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Chiral comprehensive two-dimensional gas chromatography with electron-capture detection applied to the analysis of chiral polychlorinated biphenyls in food samples

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Abstract

The feasibility of comprehensive two-dimensional gas chromatography with electron-capture detection (GC × GC–ECD) for the enantioseparation of chiral PCBs from other possible interfering compounds has been evaluated. Three commercially available enantioselective β-cyclodextrin-based capillary columns (Chirasil-Dex, BGB-172 and BGB-176SE) have been tested as first-dimension columns. Three non-enantioselective stationary phases (HT-8, BPX-50 and Supelcowax-10) were combined with the enantioselective columns to allow the unambiguous determination of the enantiomers of the target chiral PCBs. Each enantioselective first-dimension column tested was able to separate into enantiomers different PCB congeners, but in all cases, the use of Supelcowax-10 as second-dimension column provided the most satisfactory results. The Chirasil-Dex × Supelcowax-10 column combination allowed the determination of the enantiomeric fraction (EF) of PCBs 84, 91, 95, 132, 136, 149, 174 and 176 in the working standard solution, while that of congener 135 was hindered. The BGB-172 × Supelcowax-10 column set allowed a proper EF determination of congeners 45, 84, 131, 132, 135, 171, 174 and 183, while that of PCB 91 was interfered with co-elutants. The column combination BGB-176SE × Supelcowax-10 allowed the determination of all congeners that this enantioselective stationary phase was able to separate into enantiomers, i.e. PCBs 45, 91, 95, 136, 149 and 176. These column combinations have also been evaluated for the simultaneous determination of the 12 congeners with a toxic equivalency factor assigned by the WHO (PCBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189) and the seven indicator congeners (PCBs 28, 52, 101, 118, 138, 153 and 180), and evaluated for the analysis of food samples.

Keywords: Gas chromatography; Chiral comprehensive two-dimensional; Polychlorinated biphenyls; Food analysis; Enantiomer separation

1. Introduction

The analysis of chiral polychlorinated biphenyls (PCBs) and the determination of their enantiomeric fraction (EF) has become a relevant issue in recent years, since the non-racemic composition might be an evidence of biotransformation processes or selective bioaccumulation in living organisms [1]. Seventy-eight out of the 209 possible PCB congeners display axial chirality in their non-planar conformations and, among them, Kaiser predicted that 19 tri- and tetra-*ortho* substituted PCBs would present stable enantiomers at room temperature due to restricted rotation around the central C–C bond [2]. Although PCBs were released into the environment as racemates, studies showed that many organisms are able to selectively accumulate and/or degradate their enantiomers [3,4]. Some studies have also pointed to different biological and toxic behaviour for each of the enantiomers [5].

Until now, no single enantioselective capillary column has been able to separate simultaneously all 19 chiral PCBs into enantiomers. Therefore, complete information about all congeners can be only obtained by the combined used of selected capillary columns. Since Chirasil-Dex was reported to be efficient for the enantioseparation of chiral PCBs [6], this column has been one of the most commonly used for this type of analysis [7,8]. *tert*-Butyldimethylsilyl substituted cyclodex-

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trins such as BGB-172 and BGB-176SE have also shown the ability to resolve into enantiomers those PCBs presenting a 2,3,4,6-substitution pattern [9]. In a previous study, the combined use of the above mentioned enantioselective capillary columns was able to separate into enantiomers 15 out of the 19 chiral PCBs [10].

When analysing real samples by conventional one-dimensional chiral gas chromatography (GC) co-elution problems increase and the use of multidimensional techniques becomes mandatory [8,11]. Up to now, heart-cut multidimensional GC (MDGC) has been the technique mainly employed [8]. Meanwhile, and despite the profuse use of comprehensive two-dimensional GC (GC × GC) for the unambiguous determination of toxic and persistent PCBs in complex mixtures [12,13], examples dealing with the analysis of chiral PCBs are still scarce. Recently, Harju et al. reported the separation into enantiomers of selected chiral PCBs in grey seal tissues using GC × GC with Chirasil-Dex as the first-dimension column and two non-enantioselective stationary phases as second-dimension, i.e. LC-50 and VF-23ms [14]. Chirasil-Dex combined with Supelcowax-10 and VF-23ms as second-dimension columns has also been applied to the determination of chiral PCBs in cheese samples [10].

The aim of the present paper is to gain insight into the possibilities that GC × GC with electron-capture detection (ECD) offer for accurate EF determination of chiral PCBs with the simultaneous determination of environmentally relevant PCBs, i.e. the 12 toxic coplanar congeners and the seven indicator PCBs. Three enantioselective stationary phases and three non-enantioselective columns were tested as first- and second-dimension columns, respectively. The relative merits of the column combinations that provided the best results were evaluated for the analysis of fatty food samples.

