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Enhanced selectivity in the analysis of chlorobiphenyls on a carborane phenylmethylsiloxane copolymer gas chromatography phase (HT-8)

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Abstract

The congener specific analysis of polychlorinated biphenyls (PCBs) on a new chemically bonded stationary GC phase, HT-8 (1,7-dicarba-closo-dodecarborane phenylmethyl siloxane) is investigated. The incorporation of carborane in the polysiloxane backbone enhances selectivity and temperature stability of the phase. On a 60 m × 0.25 mm I.D. fused-silica column coated with 0.25 μm HT-8, a total of 106 congeners in Aroclor mixtures elute as separated peaks. By the use of mass spectrometric detection 138 congeners can be analysed without interference from coeluting PCBs. In literature, no other GC phase has been reported with such a high PCB selectivity. An interesting feature of HT-8 is its near-baseline separation of the critical pair CB138/CB163. Separations for these congeners on other GC phases are reviewed. The performance of a thin film (0.15 μm) version of the HT-8 column with fast temperature programming is tested. It is concluded that congener-specific rapid screening of seven indicator PCBs (CB28, CB52, CB101, CB118, CB138, CB153 and CB180) with a total GC run-time of 13 min is feasible with an accuracy sufficient for most purposes.

1. Introduction

The analysis of polychlorinated biphenyls (PCBs) as separated individual compounds is one of the most challenging tasks in capillary column gas chromatography (HRGC). A total of 209 possible chlorobiphenyl (CB) configurations (congeners) exists and around 150 have been reported at significant concentrations in the environment and technical PCB mixtures [1]. Although many stationary phases have been tested over the past 15 years the ideal phase for PCB separations is still to be found. Certain CB congeners have been given special priority owing to their toxicity and occurrence in the environment [2] (CBs: 18, 37, 44, 49, 52, 70, 74, 77, 81, 87, 99, 101, 105, 114, 118, 119, 123, 126, 128, 138, 151, 153, 156, 157, 158, 167, 168, 169, 170, 177, 180, 183, 187, 189, 194 and 200). This list of priority CBs is based on toxicity data and structure–activity relationships obtained in the early eighties. Since then, one of these congeners (CB168) has never been reported in biological or environmental samples and its status as priority CB seems unjustified. On the other hand another congener with a toxic potential and not

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in the priority list (CB163) has been detected in significant concentrations (20–30% of CB138) in technical, biological and environmental samples [3–5]. CB163 is a toxic congener of the phenobarbital-type [6]. As a logical consequence CB163 should take the place of CB168 among the priority congeners. In the present paper we have adopted this new list. The separation of the 36 priority congeners from the bulk of PCBs is complicated by their relatively low particular concentrations compared with the bulk of PCBs and/or by the high number of possible coelutants. A set of seven CBs (28, 52, 101, 118, 138, 153 and 180) is of particular interest for many laboratories due to their incorporation in the legislation of some European countries and international bodies. These congeners have been selected as indicator CBs on the basis of their presence at high concentrations in technical PCB mixtures and in the environment.

The investigation of any new stationary phase for PCB analysis includes the complete characterisation of the elution profile of technical and environmental PCB mixtures. Ideally, all 209 CBs should be used as individual standards for the determination of their retention times. However, all these congeners are not commercially available and have to be synthesised especially for this purpose. Thus, to date only one stationary phase, SE-54 (5% diphenyl, 1% vinyl dimethyl polysiloxane, [7]) has been investigated in this way. The PCBs found in the total environment stem from technical PCB mixtures originally produced by catalytic chlorination of biphenyl. By this process CB congeners which have a thermodynamically unfavourable chloro-substitution pattern (3-, 2,6-, 3,4-, 3,5-, 2,3,6-, 2,4,6-, 3,4,5- and 2,3,4,6-) in both rings are produced at very low yields. Therefore, these congeners are only found in the environment at insignificant concentrations. This fact allows for the use of a limited number of CB congeners for investigations of HRGC stationary phases. A series of Aroclor mixtures have been characterised with respect to qualitative and quantitative compositions using all 209 PCBs as authentic reference compounds in combination with multidimensional GC [8]. As an alternative to the use of all 209 PCBs, these Aroclor mixtures can be used as secondary standards for investigations of HRGC stationary phases.

The list of HRGC phases with well characterised PCB elution profiles include: 50% dioctyl dimethylsiloxane [9], 10% methyl-octadecyl polysiloxane [10], 20% methyl-octadecyl polysiloxane [10], 50% methyl-octadecyl polysiloxane [10], dimethylsiloxane [11], 5% diphenyl–1% divinyl dimethylsiloxane [7], 5% diphenyl dimethylsiloxane [11], 7% diphenyl dimethylsiloxane [12], 15% diphenyl dimethylsiloxane [13], 50% diphenyl dimethylsiloxane [13], 1,7-dicarbacloso-dodecarborane dimethyl polysiloxane [11], poly(dimethylsiloxane carbonate) [12], 14% cyanopropyl–1% vinyl methylsiloxane [11], 70% cyanopropyl dimethylsiloxane [13], 100% cyanopropylsiloxane [11]. With these phases, no single column has been found able to resolve all 209 congeners, neither the ca. 150 congeners present in technical mixtures, nor the 36 priority congeners and most surprisingly, not even the set of seven indicator congeners.

The main feature for the separation of CB congeners in HRGC is their difference in vapour pressure. All 209 PCBs are highly non-polar and interact only weakly with the stationary phase. As a consequence, the elution profiles of PCB mixtures are quite similar for most stationary phases. Significant changes in the retention of PCBs is mainly seen with highly polar (electron donating) groups incorporated in the stationary phase or with highly non-polar long chain aliphatics in the phase. The drawback of such phases is their low chemical stability which requires that the analysis is performed with mild temperature programmes which cause undesirable long run-times. One exception is the carborane-polydimethylsiloxane copolymer. The incorporation of a large carborane group in the siloxane backbone in this phase markedly changes the elution profile of PCB mixtures and improves the temperature stability of the phase. In the pioneer days of PCB separations with glass capillary columns coated with a liquid phase good results have been obtained with a carborane-containing phase (Dexsil 410, carborane-cyanoethylmethylsiloxane) [14]. Al-

