Optimization of the gas chromatographic analysis of a standard mixture of polychlorodibenzo-*p*-dioxins and polychlorodibenzofurans

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ABSTRACT

The separation of fifteen standard isomers of polychlorodibenzofurans (PCDFs) and polychlorodibenzo-*p*-dioxins (PCDDs) with a 2,3,7,8-substitution pattern by high-performance gas chromatography using capillary columns with bonded phases was studied in order to obtain better resolutions than those usually reported. The modified simplex method was used to improve the overall separation achievable with the analytical procedure by optimizing the experimental conditions that affect chromatographic resolution.

INTRODUCTION

Polychlorinated dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) are two series of tricyclic, planar, aromatic compounds. Each series consists of a number of chloro homologues (mono- to octachlorinated) with a variable number of isomers for each group (135 PCDFs and 75 PCDDs) [1].

Because of the biological activity associated with small amounts of these compounds, specially the 2,3,7,8-substituted isomers, acceptable analytical methods have to be capable of providing qualitative identification and accurate quantification at low parts per 10^{12} (pg/g) levels in different matrices [2–4].

High-performance gas chromatography with capillary columns provides effective separations of many PCDF and PCDD isomers and a number of stationary phases have been used [5]. Optimization of the chromatographic separations requires great experimental effort as the number of variables involved increases. In such situations, it is essential to apply an optimization method for the simultaneous handling of several experimental variables in order to resolve as much as possible the chloro homologues and the isomers of PCDDs and PCDFs. The sequential simplex method [6] begins with a patterned set of experiments involving 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 sequential simplex method has been broadly recognized as a very efficient empirical optimization method [7,8] which can attain an optimum in a minimum number of experimental runs.

   The objective of this work was the development of a GC method for analysing different complex matrices (biological materials, fly ash, etc.), resolving as much as possible the isomers of PCDDs and PCDFs. As a first step we used a mixture of 2,3,7,8-substituted isomers of PCDDs and PCDFs (tetra- to octadioxins and -furans). The optimization of the experimental conditions affecting the GC separation was performed by means of the modified simplex method.

EXPERIMENTAL

   All measurements were carried out on a Perkin-Elmer Model 8310B gas chromatograph equipped with a [43]Ni electron-capture detector. A bonded-phase BP-5 fused-silica column (50 m × 0.22 mm I.D.) with a 0.25-μm film thickness (SGE, Victoria, Australia) was used. The BP-5 stationary phase is polyphenylsiloxane–polymethylsiloxane (5:95). The oven temperature programming was the object of optimization and Table I shows the variables included in the study. The detector temperature was 300°C and the injector temperature (splitless mode) was 280°C.

   The PCDDs and PCDFs standards used were all purchased from Cambridge Isotope Labs. (Woburn, MA, U.S.A.) and Wellington Labs. (Ontario, Canada). Table II shows the mixture of 2,3,7,8-substituted isomers of PCDDs and PCDFs (tetra- to octachlorinated) used at a 1 ng/μl concentration of each in benzene.

Response function
   The effect of modifying the experimental conditions on the overall performance of the method was evaluated in terms of the differences between the retention times of each chromatographic peak achievable in each analytical run. We are mainly interested in separations between chloro homologue groups, and therefore the

TABLE I

VARIABLES INCLUDED IN THE OPTIMIZATION STUDY, EXPERIMENTAL RANGES AND STARTING VALUES (BASE LEVEL)

Variable	Min.	Max.	Base
First oven temperature, T1 (°C)	90	150	100
First temperature gradient, G1 (°C/min)	15	30	20
Second oven temperature, T2 (°C)	160	200	180
Second temperature gradient, G2 (°C/min)	1	3	2
Third oven temperature, T3 (°C)	240	260	45

