Journal of Chromatography, 607 (1992) 271–278
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 317

Simplex optimization of the analysis of polychlorinated biphenyls

Application to the resolution of a complex mixture of congeners of interest on a single gas chromatographic column

B. Jiménez*

Instituto de Química Orgánica General, CSIC, Juan de la Cierva 3, 28006 Madrid (Spain)

J. Tabera

Instituto de Fermentaciones Industriales, CSIC, Juan de la Cierva 3, 28006 Madrid (Spain)

L. M. Hernández and M. J. González

Instituto de Química Orgánica General, CSIC, Juan de la Cierva 3, 28006 Madrid (Spain)

ABSTRACT

The modified sequential simplex method was applied to optimize the oven temperature programming of a single-column gas chromatographic procedure developed to determine polychlorinated biphenyl congeners in a mixture of technical Aroclors. Both the overall resolution of fourteen congeners of interest and the time of analysis were taken into account in the optimization. Successful separations of some clusters usually unresolved with a single column have been obtained.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants. They appear as complex mixtures of theoretically 209 congeners which exhibit wide differences in their toxic and biological effects.

Several studies have indicated that the toxic nature of technical PCB mixtures may be associated with the presence of trace levels of particular toxic PCB congeners having four or more chlorine atoms at both *para* and *meta* positions in the biphenyl rings but no chlorine atoms in *ortho* positions [1]. Among the twenty possible coplanar PCB congeners, 77, 126 and 169 (numbers from Ballsmiter and Zell [2]) were found to be the most toxic [1,3–5]. Recent quantitative structure–activity relationship (QSAR) studies indicated that not only non-*ortho*-chlorine-substituted PCBs but also a few mono- and di-*ortho* analogues of coplanar PCBs possess comparable toxic potential [6,7].

The complex composition of PCB mixtures in environmental samples is characterized by a large number of peaks detected by electron-capture detection (ECD) after elution from a capillary gas chromatographic (GC) column. The evaluation of

0021-9673/92/$05.00

their constituents is not yet possible for all peaks [8]. For accurate data, each congener to be determined should appear as a single peak, well resolved from other compounds.

Our objective was the development of a chromatographic method with a single BP-5 column that will resolve as completely as possible the set of fourteen selected congeners (Table I) from different complex matrices. The total resolution of these isomers is very difficult and involves a great experimental effort. In that situation, it is of great importance to apply a suitable optimization procedure for the simultaneous handling of experimental variables. The sequential simplex method [9] has been broadly recognized as a very efficient empirical optimization procedure [10,11] which can attain an optimum in a reduced number of experimental runs. This method has been successfully used in chromatographic research [12,13].

As a first step to attain our objective, in this work we optimized the chromatographic separation of the fourteen above-mentioned PCB congeners in a mixture of Aroclors of different chlorination grade by means of the modified sequential simplex method [14].

TABLE I

SET OF PCB CONGENERS SELECTED FOR ANALYSIS

Congener number[a]	Structure	Closely eluting congeners[b]
101	2,2',4,5,5'	90 (5)
77	3,3',4,4'	101 (5)
151	2,2',3,5,5',6	82 (5)
118	2,3',4,4',5	123 (5), 149 (6)
153	2,2',4,4',5,5'	105 (5), 132 (6)
105	2,3,3',4,4'	153 (5), 132 (6)
138	2,2',3,4,4',5'	160 (6), 158 (6)
126	3,3',4,4',5	129 (6), 178 (7)
167	2,3',4,4',5,5'	– – –
156	2,3,3',4,4',5	202 (8), 171 (7)
180	2,2',3,4,4',5,5'	– – –
169	3,3',4,4',5,5'	– – –
170	2,2',3,3',4,4',5	190 (7)
194	2,2',3,3',4,4',5,5'	– – –

[a] According to ref. 2.

[b] Chlorine numbers are given in parentheses.

EXPERIMENTAL

PCB samples

The PCB congeners for this study were selected on the bases of toxicity and/or abundance in environmental samples. A 1:1:1:1 (v/v) mixture of Aroclor 1242, 1248, 1254 and 1260 was chosen because their great similarity to environmental samples. In this mixture we identified all the congeners shown in Table I except 126 and 169 which were added in an equal but different mass than the others. All fourteen congeners were identified by comparison of their retention times with those of standards.

