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Isolation of toxic polychlorinated biphenyls by electron donor–acceptor high-performance liquid chromatography on a 2-(1-pyrenyl)ethyldimethylsilylated silica column

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SUMMARY

A rapid and simple liquid chromatographic method for the isolation of toxic planar polychlorinated biphenyls from their formulations by electron donor–acceptor high-performance liquid chromatography using a 2-(1-pyrenyl)ethyldimethylsilylated silica column is described. The separation takes less than 15 min and a complete analysis, including quantitation by gas chromatography–mass spectrometry or gas chromatography with electron-capture detection, may be completed in 60 min.

Retention data for 105 individual polychlorinated biphenyl congeners are presented and the retention behaviour, as well as the mechanisms of separation, are discussed.

INTRODUCTION

Increasing attention is being centred on the toxicity of polychlorinated biphenyls (PCBs), especially on the congeners that show the same type of toxicity as polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs). The topic has been extensively reviewed by Safe[1]. Certain non-*ortho*-chlorinated PCBs (0-*ortho*-PCBs) show particulary high "dioxin-like" toxicity, *viz.* 3,3′,4,4′-tetrachlorobiphenyl (TeCB, IUPAC 77), 3,3′,4,4′,5-pentachlorobiphenyl (PeCB, IUPAC 126) and 3,3′,4,4′,5,5′-hexachlorobiphenyl (HxCB, IUPAC 169), which all are approximately isosteric with the extremely toxic 2,3,7,8-substituted PCDDs and PCDFs. Considerable toxicity is also attributed to some mono-*ortho*-chlorinated PCBs (1-*ortho*-PCBs), especially 2,3,3′,4,4′-PeCB (IUPAC 105), 2,3′,4,4′,5-PeCB (IUPAC 118) and 2,3,3′,4,4′,5-HxCB (IUPAC 157)[2].

To assess residue levels of these compounds in technical PCB formulations and environmental samples, comprehensive analytical methods are used. Most of the

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methods include activated charcoal columns to isolate toxic 0-*ortho*-PCBs[3–6]. Using the method of Jensen and Athanasiadou[6] it is also possible to isolate the 1-*ortho*-PCBs of interest. However, this method is very laborious since it requires the use of multiple charcoal column chromatography to obtain sufficient purity of the 0- and 1-*ortho* fractions. Furthermore, the activated charcoal columns have some serious drawbacks, such as low efficiency, elution profiles with severe tailing, irreversible adsorption, batchwise variations of the adsorbents, and difficulties in using UV detectors because aromatic mobile phases are used.

Recently, Fischer and Ballschmiter[7] reported that all the toxic 1-*ortho*-PCBs can be analysed in PCB mixtures and environmental samples by gas chromatography–mass spectrometry (GC–MS) using an SB-Octyl 50 fused-silica capillary column (Lee Scientific). However, this method does not allow the quantitation of 0-*ortho*-PCBs in technical PCB formulations, since the 0-*ortho*-PCBs are present at such low levels (μg/g of PCB) that it would be necessary to analyse enormous amounts of PCBs. This would exceed the loading limit of the GC column.

The aim of the present study was to investigate if electron donor–acceptor (EDA) high-performance liquid chromatography (HPLC) on a PYE column could be used to separate toxic 0- and 1-*ortho*-PCBs from PCB mixtures, and thereby make it possible to analyse these PCB congeners accurately and simply.

EXPERIMENTAL

Chemicals

All solvents used were of high purity, hexane was of HPLC grade (Rathburn, Walkerburn, U.K.) and isooctane and *n*-undecane were distilled.

Most of the individual PCB congeners used were provided by Dr. Åke Bergman (Wallenberg Laboratory, University of Stockholm). 2,2′,4,6-TeCB, 2,3′,5′,6-TeCB and 2,3,3′,4,4′,6-HxCB were gifts from Prof. Stephen Safe (Texas A&M University, U.S.A.) and [$^{13}C_{12}$]-labelled 3,3′,4,4′-TeCB, 3,3′,4,4′,5-PeCB and 3,3′,4,4′,5,5′-HxCB were purchased from Cambridge Isotope Laboratories (Woburn, MA, U.S.A.). All PCB congeners were dissolved in isooctane prior to use.

The two technical products investigated, Chlophen A50 and Halowax 1014, were produced by Bayer (F.R.G.) and Koppers Chemical (U.S.A.), respectively.