2. Experimental

2.1. Samples and chemicals

All reagents used were of trace analysis grade. *n*-Hexane was supplied from Merck (Darmstadt, Germany), acetone from SDS (Peypin, France), silica gel from Merck and granular anhydrous sodium sulphate from J.T. Baker (Deventer, The Netherlands). A working standard solution containing 65 PCBs (Table 1) was prepared from individual PCB standards purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The 19 chiral PCBs (PCB No. 45, 84, 88, 91, 95, 131, 132, 135, 136, 139, 144, 149, 171, 174, 175, 176, 183, 196 and 197) [15], the toxic coplanar PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189) [16] and the seven indicator congeners (PCB No. 28, 52, 101, 118, 138, 153 and 180) [8] were included in the mixture, which contained 250 ng/ml of each congener in isooctane.

Milk, cheese and salmon samples were purchased from supermarkets in Madrid and prepared according to a previous described method [17]. Briefly, the extraction was carried out by matrix solid-phase dispersion of the sample on granular anhydrous sodium sulphate and silica gel. Acetone:*n*-hexane (1:1, v/v) was used as elution solvent. Further clean-up consisted of the elution of the fatty extract throughout multilayer columns containing silica gel modified with sulphuric acid and sodium hydroxide. The collected extract was then concentrated under a gentle nitrogen stream, reconstituted in isooctane and directly subjected to GC analysis. The salmon extract was further fractionated on carbon SPE cartridges to separate non-*ortho* PCBs and PCDD/Fs from the bulk of PCBs.

Table 1
List of the PCBs included in the standard mixture used in this study

PCB	Cl position	PCB	Cl position	PCB	Cl position
Tri-CBs		**114**	2,3,4,4′,5	**167**	2,3′,4,4′,5,5′
18	2,2,′5	**118**	2,3′,4,4′,5	*169*	3,3′,4,4′,5,5′
28	2,4,4′	**119**	2,3′,4,4′,6	Hepta-CBs	
31	2,4,5	**122**	2′,3,3′,4,5	**170**	2,2′,3,3′,4,4′,5
33	2′,3,4	**123**	2′,3,4,4′,5	**171**	2,2′,3,3′,4,4′,6
Tetra-CBs		**124**	2′,3,4,4′,5	**173**	2,2′,3,3′,4,5,6
45	2,2′,3,6	*126*	3,3′,4,4′,5	**174**	2,2′,3,3′,4,5,6′
47	2,2′,4,4′	Hexa-CBs		**175**	2,2′,3,3′,4,5′,6
52	2,2′,4,6′	**128**	2,2′,3,3′,4,4′	**176**	2,2′,3,3′,4,6,6′
55	2,3,3′,4	**129**	2,2′,3,3′,4,5	**178**	2,2′,3,3′,5,5′,6
66	2,3′,4,4′	**131**	2,2′,3,3′,4,6	**180**	2,2′,3,4,4′,5,5′
74	2,4,4′,5	**132**	2,2′,3,3′,4,6′	**183**	2,2′3,4,4′,5′,6
77	3,3′,4,4′	**135**	2,2′,3,3′,5,6′	**187**	2,2′,3,4′,5,5′,6
80	3,3′,5,5′	**136**	2,2′,3,3′,6,6′	**189**	2,3,3′,4,4′,5,5′
81	3,4,4′,5	**138**	2,2′,3,4,4′,5′	**190**	2,3,3′,4,4′,5,6
Penta-CBs		*139*	2,2′,3,4,4′,6	Octa-CBs	
84	2,2′,3,3′,6	**141**	2,2′,3,4,5,5′	**194**	2,2′,3,3′,4,4′,5,5′
88	2,2′,3 4,6	**144**	2,2′,3,4,5′,6	**196**	2,2′,3,3′,4,4′,5,6′
91	2,2′,3,4′,6	**149**	2,2′,3,4′,5′,6	**197**	2,2′,3,3′,4,4′,6,6′
95	2,2′,3,5′,6	**153**	2,2′,4,4′,5,5′	**200**	2,2′,3,3′,4,5,6,6′
99	2,2′,4,4′,5	*155*	2,2′,4,4′,6,6′	**201**	2,2′,3,3′,4,5′,6,6′
101	2,2′,4,5,5′	**156**	2,3,3′,4,4′,5	**202**	2,2′,3,3′,5,5′,6,6′
105	2,3,3′,4,4′	**157**	2,3,3′,4,4′,5′	Nona-CBs	
110	2,3,3′,4′,6	**164**	2,3,3′,4′,5′,6	**206**	2,2′,3,3′,4,4′,5,5′,6

Relative abundances of the studied congeners in Aroclors 1242, 1254 and 1260, according to [19]: bold and underlined, levels >1.0% (w/w); bold, range 1.0–0.05% (w/w); and italics, trace or undetected.