Capillary gas chromatography

All measurements were carried out on a Perkin-Elmer Model 8600 gas chromatograph equipped with a nickel-63 electron-capture detector. A fused-silica column (50 m × 0.22 mm I.D.) with a 0.25-μm film thickness of the chemically bonded phase BP-5 (5% phenyl–95% polymethylsiloxane) (SGE, Victoria, Australia) was used. The carrier gas (nitrogen) flow-rate was 40 ml/min. The oven temperature programme was subjected to optimization. The detector temperature was 300°C and the injector temperature (splitless mode) was 280°C.

Selection of variables

The temperature programming consisted of three stages. Based on previous experience, we considered the first, second and third oven temperatures and the second temperature gradient as the four variables to work with. In order to select the most influential of them to include in the optimization study, a fractional factorial 2^{4-1} experimental design [15] was performed as explained under Results and Discussion. Estimates of the main effect of each variable were calculated according to the equation

$$E(i) = \frac{1}{4}\sum_{j} X_{ij} Y_j \qquad (1)$$

where X_{ij} is the coded level (-1 for the low level and 1 for the high level) of the variable i in run j, and Y_j is the response value of run j.

The response function

The effect of modifying the experimental condi-

tions on the overall performance of the analytical method was evaluated in terms of the differences between the retention times of each chromatographic peak achievable in each analytical run. The time spent in the analysis was also taken into account. The response function was designed as a summation of inverse differences in retention times in order to magnify the influence of low values of those differences and so penalize bad resolutions. The inverse of analysis time is affected by a negative sign, so a longer analysis time leads to higher response values. The optimization of the chromatographic method is then equivalent to the minimization of the response function.

Several chromatographic response functions (CRFs) developed to assign a numerical value to the quality of each chromatogram, as is needed in empirical optimization studies, have been reported [16–18]. Most of them are based on criteria such as the peak-to-valley ratio, fractional peak overlap, separation factor or resolution, which require high-performance data treatment. When a sufficiently complex mixture is analysed, this data elaboration cannot be performed manually. Although we considered some of these CRFs, the complexity of our PCB mixture and the limitations of our GC equipment led us to formulate the response function (eqn. 2) in terms of retention time, a characteristic well measured in our chromatograms.

As we were interested in the fourteen PCB congeners shown in Table I, the summation of the response function was extended to fourteen terms. Each of these terms corresponds to the chromatographic peak of a selected PCB congener, and is the sum of two differences in retention time: that with the preceding peak and that with the following peak in the chromatogram. Some separations were more desirable than others owing to known special difficulties in achieving them or to special significance of the congener involved (toxicity, abundance, etc.), therefore the influence of each difference in retention time over the response function was modulated by means of weighing factors. Another weighting factor was applied to the term accounting for analysis time in order to equilibrate the reward for high resolutions with the penalty for long analysis time. Our scope was to consider more valuable the attainment of new peaks or better resolutions than shortening of the analysis time. Therefore, the weighing factor of the term accounting for the time of analysis was fixed in order to affect only the ranking between chromatograms with not very different resolution performances. The response function designed for this study is

$$FR = \sum_{i=1}^{14} \left(\frac{p_{i1}}{t_{r_i} - t_{r_{i-1}}} + \frac{p_{i2}}{t_{r_{i+1}} - t_{r_i}} \right) - \frac{q}{t_f} \qquad (2)$$

where t_{r_i}, $t_{r_{i-1}}$ and $t_{r_{i+1}}$ are the corrected retention times of the ith selected peak, its preceding peak and its following peak, respectively, t_f is the final analysis time, p_{i1} (p_{i2}) are the weighing factors assigned to the differences in retention time between each selected peak and the preceding (following) peak, respectively, and q is the weighing factor assigned to the final analysis time. The value of q was fixed at 6000, as will be explained later. The weighing factors p_{i1}, p_{i2} used in this work are shown in Table II.