Liquid chromatography

The HPLC system consisted of a Waters 590 programmable solvent-delivery module, a Rheodyne 7125 injector equipped with a 20- or 50-μl loop, a Hitachi 655A-22 UV-absorbance detector and a 150 × 4.6 mm I.D. Cosmosil 5-PYE column [2-(1-pyrenyl)ethyldimethylsilylated silica gel, particle size 5 μm, Nacalai Tesque, Kyoto, Japan]. A PC-based laboratory data system was used to record, store, process and plot the data.

Gas chromatography

GC analysis was performed on a Hewlett-Packard 5890 equipped with a fused-silica capillary column (50 m × 0.20 mm I.D., 0.33 μm film thickness, 5% phenylmethylsilicone phase; Hewlett-Packard, Ultra 2), an electron-capture detector and a Nelson 2600 laboratory data system. Helium was used as carrier gas at

a flow-rate of 0.3 ml/min. The temperature programme was as follows: 70°C (4 min, 90 s splitless) to 180°C at 30°C/min, hold for 2 min, then to 300°C at 2°C/min, then isothermal for 10 min.

Gas chromatography–mass spectrometry

GC was performed on a Hewlett-Packard HP5890 equipped with the same type of column as above. Helium was used as carrier gas at a head pressure of 13 p.s.i. The oven was temperature-programmed from 90°C (2 min, 1 min splitless) to 200°C at 20°C/min, then to 280°C at 3°C/min, then isothermal for 15 min. A Hewlett-Packard HP5970B mass selective detector was used for the MS determination. It was controlled by a Hewlett-Packard HP59970A work-station. Electron impact (EI) ionization was performed with an electron energy of 70 eV, and an ion source and transfer line temperature of 290°C. The detector was turned in the selected ion monitoring (SIM) mode, and two of the most intense ions in each molecular ion cluster were monitored.

Methodology

Injections of 20 μl, containing 20–200 ng, of the PCB congeners listed in Table I were made within a three-day period. Hexane was used as the mobile phase at a flow-rate of 0.70 ml/min and the UV detector was set at 254 nm. Retention and dead times (measured at the front edge of the baseline dip caused by the injection) were recorded, and capacity factors (k') were calculated. To estimate the reproducibility of the k' measurements, 2,3,3′,4′,6-PeCB (IUPAC 110) was injected every tenth injection.

To investigate if the PYE column could be used to separate PCB mixtures into fractions containing mainly 2–4-*ortho*-PCBs, toxic 1-*ortho*-PCBs and toxic 0-*ortho*-PCBs, a technical PCB product, Chlophen A50, was fractionated.

Prior to fractionation, a window-defining mixture consisting of 2,3′,4,4′,5-PeCB, 3,3′,4,4′-TeCB and 3,3′,4,4′,5,5′-HxCB was injected. In accordance with the chromatogram obtained appropriate cutting points for the fractions were established. Following calibration, the injector was thoroughly rinsed with isooctane to ensure that no residual PCBs remained. Injections of 20 μl, containing 10, 50 and 250 μg of Chlophen A50, respectively, were made and fractions were collected according to Fig. 1.

The identification of the PCB congeners present in the fractions was based on the knowledge of the composition of the technical product and the retention order on the PYE column (fraction 1) as well as on comparisons of retention times and mass spectra with pure reference compounds (fractions 2 and 3).

Quantitative analysis of toxic 0-*ortho*-PCBs present in Chlophen A50 was also performed. A solution of 150 μg of Chlophen A50 in isooctane were spiked with 99.0 ng of [$^{13}C_{12}$]3,3′,4,4′-TeCB, 11.9 ng of [$^{13}C_{12}$]3,3′,4,4′,5-PeCB and 0.405 ng of [$^{13}C_{12}$]3,3′,4,4′,5,5′-HxCB, and 150 μl of *n*-undecane were added as a keeper. The solution was reduced to 150 μl with a gentle stream of nitrogen. The spiking levels corresponded to the indigenous levels of these congeners as reported by Jensen and Athanasiadou[6]. Four 25-μl aliquots of the solution were injected using a 50-μl loop, and fractions were collected as described above. Corresponding fractions were combined and reduced to 50 μl by a gentle stream of nitrogen, and 2-μl aliquots were analysed by GC–MS. The isotope dilution method was used for quantitation.