2.2. GC × GC system

An Agilent HP6890 (Agilent Technologies, Palo Alto, USA) with the KT2003 loop modulator (Zoex, Lincoln, Nea, USA) installed inside the oven was used for analysis. The system consisted of one cold and one hot jet that made the modulation of the first-dimension eluting compounds possible. The modulation took place in the so-called modulator loop, a 1.5 m deactivated fused silica column (0.10 mm I.D., 0.10 μm film thickness, Supelco, Bellefonte, PA, USA). Columns were connected to the modulator loop via mini press-fits (Techrom, Purmerend, The Netherlands). Details of the loop modulator principles have been described elsewhere [18]. Liquid nitrogen was used to create the cold jet, while the temperature of the hot jet heater had an offset of 80 °C over the temperature of the main oven during the chro-

Table 2
Details on the experimental conditions used with the column combinations investigated

Column combination	Second dimension length (m)	Head pressure (psi)	Temperature off-set[a] (°C)	Column code
Chirasil-Dex				
×HT-8	1	40	0	A
×HT-8	2	54	0	B
×HT-8	2	54	40[b]	C
×BPX-50	1	40	40[b]	D
×Supelcowax-10	1	40	40[b]	E
BGB-172				
×HT-8	2	62	0	F
×BPX-50	2	61	40[c]	G
×Supelcowax-10	1	45	40[c]	H
×Supelcowax-10	2	62	40[c]	I
BGB-176SE				
×Supelcowax-10	2	61	40[d]	J

[a] Temperature off-set between the main and the second-dimension oven.
[b] 200 °C (4.8 min), 0.5 °C/min to 225 °C (28 min), 10 °C/min to 240 °C (40 min), 1 °C/min to 270 °C.
[c] 210 °C (15.2 min), 10 °C/min to 230 °C (15 min), 10 °C/min to 250 °C (54 min), 1 °C/min to 280 °C.
[d] 200 °C (15.5 min), 1 °C/min to 220 °C (15 min), 1 °C/min to 240 °C (25 min), 1 °C/min to 270 °C.

matographic run. A secondary oven that allowed working with an offset of temperature between the two columns was also installed. Helium was used as carrier gas in the constant flow mode. Table 2 summarises the initial column head pressure used in the different sets of experiments carried out. The micro-ECD (μ-ECD) was maintained at 300 °C throughout the study and nitrogen was used as make-up gas at a flow rate of 150 mL/min. In all instances the modulation period was set at 6 s with a 250 ms hot jet pulse duration. Injections were performed in the splitless mode (1 μL; splitless time, 0.75 min) at 250 °C. Data acquisition rate was set at 50 Hz.

2.3. Columns

Three enantioselective β-cyclodextrin-based (β-CD) columns were tested as first-dimension columns: the permethylated Chirasil-Dex (2,3,6-tri-*O*-methyl β-CD, 25 m × 0.25 mm I.D., 0.25 μm film thickness; Varian-Chrompack, Middelburg, The Netherlands) and two *tert*-butyldimethylsilyl β-CDs, BGB-172 (25% 2,3,6-*tert*-butyldimethylsilyl β-CD, 30 m × 0.25 mm I.D., 0.18 μm film thickness; BGB Analytik, Adliswil, Switzerland) and BGB-176SE (20% 2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl β-CD, 30 m × 0.25 mm I.D., 0.25 μm film thickness; BGB Analytik). Three non-enantioselective columns with different polarity were tested as second-dimension columns: HT-8 (1 or 2 m × 0.10 mm I.D., 0.10 μm film thickness; SGE, Darmstadt, Germany), BPX-50 (1 or 2 m × 0.10 mm I.D., 0.10 μm film thickness; SGE) and Supelcowax-10 (1 or 2 m × 0.10 mm I.D., 0.10 μm film thickness; Supelco).

Slow temperature ramping in the first-dimension oven was chosen to improve the resolution between the PCB enantiomers. Under the experimental conditions proposed, a minimum of four modulations per peak was obtained when a modulation period of 6 s was applied. Although wrap-around was observed due to the slow ramps and the relatively low maximum temperature limit of the enantioselective columns, it is important to note that co-elution between analytes from different modulation periods was not observed and the separation obtained in the first dimension was preserved. The main oven was programmed differently depending on the enantioselective column: Chirasil-Dex: 90 °C (2 min), at 25 °C/min to 160 °C, at 0.5 °C/min to 185 °C (28 min), at 10 °C/min to 200 °C (40 min), at 10 °C/min to 210 °C (19 min), at 10 °C/min to 230 °C; BGB-172: 90 °C (2 min), at 25 °C/min to 170 °C (10 min), at 10 °C/min to 190 °C (15 min), at 10 °C/min to 210 °C (54 min), at 10 °C/min to 220 °C (23 min), at 10 °C/min to 240 °C; and BGB-176SE: 90 °C (2 min), at 20 °C/min to 160 °C (10 min), at 1 °C/min to 180 °C (15 min), at 1 °C/min to 200 °C (25 min), at 2 °C/min to 220 °C (40 min), at 5 °C/min to 230 °C. The second-dimension oven had a temperature program similar to that of the main oven but, when required, with an offset of temperature (Table 2).

2.4. Data analysis

ChemStation software was used for acquiring the raw data that was further exported as comma separated values (CSV) to the GC Image v1.3 (University of Nebraska, Lincoln, USA) for further data analysis. The software allowed automated baseline correction and peak volume determination. Chiral PCBs were identified matching a template generated from the standard solution containing the congeners studied.