Evolution of the response function

Throughout the optimization process, new peaks appeared in some chromatograms. When these peaks were contiguous with the fourteen selected ones, they could emanate from the PCBs of interest and we considered them as previously co-eluting with these PCBs. Once such a peak had appeared it could no longer be ignored, and any other chromatogram without this peak must be evaluated as not properly resolving the selected congener. The values $t_{r_{i-1}}$ and/or $t_{r_{i+1}}$ in eqn. 2 corresponded to the new peak/peaks and, if these do not exist, the differences in retention time, $t_{r_i} - t_{r_{i-1}}$ and/or $t_{r_{i+1}} - t_{r_i}$, would be equal to zero[a]. Therefore, the occurrence of new peaks contiguous with any of those of the PCB congeners of interest forced the recalculation of the response value of old chromatograms in order to make good comparisons. Therefore even though the expression for the response function did not vary, the terms included in it did vary. As far as we know, no simplex optimizations with such an evolutionary response function have been reported previously. This adaptation of the

[a] In these cases, to avoid "division by zero error" when running the calculation program, a very small value of 0.01 was assigned to these differences.

TABLE II

WEIGHING FACTORS ASSIGNED TO THE PEAKS CONTIGUOUS WITH THE SELECTED PCB CONGENERS

PCB congener	p_{i1}	p_{i2}
101	1	1
77	3	1
151	1	1
118	1	4
153	1	2
105	2	1
138	1	3
126	1	3
167	2	1
156	4	1
180	3	3
169	1	1
170	1	2
194	1	1

response function to new knowledge obtained from experimentation could be valuable for other workers.

Simplex optimization

The sequential simplex method [9] begins with a patterned set of experiments in all the variables of interest. The pattern is an equilateral triangle in two variables, a regular tetrahedron in three variables or a simplex (*i.e.*, a regular multi-dimensional figure) in four or more variables. The effects on the performance of the process of changes in operating variables are measured according to a previously defined criterion or response function; from the results, the directions in which further changes should be made to obtain an improvement in process performance are inferred. The resulting new values of the variables are then tested and the procedure is repeated until no further improvement can be achieved.

The initial experimental design was established according to Spendley *et al.* [19]. Physical values of factors were calculated from their mathematical co-ordinates by using

$$x_{\text{phys}} = x_0 + x_{\text{math}} \cdot SOC \qquad (3)$$

where x_{phys} is the physical value of the variable x, x_{math} the corresponding mathematical coordinate, x_0 its base level (starting physical value) and SOC the step of change of each variable.

The initial and successive simplex were moved in the directions given by the rules of movement of the modified simplex method [14] and the corresponding responses were subsequently evaluated. In all instances, two replicates of each analysis were carried out. The coordinates of each new vertex were calculated according to the expression

$$V_i^* = C + \alpha(C - V_i) \qquad (4)$$

where V_i^* is the new vertex, C the centroid of the retained vertices in the movement, V_i the rejected vertex and α a factor with different values depending on whether a reflection ($\alpha = 1$), an expansion ($\alpha > 1$) or a contraction ($\alpha < 1$) was performed.

It should be pointed out that the self-directing nature of this optimization method makes possible a boundary violation (*i.e.*, a movement outside the feasible experimental conditions); then, the corresponding vertex must be rejected without experimentation and so the simplex is subsequently forced to move back inside the boundaries by applying an $\alpha = -0.5$ factor [20].

RESULTS AND DISCUSSION

Table III summarizes the experiments carried out and the results obtained in the fractional factorial 2^{4-1} experimental design. The experimental runs

TABLE III

EXPERIMENTAL RUNS, RESULTS AND ESTIMATES OF MAIN EFFECTS FOR THE 2^{4-1} FRACTIONAL FACTORIAL DESIGN

Run	*T*1 (°C)	*T*2 (°C)	*R*2 (°C/min)	*T*3 (°C)	Response
1	80	160	1	230	28.00
2	120	160	1	270	23.00
3	80	200	1	270	30.10
4	120	200	1	230	33.00
5	80	160	3	270	735.60
6	120	160	3	230	36.10
7	80	200	3	230	320.80
8	120	200	3	270	1007.30