TABLE II

PCDD AND PCDF ISOMERS IN THE MIXTURE USED FOR OPTIMIZATION

Tetra-	Penta- (Pe)	Hexa- (Hx)	Hepta- (Hp)	Octa- (O)
2,3,7,8-TCDF	1,2,3,7,8-PeCDF	1,2,3,4,7,8-HxCDF	1,2,3,4,6,7,8-HpCDD	OCDD
2,3,7,8-TCDD	2,3,4,7,8-PeCDF	1,2,3,6,7,8-HxCDF		OCDF
	1,2,3,7,8-PeCDD	1,2,3,7,8,9-HxCDF		
		1,2,3,4,7,8-HxCDD		
		1,2,3,6,7,8-HxCDD		
		1,2,3,7,8,9-HxCDD		
		2,3,4,6,7,8-HxCDF		

differences in retention times between the first peak of a group and the last peak of the preceding group were weighted with a factor of 2 for the hepta–hexa and octa–hepta pairs and with a factor of 3 for the penta–tetra and hexa–penta pairs. Hence the response function can be expressed as

$$Y = \sum f(t_{Ri+1} - t_{Ri}) \tag{1}$$

where t_{Ri} is the retention time of the ith chromatographic peak and f is the weighting factor, equal to 1 for the peaks in the same group and to 2 or 3 for the extreme peaks in the above-mentioned groups. As is obvious, the objective was to maximize the value of the response function.

Optimization method
        The variables subjected to the optimization procedure, their experimental ranges and starting values are shown in Table I.
        The initial experimental design was established according to Spendley *et al.* [9]. Physical values of factors were calculated from their mathematical coordinates by applying

$$x_{phys} = x_0 + x_{math} \cdot \frac{x_2 - x_1}{s} \tag{2}$$

where x_{phys} is the physical value of the variable x, x_{math} is the corresponding mathematical coordinate, x_0 is its base level (starting physical value), x_1 and x_2 are the lower and upper limits of the range studied, respectively, and s is the number of mathematical units in which the range has been divided.
        The initial simplex was moved in the directions given by the rules of movement of the modified simplex method [10] and the response function was subsequently evaluated. In this way, different sets of variables were tested until no further improvement was achievable. In all instances, two replicates of each analysis were carried out. The coordinates of a new vertex were calculated according to the expression

$$V_i^* = C + \alpha (C - V_i) \tag{3}$$

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 the optimization method makes possible a boundary violation (*i.e.*, a movement outside the experimental range initially established). In such a case, the corresponding vertex must be rejected before experimentation and the simplex forced to move back inside the boundaries by applying a factor $\alpha = -0.5$ [11].

RESULTS AND DISCUSSION

Table III summarizes the sets of values tested for optimizing the peak resolution in the chromatogram. The base level of each variable (*i.e.*, the starting point of the optimization study) is also included (vertex No. 1). The *s* value was set at 3. The values attained for the response function (eqn. 1) in each chromatogram are included in the response column in Table III.

The optimization study was initiated by performing at random the first seven

TABLE III

EXPERIMENTAL RUNS AND RESULTS FOR THE SIMPLEX OPTIMIZATION OF THE GC ANALYSIS OF A MIXTURE OF PCDDs AND PCDFs

Vertex No.	Simplex No.	Retained vertices	Experimental variable levels						Response, Y (min)
			T1 (°C)	G1 (°C/min)	T2 (°C)	G2 (°C/min)	T3 (°C)	IST1 (min)	
1	1		100	20.0	180	2.0	250	45.0	117.98
2	1		118	21.0	183	2.1	251	47.0	116.56
3	1		104	24.5	183	2.1	251	47.0	116.50
4	1		104	21.0	192	2.1	251	47.0	116.42
5	1		104	21.0	183	2.6	251	47.0	113.79
6	1		104	21.0	183	2.1	256	47.0	108.84
7	1		104	21.0	183	2.1	251	54.0	120.94
8	2	1, 2, 3, 4, 5, 7	107	21.8	185	2.2	246	48.6	157.93
9	2	1, 2, 3, 4, 5, 7	109	22.2	186	2.3	241	49.5	165.45
10	3	1, 2, 3, 4, 7, 9	109	22.2	186	1.7	247	49.5	162.22
11	4	1, 2, 3, 7, 9, 10	111	22.7	175	2.0	247	50.4	162.37
12	5	1, 2, 7, 9. 10, 11	113	18.5	181	1.9	244	51.5	167.06
13	5	1, 2, 7, 9. 10, 11	118	15.5	181	1.8	241	53.8	174.91
14	6	1, 7, 9, 10, 11, 13	99	20.3	181	1.8	241	53.8	174.67
15	7	7, 9, 10, 11, 13, 14	116	21.3	183	1.9	239	58.6	182.39
16	7	7, 9, 10, 11, 13, 14	124	22.0	185	1.9	240	65.5	198.69
17	8	9, 10, 11, 13, 14, 16	119	20.1	182	1.7	232	53.5	—
18	9	9, 10, 11, 13, 14, 16	108	20.9	182	2.0	247	53.9	164.53
19	10	9, 11, 13, 14, 16, 18	114	20.9	182	2.0	246	53.8	—
20	11	9, 11, 13, 14, 16, 18	110	21.4	184	1.8	245	52.0	166.42
21	12	9, 13, 14, 16, 18, 20	112	18.1	192	1.9	237	59.1	—
22	13	9, 13, 14, 16, 18, 20	111	21.5	179	2.0	244	52.5	167.11
23	14	9, 13, 14, 16, 20, 22	116	20.1	182	1.8	235	55.1	—
24	15	9, 13, 14, 16, 20, 22	110	20.7	182	2.0	244	54.2	169.20