though the separation efficiency of such columns cannot be compared directly with that of modern fused-silica capillary columns which have chemically bonded phases capable of operating at high temperatures and less active sites, it is interesting to note this early preference for phases containing carborane in PCB separations. In our laboratory we have thoroughly tested the retention characteristics of a chemically bonded 1,7-dicarba-closo-dodecarborane-dimethylsiloxane (erroneously named 1,2-dicarba- [11]) and have come to the conclusion that this phase is the optimal choice either used alone for congener-specific rapid screening [15] or used in a series coupled with 5% diphenyl-dimethylsiloxane for analysis of the highly toxic planar CBs [16,17], the 36 priority CBs [18], the seven indicator CBs [13] and complex mixtures of CBs and organochlorine pesticides in biological samples [19]. The present paper reports the separation of 168 individual CB congeners in technical PCB mixtures on a new commercially available carborane containing stationary phase (1,7-dicarba-closo-dodecarborane phenylmethyl siloxane, HT-8). A thick (0.25 μm) and a thin (0.15 μm) film are tested. Emphasis is given to the accurate analysis and the rapid screening of the seven indicator CBs in technical and biological samples.

2. Experimental

2.1. Materials

All individual 209 CBs have been given a IUPAC number to replace their rather long chemical names. For three congeners (CB199, CB200 and CB201) their original number [20] has been found to be in disagreement with the IUPAC numbering system [21]. In the present paper the new numbers [21] will be used for these three CBs (i.e. CB201, CB199 and CB200, respectively).

A total of 149 CBs were available for this study. Of these, 105 were available as either authentic reference standards or certified standard mixtures. Individual CBs were obtained either as neat crystals from the Community Bureau of Reference (BCR, Brussels, Belgium) (CBs: 18, 20, 28, 35, 52, 101, 105, 118, 128, 138, 149, 153, 156, 170 and 180), from Promochem (Germany) (CBs: 8, 37, 42, 44, 48, 49, 53, 55, 60, 69, 70, 74, 75, 81, 84, 87, 92, 100, 110, 114, 115, 119, 123, 124, 136, 141, 151, 158, 167, 171, 172, 183, 187, 189, 194, 202), from Ultra Scientific (USA) (CBs: 1, 2, 3, 4, 6, 10, 11, 13, 16, 26, 66, 80, 95, 133, 185, 190), or were synthesised and purified as described previously (CBs: 77, 122, 126, 163, 169) [3,22]. Certified CB standard solutions were obtained from the National Research Council, Canada (CLB-1A, CBs: 18, 31, 40, 44, 49, 54, 77, 86, 87, 121, 153, 156, 159, 209; CLB-1B, CBs: 15, 52, 60, 103, 105, 128, 143, 154, 173, 182, 202, 205, 207, 208, 209; CLB-1C, CBs: 15, 114, 129, 137, 153, 171, 183, 185, 189, 191, 199, 201, 203, 206, 209; CLB-1D, CBs: 15, 101, 118, 138, 141, 153, 170, 180, 187, 194, 195, 196, 199, 209) and from Supelco (Italy) (DCMA, CBs: 1, 11, 29, 47, 121, 136, 185, 194, 206 and 209). A further 39 CB congeners had previously been synthesised in well defined mixtures [13] (CBs: 17, 19, 23, 24, 25, 27, 29, 34, 36, 43, 45, 46, 48, 57, 59, 63, 64, 67, 68, 71, 74, 83, 84, 89, 90, 91, 97, 99, 107, 110, 118, 125, 130, 132, 134, 138, 147, 152, 155, 160, 165, 177, 178, 179, 183, 193) and 5 CB congeners were obtained from Dr. J. de Boer (The Netherlands Institute for Fisheries Research) (CBs: 33, 56, 82, 85 and 157).

Technical PCB mixtures were obtained in 2,4,4-trimethyl pentane from Supelco (Milan) as the Aroclor lots LA12790 (A1232), LA13646 (A1242), LA13647 (A1248), LA13614 (A1254), LA13576 (A1260), LA12791 (A1262) and a mixture of four Aroclors (A1016, A1232, A1248 and A1262, 1:1:1:1).

For the investigation of the magnitude of the ECD interference from coeluting CB congeners on the HT-8 column for the set of seven indicator CBs selected biological sample extracts were analysed. The biological sample extracts were obtained from previous investigations in our laboratory (stored in freezer at −20°C) and represent environmentally contaminated samples: Eel and tuna from the Mediterranean Sea

[19], seal from the Kattegat Sea [23] and human milk from Italy [24].

The HT-8 GC columns were kindly supplied by Scientific Glass Engineering as 60 m × 0.25 mm I.D. fused-silica open tubular columns coated with 1,7-dicarba-closo-dodecarborane phenylmethyl siloxane. Two sizes of film thickness were investigated (0.15 μm and 0.25 μm). For the investigation of the magnitude of the ECD interference from coeluting CB congeners on the HT-8 column biological sample extracts were re-analysed on a 15% diphenyl dimethylsiloxane (0.20 μm film, 50 m × 0.25 mm I.D.) coated fused-silica open tubular SIL-13 column (Chrompack) and a 5% diphenyl dimethyl siloxane (0.25 μm film, 25 m × 0.25 mm I.D.) coated fused-silica open tubular SIL-8 column (Chrompack) series coupled with a 1,7-dicarba-closo-dodecarborane dimethyl siloxane (0.1 μm film, 25 m × 0.2 mm I.D.) coated fused-silica open tubular HT-5 column (Scientific Glass Engineering). These two columns have recently been fully characterised with respect to PCB elution profiles [13].

2.2. Gas chromatography

GC analysis was performed with a pressure controlled Hewlett-Packard 5890A II gas chromatograph (GC) equipped with a ^{63}Ni electron capture detector (ECD) and a HP7673A autosampler. The electron capture detector (ECD) was operated at 300°C, purged with 60 ml/min of 10% methane in argon. The hydrogen carrier gas flow was 45 cm/s at 90°C and held constant by the pressure controlled inlet throughout the whole temperature programme. Detection limits were 0.2–1 pg depending on the column bleed. Aliquots (1.0 μl) of the standards and extracts in 2,4,4-trimethyl pentane were on-column injected at 90°C. The Aroclors were injected at concentrations around 8 ng/μl (total PCB). The investigated columns were installed in the GC oven together with a 2 m × 0.53 mm I.D. fused-silica pre-column using a glass press-fit connector (MEGA, Milan).

Chromatographic data were acquired on an HP Vectra i386/i387 personal computer with the Chemstation Hewlett-Packard 3365 software.