Main effects:
$E(T1) = -4$; $E(T2) = 142$; $E(R2) = 497$; $E(T3) = 345$

TABLE IV

VARIABLES INCLUDED IN THE SIMPLEX OPTIMIZATION

Variable	Base level	Step of change
2nd oven temperature, *T*2 (°C)	160	5
2nd temperature gradient, *R*2 (°C/min)	1	1
3rd oven temperature, *T*3 (°C)	270	5

were carried out in randomized order. Each response value was the average of two replicates. Because the 2^{4-1} design is of resolution IV, its confounding structure leads to estimation of first-order (main) effects together with third-order (3rd interactions) effects. Hence the estimation of main effects is of high precision by assuming, as is usually done, that the effects of interactions of order higher than 2 are negligible. The response function used was eqn. 2, which was tested against these first experimental results. The value of weighing factor q was varied in the testing, and finally adjusted to 6000 in order to obtain a ranking of response values as

TABLE V

EXPERIMENTAL RUNS AND RESULTS FOR THE SIMPLEX OPTIMIZATION

Vertex No.	Simplex No.	Retained vertices	Experimental variables levels			Response[a]			
			*T*2 (°C)	*R*2 (°C/min)	*T*3 (°C)	Y_1	Y_2	Y_3	Y_4
1	1	–	160	1.0	270	26.50	622	721	1117
2	1	–	165	1.2	271	34.25	629	728	1123
3	1	–	161	1.9	271	63.49	655	754	1145
4	1	–	161	1.2	275	33.51	628	727	1122
5	2	1,2,4	163	0.4	273		20	119	518[b]
6[c]	2	1,2,4	163	−0.4[d]	273		–	–	–
7	3	1,4,5	158	0.5	274		320	420	818[b]
8[e]	4	1,5,7	159	0.01	270			–	–
9	4	1,5,7	161	0.9	273			621	1017
10	5	5,7,9	161	0.2	277				304[b]
11[c]	5	5,7,9	161	−0.2[d]	280				–
12[e]	6	5,7,10	160	−0.2	275				–
13	6	5,7,10	161	0.6	274				
14[e]	7	5,7,10	160	0.07	275				–
15	7	5,7,10	161	0.5	274				611
16	8	5,10,15	165	0.2	275				305[b]
17[e]	9	5,10,16	165	0.02	276				–
18	9	5,10,16	162	0.4	275				413
19	10	10,16,18	162	0.2	278				305
20[e]	11	10,16,19	163	0.004	279				–
21	11	10,16,19	162	0.30	276				105[b]
22	12	10,16,21	163	0.30	273				110
23	13	10,21,22	159	0.30	275				109
24	14	21,22,23	162	0.40	273				411
25[f]	14	21,22,23	161	0.30	276				109

[a] Successive responses originating from the appearance of new peaks contiguous with the selected ones in vertices Nos. 5, 9 and 10.
[b] Value checked in the $(k + 1)$th simplex from the first occurrence of the vertex.
[c] Obtained from expansion $\alpha = 2$.
[d] Vertex not better than preceding $\alpha = 1$ vertex. Rule of expansion failure rather than rule of boundary violation applied. Vertex excluded from the new simplex.
[e] Boundary violation. Impossible to apply temperature gradients below 0.1°C/min. Next vertex obtained with $\alpha = -0.5$.
[f] Obtained from contraction $\alpha = -0.5$.

coincident as possible with that obtained from a careful inspection of chromatograms. The estimated main effects are also included in Table III. From their values, the variables $T2$, $R2$ and $T3$ were selected to be simplex optimized. Base levels and steps of change of these variables are shown in Table IV. The first oven temperature, $T1$, was set at 120°C. Previously, the first temperature gradient, $R1$, was set to 20°C/min.

Table V summarizes the sets of experimental values tested throughout the optimization procedure. The starting point (vertex No. 1) is that of the best results in the factorial design. Values obtained for the response function (eqn. 2) are included in the response columns of Table V. As explained previously, these response values were recalculated each time a new peak contiguous with the fourteen selected peaks appeared. The decisions concerning the simplex movements were based, at any time, on the actual values of response; however, no discrepancies have been detected with the movements that would be performed if definitive response (Y_4) were available from the starting point. This agreement in ranking has made easy the optimization progress with evolutionary response function. If a change in the response used would cause a change in the order of desirability of vertices involved in already performed movements, some unfavourable situations could occur. The optimum could be attained, but some erratic movements would be carried out with the older response functions and a less economical search would be made.

