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High-resolution separation of polychlorinated biphenyls by comprehensive two-dimensional gas chromatography

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Abstract

Comprehensive two-dimensional gas chromatography (GC×GC) with micro electron-capture detection (μECD) has been optimised for the separation of polychlorinated biphenyl congeners with emphasis on the separation of 12 toxic non- and mono-*ortho* chlorinated biphenyls (CBs), viz. CBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189. The selection of the first- and second-dimension columns and the temperature programme optimisation were carried out with a mixture of 90 CBs and the results are compared with those of one-dimensional GC. A complete separation of all 12 priority CBs was obtained with two column combinations, HP-1–HT-8 and HP-1–SupelcoWax-10. With the HP-1–HT-8 column set, ordered structures show up in the two-dimensional plane, with the number of chlorine substituents and their position (*ortho* vs. non-*ortho*) being the main parameters of interest. This can help with congener identification. Estimated detection limits are excellent, i.e. about 10 fg. To illustrate the potential and the versatility of GC×GC–μECD, a cod liver extract and a standard mixture of the 17 most toxic polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans together with 90 CBs were analysed as an application.

Keywords: Fish; Food analysis; Gas chromatography, comprehensive two-dimensional; Polychlorinated biphenyls; Polychlorinated dibenzodioxins; Polychlorinated dibenzofurans

1. Introduction

Since the late 1960s, when the detrimental health effects of polychlorinated biphenyls (PCBs) became clear [1], there has been a continual need for their reliable measurement in food samples. PCB analyses mainly focus on the determination of marker congeners (CBs 28, 52, 101, 118, 138, 153 and 180), which are the congeners predominantly found in humans and in foodstuff of animal origin. In recent years, most attention has been devoted to the non- and mono-*ortho*-substituted congeners, because these CBs show the same type of toxicity as polychlorinated dibenzo-*p*-dioxins and dibenzofurans [2]. Since the World Health Organization (WHO) has recommended an acceptable daily intake for dioxins, furans and PCBs which includes non-*ortho* (77, 81, 126 and 169) and mono-*ortho* (105, 114, 118, 123,

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156, 157, 167 and 189) substituted CBs, and since this recommendation has been adopted by the European Scientific Committee for Food (SCF, Brussels, Belgium), it is likely that these non- and mono-*ortho* CBs will be included in on-going and future food monitoring programmes. Consequently, there is a pronounced need for unambiguous measurements of these CBs.

PCBs are routinely analysed by one-dimensional capillary gas chromatography (GC). The separation of non- and mono-*ortho* congeners from the bulk of the CBs is complicated by the high number of co-eluting compounds and also by the very low concentrations compared to the bulk of PCBs. As a result, pre-separation by other means, e.g. column liquid chromatography (LC), is necessary [3–5]. Moreover, if polychlorinated dibenzo-*p*-dioxins and dibenzofurans are present in the sample, the use of high-resolution mass spectrometry detection is inevitable [6]. This makes the procedure complicated and the analysis time-consuming and expensive. PCBs can also be separated by multi-dimensional GC heart-cutting methods [7,8]. These methods enhance the separation power of capillary GC, but are limited to the analysis of only a few discrete, critical regions of the chromatogram, and analysis times easily become very long.

Comprehensive two-dimensional gas chromatography (GC×GC) is another two-dimensional method that is used to analyse complex mixtures. In GC×GC, two independent separations are applied to an entire sample. The sample is first separated on a high-resolution column under programmed-temperature conditions. The effluent of this column then enters a thermal (or cryo) modulator, which retards (or traps) each subsequent small portion of eluate, focuses these portions and introduces them into a second column for further separation. The second separation is made to be fast enough (e.g. 5–10 s) to permit the continual introduction of subsequent, equally small fractions from the first column without mutual interference. The resulting chromatogram is in fact a surface in a three-dimensional space, of which two axes represent the retention times of the solutes on the first and the second column, respectively, while the third axis represents the detector response. The advantages of GC×GC are the large peak capacity, which is close to the product of the peak capacities of the two individual columns [9], an increase in the S/N ratios as a result of the focusing effect of the modulation [10] and a time of analysis which is the same as for a conventional one-dimensional run.

Since the introduction of GC×GC in 1991 [11], this technique—almost invariably combined with flame ionisation detection (FID)—has mainly been used in the field of petrochemical and related environmental analyses [12–14], obviously because of the complexity of the samples encountered and also because the sample mixtures are reasonably well characterised. Recently, GC×GC has also been used for characterisation of essential oils [15,16] and fatty acid methyl esters [17]. The theory of the separation of PCBs by GC×GC was discussed by Phillips and Xu [18], and recently Haglund et al. [19] used a GC×GC method for toxic CB congener analysis and Harju and Haglund [20] for separation of atropisomeric PCBs. However, to date, no convincing GC×GC results for CB congener analysis has been published with electron-capture detection (ECD). The present paper discusses the optimisation of a GC×GC procedure for the high-resolution separation of PCBs, with special emphasis on the separation of the 12 non- and mono-*ortho* CB priority congeners included in the WHO list. The main parameters are the selection of the GC column combination, and the temperature programming.

2. Experimental

2.1. Samples and reagents

A standard mixture containing 90 CBs was prepared by mixing standard solutions of each CB congener (Promochem, Wesel, Germany) in isooctane of nanograde quality (Promochem). The numbering and substitution pattern of used congeners according to IUPAC is given in Table 1. The final congener concentration in this mixture was approximately 1–3 ng/ml. For determination of detection limits, a standard mixture with exact known concentrations was used, containing 31 CB congeners (28, 31, 44, 47, 49, 52, 56, 66, 74, 85, 87, 97, 99, 101, 105, 110, 118, 128, 137, 138, 141, 149, 151, 153, 156, 170, 180, 187, 194, 202, 206). A standard