3. Results and discussion

Tables 3 and 4 summarise the potential co-elutions between the target chiral and achiral PCBs studied and those

Table 3
List of the potential interfering congeners in one-dimensional GC for the chiral PCBs under study in the enantioselective capillary columns evaluated

Congener	Chirasil-Dex	BGB-172	BGB-176SE
45	nr[a]	**52**	**33**
84	**99**, **119**	*55*, **119**	nr
91	–	**101**	**74**
95	**74**	nr	*80*, *88*, *155*
131	nr	**123**	nr
132	**141**, **176**	–	nr
135	**110**, **139**	**110**, **136**	nr
136	–	nr	–
149	**77**, **124**	nr	**77**, *81*, **124**, **139**
171	nr	**173**, **197**	nr
174	**197**, **202**	–	nr
176	**132**, **141**	nr	**141**
183	nr	–	nr

See Table 1 for font code identification.

congeners included in the working standard solution, in the three enantioselective capillary columns evaluated. In order to solve those co-elutions, three non-enantioselective second-dimension columns with different retention characteristics were tested. HT-8 is a capillary column commonly used for the analysis of PCBs and provides highly structured chromatograms based on the *ortho*-substitution degree [20]. Moderately polar polysiloxanes such as BPX-50 (50% phenylmethylpolysiloxane) and the polar polyethylene glycol capillary column Supelcowax-10 were also tested. Experimental conditions such as the temperature offset between the main and secondary oven and the length of the second-dimension were optimised as regards potential to separate the target congeners from the interfering PCBs.

Table 4
List of the potential interfering congeners in one-dimensional GC for the priority PCBs (the 12 coplanar congeners and the seven indicator PCB) in the enantioselective capillary columns evaluated

Congener	Chirasil-Dex	BGB-172	BGB-176SE
Coplanar PCBs			
77	**149**	**122**	*81*, **124**, **139**, **149**
81	–	**139**	**77**, **124**, **139**, **149**
105	–	–	–
114	–	**176**	–
118	–	–	**123**, **131**
123	–	**131**	**118**, **131**
126	**167**	**167**, **202**	–
156	–	**180**	–
157	–	–	**180**
167	*126*	*126*, **202**	**128**
169	**170**, **190**, **196**, **200**	–	–
189	–	–	–
Indicator PCBs			
28	–	–	–
52	–	**45**	–
101	*55*	**91**	–
138	–	–	–
153	–	–	–
180	–	**156**	**157**

See Table 1 for font code identification.

3.1. Chirasil-Dex column sets

Chirasil-Dex is able to resolve into enantiomers congeners 84, 91, 95, 132, 135, 136, 149, 174 and 176 [10]. In addition to the PCB enantiomers that elute without interferences in Chirasil-Dex using one dimensional GC, i.e. enantiomers of congeners 91 and 136 (Table 3), PCBs 84, 132 and 176 enantiomers were also separated from their respective interfering congeners using GC × GC with a 1 m × 0.1 mm, 0.1 μm film thickness HT-8 as second-dimension column (column set A, Table 2). On the contrary, enantiomers of congeners 95, 135, 149 and 174 still showed co-elution problems. In an attempt to improve the separation of these particular chiral congeners, a 2 m second-dimension was used (column set B) and a complete resolution between the enantiomers and co-eluting congeners was then observed in the case of PCBs 135, 149 and 174, but not for the critical pair 95/74 which remained unresolved. However, some peak widening was observed when increasing the length of the second-dimension column from 1 to 2 m. This shortcoming was solved by applying a temperature offset between both ovens of 40 °C (column set C) but this approach led to new co-elution problems for PCBs 135, 149 and 174.

In order to evaluate the influence of the second-dimension stationary phase characteristics to solve the co-elution problems before detected, a moderately polar phase, BPX-50, was installed. A 1 m column with no temperature offset was tested and a satisfactory separation was observed for all chiral PCBs investigated. However, due to the stronger retention of the analytes in this stationary phase, broader peaks were obtained and different temperature offsets were again assayed to speed up the second-dimension separation. The best results were obtained when applying an offset of 40 °C (column set D). However, under these conditions PCB 135 enantiomers co-eluted with PCBs 139 and 110, and the enantiomers of congeners 132 and 174 were only partially resolved from their respective inferences, i.e. 141 and 197/202. No further improvement was achieved when using a 2 m second-dimension instead.

Finally the polyethylene glycol phase Supelcowax-10 was tested as second-dimension column. Because of the high retention of the target compounds in this stationary phase, an offset of temperature between both ovens was applied (Column set E). Among the studied congeners, just the critical pair PCB 135/139 remained unresolved while the rest of target chiral PCBs eluted well separated from interferences (Fig. 1A). Consequently, this column combination, Chirasil-Dex × Supelcowax-10, was selected for further evaluation of its feasibility for simultaneous determination of the 12 coplanar and the seven indicator PCBs. Satisfactory results were also obtained for these environmentally relevant PCBs (Fig. 1A) as all eluted free from their potential interferences listed in Table 4.