experiments defined in Table III, which constitute the initial simplex. The assessment of the values obtained for the response function in each analysis allows the worst vertex to be rejected (No. 6). A new simplex was then formed with the retained vertices and a new one resulting from the mirror image of the rejected vertex ($\alpha = 1$). The procedure must be repeated to move from one simplex into another by rejecting the worst observation and by utilizing an adequate α value.

It should be noted that vertices Nos. 9, 13 and 16 were obtained from eqn. 3 with $\alpha = 2$ because their preceding $\alpha = 1$ vertices (Nos. 8, 12 and 15, respectively) were the best in their simplexes and then an expansion is indicated. Vertices Nos. 9, 13 and 16 achieve higher response values than the $\alpha = 1$ vertices, and therefore were maintained, rather than vertices Nos. 8, 12 and 15, to form the next simplex. In vertices Nos. 17, 19, 21 and 23 boundary violations in variable T3 occur, and therefore they were rejected without previous experimentation and a contraction was performed in the subsequent
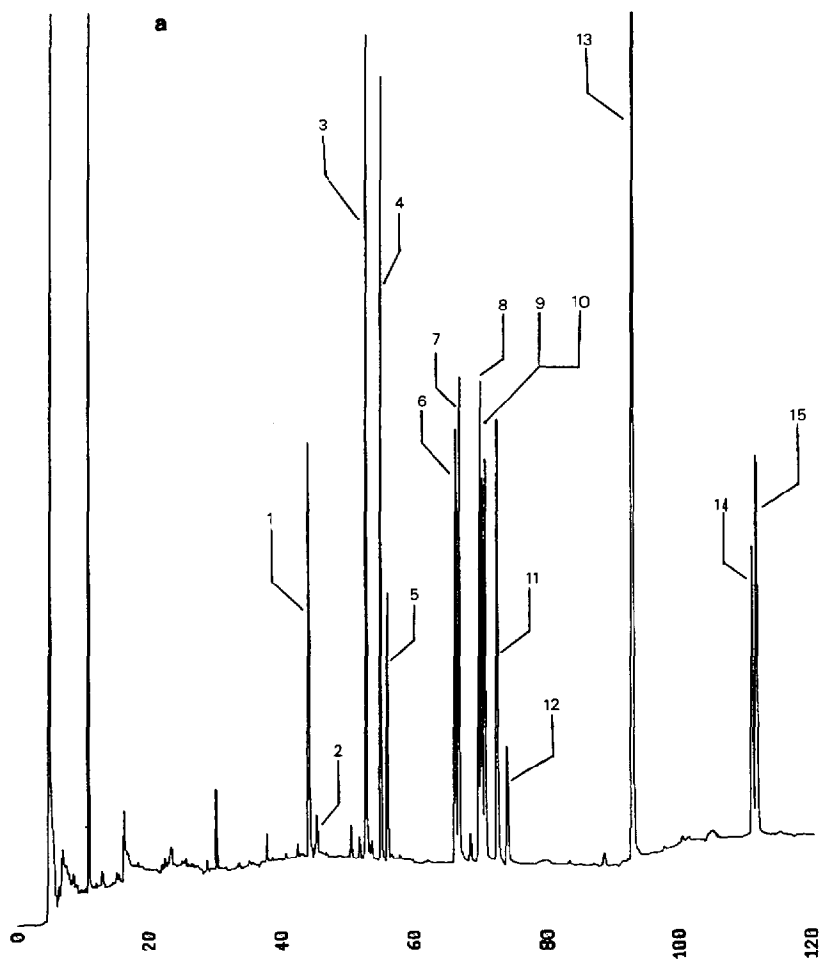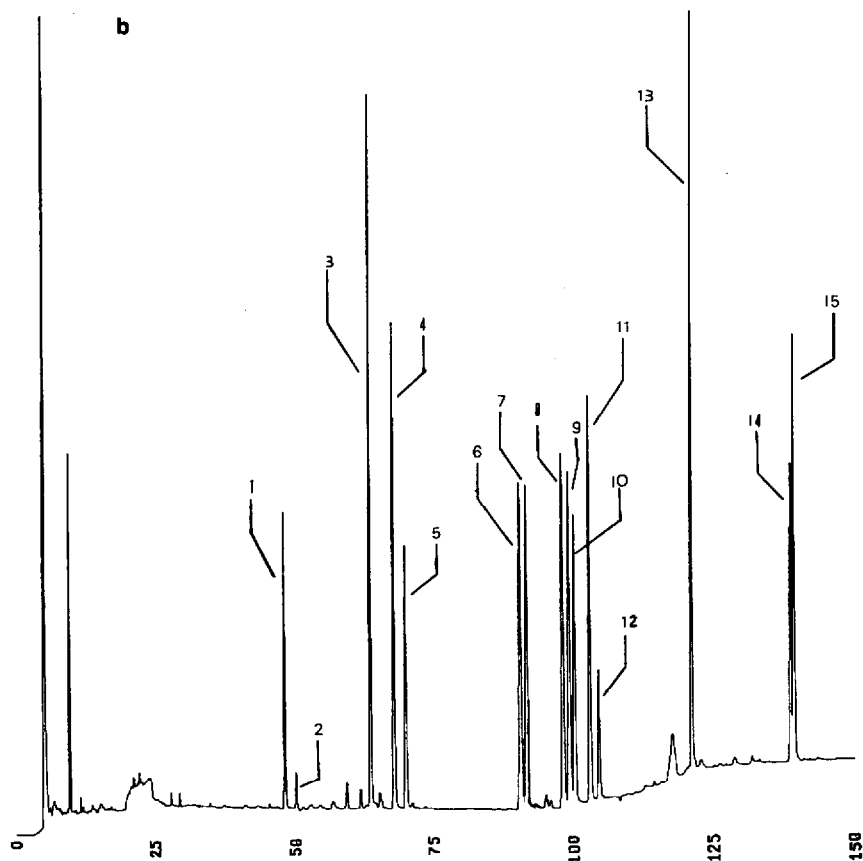