Table 1
Numbering and substitution pattern of congeners used according to IUPAC

No.	Structure	No.	Structure	No.	Structure
Dichlorobiphenyl		74	2,4,4′,5	137	2,2′,3,4,4′,5
4	2,2′	75	2,4,4′,6	138	2,2′,3,4,4′,5′
5	2,3	77	3,3′,4,4′	140	2,2′,3,4,4′,6′
7	2,4	78	3,3′,4,5	141	2,2′,3,4,5,5′
10	2,6	80	3,3′,5,5′	149	2,2′,3,4′,5′,6
11	3,3′	81	3,4,4′,5	151	2,2′,3,5,5′,6
12	3,4	*Pentachlorobiphenyl*		153	2,2′,4,4′,5,5′
Trichlorobiphenyl		82	2,2′,3,3′,4	154	2,2′,4,4′,5,6′
16	2,2′,3	84	2,2′,3,3′,6	155	2,2′,4,4′,6,6′
24	2,3,6	85	2,2′,3,4,4′	156	2,3,3′,4,4′,5
26	2,3′,5	87	2,2′,3,4,5′	157	2,3,3′,4,4′,5′
28	2,4,4′	88	2,2′,3,4,6	163	2,3,3′,4′,5,6
29	2,4,5	92	2,2′,3,5,5′	167	2,3′,4,4′,5,5′
31	2,4′,5	95	2,2′,3,5′,6	169	3,3′,4,4′,5,5′
33	2′,3,4	97	2,2′,3′,4,5	*Heptachlorobiphenyl*	
37	3,4,4′	99	2,2′,4,4′,5	170	2,2′,3,3′,4,4′,5
Tetrachlorobiphenyl		101	2,2′,4,5,5′	171	2,2′,3,3′,4,4′,6
40	2,2′,3,3′	103	2,2′,4,5′,6	173	2,2′,3,3′,4,5,6
44	2,2′,3,5′	105	2,3,3′,4,4′	180	2,2′,3,4,4′,5,5′
47	2,2′,4,4′	110	2,3,3′,4′,6	183	2,2′,3,4,4′,5′,6
49	2,2′,4,5′	114	2,3,4,4′,5	185	2,2′,3,4,5,5′,6
50	2,2′,4,6	116	2,3,4,5,6	187	2,2′,3,4′,5,5′,6
52	2,2′,5,5′	118	2,3′,4,4′,5	189	2,3,3′,4,4′,5,5′
53	2,2′,5,6′	119	2,3′,4,4′,6	*Octachlorobiphenyl*	
54	2,2′,6,6′	121	2,3′,4,5′,6	194	2,2′,3,3′,4,4′,5,5′
56	2,3,3′,4′	123	2′,3,4,4′,5	195	2,2′,3,3′,4,4′,5,6
60	2,3,4,4′	124	2′,3,4,5,5′	198	2,2′,3,3′,4,5,5′,6
61	2,3,4,5	126	3,3′,4,4′,5	201	2,2′,3,3′,4,5′,6,6′
65	2,3,5,6	*Hexachlorobiphenyl*		202	2,2′,3,3′,5,5′,6,6′
66	2,3′,4,4′	128	2,2′,3,3′,4,4′	*Nonachlorobiphenyl*	
69	2,3′,4,6	129	2,2′,3,3′,4,5	206	2,2′,3,3′,4,4′,5,5′,6
70	2,3′,4′,5	136	2,2′,3,3′,6,6′	207	2,2′,3,3′,4,4′,5,6,6′
72	2,3′,5,5′			208	2,2′,3,3′,4,5,5′,6,6′

mixture of 90 CB congeners and 17 polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) was prepared by mixing a standard mixture of 90 CBs in isooctane with a standard mixture of 2,3,7,8-TCDD, 2,3,7,8-TCDF (T = tetra), 1,2,3,7,8-PeCDD, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF (Pe = penta), 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF (Hx = hexa), 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF (Hp = hepta), OCDD and OCDF (O = octa) in *n*-nonane (Cambridge Isotope Labs, Andover, MA, USA). The final concentration of the CB congeners was approx. 1 ng/ml, of the tetra-CDD and CDF, 0.8 ng/ml, the penta- to hepta-CDD and CDF, 4 ng/ml and octa-CDD and CDF, 8 ng/ml.

A cod (*Gadus morhua*) liver sample was taken from a mixed sample of animals caught in the southern North Sea in 1987. Clean-up and fractionation was done by putting an aliquot containing not more than 250 mg fat on top of a 15 g alumina·9% H_2O column (Merck No. 1076, Darmstadt, Germany). The column was eluted with 170 ml *n*-pentane of nanograde quality (Promochem) to collect the CB fraction, while the fat remained on the column. After the addition of 2 ml of isooctane as a keeper, the eluate was concentrated to about 2 ml in a rotary evaporator. The concentrate was transferred to a silica column (1.6 g silica·$2H_2O$; Merck,

Kieselgel 60, No. 7754) and eluted with of 11 ml isooctane [21].

2.2. GC analysis

One-dimensional GC analysis was carried out on a HP 6890 GC (Hewlett-Packard, Wilmington, DE, USA), with a standard ECD system, a split/splitless inlet, and CP-Sil 8 (5% phenyl-, 95% dimethylpolysiloxane) 50 m×0.15 mm, 0.30 μm fused-silica capillary column (Chrompack, Middelburg, Netherlands). Hydrogen (Hoek Loos, Schiedam, Netherlands) with a purity of 99.999% was used as carrier gas at an inlet pressure of 45 p.s.i. (1 p.s.i.=6894.76 Pa). The ECD system was operated at 300 °C, with 99.999% pure nitrogen (Hoek Loos) as make-up gas at a flow-rate of 60 ml/min. Samples were injected manually (1 μl) into the split/splitless inlet port equipped with a deactivated single-tapered liner (4 mm I.D., no glass wool) and operated at 250 °C in the splitless mode. The purge time was 2 min. The temperature programme was 90 °C (3 min), at 30 °C/min to 150 °C, and then at 1 °C/min to 250 °C (40 min).

2.3. GC×GC analysis

The GC×GC system was built from a HP 6890 system (Hewlett-Packard) equipped with a thermal modulator assembly (Zoex, Lincoln, NE, USA). Since principles and working characteristics of the thermal sweeper modulator are extensively described elsewhere [22], only a brief description is given here. The modulator assembly, mounted in the GC oven, consists of a rotating slotted heater, a holder of the modulator tube, and a separate oven for temperature programming of the second-dimension column. The modulator tube (essentially a thick-film stationary phase column) is used to serially connect the two GC columns. The slotted heater periodically rotates over the modulator tube to desorb and focus the analyte fractions and inject them into the second column. The second column generates a high-speed chromatogram while the modulator tube accumulates the next portion of sample eluting from the first column.

Helium gas (Hoek Loos) with a purity of 99.999% was used as carrier gas through the GC×GC system at an inlet pressure of 50 p.s.i. A micro electron-capture detection (μECD) system was operated at 300 °C, with 99.999% pure nitrogen (Hoek Loos) as make-up gas at a flow-rate of 60 ml/min; 1-μl samples were injected manually into a split/splitless inlet port equipped with a deactivated single-tapered liner (4 mm I.D., no glass wool) and operated at 300 °C in the splitless mode. The purge time was 2 min. A 30 m×0.32 mm, 0.25 μm HP-5 (5% phenyl-, 95% dimethylpolysiloxane) and a 30 m×0.25 mm, 0.25 μm HP-1 (100% dimethylpolysiloxane) fused-silica column, both from Hewlett-Packard, were used as first-dimension columns. Three columns were used as second-dimension columns: 1 m×0.1 mm, 0.1 μm BPX-50 (50% phenyl (equiv.) polysilphenylene-siloxane), 1 m×0.1 mm, 0.1 μm HT-8 (8% phenyl (equiv.) polycarborane-siloxane), both from SGE International (Ringwood, Australia) and 1 m×0.1 mm, 0.1 μm SupelcoWax-10 (polyethylene glycol) from Supelco (Bellefonte, PA, USA). A 7 cm×0.1 mm, 3.5 μm 007-1 (100% methylpolysiloxane) capillary column (Quadrex, Deerfield, IL, USA) was used as modulator tube. The head of the first column was connected directly to the injector and its end to the modulator tube via a short piece of deactivated column. The head of the second-dimension column was connected to the modulator tube via a short piece of deactivated column and its end to the detector, also via a deactivated column. This set-up enabled the entire column to be located in a separate oven. Micro and mini press-fits (Techrom, Purmerend, Netherlands) were used to connect the columns. The slotted heater temperature was set 100 °C above the temperature of the first oven. The GC×GC operating programme, ver. 2.0z (Zoex) was used for the sweeper control and data acquisition. The modulation period was 6.5 s at a rotating speed of 0.15 revolutions s^{-1}, and with a pause time of 0.4 s. The data acquisition rate was 50 Hz. Transform software (Fortner Research, Sterling, VA, USA) was used for data visualisation.

