Journal of Chromatography, 485 (1989) 311–323
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 016

UNIFORM SHELL DESIGNS FOR OPTIMIZATION IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

YUZHU HU[a] and D. L. MASSART*
Pharmaceutical Institute, Vrije Universiteit Brussels, Laarbeeklaan 103, B-1090 Brussels (Belgium)

SUMMARY

An experimental design method, the uniform shell or Doehlert matrix design, is proposed for the optimization of high-performance liquid chromatography. Seven experiments are necessary for a two-factor design and yield a quadratic model with interaction. The principle of the methods is described and examples of applications are given.

INTRODUCTION

Factorial designs[1–3] are a class of experimental designs which are able to offer a large amount of information from a small number of experiments. They are often used for the optimization of the mobile phase or the chromatographic conditions in general. When one wants to optimize the solvent composition, one applies mixture designs. Otherwise, one uses designs for process variables, such as two- or more-level designs. This paper describes an efficient design for process variables that does not appear to have been used previously in chromatography.

Several workers[4–6] have described two-level factorial designs for the optimization of high-performance liquid chromatography (HPLC). Two-level factorial designs are the simplest factorial designs, as one only needs to carry out four experiments for two factors (see Fig. 1a). For k factors, 2^k experiments are required for a complete factorial design. For two factors this model is represented by

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 \quad (1)$$

With two-level factorial designs, each factor is generally investigated at two levels: an upper (+) and a lower (−) level, which then define the limits of the experimental domain. This model requires the assumption of no curvature within the experimental domain. To describe a maximum or minimum within the experimental domain, one must estimate quadratic curvature, which requires at least three levels for each factor.

[a] On leave from the China Pharmaceutical University, Nanjing, China.

0021-9673/89/$03.50

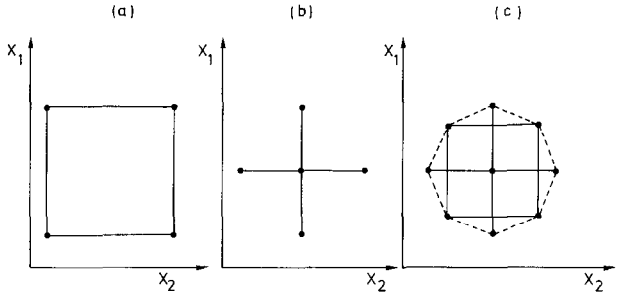

Fig. 1. Some two-factor factorial designs. (a) A two-level design; (b) a star design; (c) a central composite design.

A class of experimental design, called star designs (see Fig. 1b) can describe the second-order curvature effect. The model is given by

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (2)$$

For a k-factor star design, one needs $2k + 1$ factor combinations so that it can be used to estimate the $2k + 1$ parameters in eqn. 2. However, the model in eqn. 2 does not contain the interaction term ($b_{12}X_1X_2$ in two-level designs).

The combination of a two-level factorial design with a star design yields a central composite design when the centres of the two separate experimental designs coincide (see Fig. 1c). In Box *et al.*'s two-factor design[2], the distance from the star point to the centre in the star design equals $\sqrt{2}$ times half the length of the edge of the square required for a (coded) two-level design. It forms an ortho-octagon. The central point of the central composite designs is often replicated to be able to estimate experimental error.

The fitted model for two factors is

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (3)$$

For a k-factor design, the central composite design needs $2^k + 2k + 1$ experiments.

In this paper, we introduce an experimental design into the optimization of HPLC, which is also able to fit the second-order polynomial of eqn. 3. This method was proposed by Doehlert[7], and we therefore call it the Doehlert matrix design; he called it the uniform shell design. The feasibility of the application of Doehlert matrix designs in HPLC was studied and demonstrated by using real data.

Method development in HPLC emphasizes selectivity optimization, *i.e.*, the control of the elution order of the components so that maximum resolution and/or minimum separation time can be obtained[8]. Many factors can affect the chromatographic behaviour of components, *e.g.*, the content of organic modifier, pH, ionic strength of the mobile phase, temperature, stationary phase and length of the column. With so many factors, one feasible way of working is to use a fractional factorial design[1–3] to study the main effect of the factors, then to choose the factors with the greatest effect for further study. Some papers[6,9–12] have described designs with three

or four factors. In our view, it is often unnecessary to use complex experimental designs in practice, and two-factor design should be able to solve most practical problems in a satisfactory way, because there are often only one or two dominant factors. Therefore, this study centres on the two-factor factorial designs. Doehlert matrix design can, however, be applied to as many factors as one wants.

