Journal of Chromatography, 282 (1983) 287 295
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 162

COMPARISON OF RESOLUTION OPTIMIZATION METHODS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

JOSEP RAFEL
S. A. Cros, Research Centre, 158 Julio Galve, Badalona (Spain)

SUMMARY

An essential attribute of good chromatographic methods is the achievement of optimal resolution of the components of interest in a reasonable time. The resolution of pairs of incompletely separated peaks in each sample is evaluated by the Purnell equation (R. E. Kaiser and E. Oelrich, *Optimization in HPLC*, Hüthig, Heidelberg, 1981). The resolution of nitroaromatics and flavone derivatives has been optimized by three different methods and the results compared. An empirical function, obtained in a factorial design, relates resolution to several factors, such as eluent composition and system temperature. A general time constraint of 16 min is introduced.

INTRODUCTION

In a high-performance liquid chromatographic (HPLC) separation, the first problem to be solved for a given column and eluent system, usually selected on the basis of literature information, is the choice of the kind and number of experiments to be performed. To begin with an experimental factorial design[1,2] is set up for studying the following factors: eluent composition (X_1), system temperature (X_2) and flow-rate (X_3). By analysis of the data obtained, the resolution (R) may be expressed as a function of these factors. Then, several optimization methods can be employed.

It was the purpose of this work to compare the following methods: (a) the simplex method[3] with a convenient empirical feed-back strategy, which is a common procedure in analytical chemistry[4] and is most widely used in chromatography[5]; (b) the extended Hooke–Jeeves direct search method[6], which is applicable to such problems when the variables are defined in the experimental range; this method is valid for any number of factors through a Fortran program[7], run on an IBM 360 computer, and a flow chart is presented in Fig. 1; and (c) the Box–Wilson steepest ascent path[8], which determines the fastest way to optimal conditions.

EXPERIMENTAL

Nitroaromatics and the flavone derivatives rutin and quercetin were obtained from Merck (Darmstadt, F.R.G.) and kaempferol from Fluka (Buchs, Switzerland).