3. Results and discussion

3.1. One-dimensional GC

A one-dimensional chromatogram reconstructed by a mathematical operation from a GC×GC chro-

matogram is frequently used to evaluate the (dis)-advantages of GC×GC compared with one-dimensional GC. However, such a comparison is often not valid, because in GC×GC the first-dimension column frequently has a lower efficiency than the column normally used in a one-dimensional separation. Moreover, the final efficiency of the reconstructed chromatogram is strongly affected by the fact that the peaks consist of only 4–10 data points. Therefore, in this study, a one-dimensional GC chromatogram was obtained on a highly efficient column (CP-Sil 8, 50 m×0.15 mm, 0.30 μm) routinely used for PCB analysis in our laboratory. The temperature programme was similar to that used for GC×GC separation (see Section 2). As can be observed in Fig. 1, for the total number of 90 CBs present in the injected mixture, 74 peaks attributed to PCBs show up in the chromatogram. In other words, 60 congeners elute from this column as at least partially resolved peaks and 14 peaks contain two or three co-eluting CBs. More importantly, from among the 12 CBs mentioned in the WHO list, seven (CBs 105, 114, 118, 126, 167, 169 and 189) elute as resolved peaks, while five (CBs 77, 81, 126, 156 and 157) co-elute with other congeners. We would like to stress here that PCBs exist at approximately the same concentration in this chromatogram, which is a situation not encountered in ''real world'' samples. Separation problems are magnified when concentration differences exist between closely eluting congeners.

3.2. GC×GC analysis

3.2.1. Selection of first-dimension column

Selecting the type of stationary phase for the first column in comprehensive GC is relatively simple: for reasons that have been widely discussed in the literature [23], a non-polar phase is generally preferred. A 5% phenyl-, 95% dimethylpolysiloxane stationary phase is only slightly polar and is often used for routine one-dimensional GC separations of

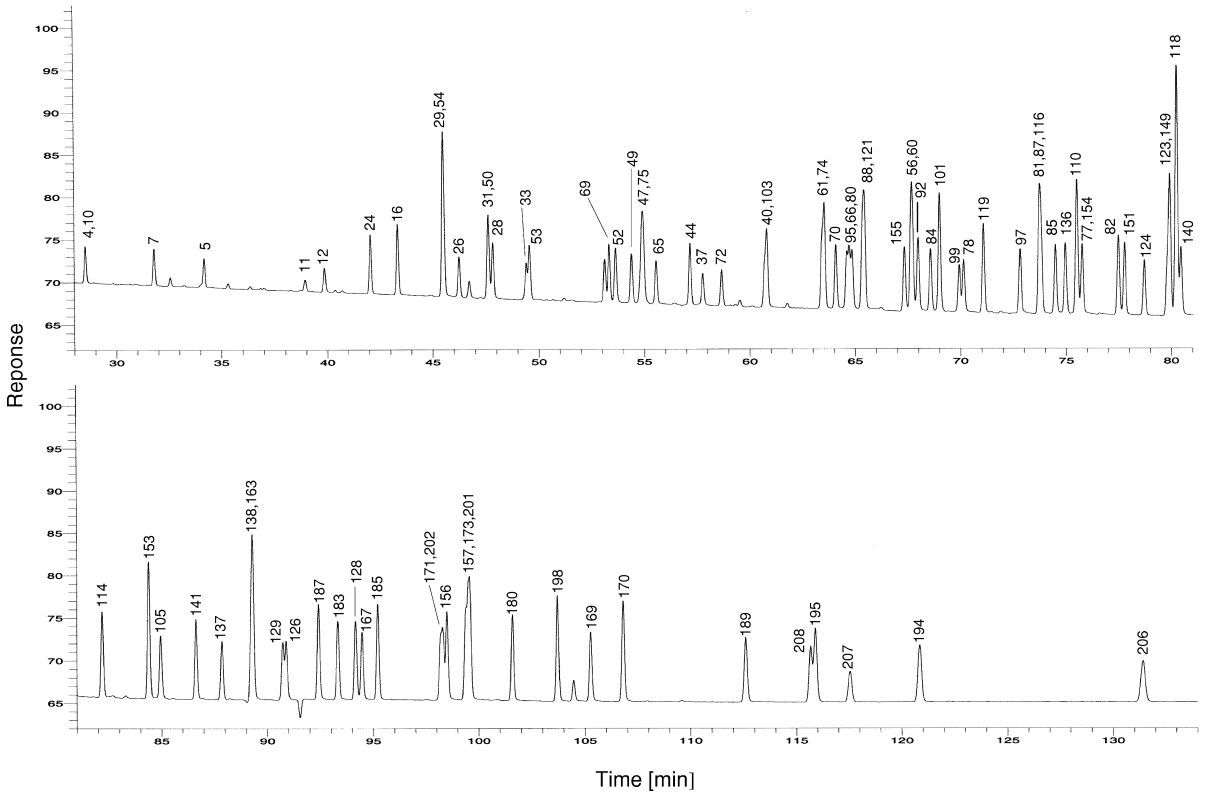

Fig. 1. GC–ECD chromatogram of the mixture of 90 CBs.

PCBs; therefore, retention characteristics for many PCB congeners are available. Consequently, this phase was our first choice (HP-5, 30 m×0.32 mm, 0.25 μm). However, with this column as part of the set-up, breakthrough of all PCBs was observed in the modulator tube. The insufficient retardation in the modulator was caused by the lower retention power of the stationary phase in that tube, 100% dimethylpolysiloxane, compared with the first-dimension column. The analytes were eluted from the first column into the modulator at a temperature which was ca. 20 °C higher than the elution temperature of the 100% dimethylpolysiloxane used in the modulator. With this phase combination, not even a 3.5-μm-thick stationary phase can retain the analytes for the required 6.5-s modulation time.

When a 100% dimethylpolysiloxane stationary phase was used as first-dimension column (HP-1, 30 m×0.25 mm, 0.25 μm), expectedly the breakthrough was not observed anymore. Actually, the 3.5-μm-thick stationary phase in the modulator could now retain the analytes even for 13 s without any breakthrough being observed. It will be clear that another option would be to couple the 5% phenyl-, 95% dimethylpolysiloxane first-dimension column to a modulator with the same stationary phase. However, in that case, the required final temperature of the first oven and the sweeper would be ca. 20 °C higher than for the 100% dimethylpolysiloxane phase and, consequently, higher than the maximum temperature of the thick phase in the modulator tube. Here, one should add that, although a 5% phenyl-, 95% dimethylpolysiloxane stationary phase is not suitable as a first column for PCB analysis by means of GC×GC with a sweeper modulator, it can be used without any problem in combination with a cryogenic modulator such as a jet cooling system [24] or a longitudinal modulating cryogenic system [25].