The GC conditions for the HT-8 columns were varied from a slow temperature program (which furnished overall optimum separations) to ballistic heating (which gave acceptable separations in the shortest possible time). The temperature programmes used are listed in Table 1 for the thick and the thinner film, respectively. The analysis of biological extracts on HT-8, SIL-13 and SIL-8/HT-5 were done with the slowest temperature programme.

2.3. Mass spectrometry

For confirmation purposes, the characterisation of the PCB elution profiles on the HT-8 columns was repeated with GC–MS.

A Carlo Erba QMD-1000 quadrupole mass spectrometer interfaced to a Hewlett-Packard 5890A gas chromatograph equipped with an on-column detector and an HP7673A autosampler. Selected-ion monitoring of the main ion for each chlorination class was carried out as follows (m/z): Mono-CBs 186, di-CBs 222, tri-CBs 256, tetra-CBs 292, penta-CBs 326, hexa-CBs 360, hepta-CBs 396, octa-CBs 432, nona-CBs 466 and deca-CB 500. The detection limit obtained with the QMD-1000 was around 15 pg (m/z 292 for CB 77 at a signal-to-noise ratio of 3). The Aroclors were injected in (1 μl) 2,4,4-trimethyl pentane at concentrations of 0.8 μg/μl (total PCB) and the individual PCB standards at concentrations around 1–2 ng/μl. The HP-5890A gas chromatograph was equipped with columns of identical dimensions as for GC–ECD analysis and run under identical conditions, the only exception being that the carrier gas was helium (at 24 cm/s). The use of helium in stead of hydrogen as carrier gas did not alter the gas chromatographic elution of the CB congeners significantly.

3. Results and discussion

3.1. Characterisation of PCB elution profiles on HT-8

The identification of the peaks in the chromatograms of a mixture of four Aroclors

Table 1
Temperature programmes used for rapid analyses of the seven indicator CBs on HT-8

Run time (min)	Isothermal-1		Ramp-1 (°C/min)	Isothermal-2		Ramp-2 (°C/min)	Isothermal-3	
	(°C)	(min)		(°C)	(min)		(°C)	(min)
Film thickness 0.25 μm								
55	90	2	20	170	7.5	3	275	10
48	90	2	20	170	5	4	275	10
41	90	2	20	170	2.5	5	275	10
35	90	2	20	170	0	6	280	10
26	90	1	30	170	0	8	300	5
21	90	1	30	170	0	12	300	5
18	90	1	30	170	0	15	310	5
16	90	1	30	170	0	20	320	5
Film thickness 0.15 μm								
48	90	2	20	170	7.5	3	275	10
39	90	2	20	170	5	4	275	10
33	90	2	20	170	2.5	6	275	10
27	90	1	20	170	0	8	280	10
21	90	1	30	170	0	10	310	8
18	90	1	30	170	0	12	310	5
16	90	1	30	170	0	15	310	5
13	90	1	30	170	0	20	320	5

(A1016, A1232, A1248 and A1262, 1:1:1:1) injected on the HT-8 columns (Figs. 1 and 2) was based on injections of the 149 available authentic reference compounds. Analysis of Aroclor mixtures has shown the presence of 20 other CB congeners at detectable concentrations (CBs: 5, 7, 9, 21, 22, 32, 41, 51, 96, 102, 131, 135, 144, 146, 174, 175, 176, 197, 198, 200) [8]. For these CBs, peaks in the chromatograms of Figs. 1 and 2 were tentatively assigned based on GC–MS analysis of the individual Aroclor mixtures. The selected-ion chromatograms obtained with the HT-8 column were very similar to selected-ion chromatograms obtained previously with a HT-5 column [11,15] and no problems in tentative peak assignments were encountered.

A useful tool for peak assignments in GC of CB congeners has proven to be the construction of single ring indices (SRIs) for each possible phenyl ring substitution pattern [25]. With this approach the assumption is made that the retention index (RI) for each specific CB congener can be expressed as the sum of the two halves of the molecule. Because of the symmetry of the CB molecule, there are twenty possible "halves"; these single-ring substitution patterns are shown in Table 2. All retention time data was linearly transformed into RI data relative to CB16 (RI = 44) and CB180 (RI = 144). The 20 possible SRIs were obtained as the half values of the respective RIs for each of the corresponding twenty symmetric CB congeners.

The tentative peak assignments for the 20 CBs for which no authentic standards were available were confirmed by the SRI approach: The RIs of the 149 identified peaks in the chromatogram of Fig. 1 were significantly correlated with the RIs computed by the sum of the SRIs ($r^2 = 0.998$, mean standard error of estimate 1.8) and the observed RIs for the 20 tentatively assigned peaks were in good agreement with the computed RIs (with a deviation less than 2).

In Table 2 the SRIs for HT-8 are compared to those of other carborane stationary phases, i.e. HT-5 (data from previous studies in our laboratory [11,15]) and Dexsil 410 (reconstructed data from [14]) and from phases which do not contain the carborane group i.e. SE54 (5% diphenyl, 1% vinyl dimethyl polysiloxane, [7]), SIL-5 (dimetylsiloxane, [11]) and SIL-8 (5% diphenyl