0021-9673/83/$03.00

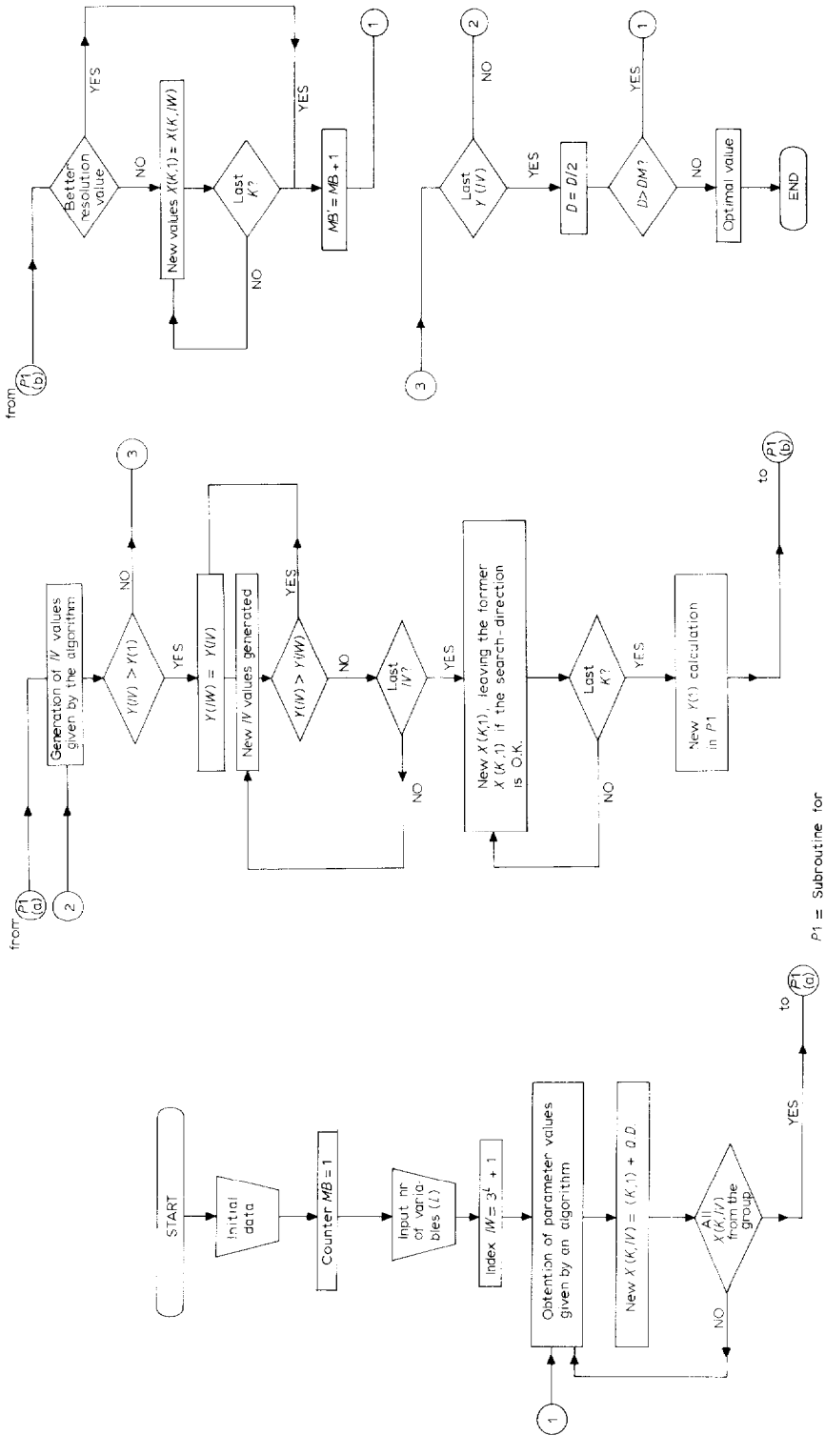

Fig. 1. Flow chart.

All other chemicals and solvents were of the purest grades available from commercial sources.

Solutions containing: nitrobenzene, 3,4-dinitrotoluene, 2-nitrotoluene and 3-nitrotoluene in methanol at concentrations around 0.01% and rutin, quercetin and kaempferol at concentrations around 0.05% were employed.

Liquid chromatography

A Hewlett-Packard HP 1084 liquid chromatograph, with a standard variable-wavelength detector and a reversed-phase column Supelco LC-8, ref. 5-28-5, of 5-μm particle size) were used.

For the nitroaromatics, an elution system containing water (solvent A) and methanol (solvent B) was used, and the wavelength was fixed at 254 nm. For the flavone derivatives a system containing acetic acid–methanol–water (5:5:90) (solvent A) and acetic acid–methanol–water (5:90:5) (solvent B) was used.

TABLE I

LOWER (−1), central (0) and upper (+1) VALUES FOR NITROAROMATICS AND FLAVONE DERIVATIVES

Factors: coded values (X')	*Nitroaromatics*			*Flavone derivatives*		
	−1	*0*	*1*	*−1*	*0*	*1*
Eluent B (%)	40	50	60	25	37.5	50
Temperature (°C)	35	53.5	70	35	53.5	70
Flow-rate (ml/min)	1	1.5	2	1	1.5	2

TABLE II

DESIGN MATRIX (STARTING 2^3 FACTORIAL DESIGNS) FOR NITROAROMATICS AND FLAVONE DERIVATIVES

Coded values for			*"Off-line" calculated resolution*	
Eluent B (%) (X'_1)	*Temperature (X'_2)*	*Flow-rate (X'_3)*	R_A*	R_B**
−1	−1	−1	2.81	4.4
+1	−1	−1	2.01	1.1
−1	+1	−1	2.41	4.2
+1	+1	−1	0.76	1.5
−1	−1	+1	2.77	5.3
+1	−1	+1	1.45	2.5
−1	+1	+1	1.53	4
+1	+1	+1	0.73	0.8

* Nitroaromatics.