3.2.2. Selection of second-dimension column

Selecting the second column is a critical step in GC×GC method development. Most importantly, the separation in this column has to be fast enough to permit the rapid introduction of the effluent fractions from the first column without mutual interference. To satisfy this requirement very fast capillary columns—1 m long with an internal diameter of 0.1 mm and a film thickness of 0.1 μm—are normally used. Secondly, the separation mechanism in the second-dimension column has to be substantially different from that in the first column. Only then will GC×GC offer an orthogonal separation or, in other words, a seriously increased peak capacity. In this study, three stationary phases were tested: 50% phenyl (equiv.) polysilphenylene-siloxane (BPX-50), 8% phenyl (equiv.) polycarborane-siloxane (HT-8) and polyethylene glycol (SupelcoWax-10). The BPX-50 stationary phase was chosen because it is often used by GC×GC community in the second column, the HT-8 phase was selected because it is considered one of the best stationary phases for CB congener analysis [26,27] and polyethylene glycol was tested as a very polar stationary phase. As will be shown below, with each of these phases, a distinctly different elution pattern was obtained.

Fig. 2 presents the chromatogram obtained with the HP-1–BPX-50 column combination under optimised conditions (see next section). From the 90 CBs present in the test mixture, 71 congeners now elute as resolved peaks and only nine peaks contained two or, in a single instance, three co-eluting CBs (4–10, 28–31–50, 37–44, 61–74, 56–60, 77–136, 118–149, 126–129, 156–171). This is a distinct improvement over the result of the one-dimensional GC run with the 60 partially resolved peaks. However, with the 12 non- and mono-*ortho* CBs of WHO relevance, the gain was rather modest: only CB 157 now was resolved and could be taken off the list of co-eluting compounds.

A much better separation was observed in the case of the HT-8 phase (Fig. 3). Now, 78 congeners elute as resolved peaks, and only six co-eluting pairs remain, the CB pairs 4–10, 28–31, 52–69, 74–61, 56–60 and 138–163. In addition, all 12 non- and mono-*ortho* CBs of interest are baseline-separated with the HP-1–HT-8 combination. Another notable difference with the earlier column combination is that one can now clearly observe an ordered structure of the two-dimensional chromatogram, as is indicated by the dotted lines in Fig. 3. Each group of peaks comprises CBs with the same number of chlorine substituents in the molecule; the position of each CB congener within a group is determined by the substitution pattern on the biphenyl skeleton. The most relevant observation for many real-life studies

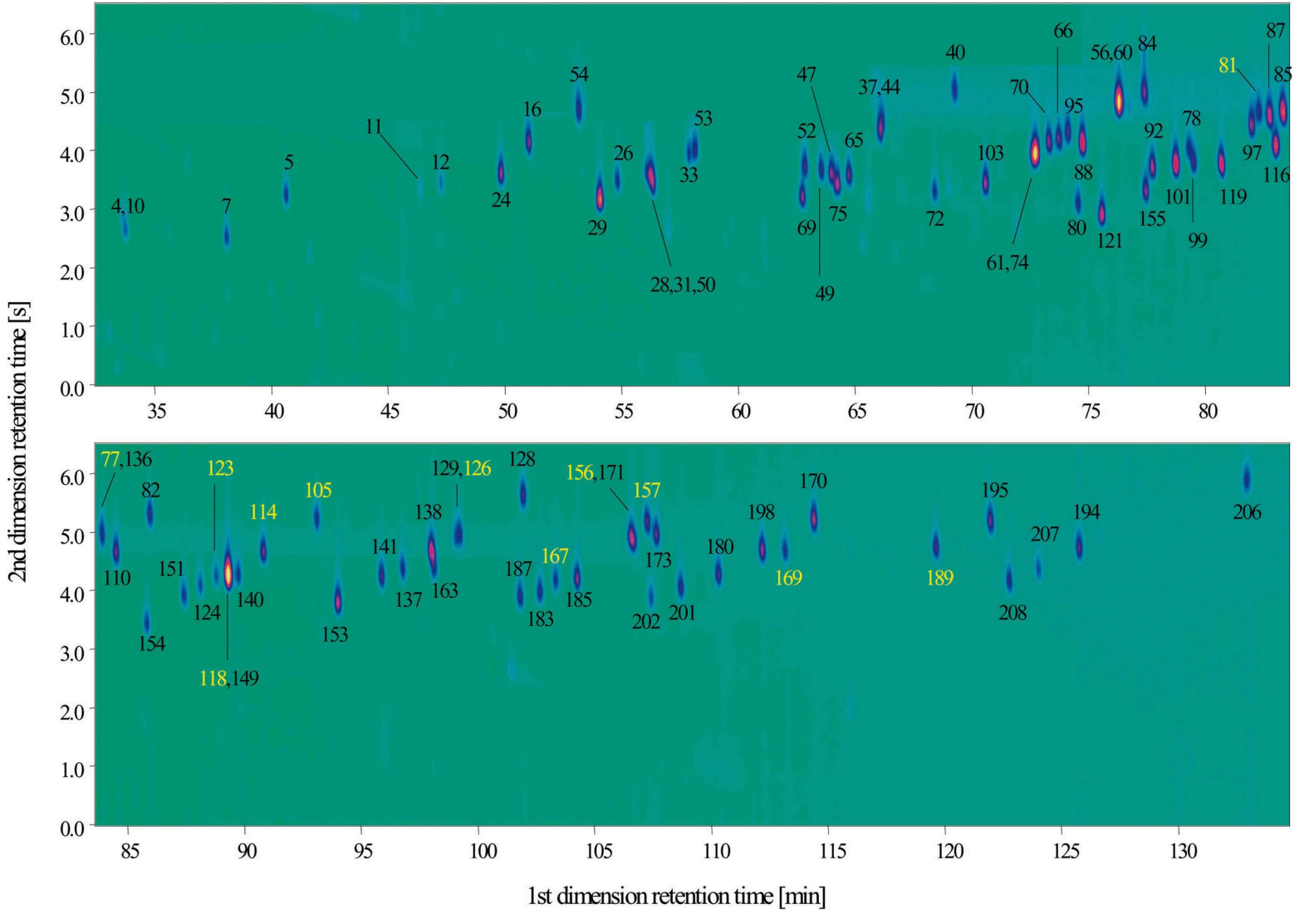

Fig. 2. GC×GC–μECD chromatogram of the mixture of 90 CBs with HP-1–BPX-50 column combination. Temperature programme: 90 °C (2 min), at 5 °C/min to 110 °C, then at 1 °C/min to 240 °C (25 min) for the first oven, and 110 °C (2 min), at 5 °C/min to 130 °C, then at 1 °C/min to 260 °C (25 min) for the second oven. In this and other figures, the yellow numbers represent the 12 priority non- and mono-*ortho* CBs of WHO relevance.