A technical polychlorinated naphthalene (PCN) product, Halowax 1014, has been characterized by charcoal fractionation on Amoco PX-21A, GC–MS and GC

TABLE I

INDIVIDUAL k' VALUES FOR SOME PCB CONGENERS ON A PYE COLUMN

The lines indicate the positions of the cutting points for the 1- and 0-*ortho*-PCB fractions, respectively. The chromatographic conditions are described under Experimental.

k'	*IUPAC No.*	*Structure*	k'	*IUPAC No.*	*Structure*
0.28	98	2 4 6–23	0.70	64	23 6– 4
0.30	155	2 4 6–2 4 6		135	23 5 –23 6
0.34	29	2 45 –		149	23 6–2 45
0.35	152	23 56–2 6	0.71	58	23 – 3 5
0.36	209	23456–23456		83	23 5 –23
0.37	207	23456–234 6		196	2345 –234 6
	197	234 6–234 6	0.72	84	23 6–23
0.38	10	2 6–	0.73	97	2 45 –23
	1	2 –	0.76	168	2 4 6– 345
0.39	7	2 4 –		201	2345 –23 56
0.40	9	2 5 –	0.77	153	2 45 –2 45
	50	2 4 6–2	0.78	85	234 –2 4
	200	234 6–23 56		171	234 6–234
0.41	2	3 –	0.80	61	2345 –
0.42	202	23 56–23 56		70	2 5 – 34 6
0.44	5	23 –	0.81	174	2345 –23
	12	34 –	0.82	80	3 5 – 3 5
0.46	30	2 4 6–	0.83	132	234 –23 6
	146	23 5 –2 45	0.84	87	234 –2 5
0.48	3	4 –		120	2 45 – 3 5
	69	2 4 6– 3		177	23 56–234
	75	2 4 6– 4		206	23456–2345
0.49	24	23 6–	0.85	82	234 –23
0.50	62	234 6–	0.86	66	2 4 – 34
	176	234 6–23 6	0.87	71	2 6– 34
0.53	102	2 45 –2 6		94	23 5 –2 6
0.54	47	2 4 –2 4	0.88	56	23 – 34
	53	2 5 –2 6	0.89	137	2345 –2 4
	165	23 56– 3 5	0.90	15	4 – 4
0.55	21	234 –	0.92	172	2345 –23 5
	73	2 6– 3 5	0.94	110[a]	23 6– 34
0.57	46	23 –2 6	0.98	107	23 5 – 34
	140	234 –2 4 6		180	2345 –2 45
0.59	49	2 4 –2 5	1.00	106	2345 – 3
0.60	31	2 5 – 4	1.04	122	345 –23
	42	23 –2 4		158	234 6– 34
0.61	28	2 4 – 4	1.09	123	345 –2 4
0.62	14	3 5 –	1.11	118	2 45 – 34
	52	2 5 –2 5		138	234 –2 45
	144	234 6–2 5	1.12	128	234 –234
0.63	72	2 5 – 3 5	1.19	159	2345 – 3 5
	183	234 6–2 45	1.24	194	2345 –2345
0.64	90	23 5 –2 4	1.26	193	23 56– 345
0.65	187	23 56–2 45	1.28	170	2345 –234
0.66	86	2345 –2	1.31	167	2 45 – 345
0.67	4	2 –2	1.36	105	234 – 34
	44	23 –2 5	1.58	156	2345 – 34
	92	23 5 –2 5	1.74	157	234 – 345
0.68	99	2 45 –2 4	1.84	189	2345 – 345
0.69	40	23 –23	1.99	77	34 – 34
	95	23 6–2 5	2.73	126	345 – 34
	101	2 45 –2 5	3.52	169	345 – 345
	116	23456–			

[a] IUPAC 110; $k' = 0.94 \pm 0.01$, $n = 10$.