** Flavone derivatives.

TABLE III

NITROAROMATICS SIMPLEX OPTIMIZATION

Vertex	*Coded values for*			*Resolution (R)*	*Vertices retained from previous simplex*
	Eluent B (%) (X'_1)	*Temperature (X'_2)*	*Flow-rate (X'_3)*		
1	0	0	0	2.3	–
2	0.9428	0.2357	0.2357	2.2	–
3	0.2357	0.9428	0.2357	2.4	–
4	0.2357	0.2357	0.9428	2.4	–
5	−0.6285	0.5499	0.5499	2.8	1, 3, 4
6	−0.1047	1.1523	1.1523	2.5	3, 4, 5
7	−0.5674	0.3491	0.8610	2.7	4, 5, 5
8	−0.6285	0.5499	0.5499	2.8	*
9	−1.1028	1.1318	0.7660	2.8	5, 6, 7**,***
10	0.2357	0.2357	0.9421	2.4	5, 7, 9

* At this point one duplication experiment is performed, to ensure that resolution in vertex 5 is not obtained from an erroneously high response.

** At this point, an optimum can be obtained with simplexes of the chosen size. In case there are no instrumental limitations, smaller step sizes are possible.

*** Optimal resolution achieved (R = 2.8) for eluent B = 35.3%, temperature = 43°C and flow-rate = 1.14 ml/min.

TABLE IV

NEW LOWER AND UPPER LIMITING VALUES FOR THE FACTORS IN THE HOOKE-JEEVES OPTIMIZATION

Coded values for	*Nitroaromatics*		*Flavone derivatives*	
	Lower	*Upper*	*Lower*	*Upper*
Eluent B (%) (X'_1)	−1.25	1.5	−1.25	1.5
Temperature (X'_2)	−1.125	1.5	−1.125	1.5
Flow-rate (X'_3)	−1	1.5	−1.125	1.5

TABLE V

OPTIMAL CONDITIONS IN THE HOOKE JEEVES OPTIMIZATIONS

Parameter	*Nitroaromatics*	*Flavone derivatives*
Eluent B (%)	37.5	21.9
Temperature (°C)	33	33
Flow-rate (ml/min)	1	1.43
Expected resolution	3.21	5.21
Resolution obtained in the confirmation experiment	3.16	5.47

TABLE VI

EXPERIMENTAL MATRIX AND OPTIMAL CONDITIONS OBTAINED IN THE BOX WILSON STEEPEST ASCENT PATH METHOD FOR NITROAROMATICS

μ (slope)	Coded values for			Resolution*
	Eluent B (%)	Temperature	Flow-rate	
0.6	−0.4	−0.38	−0.03	1.67
0.4	−0.6	−0.58	−0.05	2.03
0.3	−0.8	−0.77	−0.07	2.13
0.24	−1	−0.96	−0.08	2.72
0.2	−1.2	−1.15	−0.10	3.06
0.17	−1.4	−1.35	−0.12	2.99
0.15	−1.6	−1.53	−0.13	2.85

* Optimal resolution: R = 3.06 for eluent B = 38%, temperature = 32°C and flow-rate = 1.45 ml/min.

TABLE VII

SIMPLEX OPTIMIZATION FOR FLAVONE DERIVATIVES

Vertex	Coded values for		Resolution (R)	Vertices retained from previous simplex
	Eluent B (%) (X'_1)	Temperature (X'_2)		
1	0	0	5.2	–
2	0.9659	0.2591	2.2	–
3	0.2591	0.9659	5.3	–
4	−0.7068	0.7068	5.5	1, 3
5	−0.4477	1.6727	5.4	3, 4
6	−1.4136	1.4136	*	4, 5
7	−0.7068	0.7068	5.6	**
8	−1.6727	0.4477	*	4***,§, 5

* Considered as the worst value in the current simplex run. Later retention time greater than the time constraint.

** At this point one duplication experiment is performed, to ensure that resolution in vertex 4 is not obtained from an erroneously high response.

*** At this point, an optimum can be attained with simplexes of the chosen size. In case there are no instrumental limitations, smaller step sizes are possible.

§ Optimal resolution achieved (R = 5.55) for eluent B = 22.5%, temperature = 39.5°C and flow-rate = 2 ml/min (fixed at this value during the whole simplex optimization).

TABLE VIII

EXPERIMENTAL MATRIX AND OPTIMAL CONDITIONS OBTAINED IN THE BOX-WILSON STEEPEST ASCENT PATH METHOD FOR FLAVONE DERIVATIVES

μ (slope)	Coded values for			Resolution*
	Eluent B (%)	Temperature	Flow-rate	
3	−0.5	−0.12	0.06	2.81
2.14	−0.7	−0.16	0.08	3.42
1.67	−0.9	−0.21	0.11	4.55
1.5	−1	−0.23	0.12	**

* Optimal resolution: R = 4.55 for eluent B = 26.3%, temperature = 49°C and flow-rate = 1.60 ml/min.