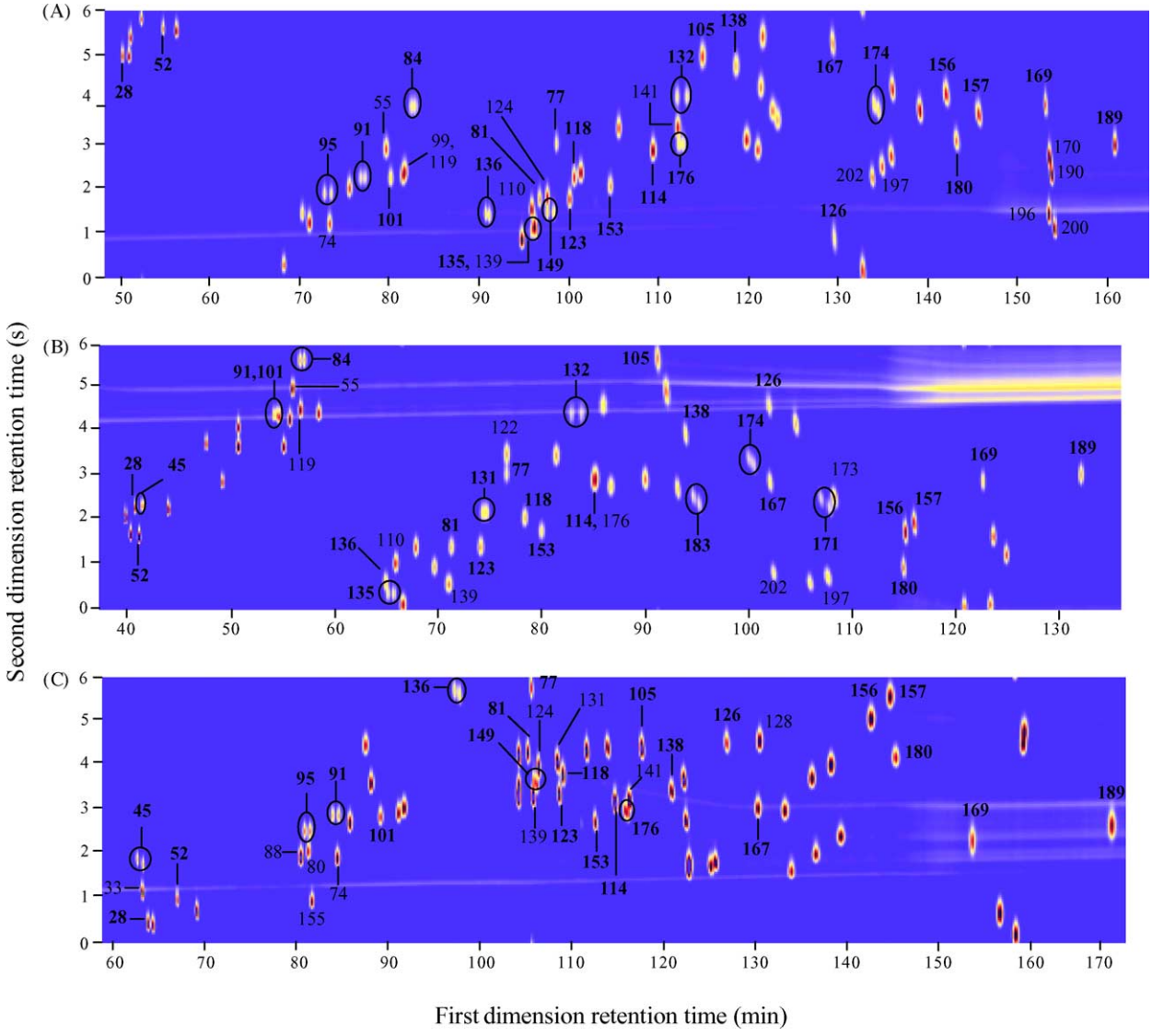

Fig. 1. GC × GC-μECD chromatograms of the working PCB standard solution with (A) Chirasil-Dex × Supelcowax-10; (B) BGB-172 × Supelcowax-10 and (C) BGB-176SE × Supelcowax-10.

3.2. BGB-172 column sets

Under the experimental conditions proposed in the present study, BGB-172 has been reported to separate into enantiomers congeners 45, 84, 91, 131, 132, 135, 171, 174 and 183. Although a partial separation has also been reported for PCBs 144, 149 and 175 using this column [10], such a separation requires a very slow ramp of temperature that results in unfeasible analysis times for the present study. A 2 m HT-8 was first assayed as second-dimension column (column set F) to resolve the interferences listed in Table 2. With this combination, besides congeners 132, 174 and 183 that eluted without interferences, the enantiomers of PCBs 45, 84, 91 and 135 eluted free from their respective coelutants. On the other hand, the first enantiomer of PCBs 131 co-eluted with the mono-*ortho* PCB 123, while the second enantiomer of PCB 171 was partially interfered by congener 197. Increasing the polarity of the second-dimension column (column set G), combined with the use of an offset of 40 °C, did not result in a better resolution of the target analytes from their interferences than that obtained with column set F. In fact, PCB 91 and 171 were found to partially co-elute with congeners 101 and 197, respectively, and PCB 135 enantiomers were completely hampered by congeners 136 and 110, respectively. When using a 1 m Supelcowax-10 as second-dimension column (column set H), a proper determination of the two enantiomers of PCBs 45, 84, 131, 132, 174 and 183 was obtained. However, congeners 91, 135 and 171 showed partial co-elution with PCBs 101, 136 and 173, respectively. In order to solve these co-elutions, the length of the second-dimension was increased to 2 m (column set I). Under these conditions the co-elutions reported for congeners 135 and