Fig. 1.

Fig. 1. High-performance GC separation of a mixture of standards PCDDs and PCDFs. (a) Initial conditions, (b) after simplex optimization. Peaks: 1 = 2,3,7,8-TCDF; 2 = 2,3,7,8-TCDD; 3 = PeCDF; 4 = PeCDF; 5 = PeCDD; 6 = HxCDF; 7 = HxCDF; 8 = HxCDF; 9 = HxCDD; 10 = HxCDD; 11 = HxCDD; 12 = HxCDF; 13 = 1,2,3,4,6,7,8-HpCDD; 14 = OCDD; 15 = OCDF. Time scale in min.

simplexes by applying eqn. 3 with $\alpha = -0.5$ as mentioned before. A re-incidence of boundary violations in addition to no significant improvements in response registered in the last vertices led us to end the search and to establish that the experimental conditions of vertex No. 16 were the optimum for our objective.

Fig. 1 shows the initial and final chromatograms resulting from optimizing the GC conditions. It is evident that the optimization process allows a significant increase in the overall peak separations, mainly between penta–tetra and hexa–penta groups, as was desired. It must be emphasized that in only 20 experimental runs (24 vertices generated minus 4 rejected without experimentation) an optimum zone was achieved with six variables involved in the optimization study.

CONCLUSIONS

The application of the modified simplex method to the variables involved in the

oven temperature programming results in an improvement of the overall peak separation of PCDF and PCDD isomers. As a direct consequence, a better differentiation from interferences generally due to some much more abundant compounds (*e.g.*, polychlorobiphenyls) of similar structural characteristics, present in natural samples, would be obtained.

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