** Time constraint exceeded.

TABLE IX

COMPARISON OF RESULTS FOR NITROAROMATICS

Parameter	*Optimization method*			M^*	D_1**	*Values in each method within the range* $M \pm 2(D_1)$ *in***		
	(A) Simplex	*(B) Hooke–Jeeves*	*(C) Box Wilson*			*A*	*B*	*C*
Resolution	2.80	3.16	3.06	3.01	0.18	+	+	+
Eluent B (%)	35.3	37.5	38	36.93	1.44	–	+	+
Temperature (°C)	43	33	32	36	6	–	+	+
Flow-rate (ml/min)	1.14	1	1.45	1.20	0.23	+	+	+

RESULTS

Separation of nitroaromatics

The optimization of the resolution between 2-nitrotoluene and 3-nitrotoluene was carried out by setting up a starting two-level factorial design. The limiting values for each factor in this factorial design are indicated in Table I, where the coded X' values (-1, 0, 1) indicate the lower, central and upper values, respectively. The corresponding experimental matrix is shown in Table II. The following empirical equation was obtained:

$$R = 1.81 - 0.57\,X'_1 - 0.45\,X'_2 - 0.19\,X'_3 \qquad (1)$$

TABLE X

COMPARISON OF RESULTS FOR FLAVONE DERIVATIVES

Parameter	*Optimization method*			M^*	D_1**	*Values in each method within the range* $M + 2(D_1)$ *in****		
	(A) Simplex	*(B) Hooke–Jeeves*	*(C) Box–Wilson*			*A*	*B*	*C*
Resolution	5.55	5.49	4.55	5.19	0.56	+	+	+
Eluent B (%)	22.5	21.9	26.3	23.57	3.38	+	+	+
Temperature (°C)	39.5	33	49	40.5	8.5	+	+	+
Flow-rate (ml/min)	2	1.43	1.6	1.68	0.29	+	+	+

N^*	$D_2{}^*$	*Values in simplex method within the range* $N \pm 2\,(D_2)$	P^*	$D_3{}^{**}$	*Values in Hooke–Jeeves method within the range* $P \pm 2\,(D_3)^{***}$	T^*	$D_4{}^{**}$	*Values in Box–Wilson method within the range* $T \pm 2\,(D_4)^{***}$
3.11	0.07	–	2.93	0.18	+	2.98	0.25	+
37.75	0.35	–	36.65	1.91	+	36.4	1.56	+
32.5	0.71	–	37.5	7.8	+	38	7.1	+
1.23	0.32	+	1.30	0.22	+	1.07	0.10	–

* M = Mean value, methods A, B and C; N = = mean value, methods B and C; P = mean value, methods A and C; T = mean value, methods A and B.

** D_1 = Standard deviation values obtained in methods A, B and C; D_2 = standard deviation values obtained in methods B and C; D_3 = standard deviation values obtained in methods A and C; D_4 = standard deviation values obtained in methods A and B.

*** + = Yes, they are; – = no, they are not.

Subsequently, three different optimization methods were used and compared, as follows.

(A) Simplex method[3]. The corresponding starting values in the first simplex run (40% methanol, 40°C and 1 ml/min) were choosen close to the best values found in the factorial design. The step sizes were 7.5% methanol, 5°C and 0.2 ml/min. The results of the simplex matrix used are listed in Table III.

(B) Extended Hooke–Jeeves method[6]. Here a Fortran program[7] extends the Hooke–Jeeves method. It starts from eqn. 1 and is valid for any number of factors

N^*	$D_2{}^{**}$	*Values in simplex method within the range* $N \pm 2\,(D_2)$	p^*	$D_3{}^{**}$	*Values in Hooke–Jeeves method within the range* $P \pm 2\,(D_3)^{***}$	T^*	$D_4{}^{**}$	*Values in Box Wilson method within the range* $T \pm 2\,(D_4)^{***}$
5.01	0.65	+	5.05	0.71	+	5.51	0.06	+
24.1	3.11	+	24.4	2.68	+	22.5	0.42	–
41	11.3	+	44.25	6.71	+	36.25	4.6	–
1.52	0.12	–	1.8	0.28	+	1.72	0.40	+

* M = Mean value, methods A, B and C; N = mean value, methods B and C; P = mean value, methods A and C; T = mean value, methods A and B.