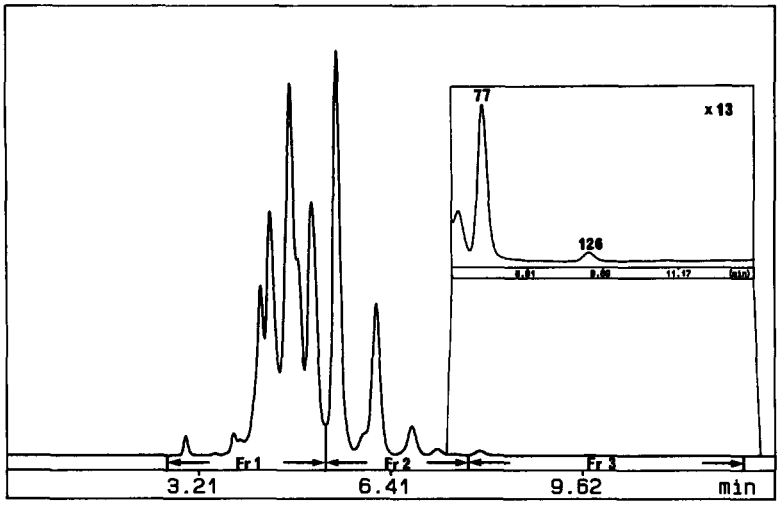

Fig. 1. HPLC chromatogram showing the fractionation of Chlophen A50 on a 150 × 4.6 mm I.D. PYE column. The numbered peaks correspond to 3,3',4,4'-TeCB (IUPAC 77) and 3,3',4,4',5-PeCB (IUPAC 126). The chromatographic conditions are described in Experimental.

with electron-capture detection (ECD), as described elsewhere[8]. To compare the selectivity of the Amoco PX-21A and the PYE phases, 20 μg of Halowax 1014 dissolved in 20 μl of isooctane were injected and fractions were collected according to Fig. 2. GC–ECD analysis of the fractions was performed according to methods described elsewhere[8].

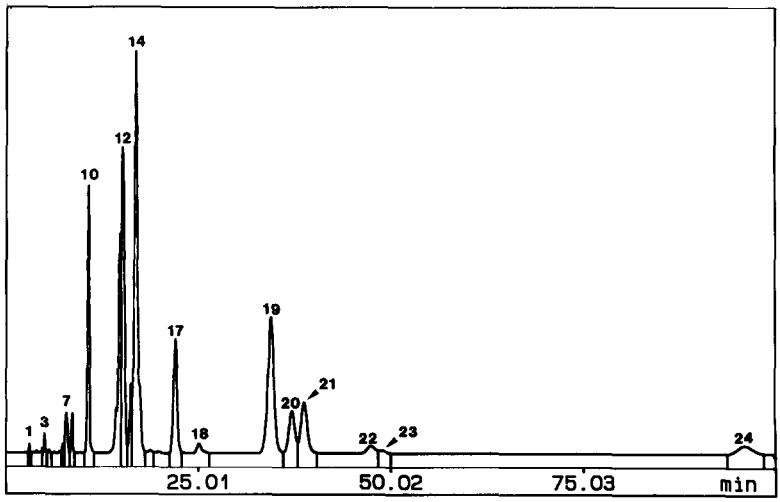

Fig. 2. HPLC chromatogram showing the fractionation of Halowax 1014. The fractions contain the following compounds or compound classes: 1–6, tri-; 7–10, tetra-; 11–16, penta- and early eluting hexa-; 17, 1,2,3,4,5,6,8-hepta-; 18, octa-; 19–23, late eluting hexa- and 24, 1,2,3,4,5,6,7-heptachloronaphthalene. The chromatographic conditions are described in Experimental.

RESULTS AND DISCUSSION

Chromatography of reference compounds

The results of the k' measurements of individual PCB congeners are summarized in Table I. The instrumental error of the k' measurements was *ca.* 1% (IUPAC 110; $k' = 0.94 \pm 0.01, n = 10$). The data in Table I were used to create a three-dimensional plot, in which the k' values were plotted *versus* the number of chlorines and *versus* the number of *ortho* chlorines (Fig. 3). From this plot some general features of the retention on the PYE column become evident. Retention tends to increase with the degree of chlorination and to decrease with increasing number of *ortho* chlorines. The same phenomenon has been described for the retention of PCBs on an activated charcoal (Amoco PX-21) column[3].

Furthermore, since the retention varies considerably between isomers with the same number of total as well as *ortho* chlorines, there must be some other characteristic affecting the retention, probably the substitution pattern. To study the influence of the substitution on the retention, the k' values of biphenyls with the same substitution in one ring and different substitution in the other have been compared. Some trends revealed by the comparison are summarized in Table II. It seems as if half-ring structures with the chlorines close together are more retentive than those with the chlorines spread over the rings, *e.g.*, 2,3,4-substitution is more retentive than 2,3,5-substitution.

The same type of selectivity of the PYE phase has been described for the reversed-phase separation of tetrachlorodibenzo-*p*-dioxins[9].