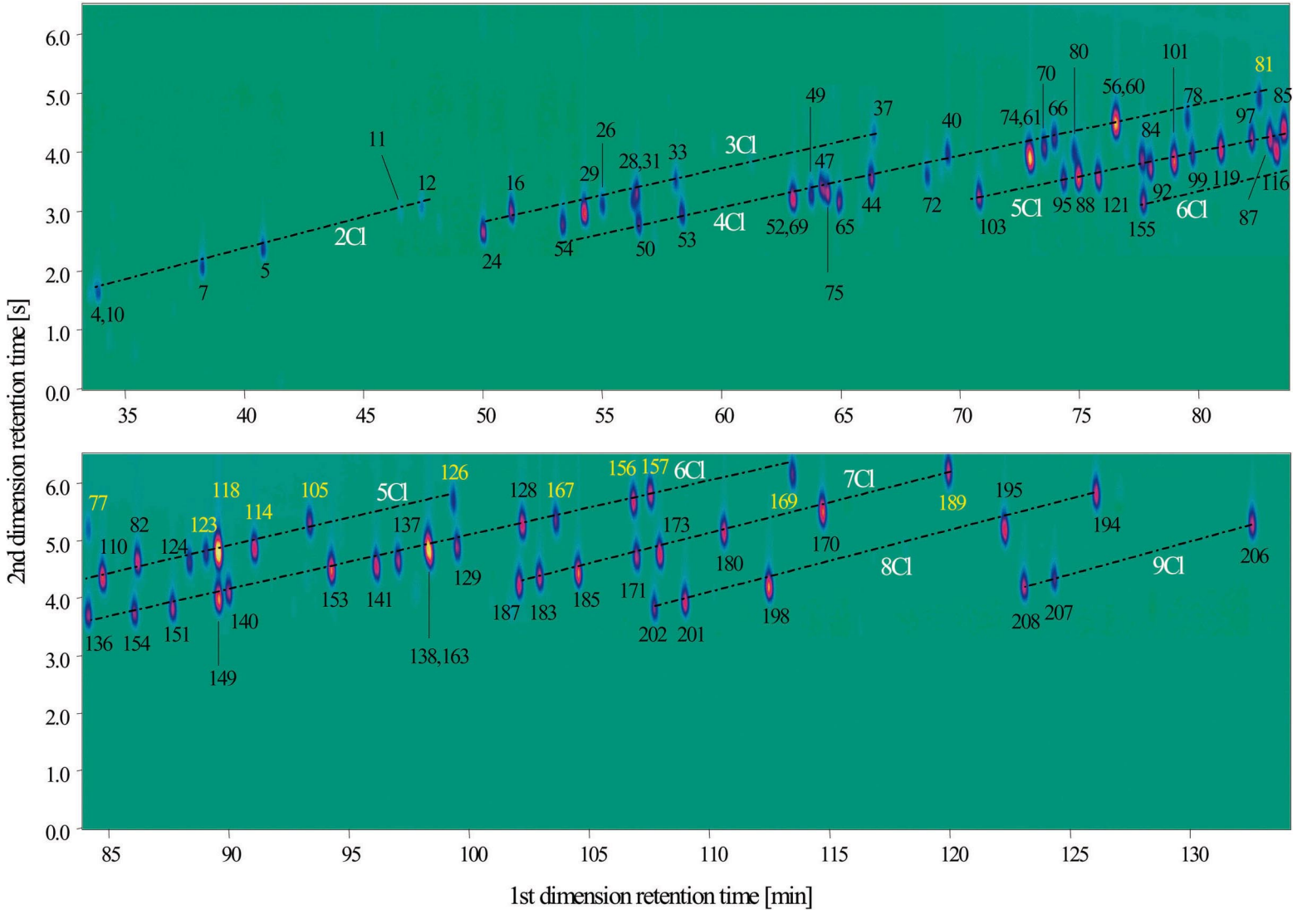

Fig. 3. GC×GC–μECD chromatogram of the mixture of 90 CBs with HP-1–HT-8 column combination. Temperature programme: 90 °C (2 min), at 5 °C/min to 110 °C, then at 1 °C/min to 240 °C (25 min) for the first oven, and 110 °C (2 min), at 5 °C/min to 130 °C, then at 1 °C/min to 260 °C (25 min) for the second oven.

is that the non- and mono-*ortho* CBs have the highest retention times in the second dimension and are, therefore, situated at the end of their group, i.e. at the upper end of the chromatographic plane. The presence of an ordered structure is very useful, because it helps to predict the position of CB congeners in the two-dimensional plane. As an example, by combining published retention data on 100% dimethylpolysiloxane for 140 CB congeners present in Aroclor technical mixtures [26,28] and the information contained in the ordered structure of Fig. 3, one can predict that the WHO-list non- and mono-*ortho* CBs will be separated from all other congeners. Details can be read from Table 2.

An even better separation of the CB congeners was achieved using a polyethylene glycol stationary phase in the second column. As can be read from Fig. 4, with the HP-1–SupelcoWax-10 column set-up, no less than 84 congeners show up as resolved peaks and only three critical pairs remain, viz. CBs 28–31, 56–60 and 80–88. As well as with the HT-8 second-dimension column, all WHO-list congeners were baseline-separated and the non-*ortho* CBs were observed at the top end of the chromatographic plane as well. However, with this column combination, there is little or no ordered structure, which means that although the separation is successful, useful predictions cannot be made, which in this case detracts from the value of the GC×GC operation.

In summary, as a comparison of Figs. 2–4 shows, the polyethylene glycol phase provides the highest additional selectivity for CB separation—peaks eluted from the polyethylene glycol column are spread over essentially the entire plane while peaks

Table 2
Separation of the 12 WHO-list non- and mono-*ortho* CB congeners from 140 CBs present in technical mixtures

CB no.	Results taken from published data on 100% dimethylpolysiloxane [28]	GC×GC on HP-1–HT-8 column set[a,b]
77	Co-elution with 136	Separated
	Contiguous to 110	Separated
81	Co-elution with 86	Will be separated
	Contiguous to 97 and 87	Separated
105	Co-elution with 132	Will be separated
	Contiguous to 146	Will be separated
114	Co-elution with 134	Will be separated
	No contiguous peaks	
118	Co-elution with 149	Separated
	No contiguous peaks	
123	No co-elution	
	Contiguous to 147	Will be separated
126	Co-elution with 129	Separated
	No contiguous peaks	
156	Co-elution with 171	Separated
	No contiguous peaks	
157	Co-elution with 202	Separated
	Contiguous to 173	Separated
167	No co-elution	
	Contiguous to 183	Separated
169	No co-elution	
	No contiguous peaks	
189	No co-elution	
	No contiguous peaks	

[a] Separated: as shown in Fig. 3; Will be separated: prediction based on known first-dimension retention times [28] and the ordered structure of Fig. 3.

[b] Example: As was reported in Ref. [28], in one-dimensional GC on 100% dimethylpolysiloxane, CB 81 co-elutes with CB 86. This means that, in GC×GC, CB 86 will have the same first-dimension retention time as CB 81. However, CB 86 has five, and CB 81, four Cl substituents. Therefore, a second-dimension separation can be predicted, with CB 86 appearing at shorter second dimension retention than CB 81.