** D_1 = Standard deviation values obtained in methods A, B and C; D_2 = standard deviation values obtained in methods B and C; D_3 = standard deviation values obtained in methods A and C; D_4 = standard deviation values obtained in methods A and B.

*** + = Yes, they are; – = no, they are not.

chosen. The new ranges for the values of the factors are indicated in Table IV and the results are shown in Table V. No further experiments are required, but a confirmation test was performed.

(C) Box-Wilson method[8]. In the Box–Wilson steepest ascent path, the starting values and the step size for each factor were methanol = 50%, step size = 10%; temperature = 52.5°C, step size = 17.5°C; flow-rate: 1.5 ml/min, step size = 0.5 ml/min. The results are given in Table VI.

Separation of flavone derivatives

The optimization of resolution of quercetin and kaempferol was carried out in a similar manner to that for nitroaromatics, a starting factorial design being performed. The limiting values for each factor are indicated in Table I and the coresponding experimental matrix is shown in Table II. The following empirical equation was obtained:

$$R = 2.98 - 1.50\,X'_1 - 0.35\,X'_2 - 0.18\,X'_3 \qquad (2)$$

The same optimization methods were used as before and compared.

(A) Simplex method[3]. According to the results of eqn. 2, the flow-rate was fixed at 2 ml/min. The corresponding starting values in the first run and the chosen step size for the other factors were eluent B = 25%, step size = 4%; temperature = 350°C, step size = 5°C. The results are presented in Table VII.

(B) Extended Hooke–Jeeves method[6]. In this case the program is initiated with data from eqn. 2. The new ranges for the values of the factors are indicated in Table IV and the results are given in Table V. As above, the expected value in the confirmatory experiment agrees with the actual value.

(C) Box–Wilson method[8]. In this case the starting values and step size for each factor were eluent B = 37.5, step size = 10%; temperature = 52.5°C, step size = 17.5°C; flow-rate = 1.5 ml/min, step size = 0.5 ml/min. The results are presented in Table VIII.

DISCUSSION AND CONCLUSIONS

Second- and third-degree interactions have not been taken into account, owing to their very low importance as observed in previous experiments[9]. A time constraint was introduced, as resolutions of 1.25 usually are high enough for a good qualitative and quantitative analysis[10] and often there is no special interest in obtaining greater resolutions by an increase in analysis time.

The optimal resolutions obtained by any of the above methods are within the range ($MR \pm 2S$) (where MR = mean of optimal resolutions obtained in each method and S = standard deviation) and they give very similar values. With the Hooke–Jeeves extended method a better agreement is obtained for both optimal resolution and optimal conditions, as indicated in Tables IX and X.

In conclusion, the Hooke–Jeeves method, following a $2\,k$ factorial design, appears to be an easy and fast way of achieving optimal resolution, especially when a fast approach and a reasonable reduction in the number of experiments are required.

The time savings when using the program are increased if non-linear functions are obtained in the factorial design relating R to k factors, and also when the number of factors is large.

REFERENCES

1 G. E. P. Box, W. G. Hunter and S. Hunter, *Statistics for Experimenters. An Introduction to Design, Data Analysis and Model Building*, Wiley, New York, 1978.
2 S. N. Deming and R. L. Kong, *J. Chromatogr.*, 217 (1981) 421–434.
3 W. Spendley, G. R. Hext and F. R. Himsworth, *Technometrics*, 4 (1962) 441.
4 S. L. Morgan and S. N. Deming, *Anal. Chem.*, 46 (1974) 1170.
5 S. L. Morgan and S. N. Deming, *J. Chromatogr.*, 112 (1975) 267–285.
6 R. Hooke and T. A. Jeeves, *J. Ass. Comput. Mach.*, 8 (1961) 221–229.
7 J. Rafel, P. Andreu, V. Mans and J. M. Lema, *Ing. Quim.*, 166 (1983) 65–71.
8 G. E. P. Box and K. B. Wilson, *J. R. Stat. Soc., Ser. B*, 13 (1951) 1.
9 J. Rafel and J. M. Lema, *Afinidad*, in press.
10 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979.