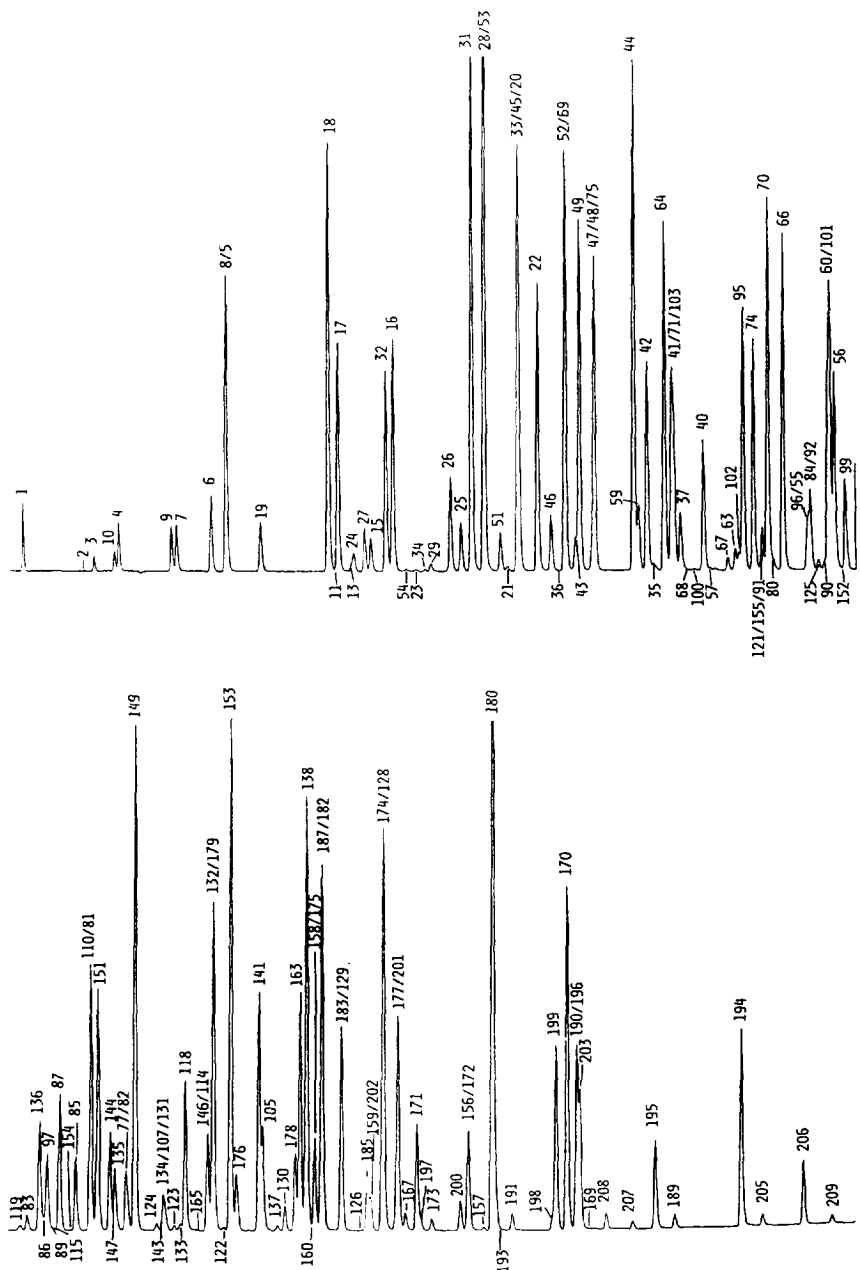

Fig. 1. GC–ECD analysis of Aroclors (A1016, A1232, A1248 and A1262, 1:1:1:1) on HT-8 (0.15 μm). Total run-time 48 min.

dimethylsiloxane, [11]). Principal component analysis (PCA) has previously been used to classify samples into groups of similar PCB congener patterns [26]. In order to classify the stationary phases into groups of similar PCB SR elution patterns PCA was carried out on the normalised SRIs in Table 2. The PCA showed that about 99% of the total variance could be explained by the first principal component and about 0.4% by the second. A plot of the first two

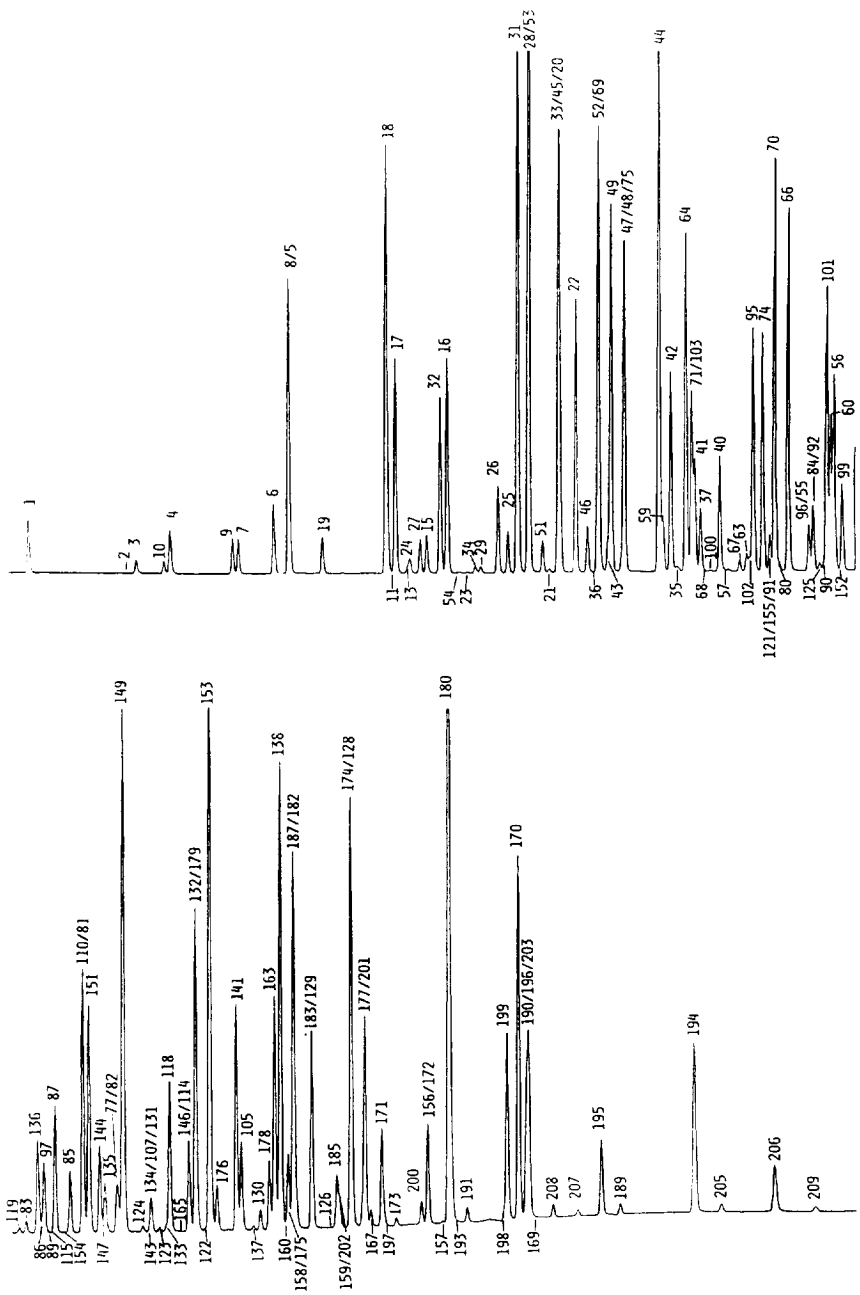

Fig. 2. GC–ECD analysis of Aroclors (A1016, A1232, A1248 and A1262, 1:1:1:1) on HT-8 (0.25 μm). Total run-time 57 min.

