Using Porous Graphitic Carbon Column for HPLC Separation and Isolation of potentially Toxic PCB congeners in Fish

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1. Introduction

It is now an established fact that individual PCB congeners do not only have different physico-chemical properties but also different toxic and biological responses, the variation of which is largely dependent on the number of chlorine atoms on the biphenyl rings and the degree of ortho substitution. Development of routine congener-specific determination, particularly of the demonstrably toxic non-ortho congeners, in biological matrices, continues to generate keen interest among the analytical chemists.

We had earlier in our laboratory succeeded in using two micro active coal columns[1] to separate the PCB congeners into three major groups - non-ortho, mono-ortho and di-ortho congeners - of which the concentrations of the non-orthos are several orders less than the most abundant di-orthos in biological samples. Recently HPLC with porous graphitic carbon (PGC) column has been used to obtain separation data on selected CB congeners [2-5] but the data were either based on pure standard solutions or procedures too tedious for routine analysis. In some cases there were difficulties in reproducing these methods. In this study an HPLC technique has been used to establish the retention characteristics of certain individual CB congeners on the PGC column according to their degree of ortho substitution. The method has been applied to the determination of a selection of CB congeners in fish.

2. Method

The HPLC system consisted of LDC ConstaMetric 3200 pump, LDC SpectroMonitor 3200 detecor operating at 254 nm, a Rheodyne 7125 valve injector with a 100 ul loop and a Gilson FC 203 fraction collector. The column was Shandon Hypercarb porous graphitic carbon (100 x 4.7 mm, 7 um particle size).
The choice of seventeen CB congeners (IUPAC No 28, 52, 77, 101, 105,110, 118, 126, 128, 138, 149, 153, 156, 158, 167, 169 and 180) selected for study was on the basis of their toxicity and levels found in environmental and food samples. The retention characteristics and separation efficacy of the HPLC system were evaluated by analysing standard mixtures as well as fish samples using different solvent systems.
The fish samples were homogenized and extracted and the fat content determined. The resulting fat extract was reconstituted in hexane and run on a sulphuric acid/silica gel

column (50% acid on gel) and eluted with 40 ml hexane. The eluate was reduced to about 500 ul which was then fractionated on a 3% water deactivated silica gel to separate the PCBs from other substances that could otherwise interfere with the subsequent PCB analysis.

For the purpose of recovery tests the following internal standards were added to all the samples : CB 53 for the di-ortho PCB congeners and CB 189 for the mono-ortho congeners. Three $^{13}C_{12}$-labelled CB standards #77, #126 and #169 were also added to some of the samples prior to silica gel clean-up.

The samples were injected into the HPLC system and after collecting the first two fractions, comprising of the di-ortho and mono-ortho congeners respectively, the column was backflushed to obtain the third fraction consisting of the non-ortho PCBs. These fractions were reduced to appropriate volumes for further analysis; congener identification and quantification were carried out as reported earlier[1]. GC/ECD analytical data were obtained with a Hewlett-Packard model 5890 Gas Chromatograph equipped with dual capillary columns and dual electron-capture detectors while GC/MS data were obtained with Finnigan SSQ 710 operating in negative ion chemical ionisation (NICI) mode and detection by SIM technique. Each series of analysis included a procedure blank.

3. Results and Discussion

Figures 1 and 2 show the elution profiles of the three congener groups , namely, the non-ortho, mono-ortho and the di-ortho, using (1) only n-hexane and (2) a mixture of hexane/DCM (4:1 v/v) as well as 100% DCM as eluents.

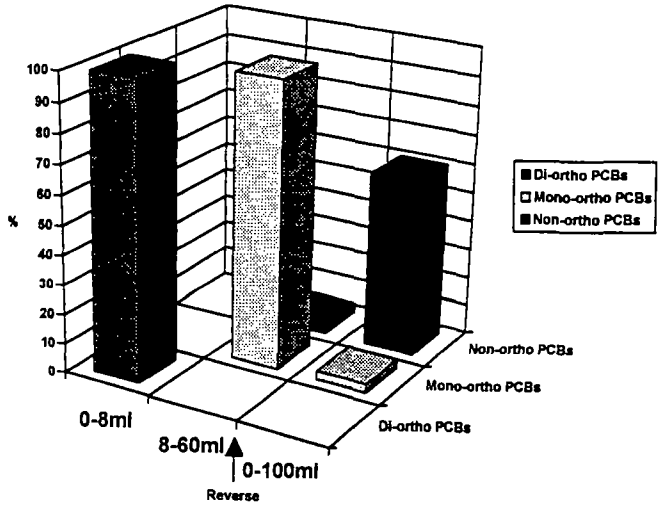

Fig. 1 Elution profiles of the three congener groups - non-ortho, mono-ortho and di -ortho using **n-hexane** as elution solvent.
Fraction 1 (0 - 8 ml): di-ortho congeners
Fraction 2 (8 -60 ml): mono-ortho plus traces of CB #77
Fraction 3 (backflushed 0 - 100 ml) : non-ortho congeners plus ca 5% of CB # 118.