The optimization study was initiated by performing the first four experiments defined in Table V, which constitute the initial simplex. The assessment of the values obtained for the response function in each analysis allows the worst vertex (No. 3) to be rejected. Then, a new simplex was formed with the retained vertices and a new one (No. 5) resulting from the mirror image ($\alpha = 1$) of the rejected vertex. As the response at vertex 5 is better than the best of the preceding simplex (vertex No. 1), an expansion is indicated ($\alpha = 2$), and the new simplex is formed with the recently obtained vertex No. 6 instead of No. 5. In vertex No. 8 a boundaryviolation occurred in $R2$ (no values lower than 0.1°C/min can be used); then the response at vertex No. 8 was considered as the worst one without experimentation, and a negative contraction ($\alpha = -0.5$) was carried out to obtain vertex No. 9. The procedure must be repeated to move from one simplex to another by rejecting the worst observation and by selecting an adequate α value.

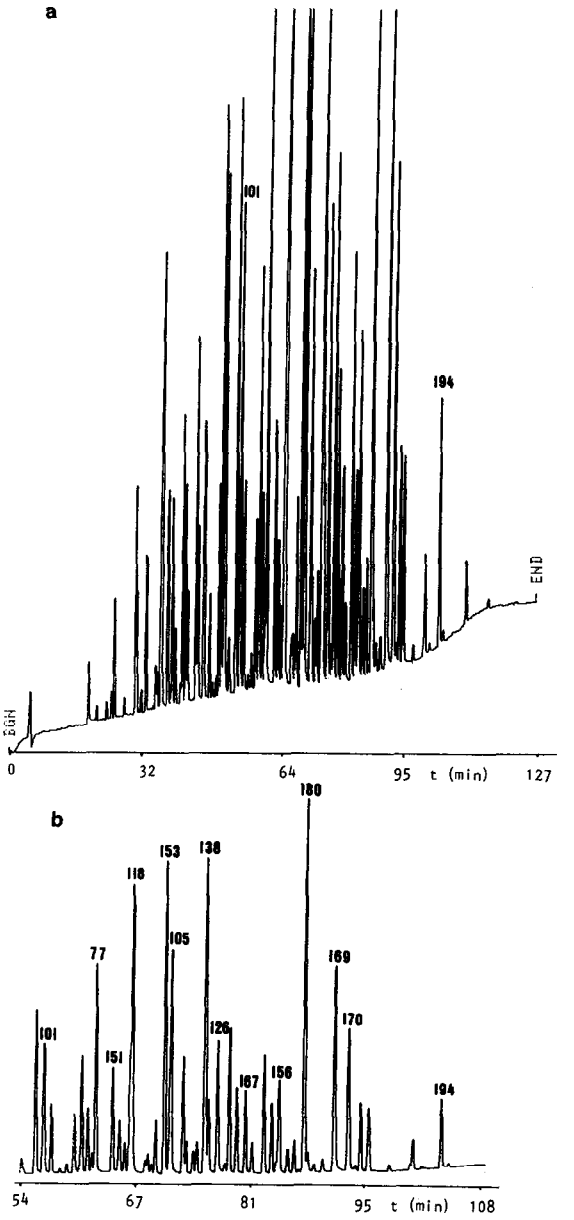

Fig. 1. High-resolution gas chromatogram of a mixture of Aroclors with PCBs 126 and 169 added. Analysis conditions of the simplex starting point (vertex No. 1). (a) Whole chromatogram. (b) Detail of the 101–194 zone.

Searching was stopped after vertex 25 because the responses in all the vertices of the last simplex (21, 22, 23 and 25) and their experimental conditions were very similar, thus indicating that a stable region had been reached and no further improvement could be expected. The calculation of a tentative new movement leads to a set of experimental values very close to that of the above mentioned vertices, which confirms the assumption of reaching a stable region. Hence, we can accept that an optimum has been attained at vertex No. 21. The value of the temperature gradient for this optimum was 0.3°C/min, which gave an analysis time of 240 min.

As far as the resolution of the PCB congeners is concerned, the results can be summarized as follows:

(a) Isomers where the peak elutes as a single peak (180, 169 and 194). Congener 180 appears in the chromatogram (Fig. 2) as a peak well resolved from the adjacent ones. This peak can be unequivocally identified in complex chromatograms because it is a characteristic large peak in commercial mixtures and environmental samples [8]. Peak 169 provides useful information on the chromatographic determination of corresponding congeners. It should be noted that this isomer was added to the mixture of Aroclors because of their toxicological interest. Despite its low ECD response, isomer 194 has been determined without ambiguity problems.