component weights is reflected in Fig. 3a. It appears that the carborane stationary phases are clearly differentiated from the non-carborane stationary phases by their negative second component. A closer study of the load distribution of the SRIs (Fig. 3b) reveals that the carborane stationary phases are special in their relatively stronger retention of the single ring structures

Table 2
Single ring indices for PCBs on carborane stationary phases

Single ring substitution pattern	HT-5	HT-8	DEXSIL 410	SE54	SIL-5	SIL-8
0-	−5.1	−13.7	−16.7	−10.3	−11.4	−6.3
2-	10.5	10.0	7.8	7.4	6.8	9.8
3-	19.7	18.7	20.0	17.9	15.9	19.0
4-	21.3	20.8	20.9	19.5	19.4	19.7
2,6-	20.9	22.7	22.7	23.8	24.1	23.0
2,5-	29.9	31.2	32.3	31.8	32.2	30.6
2,4-	33.1	32.7	33.1	32.7	33.3	31.7
2,3-	36.4	38.5	38.1	37.6	37.9	37.0
3,5-	41.7	42.0	42.4	41.4	41.9	40.4
3,4-	51.3	52.7	52.8	50.2	50.5	49.7
2,4,6-	41.4	41.5	40.6	43.5	45.2	42.9
2,3,6-	45.7	48.1	47.0	49.8	50.3	49.3
2,3,5-	54.4	55.6	55.1	56.3	56.8	55.9
2,4,5-	57.1	58.1	57.6	58.0	58.5	57.2
2,3,4-	65.2	66.3	66.1	65.7	65.0	66.0
3,4,5-	77.2	77.0	78.5	74.8	74.4	75.1
2,3,5,6-	64.4	65.6	63.7	69.1	69.4	69.2
2,3,4,6-	67.5	68.2	68.9	71.3	71.7	71.5
2,3,4,5-	86.4	85.5	89.2	85.3	84.4	86.4
2,3,4,5,6-	90.7	90.1	91.2	94.5	93.1	98.8

3,4-, 3,4,5- (which have all significant negative loading on the second component) and their relatively weaker retention of the single ring structures 2,6-, 2,4,6-, 2,3,6- 2,3,4,6,- 2,3,5,6- and 2,3,4,5,6- (which have all significant positive loading on the second component).

This similarity in retention mechanism towards PCBs is explained by the presence of the large 1,7-dicarba-closo-dodecarborane group in the copolymer. The carborane group in the stationary phase has a high affinity towards CBs with a low degree of *ortho*-substitution. This has previously been explained by a π-electron interaction between the CB moiety and 1,7-dicarba-closo-dodecacarborane caused by a high degree of rotational freedom for non- and mono-*ortho* chlorinated CBs compared to di-, tri-, and tetra-*ortho* chlorinated CBs which allows for a coplanar conformation of the former CBs and a closer contact with the carborane group [11].

3.2. Performance of the HT-8 columns (0.15 μm and 0.25 μm) at optimal conditions

The ECD chromatograms obtained for the Aroclor mixture (A1016-A1260) on HT-8 with the film thicknesses 0.15 μm and 0.25 μm are shown in Figs. 1 and 2, respectively. A series of temperature programmes and carrier gas velocities were tested. The conditions used in Figs. 1 and 2 result in GC runs of 48 and 55 min, respectively, and represent a good compromise between speed of analysis and efficiency of separations. Optimisations for specific critical separations could be obtained with slower temperature programmes. The chromatograms ob-

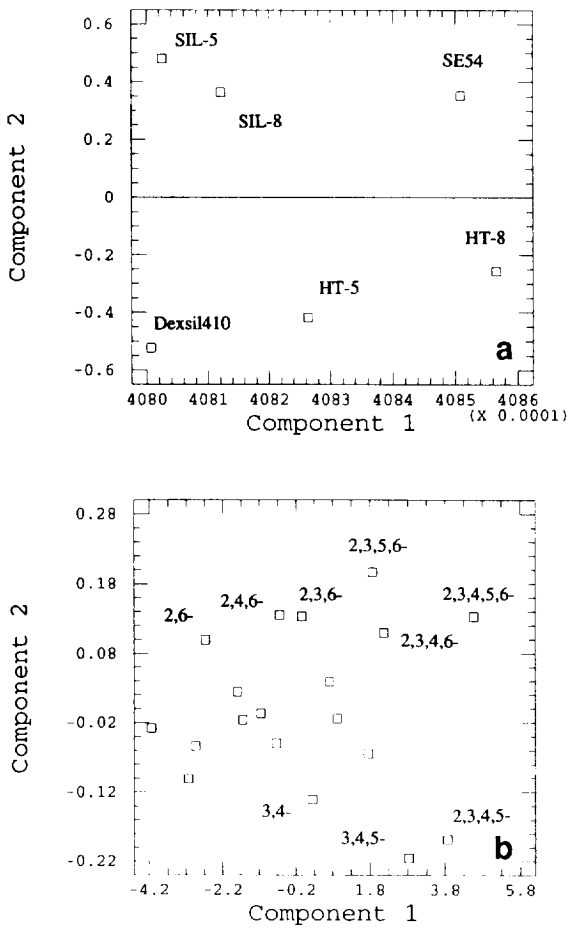

Fig. 3. Plot of first two components of (a) stationary phases, and (b) single-ring structures.

tained with the two sizes of film thickness are almost identical. A few critical separations are slightly better on the thin film (CB43/CB49, CB44/CB59, CB77/CB82/CB149, CB133/CB118, CB122/CB153, CB157/CB180, CB198/CB199, CB190/CB196/ CB203) and vice versa on the thick film (CB41/CB71, CB101/CB60, CB141/CB105).