Doehlert matrix design

Doehlert[7] proposed uniform shell designs that have an equally spaced distribution of points lying on concentric spherical shells. They have uniform space-filling properties and were shown to be more uniform than general experimental designs. For this reason, models based on this design provide a good basis for interpolation.

Doehlert described how to generate the designs for up to at least ten factors. Here we introduce his method by generating the design matrix for two factors. An experiment in two factors may be thought of as a point in two-dimensional space (X_1, X_2). We start with three such points:

(0.000, 0.000),
(1.000, 0.000),
(0.500, 0.866).

The three vertices form an equilateral triangle. This is called a simplex in two-dimensional space. These points are labelled S in Fig. 2a.

Then, subtract each point from each other point to obtain

(−1.000, 0.000),
(−0.500, −0.866),
(−0.500, 0.866),
(0.500, −0.866).

These four points are the unlabeled points in Fig. 2a. This figure is an ortho-hexagon with a centre point.

To design experiments for more factors (k), the starting simplex with $k + 1$ sides is formed by adding to the $k - 1$ simplex (with k sides) the point

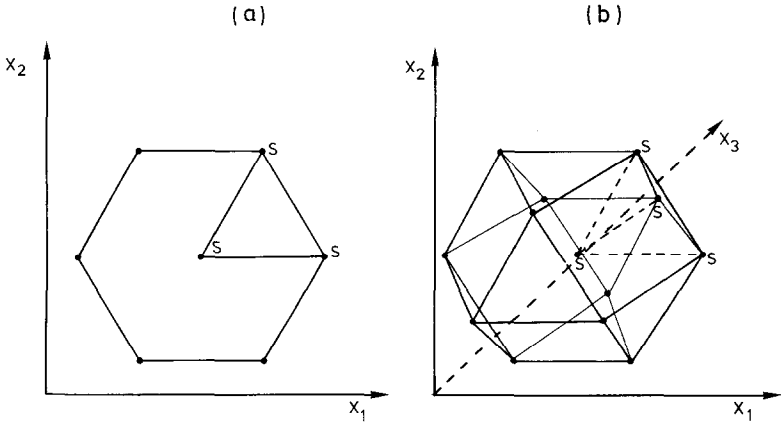

Fig. 2. Uniform shell design. (a) A hexagon (for two factors); (b) a cubooctahedron (for three factors). The points of the starting simplex are labelled S.

$[1/2, 1/2\sqrt{3}, 1/2\sqrt{6}, \ldots, 1/\sqrt{2(k-1)(k-2)}, 1/\sqrt{2k(k-1)}, \sqrt{k+1}/\sqrt{2k}]$

This line must be read starting from the right, as explained in the following example. Suppose we want to develop the simplex for three factors. This will require four corner points (four sides), each characterized by a value for the three variables. We compute the three last points of the line given above:

$1/\sqrt{2(k-1)(k-2)} = 1/\sqrt{2(3-1)(3-2)} = 0.500$

$1/\sqrt{2k(k-1)} = 0.289$

$\sqrt{k+1}/\sqrt{2k} = 0.816$

We now take the simplex for two factors, add 0.000 to each row and add the line computed above to obtain

(0.000, 0.000, 0.000),
(1.000, 0.000, 0.000),
(0.500, 0.866, 0.000),
(0.500, 0.289, 0.816).

In this way, the simplex contains $k + 1$ points, one of which is the origin. The other k points of the simplex lie on a sphere of radius 1.0 centred on the origin. Each of the points on the sphere subtracted from the other k points in the simplex forms k new points. For three factors, the design is therefore an ortho-cubooctahedron with a centre point (see Fig. 2b).