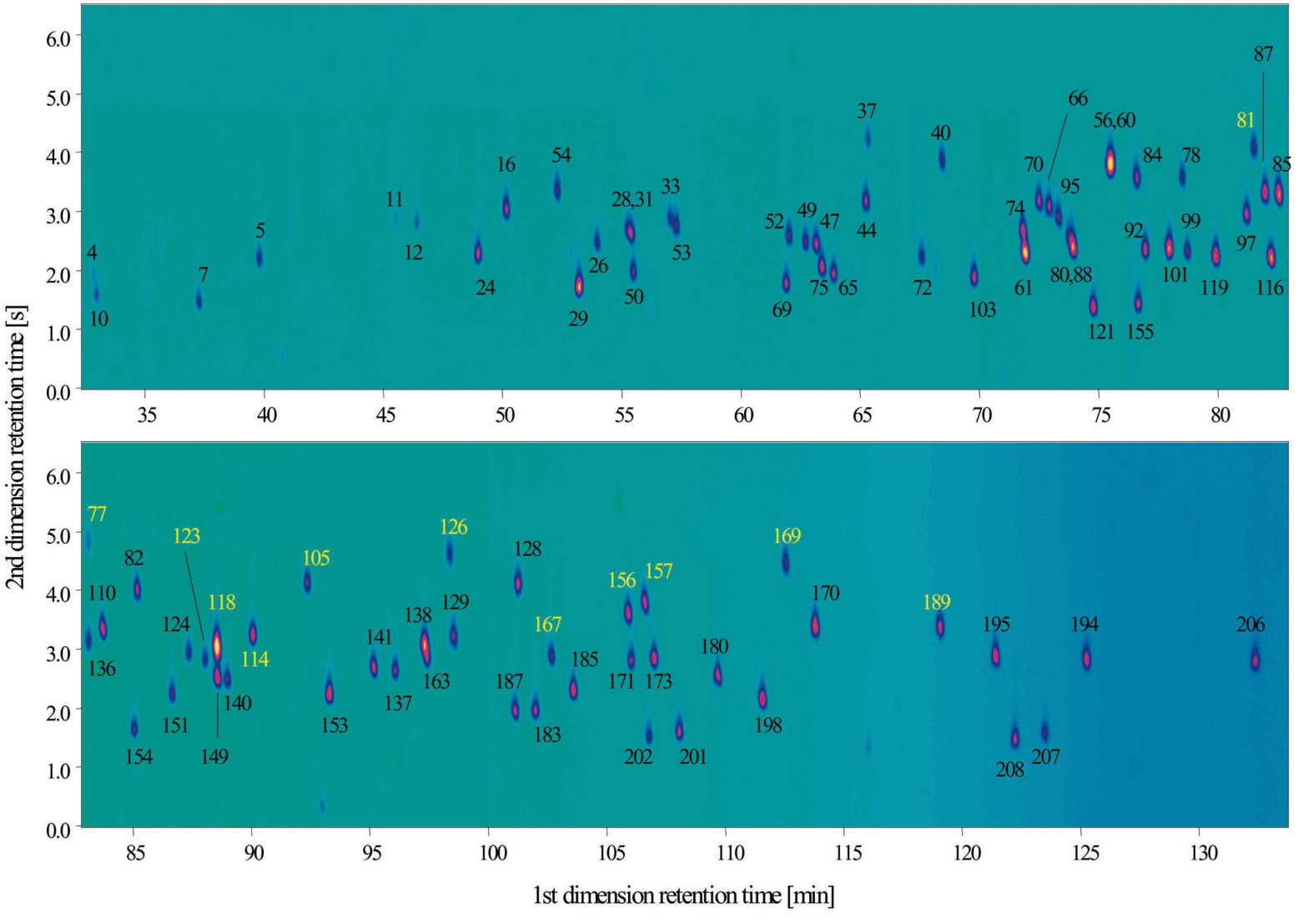

Fig. 4. GC×GC–μECD chromatogram of the mixture of 90 CBs with HP-1–SupelcoWax-10 column combination. Temperature programme: 90 °C (2 min), at 5 °C/min to 110 °C, then at 1 °C/min to 240 °C (25 min) for the first oven, and 110 °C (2 min), at 5 °C/min to 130 °C, then at 1 °C/min to 260 °C (25 min) for the second oven.

eluted from the carborane and 50% phenyl (equiv.) polysilphenylene-siloxane columns are grouped closer together—but the information content is highest for the combination with the carborane second-dimension column.

3.2.3. Temperature programme optimisation for both ovens

The parameter that had to be optimised for the first oven was the gradient. If the gradient is too shallow, the total analysis time will be much too long. But, if it is too steep, the peaks eluted from the first column will be too narrow to allow three- to fourfold modulation as is required for proper performance of the GC×GC system [29]. Four temperature gradients ($X = 0.5$, 1, 2 or 3 °C/min) were evaluated for the HP-1–HT-8 column combination using as a temperature programme: 90 °C (2 min), at 5 °C/min to 110 °C, then at X°C/min to 240 °C (25 min). The optimum was found at 1 °C/min. With steeper gradients a modulation into only one or two fractions was achieved for lower boiling PCBs. Moreover, breakthrough of these CBs was observed in the modulator, because the steeper gradient elutes compounds into the tube at higher temperature and thus prevents sufficient retardation in the modulator tube. With a 0.5 °C/min temperature gradient, the analysis time was unacceptably long, i.e. 4–5 h. The same temperature programme was used for all column sets. However for HP-1–SupelcoWax-10, the final temperature was 230 °C instead of 240 °C because of the lower temperature limit of the polyethylene glycol phase.

The use of two independently controlled ovens enables running the second column at a temperature which is different from that of the first column and, thus, to tune the retention power and selectivity of the second column independently of those of the first column. The parameter which was optimised for the second oven, was the temperature offset between the two ovens during the gradient run. This means that, in the second oven, the same temperature programme was used as in the first oven—with the second oven running ahead by a pre-set temperature difference. As can be read from Table 3, where CB 124 was used to determine peak widths and the critical pair CBs 118–149 to assess resolution, increasing the temperature difference not unexpectedly causes the peak widths to decrease because the analytes spend less time in the second column. However, if the second-oven temperature is set too high, the selectivity is adversely affected and the resolution will decrease. In other words, the optimum temperature difference reflects a compromise between peak width (or retention time) and required resolution. It was found that, for BPX-50, HT-8 and SupelcoWax-10, the optimum second-oven temperatures should be 20, 20 and 50 °C, respectively, above the first-oven temperature. With these optimum values, acceptable second-dimension retention times (from 6.5 to 13 s) and peak widths (from 300 to 400 ms at half peak height) were observed for all CB congeners and sufficient resolution was created for the critical pair, CBs 118–149. In the case of the BPX-50 column, where this critical pair is not separated, the optimum was determined only on the base of the peak width

Table 3
Optimisation of the temperature offset, ΔT, between first and second oven in the GC×GC system for three second-dimension stationary phases

ΔT (°C)	BPX-50			HT-8			SupelcoWax-10		
	$W_{1/2}$ (ms)	t_R (s)	R_s	$W_{1/2}$ (ms)	t_R (s)	R_s	$W_{1/2}$ (ms)	t_R (s)	R_s
10	470	13.78	0	440	14.37	1.5	–	–	–
20	340	10.58	0	340	11.79	1.5	–	–	–
30	290	9.96	0	280	9.62	1.3	470	15.36	1.3
40	–	–	–	–	–	–	380	12.66	1.3
50	–	–	–	–	–	–	310	10.32	1.1

$w_{1/2}$, peak width at half peak height of CB 124; t_R, second-dimension retention time of CB 124 (retention times were estimated based on peak width and position in GC×GC chromatogram); R_s, resolution of critical pair, CBs 118–149.