The selectivity of the PYE phase may be explained by a charge-transfer mechanism, in which electron-density acceptor and donor regions of the PCBs induce a change in the localization of the π-electron cloud of the pyrene moieties of the phase

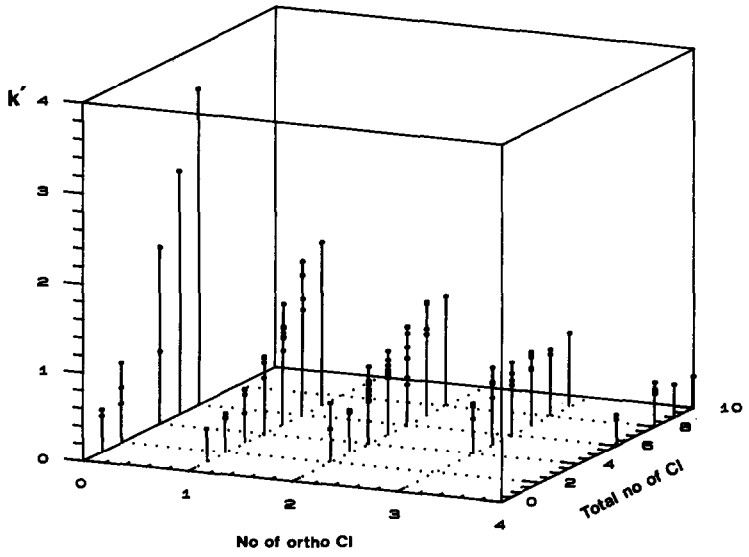

Fig. 3. Plot of k' values from Table I *versus* the number of chlorines and *versus* the number of *ortho* chlorines.

TABLE II

RETENTION ORDER ON A PYE COLUMN OF PCBs SUBSTITUTED IN THE SAME WAY IN ONE OF THE RINGS AND AS BELOW IN THE OTHER

The chromatographic conditions are described under Experimental.

Number of chlorine	*Retention order*
1	$2 < 3 \ll 4$
2	$26 < 25 \approx 24 < 23 \leqslant 35 \ll 34$
3	$246 < 236 \leqslant 235 < 245 < 234 \ll 345$
4	$2346 < 2356 \ll 2345$

so that an EDA complex is formed. This type of mechanism could account for the observed retention behaviour in the following three ways:

(1) Highly chlorinated biphenyls would be expected to form strong EDA complexes with the PYE column because chlorinated compounds are very good electron-density acceptors and polycyclic aromatic hydrocarbons, such as pyrene, are among the most effective electron donors known[10].

(2) PCBs with many *ortho* chlorines should be less retained owing to steric interaction between *ortho* chlorines (or between *ortho* chlorines and *ortho* hydrogens), leading to twisting of the biphenyl σ-bond, an increase in the distance between the biphenyl and the pyrene moieties, and thus to a weaker EDA complex.

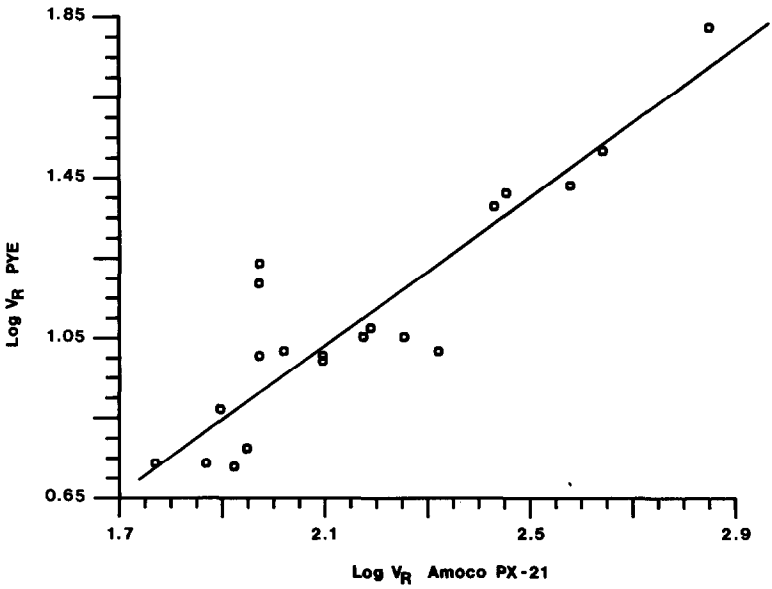

Fig. 4. Graph of log V_R of polychlorinated naphthalenes chromatographed on a 150 × 4.6 mm I.D. PYE column and on a 300 × 4.6 mm I.D. Amoco PX-21 column. The chromatographic conditions for the PYE column are described in Experimental. The chromatographic conditions for the Amoco PX-21 column were the following: mobile phase, toluene; flow-rate, 1 ml/min.