TABLE I

SOME PROPERTIES OF THE CENTRAL COMPOSITE DESIGNS AND THE DOEHLERT MATRIX DESIGN

Model	*Experimental factors (k)*	*Number of b coefficients (p)*	*Experiments (f)*	*Efficiency (p/f)*
Centred two-level design	2	4	5	0.80
	3	7	9	0.78
	4	11	17	0.65
	5	16	33	0.48
	6	22	65	0.34
	8	37	257	0.14
	10	56	1025	0.05
Central composite design	2	6	9	0.67
	3	10	15	0.67
	4	15	25	0.60
	5	21	43	0.49
	6	28	77	0.36
	8	45	273	0.16
	10	66	1045	0.06
Doehlert matrix design	2	6	7	0.86
	3	10	13	0.77
	4	15	21	0.71
	5	21	31	0.68
	6	28	43	0.65
	8	45	73	0.62
	10	66	111	0.59

The total number of points for a complete design with k factors is $k^2 + (k + 1)$, and the number of parameters to be estimated in eqn. 3 is $(k + 1)(k + 2)/2$. The Doehlert matrix design therefore permits fitting the quadratic model in eqn. 3.

A comparison of the designs discussed is listed in Table I (also see ref. 3). Clearly, the number of observations needed by the Doehlert matrix designs is less than that for the central composite designs. These numbers cannot be compared directly with the simple two-level designs as they do not take account of curvature. A feature of central composite designs is that the number of levels for each variable is always the same, whereas the number of levels using the Doehlert matrix design is not the same for all variables. One should then use the design in such a way that the variable with the most complex variables is modelled with the largest number of levels. The analyst's experience is therefore needed to decide which variable should be used at the higher number of levels.

EXPERIMENTAL

A Varian 5000 liquid chromatograph, equipped with a Gilson 231 autosampler–injection system sample loop (50 μl) and a Hewlett-Packard 1040-A diode-array detector, was used. The chromatograms were recorded and integrated with a Varian CDS 401 data system. A stainless-steel column packed with LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.) of particle size 5 μm (250 × 4 mm I.D.) was used. Unless stated otherwise, experiments were carried out at 25°C with a flow-rate of 1 ml min^{-1}.

Methanol, sodium dihydrogenphosphate monohydrate, disodium hydrogenphosphate dihydrate and 85% orthophosphoric acid were of analytical-reagent grade from Merck. Purified water was obtained from a laboratory water-purification system (Milli-Q; Millipore, Bedford, MA, U.S.A.).

All drugs were of pharmaceutical purity. Stock solutions containing 1000 μg ml^{-1} of the drugs were prepared in methanol and diluted with water to the final injected concentrations. The final injected concentration of the drugs was between 2 and 10 μg ml^{-1}.

To prepare phosphate buffers of various pH, 0.05 mol l^{-1} sodium dihydrogenphosphate monohydrate and 0.05 mol l^{-1} disodium hydrogenphosphate dihydrate were prepared. The former solution was adjusted to pH 3.0 with phosphoric acid using an Orion 501 Digital Ionalyzer. The other buffer solutions were obtained by adding 0.05 mol l^{-1} disodium hydrogenphosphate dihydrate to the pH 3.0 buffer and adjusting to the final pH values with the Ionalyzer. In this way constant ionic strength was obtained. The buffer solutions were filtered through 0.2-μm membrane filters before they were used for HPLC.

A computer program for two-level designs, central composite designs, Doehlert matrix designs each with overlapping resolution map methods was developed in Fortran 77 for an IBM-AT computer. This program is separated into two parts: (i) designing the factorial design from the given limits of each factor and (ii) calculation of the response surface from experimental values by least-squares regression. An overlapping resolution map is then obtained, and the optimum value of variables by grid searching is given. Users may change the criterion value of the resolution (the default value is 1.5) for the overlapping resolution map, and obtain the calculated resolution value at any point they wish within the domain studied.

A program for plotting the response surface was constructed in compiled QuickBasic.

RESULTS AND DISCUSSION

For illustration purposes, the method is used for two groups of pharmaceutical components. One is a mixture of six drugs with different polarity and acidity (see the components listed in Table III) and the other is a mixture consisting of five sulphonamides (see the legend of Fig. 9). In both instances, reversed-phase HPLC is applied.

In the first example, the two variables are the content of methanol (the solvent strength-adjusting component of the mobile phase) and pH, and in the second, the content of methanol and temperature.

One should first ask which model has to be used. Clearly, this depends on the relationship between the chromatographic responses modelled (retention and half-width of peak) and factors studied. One can conclude that one needs a second-order design in the pH, and one might be content with a first-order design for the methanol content in modelling k' as its natural logarithm, as one often applies the relationship

$$\ln k' = A\varphi + B \tag{4}$$

where φ is the volume fraction of the organic modifier, the coefficient A is expected to be negative and B is the natural logarithm of the capacity factor in pure water.