(to have similar conditions as with the other columns). Although the second dimension time scale in Figs. 2–6 is 0–6.5 s (because the modulation period was 6.5 s), the real retention times of the analytes in the second column are longer and wrap-around is observed. The absolute times in Table 3 were then estimated based on peak widths and positions in the GC×GC chromatogram.

We would like to emphasise that the temperature requirements for the second oven are a main limiting factor when selecting the stationary phase of the second column and setting the final temperature of the first-column temperature programme. Referring to what was briefly mentioned above, not exceeding the upper temperature limit of a stationary phase was the reason why, for the HP-1–SupelcoWax-10 column set, the final first-column temperature was set at 230 °C. The required final second-oven temperature, then, was 280 °C, which is the upper temperature limit of the polyethylene glycol phase. This explains the increasing baseline (change in background colour) observed at the end of the chromatogram of Fig. 4.

3.2.4. Applications

An extract of a cod liver sample was analysed on the HP-1–HT-8 column set to demonstrate the potential of the GC×GC technique to separate PCBs in real-life samples. A typical chromatogram is shown in Fig. 5. The analysis was carried out under chromatographic conditions which slightly differed from those used for the standard mixture (Fig. 3). In order not to expose a stationary phase in the modulator tube to high temperature and thus prolong its life-time, the final temperature of the temperature programme was 220 °C as against 240 °C in the earlier example. Consequently, CBs 189, 195, 208, 207, 194 and 206 eluted somewhat late, i.e. during the final isothermal part of the run. Therefore, they do not lie on the group lines.

Fig. 5 shows that for some peaks, tailing occurred (e.g. CB 112, 153, 138, 163). This was due to overloading caused by the huge concentration differences of the several congeners commonly encountered in environmental samples (except CB 112, which was added as an internal standard). In addition, the sample was not diluted to facilitate the visualisation of trace congeners. The peaks were identified using the retention times of the 90 available CB standards. Out of the 12 WHO PCBs, CBs 77, 105, 114, 118, 156, 157, 167, and 189 were found in the sample. The most important aspect is the detection of CB 77, because: (i) it is a non-*ortho* CB congener with very low concentration in real samples and (ii) the present method allows it to be separated without any pre-separation despite the very intense peak of CB 136 which has the same first-dimension retention time. The known retention pattern enables a ‘semi-identification’, i.e. to decide if a peak represents a CB congener and to determine the number of chlorine atoms in the molecule. If the peak shows up on one of the lines, then there is a high probability that it represents a CB congener; these peaks are indicated by an asterisk. The number of chlorine substituents can be read from the specific line the peak is situated on.

As another example, a mixture of 90 CBs and the 17 most toxic PCDDs and PCDFs was analysed to demonstrate the separation power of the HP-1–HT-8 column combination. As can be read from Fig. 6, 16 CDD and CDF congeners were separated from each other and also from all CBs; only one penta-CDD or -CDF co-eluted with CB 169. Some co-elutions can be seen for the Hx-CDD and Hx-CDF, however, on an enlarged picture the valley between the peaks could be observed. No definite conclusion regarding the CDD or CDF nature could be reached because no individual standards were available. It should be emphasised that the separation was achieved without any pre-separation, which is always required in conventional GC analysis. This highlights the distinct potential of GC×GC-μECD for the separation of PCDDs, PCDFs and PCBs in real samples which will be the subject of future studies.

3.3. Limits of detection

The limits of detection (LOD) of selected CB congeners were estimated for both GC×GC–μECD and GC–ECD. The data for GC×GC LODs were measured with the HP-1–HT-8 column set. As has been observed by other authors [30], the determination of LODs in GC×GC is not fully straightforward because, during processing, each first-di-

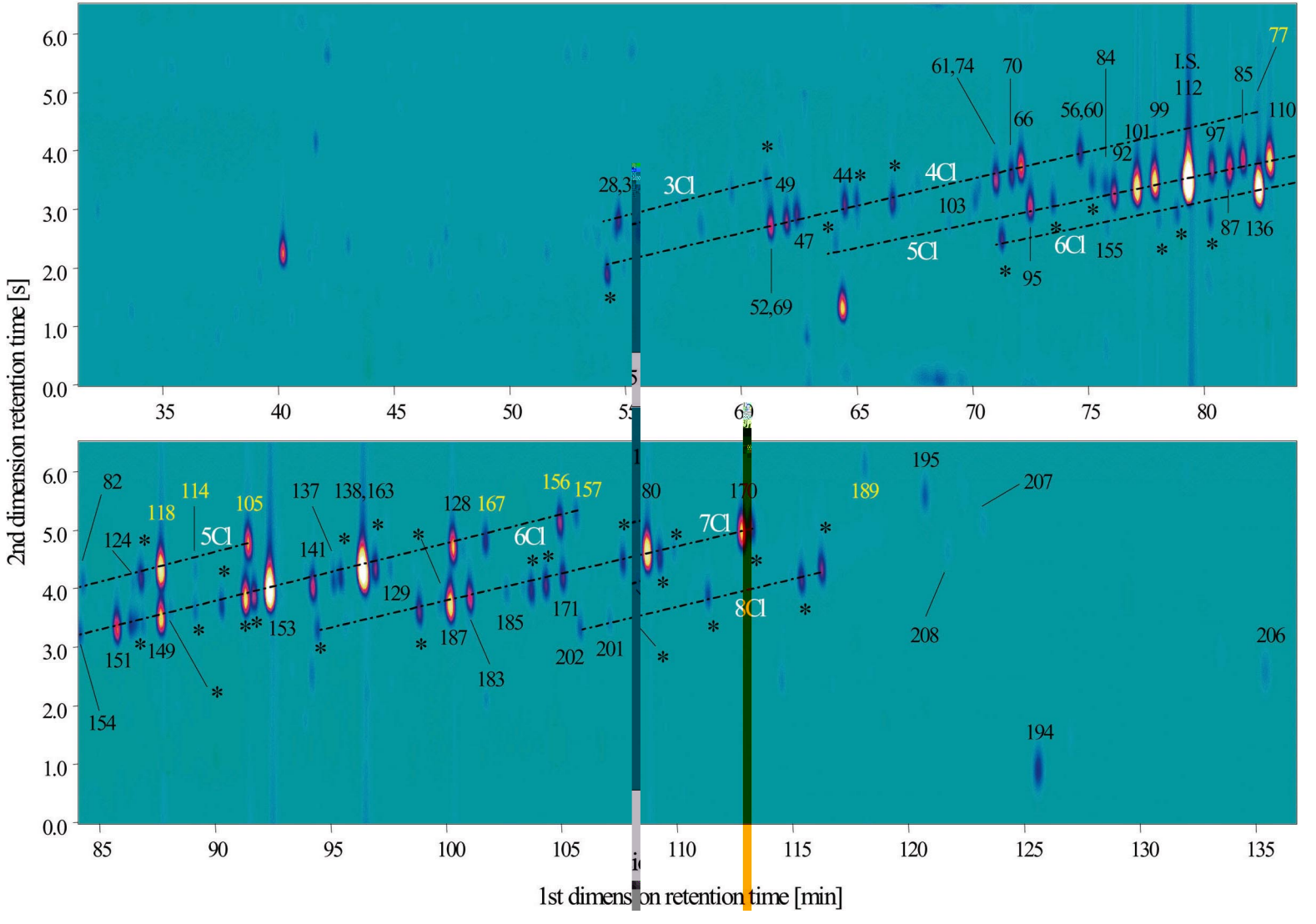

Fig. 5. GC×GC–μECD chromatogram of the cod liver sample with HP-1–HT-8 column combination. Temperature programme: 90 °C (2 min), at 5 °C/min to 110 °C, then at 1 °C/min to 220 °C (25 min) for the first oven, and 110 °C (2 min), at 5 °C/min to 130 °C, then at 1 °C/min to 240 °C (25 min) for the second oven. The inlet pressure was set at 55 p.s.i.