171 were solved, although the pair PCB 91/101 remained unresolved. According to these results, it was concluded that Supelcowax-10 was the best option as second-dimension column among those assayed, and therefore evaluated for simultaneous determination of toxic and indicator PCBs. Only one coplanar congener showed co-elution problems, that is the critical pair PCB 114/176. Concerning the indicator PCBs, as quoted above, PCB 101 was found to co-elute with the second enantiomer of PCB 91, but the rest of priority congeners were separated free from their interferences (Fig. 1B).

3.3. BGB-176SE column sets

BGB-176SE is able to separate the enantiomers of congeners 45, 91, 95, 136, 149 and 176. A partial separation has also been reported for the enantiomers of PCB 131 [10], but only using an extremely slow temperature ramp not applicable in the present study. Considering the results obtained with the other two enantioselective GC columns tested in this study, only a 2 m Supelcowax-10 was evaluated as second-dimension (column set J). All target enantiomers eluted separated from their respective interfering congeners using this column combination (Table 2). Satisfactory separation of both enantiomers of PCBs 149 from congeners 77, 81, 124 and 139 was achieved, as well as the separation of the pair PCB 176/141. All coplanar PCBs eluted well separated from the potential interfering congeners summarised in Table 4, as well as the seven indicator PCBs (Fig. 1C).

3.4. Application to food samples

The results obtained during method development pointed to column sets E (Chirasil-Dex × Supelcowax-10), I (BGB-172 × Supelcowax-10) and J (BGB-176SE × Supelcowax-10) as the most promising column combinations for chiral PCB analysis in complex real-life samples. Figs. 2 and 3 show the typical contour plots obtained by GC × GC-μECD of a salmon and two dairy products samples with the above mentioned column combinations.

A detailed inspection of the salmon extract chromatogram obtained with column set E (Fig. 2A) revealed that both enantiomers of congeners 91, 95, 132, 149 and 174 eluted without interferences. None of the enantiomers of PCB 176 were detected. In the case of PCB 135, which was found to co-elute with PCB 139, the second eluted enantiomer appears to be higher than the first eluted one. In order to rule out a possible co-elution with PCB 139 and to assign the higher abundance to an enantio-enrichment, the salmon extract was also analysed by means of heart-cut multidimensional GC (heart-cut MDGC) with the instrumental conditions described elsewhere [10]. The analysis confirmed an enrichment of the second eluted enantiomer (data not shown), although the co-elution with PCB 139 when using GC × GC cannot be disregarded. On the other hand, the second eluted enantiomer of PCB 136, which eluted separate from potential interferences in the working standard solution, was partially affected by co-elution with a non-identified organochlorinated compound present in the extract. The same happened, but to a lesser extend, with the second eluted enantiomer of congener