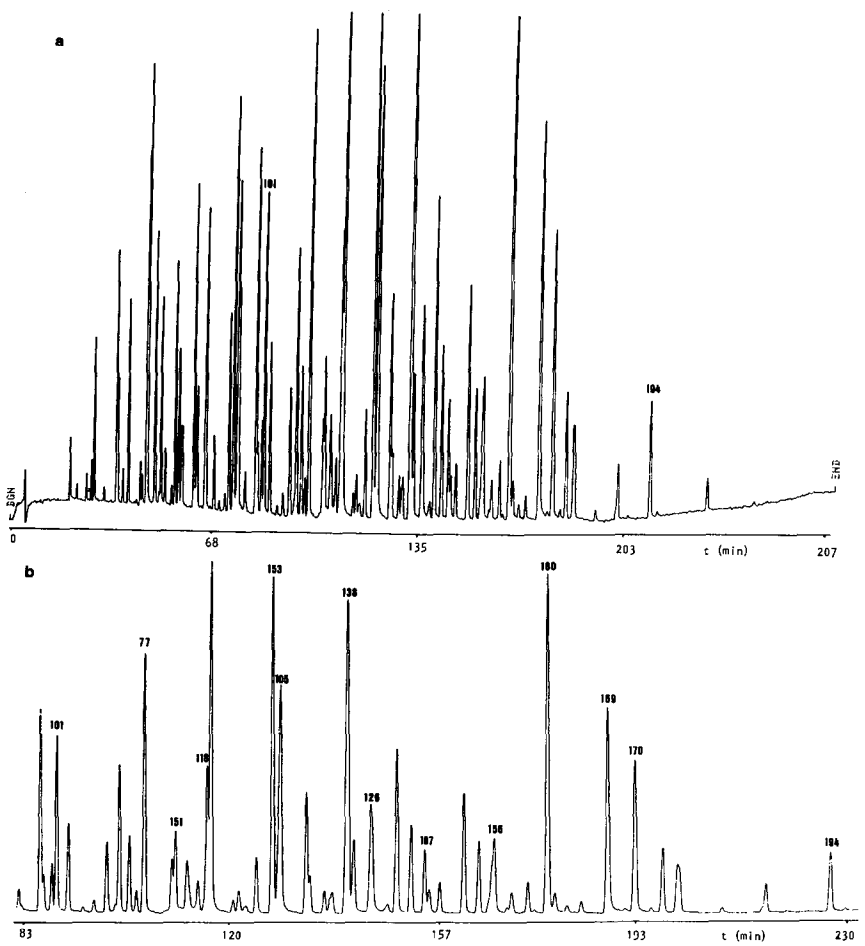

Fig. 2. High-resolution gas chromatogram of a mixture of Aroclors with PCBs 126 and 169 added. Analysis conditions of the simplex optimum (vertex No. 21). (a) Whole chromatogram. (b) Detail of the 101–194 zone.

(b) Isomers co-eluting that were resolved in this work as a single peak (see Figs. 1b and 2b). The peak corresponding to congener 101 that co-elutes with 90 [8] was split into two peaks after the optimization of the chromatographic conditions. The same splitting for the peak preceding 101 can be observed. On the left of the peak corresponding to congener 151 a new peak has appeared. On the basis that 151 is known as a congener co-eluting with isomer 82, the new peak could be tentatively identified as the congener 82. However, conclusions on the compositions of two new peaks mentioned above require the availability of Nos. 90 and 82 as reference compounds. Very good resolution was attained for the cluster 153/105 that usually appear in the literature as a set unresolved with columns similar to BP-5. The isomer 77, with four chlorine atoms, co-elutes with the pentachlorobiphenyl 110. This problem has been successfully resolved using GC–mass spectrometry (GC–MS) in the selected ion monitoring. MS allows an unambiguous identification of the two isomers.