(3) PCBs with half-ring structures with the chlorines close together offer naturally better acceptor pockets for EDA complexing than those that have half-ring structures with the chlorines spread over the rings, and are therefore more retained.

GC analysis of the fractions of the PCN product Halowax 1014 revealed that the elution order, shown in Fig. 2, is much the same from a PYE column as from an activated charcoal column (Amoco PX-21). In Fig. 4 these results are shown in the form of a log V_R (V_R = retention volume) *versus* log V_R plot. This emphasizes further the similarities in retention between the PYE column and activated charcoal columns of the PX-21 type.

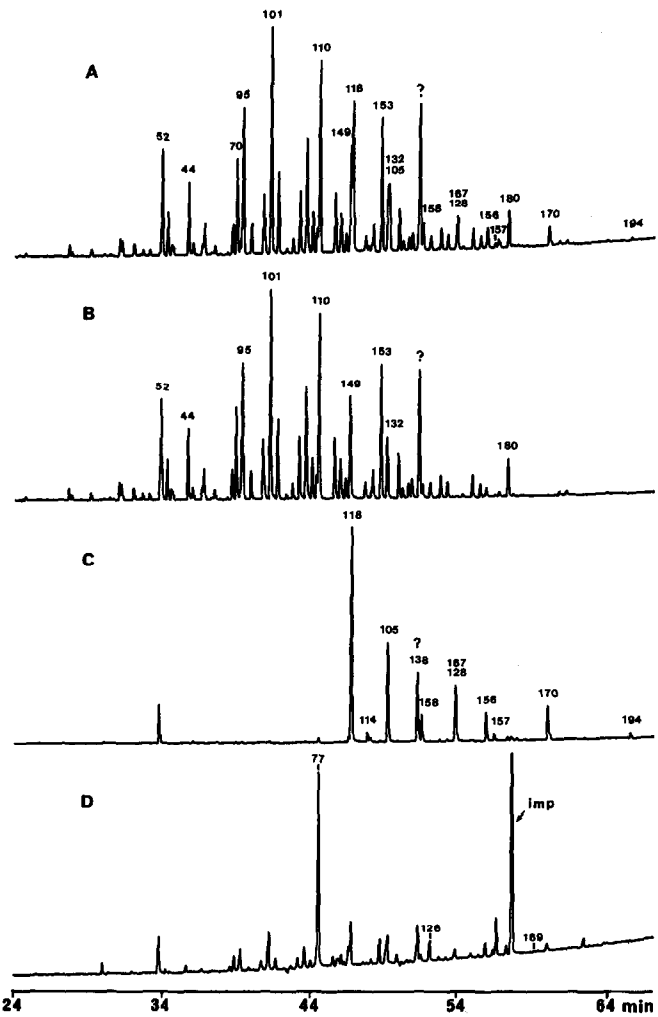

Fig. 5. Gas chromatograms of the fractions from Fig. 1: (A) unfractionated Chlophen A50; (B) fraction no. 1; (C) fraction no. 2; (D) fraction no. 3. The "?" mark denotes a peak of uncertain identity (see Results and Discussion); "imp" means impurity. The chromatographic conditions are described in Experimental.

Fractionation of Chlophen A50

GC chromatograms from the analysis of the Chlophen A50 fractions from the PYE column are shown in Fig. 5.

The peak labelled "?" in the chromatogram of unfractionated Chlophen A50 is generally thought to be 2,2',3,4,4',5-HxCB (IUPAC 138) and is supposed to elute in fraction 2 according to Table I, but since an HxCB isomer with the same retention time also eluted in fraction 2, the peak "?" was suspected to contain two different PCB congeners. Very recently, Roos *et al.*[11,12] reported that an HxCB isomer, 2,3,3',4',5,6-HxCB (IUPAC 163), is present in PCB mixtures and is interfering with the determination of IUPAC 138 when SE-54 type phases are used. However, lack of reference compounds prevented us from identifying the HxCB isomers present in fractions 1 and 2.

