137	18.7	17.0	
CB-138	1508	1341	
CB-158	27.2	n.d.	masked by CB-138 on C2
CB-166	n.d.	n.d.	
CB-128	189	171	
CB-167	n.d.	n.d.	
CB-156	n.d.	13.5	false identification
CB-157	n.d.	n.d.	masked by CB-180 on C2
CB-180	723	731	
CB-170	328	337	
CB-189	n.d.	n.d.	trace on DB-5, false identification
CB-194	99.0	88.9	
ICES[b]	4813	4672	

[a]C1 = DB-5, C2 = DB-1701. Calculated in ng/g sample. n.d. = Not detected (approx. 10 ng/g in this case).

[b]Sum of seven PCB-congeners recommended by ICES: PCB-28/52/118/153/138/180.

quantitation on DB-1701 may be more precise than on DB-5, because of the better separation of PCB-153/105 on DB-1701.

PCB-157 was better separated from PCB-180 on DB-5 than DB-1701, which indicates the DB-5 column to be preferred for quantitation. A major amount of PCB-180 may exclude the identification of a minor amount of PCB-157 on the DB-1701 columns. GC–MS analysis may solve this problem as these congeners are hexa- and heptachlorinated, respectively, and thus confirm the presence of PCB-157 in environmental samples.

PCB-158 was slightly better separated from the major peak PCB-138 on DB-5, but the separation was not better than just baseline. This was taken to implicate, that determination of PCB-158 would be complicated by the presence of PCB-138 in any case.

In Figure 6, the data are illustrated graphically for all congeners identified in the

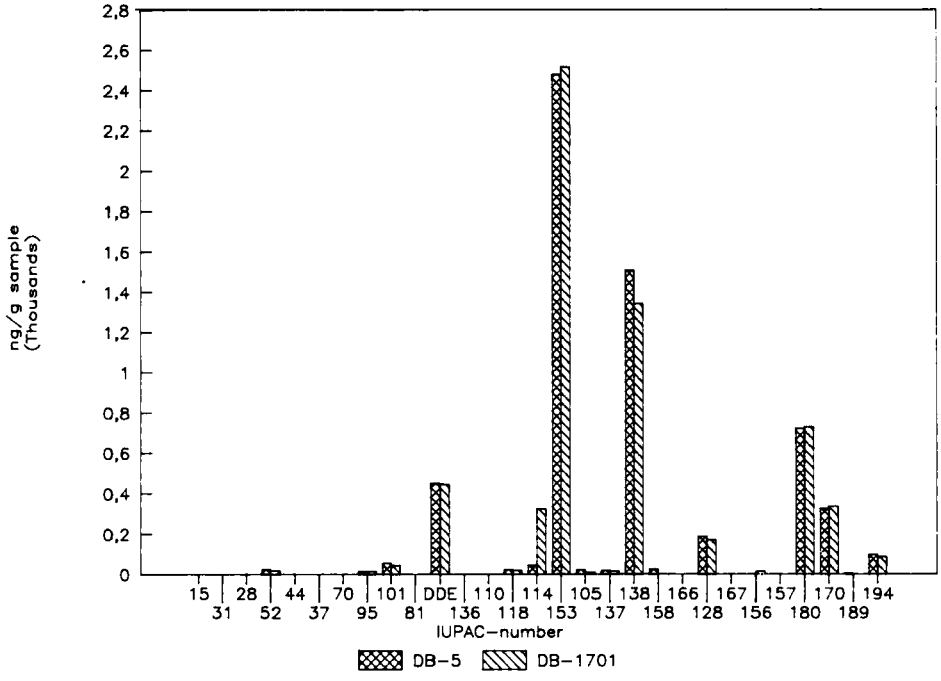

Figure 6 Quantitative data for the seal blubber extract represented for all congeners present in the sample, except PCB-3 and PCB-198, which are added to the sample as recovery surrogate standards.

sample. PCB-3 and PCB-198 were not originally present, but were added to the sample as recovery surrogate standards.[10] Quantitation of components in the seal blubber extract—when falsely identified compounds are excluded—on both columns in the dual-column system are similar, except for PCB-114 which has a major interfering co-elutant on DB-1701.

CONCLUSION

The present chromatographic system offers a convenient route to improve the quality of PCB-analysis by means of GC–ECD, by simultaneous analysis on two columns of different polarity. Retention times were more stable on the dual-column system than on the tested single-column system, and the glass *T*-split is easy to handle and works well. The DB-1701 column is a commonly-used type for confirmation of PCBs, and based on the presented data for 32 PCB-congeners, DB-1701 is suitable as a confirmation column. Analysis of a seal blubber extract revealed several identifications on DB-5, that were identified as "false" and excluded by analytical data from DB-1701. The quantitative data from analysis of the seal blubber extract indicated a PCB-congener, co-eluting with PCB-114 on DB-1701.

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