(c) Isomers with very closely eluting congeners. Peak 118, known to co-elute with 123 and 149, is only split into two peaks (Figs. 1b and 2b). The situation is similar for the peak corresponding to congener 138, which co-elutes with 160 and 158, and for which we have obtained only two peaks. From Fig. 2b it could be observed that congener 156 has a marked trend to split into two peaks, but at present the situation remains unresolved for the cluster 156/202/171 [21]. Fortunately, this situation could be easily resolved by GC–MS, as we have observed for isomer 77, because the congeners have different chlorine numbers. In spite of the fact that isomer 126 co-elutes with 129 and 178, the peak in this work consists of 126 only, because this was added individually to the mixture. A similar situation occurs with congener 169. Hence, from this work, information could be obtained on the behaviour of two toxic coplanar congeners, 126 and 169, when analysing complex samples. The peak corresponding to congener 170, which co-elutes with 190, could be considered as mainly due to 170 because of its larger proportion in commercial Aroclors.

Finally, an interesting observation can be derived for congener 167 from this work. From Fig. 2b, it can be seen that the peak that appeared as a single one in the initial chromatogram (Fig. 1b) has been split into two peaks after the optimization of the chromatographic conditions. We have not found in the literature any references to co-elution for this congener. Hence confirmation by MS and using another column with different polarity is required for this new peak, in order to obtain a deeper knowledge of the behaviour of this toxic coplanar congener.

REFERENCES

1 S. Safe, *CRC Crit. Rev. Toxicol.*, 13 (1984) 319.
2 K. Ballsmiter and M. Zell, *Fresenius' Anal. Chem.*, 302 (1980) 20.
3 H. Yoshimura, I. Wada, N. Koga, K. Nagata, Y. Yamauchi, S. Yoshihara and O. Kamata, *Fukuoka Acta Med.*, 72 (1981) 149.
4 N. Kannan, S. Tanabe, M. Ono and R. Tatsukawa, *Arch. Environ. Contam. Toxicol.*, 18 (1989) 850.
5 S. Tanabe, N. Kannan, T. Wakimoto and R. Tatsukawa, *Int. J. Environ. Anal. Chem.*, 29 (1987) 199.
6 A. Parkinson, S. Safe, L. W. Robertson, P. E. Thomas, D. E. Ryan, L. M. Reik and W. Levin, *J. Biol. Chem.*, 258 (1983) 5967.
7 S. Safe, S. Bandiera, T. Sawyer, L. Robertson, L. Safe, A. Parkinson, P. E. Thomas, D. E. Ryan, L. M. Reik, W. Levin, M. Denomme and T. Fujita, *Environ. Health Perspect.*, 60 (1985) 47.
8 J. C. Duinker, D. E. Schultz and G. Petrick, *Mar. Pollut. Bull.*, 19 (1988) 19.
9 C. Hendrix, *CHEMTECH*, August (1980) 488.
10 J. C. Berridge, *Anal. Chim. Acta*, 191 (1986) 243.
11 R. J. Fisher, *Food Technol.*, 43 (1989) 90.
12 J. Tabera, G. Reglero, M. Herraiz and G. P. Blanch, *J. High Resolut. Chromatogr.*, 14 (1991) 392.
13 J. Tabera, B. Jiménez, L. M. Hernández and M. J. González, *J. Chromatogr.*, 557 (1991) 481.
14 J. A. Nelder and R. Mead, *Comput. J.*, 7 (1965) 308.
15 G. E. P. Box and N. R. Draper, *Empirical Model-Building and Response Surfaces*, Wiley, New York, 1987, pp. 143–167.
16 H. J. G. Debets, B. L. Bajema and D. A. Doornbos, *Anal. Chim. Acta*, 151 (1983) 131.
17 P. T. Schoenmakers, *Optimization of Chromatographic Selectivity*, Elsevier, Amsterdam, 1986, pp. 119–169.
18 J. A. Crow and J. P. Foley, *Anal. Chem.*, 62 (1990) 378.
19 W. Spendley, G. R. Hext and F. R. Himsworth, *Technometrics*, 4 (1962) 441.
20 S. L. Morgan and S. N. Deming, *Anal. Chem.*, 46 (1974) 1170.
21 D. E. Schultz, G. Petrick and J. C. Duinker, *Environ. Sci. Technol.*, 23 (1989) 852.