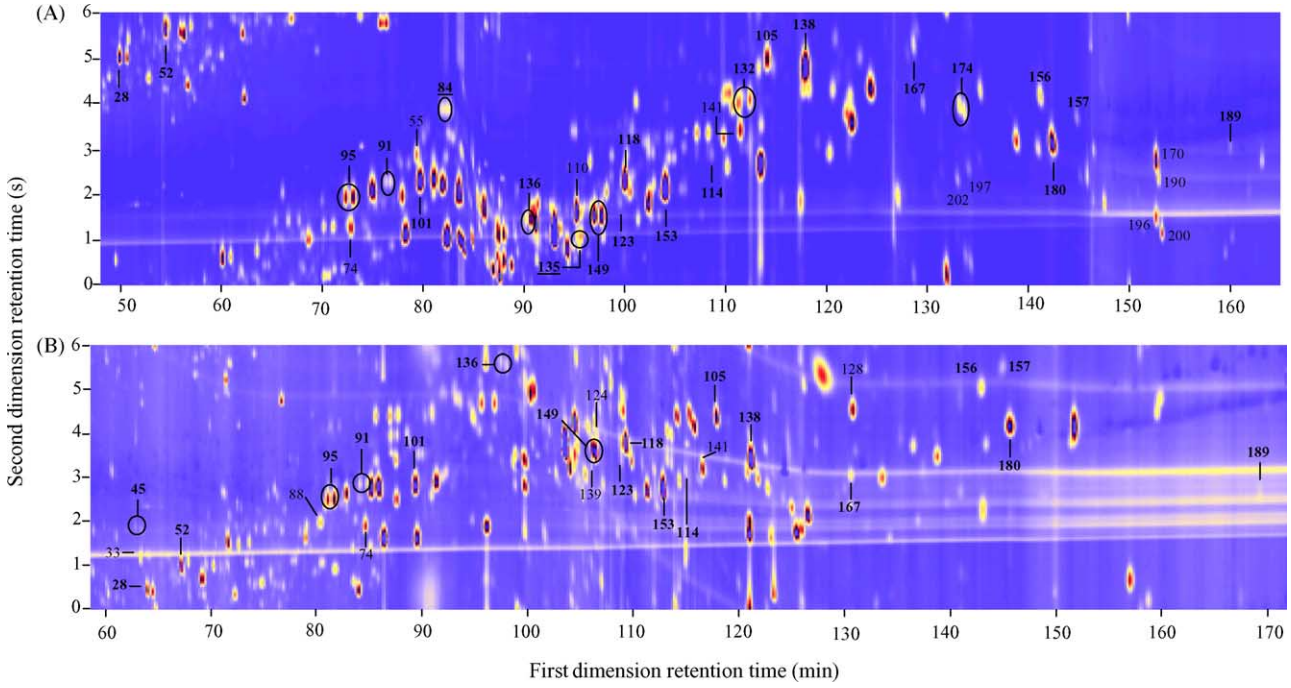

Fig. 2. GC × GC-μECD chromatograms of a salmon extract with (A) column combinations E (Chirasil-Dex × Supelcowax-10) and (B) column combination J (BGB-176 × Supelcowax-10).

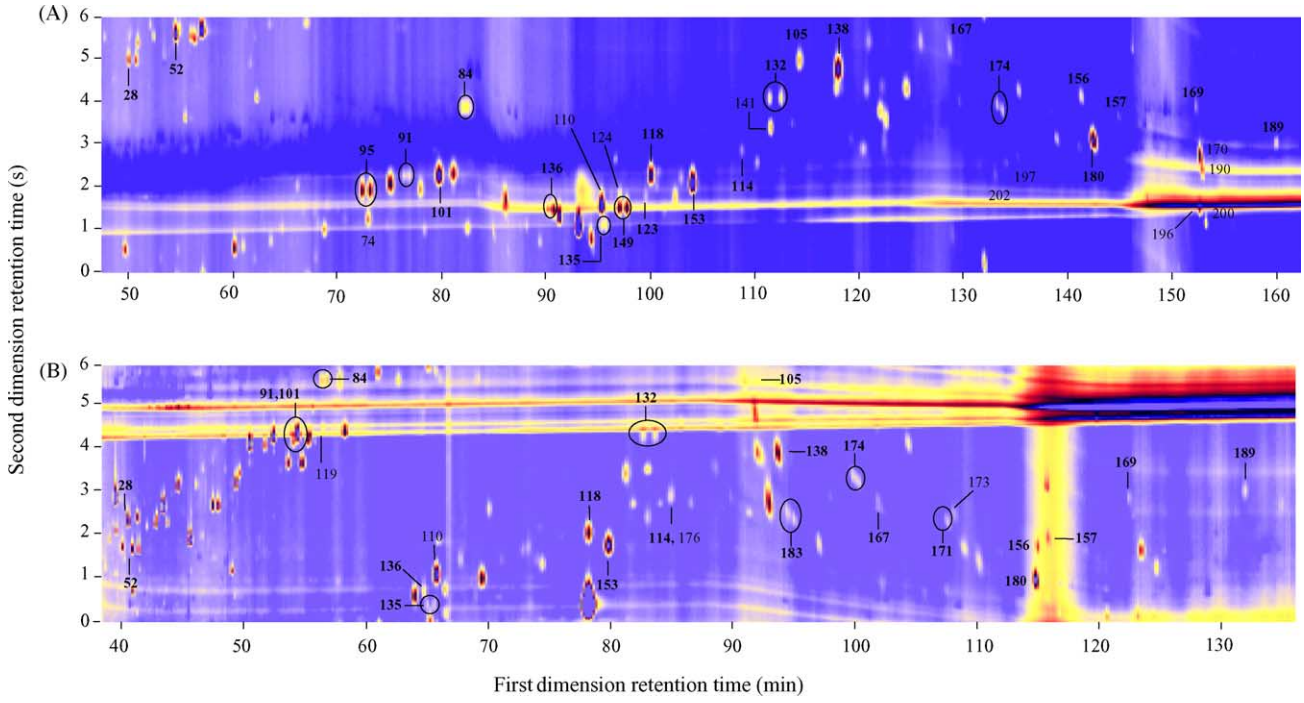

Fig. 3. GC × GC-μECD chromatograms of (A) a cheese extract with column combination E (Chirasil-Dex × Supelcowax-10) and (B) a milk extract with column combination I (BGB-172 × Supelcowax-10).

84. The use of less polar columns as second-dimension, such as BPX-50, did not solve these co-elutions (data not shown). Thereby, PCBs 95 and 135 showed an enantio-enrichment of the second eluted enantiomer (using Chirasil-Dex as first dimension column) in the salmon extract analysed. Deviations from the racemic composition have also been reported for these particular congeners in other aquatic species [1,14]. All seven indicators and the coplanar PCBs detected in the extract were identified free from interferences.