A total of 162 CB congeners have been marked in the chromatograms. Out of these approximately 140 are present in the Aroclor mixtures at detectable concentrations [8]. We have previously adopted the criterion for sufficient separation of two possibly interfering compounds as less than 10% interference from one to the other. This criterion is met for symmetric peaks when the separation is just visible and peak heights are used for quantitation [11,13,15–18]. CB congeners which can be analysed as resolved peaks on HT-8 (0.25 μm) according to this criterion are listed in Table 3. Critical separations for which the interference can be reduced to less than 10% by HRGC–MS are listed in brackets. In the table it appears that with ECD, a total of 110 congeners are sufficiently separated; using MS the number is increased to 138. Out of the 36 priority CBs, 28 can be analysed without significant interference by ECD; using MS all 36 priority congeners can be analysed.

Also for the set of seven indicator CBs is HT-8 performing well. Four congeners elute as resolved peaks (CB101, CB118, CB138 and CB153), one (CB52) coelutes with a congener (CB69) which has been proved absent at detectable concentrations in PCB mixtures [13] and one (CB180) elutes with a low-concentration congener (CB193) at its tail. Only CB28 coelutes with a significant congener (CB53); but this pair can be separated by MS. In order to get an estimate of the interference by ECD from the closely eluting congeners various technical, and biological samples were analysed with other GC columns (SIL-13 and SIL-8/HT-5) previously found to facilitate separation for these critical pairs. The absence of CB69 in any sample was confirmed. The maximum interference obtained as if perfect coelution was seen for the pairs CB53/CB28 and CB193/CB180 is shown in Table 4. It follows that the use of ECD may lead to considerable over-estimations of CB28 in technical samples. This over-estimation is reduced in biological samples at the higher end of the food chain, possibly due to the selective elimination/biodegradation of CB53 [1]. For CB180 it seems safe to conduct analysis with ECD alone, as the potential interference from CB193 is in the order of 10%.

As mentioned in the introduction, the analysis of the pair CB138/CB163 is of particular toxicological importance. To date, this has unfor-

Table 3
CB congeners in technical PCB mixtures which can be analyzed accurately[a] as resolved peaks on HT-8 (0.25 μm film) by ECD and MS

n_{Cl}	IUPAC number
mono	1, 2[d], 3
di	4, 6, 7, 9, 10, 11[d], 13[d], 15
tri	16, 17, **18**[b], 19, 21[d], 22, 23[d], 24, 25, 26, 27 (28)[c], 29, 31, 32, 34[d], 35[d], 36[d], **37**
tetra	40, 41, 42, **44**, 46, **49**, 51, **52**[c], 54[d], (55), 56, 57[d], 59, 60, 63[d], 64, 66, 67, **70**, (71), **74**, (**77**), 80[d], (**81**[d])
penta	(82), 83, 85, 86[d], **87**, 89[d], 90[d], 95, (96), 97, **99**, 100[d], **101**, 102[d], (103[d]), **105**, (107), (110), (**114**), 115[d], **118**, **119**[d], 122[d], **123**[d], 124[d], 125[d], **126**[d]
hexa	(**128**), (129), 130, (132), 133[d], 135, 136, 137[d], **138**, 141, 143[d], 144, (146), 149, **151**, 152[d], **153**, 154[d], (155[d]), (**156**), **157**[d], (**158**), 159[d], 160[d], **163**, 165[d], **167**, **169**[d]
hepta	**170**, 171, (172), 173, (174), (175), 176, (**177**), 178, (179), **180**, (**183**), 185, **187**[c], **189**, (190), 191
octa	**194**, 195, (197[d]), 199, **200**, (201), (202), 205
nona	206, 207[d], 208
deca	209

[a] Less than 10% interference with the peak height from any closely eluting congener.
[b] Priority CB embolded owing to their toxic potential and environmental occurrence [19].
[c] CB congeners in brackets indicate that interference from closely eluting congener is reduced to less than 10% by use of MS.
[d] Congener found at insignificant concentrations in technical mixtures.
[e] Coelutes with a congener found at insignificant concentrations in technical and environmental mixtures.

tunately been ignored in the majority of congener-specific PCB investigations. The reason for this must be found in the difficulties connected with the chromatographic separation of this pair. Fig. 4 shows the critical separations for CB138/CB163 and closely eluting CBs obtained with the seven best performing GC phases published in the literature (SIL-8/HT-5 [15], HT-5 [11], HT-8 [this study], SIL-13 [13], poly(carbonate methylsiloxane) [12], BPX-70 [13] and SIL-88 [11]). HT-8 shows near-baseline separation of this critical pair from any other CB. HT-5 has CB160 as interference for CB-163, but since the former is not found in technical mixtures and environmental samples this is of little importance. SIL-13 has CB158 as a shoulder on CB138. Again, the former is a minor compound and accurate measurements of CB138 can be achieved with this phase. SIL-88, poly(carbonate methylsiloxane), SIL-8/HT-5 and BPX-70, in this order, offer less separation for the critical pair, but at least CB138 can be measured without significant interference on all these phases. An additional phase described in the literature by others, 50% dioctyl dimethylsiloxane [9], has been reported to give good separations for

Table 4
ECD interference[a] (%) from coeluting congeners with the indicator PCBs CB28 and CB180 in selected technical and biological samples

	Critical pair	
	CB53/CB28	CB193/CB180
A1242	13–17%	7–9%
A1248	31–37%	9–13%
A1254	84–106%	8–10%
A1260	11-15%	8–10%
Eel	35–41%	8—10%
Tuna	7–9%	5–7%
Seal	0–1%	6–8%
Human milk	0–1%	6–8%

[a] The interference is expressed in percentage relative to quantitations based on peak heights on SIL-13 and SIL-8/HT-8 for which the absence of coeluting CBs has been proven [13]. A result equal to 0 means no interference.

