Separation of Non-ortho PCBs, Mono-ortho PCBs and Poly-ortho PCBs by HPLC, using a Porous Graphitic Carbon Column.

Engberg ,D., Storr-Hansen,E.
National Environmental Research Institute, Department of Environmental Chemistry, Frederiksborgvej 399, PO Box 358, DK-4000 Roskilde, Denmark

Abstract
A HPLC method to separate PCBs according to the number of ortho chlorine substituents is presented. Separation of 34 PCBs is achieved on a porous graphitic carbon HPLC column using n-hexane and toluene as eluents.

Introduction

In recent years increasing attention has been focused on the potential toxcity of polychlorinated biphenyls (PCBs), especially those congeners showing "dioxin-like" toxicity. The most toxic congeners, i.e. the non-ortho substituted PCBs, are routinely measured by GC/MS in environmental samples.

Certain mono-ortho substituted chlorobiphenyls, e.g. 2,3,3',4,4'-penta- (IUPAC 105) and 2,3,'4,4',5-penta- (IUPAC 118) and 2,3,3',4,4',5 hexachlorobiphenyl (IUPAC 156) show a considerable dioxin-like toxicity. These mono-ortho PCBs are analysed by GC/ECD. However, some of the mono-ortho apparently PCBs co-elute or elute close to other PCBs on the GC columns. The problem increases in environmental samples, where the congeners appear in varying concentrations. There are several methods for chromatographic separation of PCB congeners according to their degree of ortho substitution, [1] , however, most methods aim at separating the toxic non-ortho PCBs from the ortho substituted PCBs.

Experimental section

HPLC equipment. The HPLC system include a Waters 600 Multi solvent delivery system, a Waters 712 WISP autosampler, a Waters 440 UV-detector measuring the absorbance at 254 nm and a FOXY fraction collector.

Column: Shandon Hypercarb porous graphitic carbon (100 mm x 4.7 mm), particle size 7 μm, flow: 1.15 ml/min. Eluents: hexane (21 min), toluene/hexane 97/3 (15 min)

The HPLC system was tested by analysis of a standard mixture containing 34 PCB congeners (cf. Table 1) as well as pp'-DDE, pentachlorobenzene and hexachlorobenzene.

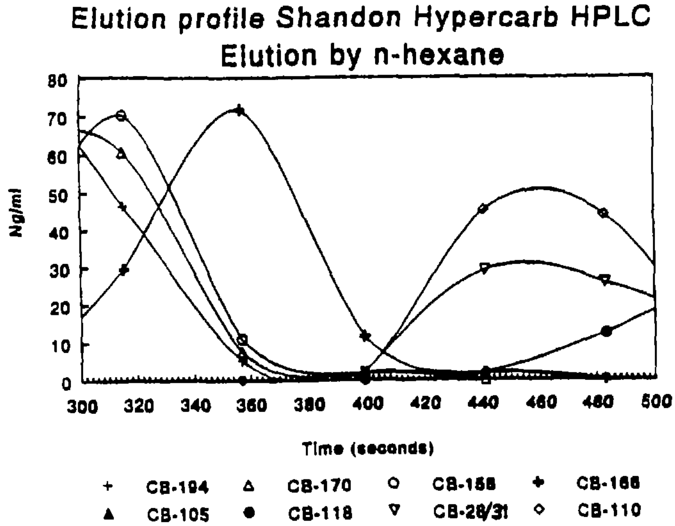
GC equipment. The single HPLC fractions were analysed by dual-column high resolution capillary gas chromatography with electron capture detection (GC/ECD),[5,6] the two columns applied were J&W DB-5 and J&W DB-1701.

Elution profiles. The elution profiles for the compounds in the standard mixture were obtained using hexane as eluent for 21 min collecting 30 fractions of 0.8 ml each followed by elution with toluene/hexane 97/3 . Elution profiles for a representative number of PCB congeners are shown in Fig. 1. It is noted that PCB-166 seldom is present in environmental samples.

Table 1
The table shows the 34 PCB congeners in the standard mixture, for which elution profiles have been examined. Rec 1 and Rec 2 are recovery surrugate standards used for GC/ECD analysis of PCBs in environmental samples.