Contrary to earlier reports[2,4] pure hexane as elution solvent proved inadequate for the efficient elution of the non-ortho CBs from the column. After 60 ml of elution ca 5% CB 118 was still retained on the column whilst the first non-ortho congener, CB 77, started eluting. 100 ml after the backflush, less than 60% of the non-ortho congeners had been eluted. A further elution with 100 ml still left about 20% retained on the column.

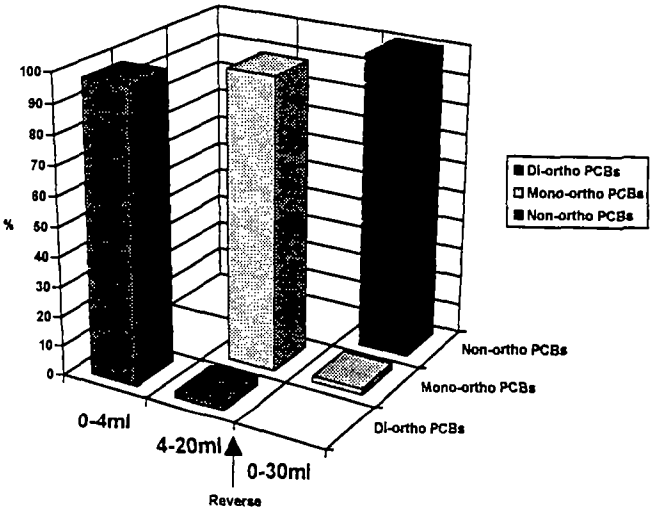

Fig. 2 Elution profile of non-ortho, mono-ortho and di-ortho congeners with **hexane/DCM mixture (4:1 v/v) and 100% DCM** as solvent systems.
Fraction 1 (0 - 4 ml hexane/DCM): di-ortho congeners
Fraction 2 (4 - 20 ml hexane/DCM) : mono-ortho plus ca 3% CB 110
Fraction 3 (backflushed 0 - 30 ml DCM) : non-ortho plus ca 2% of CB 118.

Figure 2 shows a much cleaner fraction and a higher recovery of the non-ortho congeners. Ortho-substituted CBs required only 20 ml and non-ortho congeners 30 ml (backflushed) of the solvent systems to completely elute from the column.

Results obtained from the application of this method to the determination of the same PCB congeners in herring *(Clupea harengus)* and sea trout *(Salmo trutta)* from the Baltic Sea as well as char *(Salmo salvelinus)* from Lake Vättern are shown in Table 1.
The method blanks which were run through the entire separation technique did not show any interferences in both the GC/ECD and GC/MS analyses. Replicate analyses agreed within 5-10% depending on the congener analysed.

The levels of the congeners (range and average values) indicated in the Table are in good agreement with results obtained from earlier investigations on similar samples[1,6]. The ease with which these congeners were isolated and analysed, and recoveries over 90% obtained, validates the reliability of the separation technique. Compared to activated coal column which has essentially the same separation capability as the PGC, the PGC method requires much less solvent and offers a faster separation of the PCBs.

Table 1 Levels of PCB congeners (ug/kg freshweight) in Herring and Sea trout from the Baltic Sea as well as Char from Lake Vättern ; Average values are indicated in **bold** figures.

PCB No	Herring (n = 11)		Sea trout (n = 7)		Char (n = 4)	
52	4.70 -8.21	**6.78**	3.02-4.35	**3.84**	3.88-5.17	**4.36**
101	13.0-32.8	**22.4**	12.4-21.8	**17.6**	13.6-16.5	**15.1**
110	10.1-29.2	**19.8**	9.27-17.5	**13.6**	12.3-15.4	**13.8**
149	12.4-36.3	**27.9**	15.9-27.6	**22.4**	14.0-26.4	**20.4**
153	22.1-73.9	**47.5**	32.5-74.3	**55.9**	115-206	**161**
138	16.2-54.5	**39.7**	25.2-55.0	**41.9**	84.9-161	**122**
158	1.90-6.24	**4.26**	2.47-5.15	**3.88**	9.50-14.5	**11.7**
128	2.99-9.83	**6.38**	3.72-6.55	**5.26**	15.3-23.3	**18.9**
180	5.98.24.2	**17.9**	12.4-23.8	**19.2**	45.4-88.4	**66.6**
28	1.22-2.16	**1.63**	0.66-1.07	**0.88**	1.41-2.49	**1.96**
118	10.2-32.5	**17.6**	9.75-19.3	**15.1**	38.1-52.3	**45.4**
105	3.68-11.8	**6.54**	3.53-6.65	**5.30**	11.6-21.4	**16.0**
167	0.48-1.25	**1.07**	0.53-1.62	**1.08**	3.38-4.12	**3.68**
156	1.04-4.34	**2.47**	1.49-3.43	**2.60**	8.42-15.5	**11.5**
77	0.079-0.185	**0.131**	0.100-0.150	**0.127**	0.126-0.175	**0.153**
126	0.054-0.250	**0.122**	0.047-0.125	**0.077**	0.274-0.291	**0.280**
169	0.019-0.075	**0.042**	0.025-0.075	**0.049**	0.095-0.175	**0.130**

4. References

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