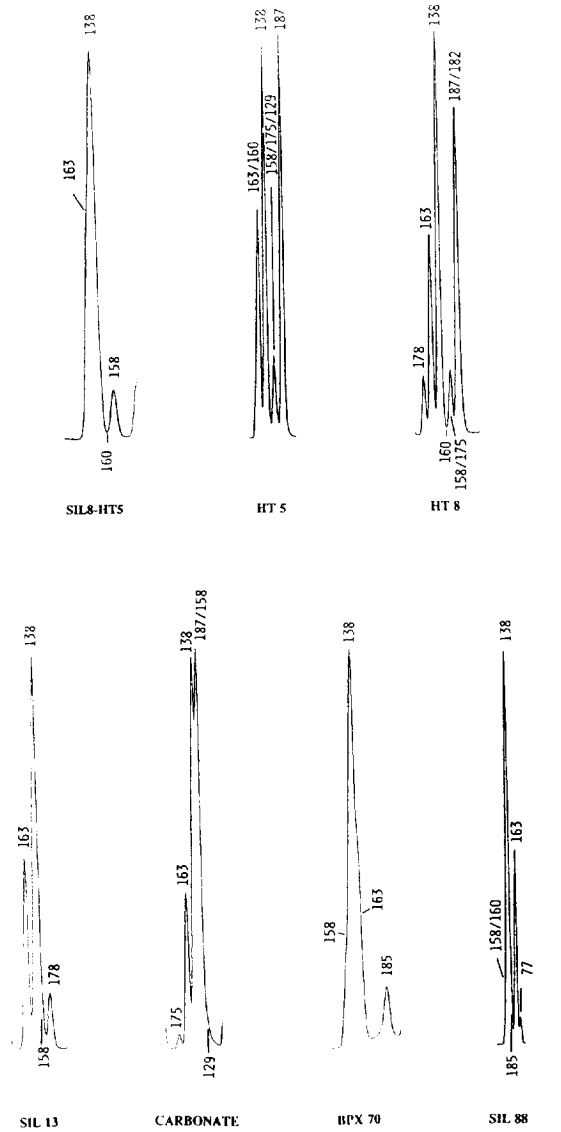

Fig. 4. Critical separations for CB138/CB163 on SIL-8/HT-5 [15], HT-5 [11], HT-8 [this study], SIL-13 [13], poly(carbonate methylsiloxane) [12], BPX-70 [13] and SIL-88 [11].

CB138. The authors did not identify a medium size peak occurring in the chromatograms just before CB138. This is most likely CB163.

With so many stationary phases offering adequate separations for the critical pair CB138/CB163 it seems unjustified that future determinations of CB138 (and CB163) be performed as the sum of the two congeners.

In biological extracts other organochlorine compounds (OCCs) than PCBs are most often also present. Especially, DDE and DDT can cause problems through coelution with PCBs [19]. The chromatogram on HT-8 (0.25 μm) of a cleaned-up duck egg extract is shown in Fig. 5. It is seen that these two OCCs do not interfere with any priority CB. DDE elutes as a resolved peak, whereas DDT coleutes with CB178. For accurate determinations of the latter OCC parallel analysis on other columns or class separation of OCCs and PCBs by liquid chromatography is necessary.

With the use of a glass press-fit connector and multi-channel data acquisition software, dual-column GC is becoming a routine method in PCB analysis. We have previously concluded that the highest number of separated CB congeners in dual-column GC (104 with ECD, 127 with MS) can be obtained with the DB-17 (50% diphenyl dimethylsiloxane) column and with SIL-8 (5% diphenyl dimethylsiloxane) series coupled with HT-5 (1,7-dicarba-closo-dodecarborane di-

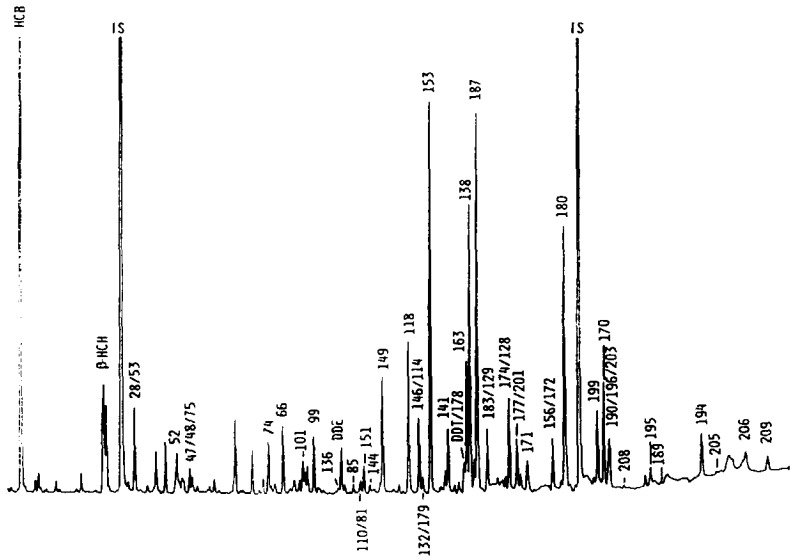

Fig. 5. GC–ECD analysis of a cleaned-up duck egg extract on HT-8 (0.25 μm).

methylsiloxane) [15]. When considering the 15 stationary phases with well characterised elution profiles mentioned in the introduction and the findings of the present study, we can now conclude that the highest number of separated CB congeners is offered by the combination of DB-17 and HT-8 which gives 106 separated CBs with ECD and 157 with MS.

3.3. Speeding up to ballistic conditions

The incorporation of carborane in the polysiloxane backbone of the stationary phase has the main function of enhancing the temperature stability of the phase. By using ballistic temperature programmes it has previously been shown for HT-5 that this advantage of carborane-containing phases can be utilised in rapid screening of CB congeners [15]. As noticed previously for HT-5 the present investigation of HT-8 shows that, speeding up to ballistic conditions naturally cause the loss of some critical separations in the complex Aroclor mixtures (Fig. 6). However, the main components of the mixtures are still separated from each other with resolution better than 30–50% even when the run-time of the chromatogram is reduced drastically.

In Table 5 the performance is shown for the two film thicknesses at different analysis speed. The interference from coeluting CBs upon speeding up of the temperature programme for the quantitation of the seven indicator CBs in A1016-1262 is measured. The interference is expressed as percentage for triplicate quantitations on HRGC phases for which the absence of coeluting CBs has been proven [13]: SIL-13 for CB28, CB52, CB138, CB153 and CB180; SIL-8/HT-5 for CB101 and CB118.