PCB IUPAC no.	No. of ortho-chlorine subs.	No. of chlorine substituents	Chlorine substitution
PCB-136	4	6	2,2',3,3',6,6'
PCB-95	3	5	2,2',3,5',6
PCB-149	3	6	2,2',3,4',5',6
PCB-198 (REC-2)	3	8	2,2',3,3',4,5,5',6
PCB-44	2	4	2,2',3,5'
PCB-52	2	4	2,2',5,5'
PCB-101	2	5	2,2',4,5,5'
PCB-110	2	5	2,3,3',4',6
PCB-128	2	6	2,2',3,3',4,4'
PCB-137	2	6	2,2',3,4,4',5
PCB-138	2	6	2,2',3,4,4',5'
PCB-153	2	6	2,2',4,4',5,5'
PCB-158	2	6	2,3,3',4,4',6
PCB-166	2	6	2,3,4,4',5,6
PCB-170	2	7	2,2',3,3',4,4',5
PCB-180	2	7	2,2',3,4,4',5,5'
PCB-194	2	8	2,2',3,3',4,4',5,5'
PCB-28	1	3	2,4,4'
PCB-31	1	3	2,4',5
PCB-70	1	4	2,3',4',5
PCB-105	1	5	2,3,3',4,4'
PCB-114	1	5	2,3,4,4',5
PCB-118	1	5	2,3',4,4',5
PCB-156	1	6	2,3,3',4,4',5
PCB-157	1	6	2,3,3',4,4',5'
PCB-167	1	6	2,3',4,4',5,5'
PCB-189	1	7	2,3,3',4,4',5,5'
PCB-3 (REC-1)	0	1	4
PCB-15	0	2	4,4'
PCB-37	0	3	3,4,4'
PCB-77	0	4	3,3',4,4'
PCB-81	0	4	3,4,4',5
PCB-126	0	5	3,3',4,4',5
PCB-169	0	6	3,3',4,4',5,5'

Figure 1
The elution profiles for a representative number of the PCB congeners. A separation of the ortho chlorine substitued congeners can be obtained at 400 s (7.7 ml of hexane), as PCB-166 seldom is present in environmental samples.



Discussion

Porous graphitic carbon (PGC) column are known to be able to separate the toxic non-ortho PCB's from other PCB's. [2,3,4]
The elution profiles with hexane as an eluent show, that the present HPLC method allows the separation of the standard mixture in three fractions:

Fraction 1 (0 -7.7 ml): Tetra-, tri- and di-ortho substituted PCB and pp'-DDE.
Fraction 2 (7.7-24 ml): All the mono-ortho substituted PCBs, the di-ortho substituted PCB-110, the non-ortho substituted standard PCB-3 and pentachlorobenzene
Fraction 3 (toluene frac.):The non-ortho substituted PCBs, hexachlorobenzene. Ca. 5% of some of the mono-ortho PCBs will be present in this fraction.

To prevent the mono-ortho PCB from eluting in the toluene fraction , a stronger eluent can be introduced before the elution with toluene.
The separation of all mono-ortho PCB in one fraction, improves the measurement of PCBs by the GC-system. The three most important separations are the separation of PCB-153 and PCB-105, the separation of PCB-118 and PCB-149, and the separation of PCB-167 and PCB-128 respectively.

The separation of all mono-ortho PCB in one fraction, improves the measurement of PCBs by the GC-system. The three most important separations are the separation of PCB-153 and PCB-105, the separation of PCB-118 and PCB-149, and the separation of PCB-167 and PCB-128 respectively.

Conclusion

Preliminary results suggest that porous graphitic carbon columns for HPLC can separate PCBs according to degree of ortho substitution. All mono-ortho PCB apparently can be collected in a single fraction. Thus this significantly will improve the measurement of the mono-ortho coplanar PCBs by GC/ECD, as major coeluting PCB congeners are eluted differently. Thus, GC separation problems commonly envisaged using a DB5 column can be eliminated.

References

1) Creaser, C. S. and Krokos, F.
Analytical Methods for the Determination of Non-ortho Substituted Chlorobiphenyls: A Review
Chemosphere (1992), Vol 25, No. 12, 1981-2008

2) De Boer, J.; Stronck, C.J.N; Van der Valk, F, Wester, P.G. and Daudt, M. J. M.
Method for the Analysis of Non-ortho Substituted Chlorobiphenyls in Fish and Marine Mammals
Chemosphere (1992), Vol. 25, Nos. 7-10, 1277-1283

3) Hong, C-S, Bush, B., Xiao J. and Fitzgerald, E. F.
Isolation and Determination of Mono-ortho and Non-ortho Substitututed PCBs (coplanar PCBs) in Human Milk by HPLC Porous Graphitic Carbon and GC/ECD
Chemosphere (1992), Vol 24. No. 4, 465-473

4) Creaser, C. S. and Al-Haddad, A.
Fractionation of Polychlorinated Biphenyls, Polychlorinated Dibenzo-p-dioxins, and polychlorinated Dibenzofurans on Porous Graphitic Carbon
Anal. Chem. (1989), 61, 1300-1302

5) Storr-Hansen, E. (1991a)
Simultaneous analysis of 32 PCB congeners on two capillary columns operated in parallel with a glass T-split.
Intern J Environ Anal Chem (1991), 43, 253-266

6) Storr-Hansen, E. (1991b)
Comparative analysis of thirty polychlorinated biphenyl congeners on two capillary columns of different polarity with non-linear multilevel multi-level calibration.
J Chromatogr (1991) 558, 2, 253-266