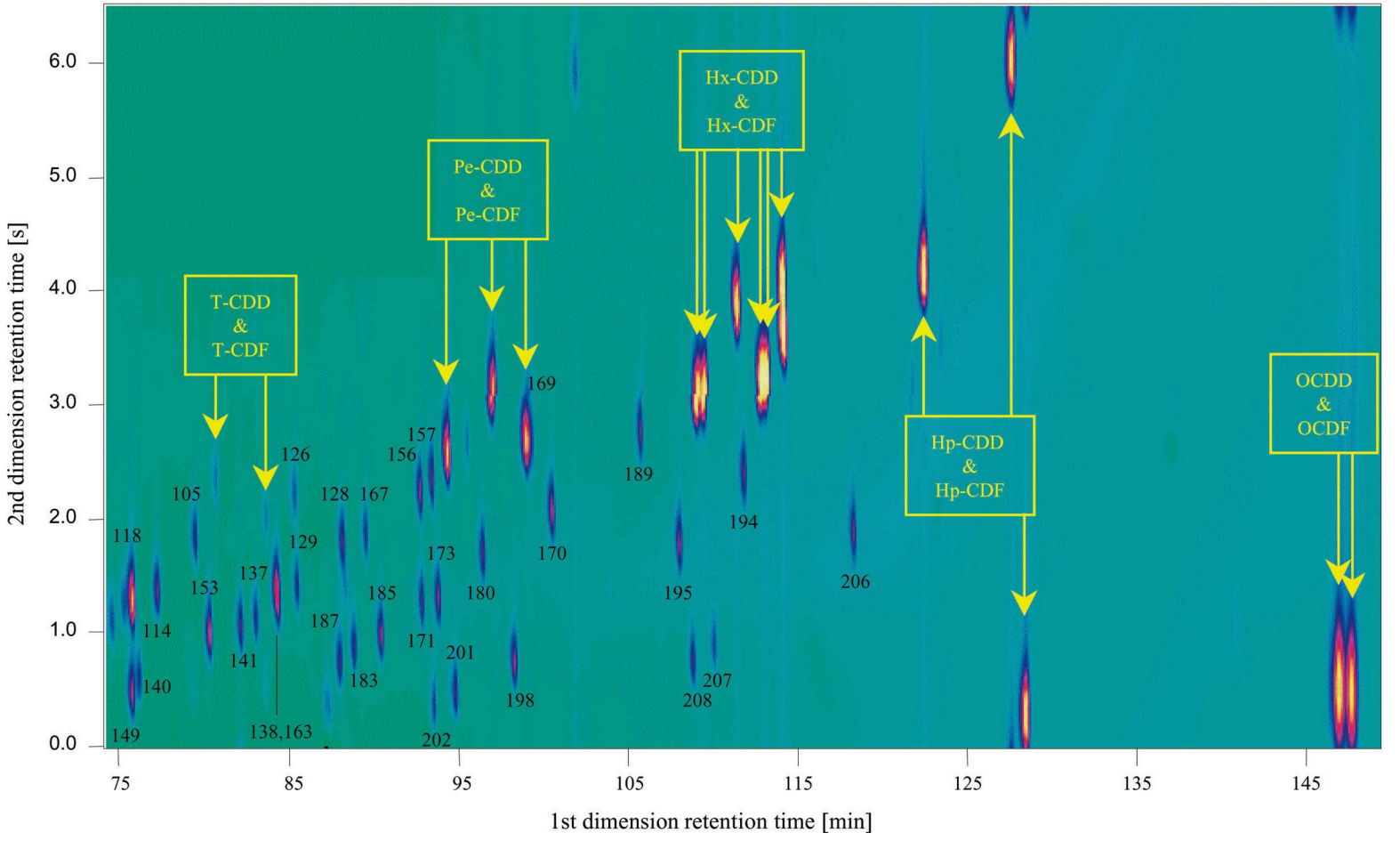

Fig. 6. Part of GC×GC–μECD chromatogram of the mixture of 90 CBs and 17 PCDDs and PCDFs with HP-1–HT-8 column combination. Temperature programme: 90 °C (2 min), at 5 °C/min to 130 °C, then at 1 °C/min to 240 °C (40 min) for the first oven, and 110 °C (2 min), at 5 °C/min to 150 °C, then at 1 °C/min to 260 °C (40 min) for the second oven.

mension peak is modulated several times (typically, two to five times). In this study, in order to arrive at a conservative estimate, a series of increasingly diluted standard CB mixtures was injected and the LODs (at signal-to-noise ratio of 3:1) were calculated only from chromatograms in which only one (or, occasionally, two equally intense) peaks was observed and the other, smaller, peaks had disappeared. The experimental results for selected CB congeners are reported in Table 4. As can be seen, under the conditions of the present study, three- to fourfold lower LODs were obtained in GC×GC compared to GC. In other words, LODs of 10 fg can generally be expected for tetra- or higher substituted CBs in comprehensive GC with μECD.

4. Conclusions

Comprehensive two-dimensional gas chromatography with two column sets (HP-1–HT-8 and HP-1–SupelcoWax-10) provides a complete separation of all 12 toxic non-*ortho* and mono-*ortho* CB congeners mentioned in the WHO list out of 90 CBs in the standard mixture. Based on published data, it can be expected that the 12 toxic congeners can also be separated from other congeners present in Aroclor. A real-life fish sample indicates that the 12 toxic congeners could be separated from the bulk PCBs without LC-based pre-fractionation. With the HP-1–HT-8 column combination, structured chromatograms are obtained with the CBs grouped together according to the number of chlorine substituents (and, additionally, the number of free *ortho* positions). These characteristics, combined with the detection limits of about 10 fg, make GC×GC with improved μECD highly suitable for congener-specific CB analysis, inclusive of non- and mono-*ortho* substituted CBs. Separation of nearly all priority PCDDs and PCDFs from the group of 90 CBs is possible in one run.

Table 4
LODs of selected CB congeners in GC×GC–μECD and GC–ECD

CB no.	No. of chlorines in molecule	LOD[a] (fg)	
		GC	GC×GC
44	4	60	20
97	5	50	10
105	5	40	10
118	5	40	10
137	6	30	10
156	6	30	10
180	7	40	10
194	8	40	10
206	9	70	20

[a] LODs determined at signal-to-noise ratio of 3:1.

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