According to Fig. 5 the PYE column produces fractions containing toxic 0- and 1-*ortho*-PCBs that are clean enough for GC–ECD analysis, although the second fraction contanis some 2-*ortho*-PCBs in addition to the 1-*ortho*-PCBs. One of these congeners, 2,2',3,3',4,4'-HxCB (IUPAC 128), interferes with the analysis of one of the 1-*ortho*-PCBs, 2,3',4,4',5,5'-HxCB (IUPAC 167). IUPAC 167 has a lower toxicity than the other 1-*ortho*-PCBs and is therefore not of particular interest. If this congener is considered important it could be separatad from IUPAC 128 on an SB-Octyl 50 column (see Introduction). Some carryover of 1-*ortho*-PCBs into the 0-*ortho* fraction can also be seen, which is probably due to deposition of PCBs on the outside wall of the outlet capillary and subsequent washing off into the following fractions. The problem may be avoided by using a fractionation valve and a separate outlet capillary for each fraction, and by washing the tips of the outlet capillaries between runs.

Quantitative analysis of the 0-*ortho* fraction showed that the concentrations of 3,3',4,4'-TeCB, 3,3',4,4',5-PeCB and 3,3',4,4',5,5'-HxCB were 1260 μg, 51.8 μg and 2.72 μg per gram of Chlophen A50, respectively. These results are of the same magnitude as those reported by Jensen and Athanasiadou[6] (660 μg of 3,3',4,4'-TeCB, 79 μg of 3,3',4,4',5-PeCB and 2.7 μg of 3,3',4,4',5,5'-HxCB per gram of Chlophen A50).

No notable effect on the separation could be seen between the different loads of Chlophen A50 studied (10–250 μg).

Comparison of the PYE column and activated charcoal columns for the isolation of toxic PCBs

Although activated charcoal columns seem to separate the compounds covered by the present investigation in almost the same way as the PYE column, there are great differences in efficiency, solvent requirements and consumption, and analysis time.

The PYE columns are highly efficient, *ca.* 45 000 theoretical plates/m, and give sharp peaks with good symmetry (asymmetry factor 1.10), which is in great contrast to the broad elution profiles with severe tailing generally observed with chromatography on activated charcoal. The high efficiency of the PYE column makes it possible accurately to isolate the toxic 1-*ortho*-PCBs, which is not possible by the generally used charcoal methods[3–5].

Toxic 0-*ortho*-PCBs are usually eluted from activated charcoal columns with stepwise gradients of cyclohexane–dichloromethane, benzene in ethyl acetate and toluene[3,4] or increasing percentages of toluene in cyclohexane[5]. In contrast, the PYE column requires only a single solvent, hexane, which provides many advantages: UV detectors can be used to detect the solutes, the fractions become easy to evaporate and the residue can be directly analysed by GC–ECD or GC–MS.

Fractionation of a technical PCB product into three fractions (containing 2–4-*ortho*-PCBs, toxic 1-*ortho*-PCBs and toxic 0-*ortho*-PCBs) takes less than 15 min if the PYE column is used. The fraction volume are only 2–3 ml and are easily reduced to volumes suitable for GC–ECD or GC–MS by a gentle stream of nitrogen. On activated charcoal columns a fractionation of PCB mixtures into two fractions (one containing the bulk of PCB and one containing toxic 0-*ortho*-PCBs) takes at least the same time. Furthermore, the evaporation of the large volumes of aromatic solvents (50–200 ml) used to elute the 0-*ortho*-PCB fraction, is very time-consuming. The risk of contamination is also increased by the use of such large amounts of aromatic solvents.

CONCLUSIONS

The retention behaviour of PCBs on the PYE column can be summarized as follows: a decreasing number of *ortho* chlorines, and thus increasing planarity, gives increasing retention; solutes with continuous acceptor regions are retained through EDA complexing with the pyrene moieties of the stationary phase; highly chlorinated PCBs are retained owing to the high electron affinity of the chlorines.

The PYE column offers a simple and powerful tool for the isolation of toxic 0- and 1-*ortho*-PCBs from technical PCB formulations.

It is worth noting that our method makes it possible to isolate and analyse 1-*ortho*-PCBs, which is not possible by the generally used charcoal methods[3–5].

Further work is needed to adapt and extend the method in such a way that toxic 0- and 1-*ortho*-PCBs can be accurately quantitated in environmental samples.

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