Of the seven indicator CBs, significant interference is evident only for CB28, which may be over-estimated by 15–30% owing to the coelution with CB53. The six other indicator CBs can be analysed with less than 10% interference at slow to fast speed (> 20 min) with the thin (0.15 μm) and the thick (0.25 μm) film. With the thick film, interference becomes significant for CB153 (coeluting with CB132 and CB179) at analysis periods of 18 min or less; for CB138 (coeluting with CB163, CB158 and CB175)) at the analysis period of 16 min. With the thin film, interference for these to indicator CBs (14–18%) is observed only at 13 min. The performance of HT-8 (0.15 μm) at high speed is even better than the previously reported performance of HT-5 (0.1

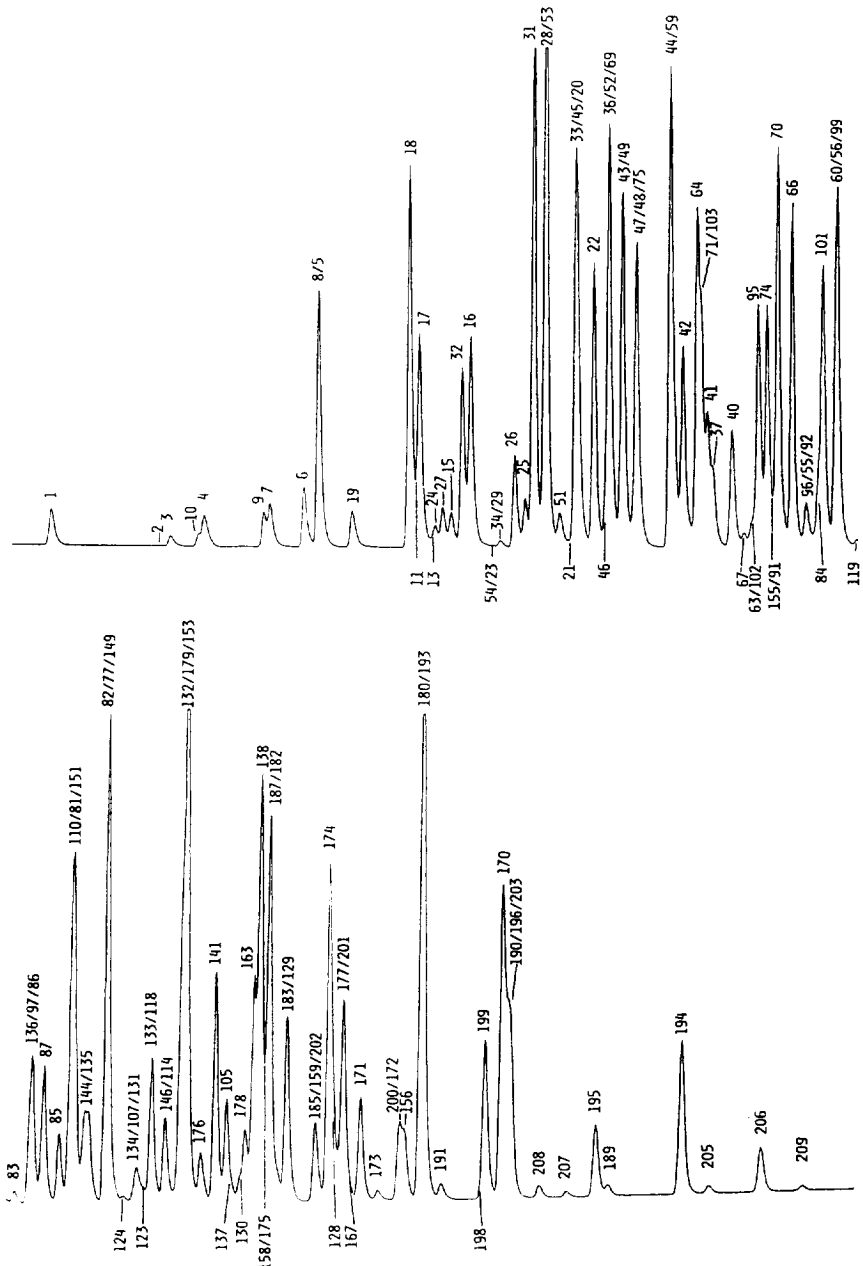

Fig. 6. Rapid GC–ECD analysis of Aroclors (A1016, A1232, A1248 and A1262, 1:1:1:1) on HT-8 (0.15 μm). Total run-time 13 min.

μm) [15] and this new phase seems to be an optimal choice for congener-specific rapid screening with total GC run-times down to 13 min.

Acknowledgements

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Table 5
The interference[a] from coeluting CBs on quantitations of the seven indicator CBs in A1016-1262 on HT-8 upon speeding up of the temperature programme (see Table 1)

Run (min)	CB28		CB52		CB101		CB118		CB138		CB153		CB180	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
55	114	2.1	100	1.5	105	3.3	101	0.5	103	2.0	101	1.7	105	2.0
48	116	2.3	99	1.6	103	2.4	101	0.1	103	0.1	100	1.8	103	2.9
41	118	3.0	98	2.7	102	4.3	102	0.9	106	5.9	98	2.6	94	4.6
35	119	4.8	97	2.8	100	3.3	103	1.1	107	5.9	97	2.9	95	3.7
26	122	4.2	96	2.8	101	4.7	104	3.1	107	4.2	97	2.1	99	2.1
21	125	5.3	98	3.3	103	5.8	105	2.4	108	3.9	105	1.5	96	0.3
18	127	5.0	101	2.9	107	5.2	104	1.1	107	9.9	116	4.7	93	2.7
16	134	1.4	99	0.3	106	2.2	105	2.1	118	13	130	7.7	93	2.5
48	114	1.3	100	0.6	105	4.9	101	0.9	103	1.1	101	0.7	105	0.3
39	115	1.4	100	0.2	101	5.8	100	0.8	104	1.4	101	0.5	105	0.6
33	116	1.2	100	0.9	101	6.8	99	1.9	104	0.5	101	0.5	105	0.1
27	117	1.0	100	1.5	98	4.8	97	1.5	104	1.7	101	0.9	105	0.8
21	119	1.6	99	1.9	99	7.4	99	1.7	101	6.9	101	0.3	104	0.3
18	123	1.7	99	2.2	99	8.1	99	0.4	107	2.7	103	0.6	106	0.7
16	125	2.7	101	0.5	101	7.4	98	0.3	108	2.6	105	1.0	104	1.1
13	129	1.8	103	1.3	103	3.9	100	4.6	114	0.1	118	1.3	102	2.6

[a] The effect is expressed in percentage relative to triplicate quantitations on HRGC phases for which the absence of coeluting CBs has been proven [13]: SIL-13 for CB28, CB52, CB138, CB153 and CB180; SIL-8/HT-5 for CB101 and CB118. A result equal to 100 means no interference.

collaboration and donation of the HT-8 GC columns. The data presented on poly(carbonate methylsiloxane) [12] were obtained with a column kindly made available by Prof. Pat Sandra.

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