When column combination I was used for the analysis of the same salmon extract, results agree with those previously reported for the working standard solutions: co-elution problems were only detected for PCB 91 that co-eluted with congener 101. The remaining chiral PCBs investigated, i.e. 45, 84, 91, 132, 135, 171, 174 and 183, were satisfactorily resolved from other organochlorine analytes present in the extract. Nevertheless, it should be pointed out that, when required, the possible enantio-enrichment of PCB 91 in real samples could be determined with column set F (using HT-8 as second-dimension), as this combination provided a complete separation of both enantiomers from other interferences present in the extract. Enantio-enrichment was observed for PCB 171 and again for PCB 135, although in this later case, contrary to that observed in Chirasil-Dex, the enantio-enrichment was detected in the first eluted enantiomer. This result was also confirmed by heart-cut MDGC, and could be related to the inversion of the elution order of PCB 135 enantiomers on BGB-172. Several studies have reported reversal elution order on different columns and regarding different chiral pollutants [21]. The elution sequence of the individual enantiomers of this particular PCB on Chirasil-Dex has been already described by Haglund and Wiberg [22], but data on BGB-172 has not been reported until now. On what concerns the priority achiral congeners detected in the extract, all eluted well separated from interferences.

When the same salmon extract was analysed using column combination J (Fig. 2B), the target chiral PCBs, i.e. PCB 45, 91, 95, 136 and 149, could be determined without interferences although the second eluted enantiomer of PCB 91 was partially overlapped by an unidentified compound present in the sample extract. Enantiomers of congener 136 eluted, in this case, without the interferences that hampered its identification when using Chirasil-Dex as first-dimension. The previously observed enantio-enrichment of the second eluted enantiomer of PCB 95 when using Chirasil-Dex as first dimension column was confirmed with this column combination. No co-elution problems were detected for the seven indicator PCBs and those coplanar congeners in the extract, but in the case of congener 189, its low concentration together with its elution in the column-bleeding band made its determination somehow problematic. This drawback could be probably resolved by using lower elution temperatures but with the cost of much longer analytical times.

As expected, less contaminated samples yielded less complex chromatograms in which identification of the target congeners was easier. As an example, Fig. 3A shows the chromatogram corresponding to a cheese extract analysed using column combination E. None of the enantiomers of PCB 176

were detected in the extract. Again, and as previously reported for the salmon sample, the second enantiomer of congener 136 appeared hampered by an interferent. However, the coelution reported above for the second eluted enantiomer of PCB 84 in the salmon sample was not really evident in the cheese extract, probably because of the low levels of the coelutant in this particular extract. These results agree with those previously published for the determination of chiral PCBs in dairy products using Chirasil-Dex as first-dimension and a shorter Supelcowax-10 as second-dimension [10], in which co-elution problems were reported for PCB 136. Slight deviations from the racemic composition were only observed for PCBs 95 and 132 in this extract. As regards the coplanar and indicator PCBs, the non-*ortho* substituted CBs 77, 81 and 126, were not detected in the extract, while PCB 169 as well as the 8 mono-*ortho* congeners and the seven indicator PCBs were accurately identified. The column-bleeding band affected none of the enantiomers investigated because they were relatively abundant, that is the case with the enantiomers of PCB 149.

Fig. 3B shows a milk extract chromatogram with column combination I. Results agree with those described for the salmon sample when using the same column set. Of the target chiral PCBs, just PCB 91 co-eluted with congener 101. Enantiomers of PCB 45 showed no detectable levels. Meanwhile, PCBs 171 and 183 showed a clear enrichment of the second and first eluted enantiomer, respectively, a result that agree with those previously reported for milk and dairy products [10,23]. Regarding the target coplanar congeners, PCBs 77, 81, 123 and 126 were again not detected, and PCB 114 was overlapped by congener 176 as expected from the results with the standard solution. The low concentration of some of the target compounds, e.g. PCBs 105, 132 and 157, made their determination problematic as they elute in the column-bleeding band.

4. Conclusions

GC × GC-μECD using an enantioselective column as first-dimension, i.e. Chirasil-Dex, BGB-172 or BGB-176SE, and several non-enantioselective capillary columns as second-dimension, i.e. HT-8, BPX-50 or Supelcowax-10, allowed the separation of most of the chiral PCBs studied from other common organochlorinated compounds found in real food samples. The best results were obtained when the enantioselective columns were combined with Supelcowax-10 as second-dimension. These sets also allowed the determination in the same chromatographic run of the toxic coplanar PCBs and the seven indicator PCBs usually analysed for monitoring purposes. Although the determination of some of these compounds can eventually be hampered by the characteristic bleeding of these enantioselective columns, the satisfactory results obtained when analysing complex extracts illustrate the potential of GC × GC-μECD for PCB enantioenrichment studies. These results also point to this technique as a valuable alternative to other (more tedious) multidimensional chromatographic approaches that often require either extra fractionation of the bulk of PCBs before analysis or multiple injections of the extract on the GC system.

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