There are three reasons for not doing this, as follows.

(1) As proposed by Schoenmakers[4], the quadratic equation of the form

$$\ln k' = A\varphi^2 + B\varphi + C \tag{5}$$

is more suitable for the description of the retention as a function of the binary mobile phase composition. In this equation, the coefficient A is expected to be positive, B large and negative and C is the natural logarithm of the capacity factor in pure water. Clearly, eqn. 5 is a better description of the relationship between k' and the volume fraction of the organic modifier φ.

The mixed second-order pH–first-order methanol design would require at least six whereas the Doehlert design requires seven experiments, which is a small price to pay for the more correct description of eqn. 3.

(2) The mixed second-order–first-order design would use three levels for the pH and two for methanol concentration, whereas the Doehlert matrix has five levels for pH and three for methanol. Clearly, the Doehlert matrix describes the experimental domain in a more comprehensive way.

(3) One not only has to model k' but also the plate height. The relationships between plate height and the variables are more complex, so that the second-order design seems advisable.

For the second example, *i.e.*, the mixture of five sulphonamides, there are more reasons to propose a first-order design as both the relationship between ln k' and methanol concentration and ln k' and temperature (as $1/T$ in the Van 't Hoff plot) are more or less linear. However, this is an approximation not only for the methanol

concentration as explained above, but also for the temperature. As explained by Poole and Schuette[13], non-linear Van 't Hoff plots may be due to several reasons, such as retention by mixed mechanisms. When a very efficient design such as the Doehlert matrix is available, it seems advisable not to depend on linearity, but to apply the second-order design anyway.

The experimental design permits one further to predict the retention and peak width of each component. In a following step, one computes an overlapping resolution map (ORM)[14,15] after dividing the experimental domain into 40 × 40 squares. The resolution of the worst separated pair, *i.e.*, R_{min}, is chosen as a chromatographic response. The threshold of the criterion value of the chromatographic response can be chosen by the user, and the overlapping resolution map can be shown on screen as well as printed out. The shaded part indicates that the required R_{min} is not respected by at least one peak pair whereas in the remaining unshaded area R_{min} is obtained for all pairs of peaks.

The overlapping map allows the user to find a permissible area for the combination of factors. The best combination of factors by grid searching, *i.e.*, the combination of factors with the best resolution (maximum R_{min}), can also be obtained. However, the optimum found by grid searching consists of one specific set of conditions, which may be inadequate because it does not allow analysis time or other possible criteria to be taken into account. Three-dimensional window diagrams or minimum resolution plots[16] can be more appropriate for a full comprehension of chromatographic behaviour within the studied domain and for selecting optimum conditions. Successful results depend on a suitable model when ORM and the minimum resolution plots are used. This is, in fact, common to all simultaneous methods.

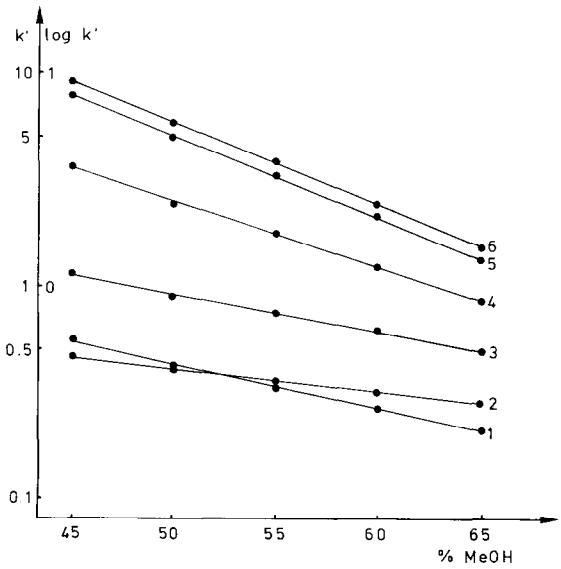

Fig. 3. Retention (log k') *vs.* the content of methanol (MeOH). Mobile phase: methanol–phosphate buffer (pH 4.5, μ = 0.05). 1 = Acetylsalicylic acid; 2 = paracetamol; 3 = caffeine; 4 = benzocaine; 5 = carbamazepine; 6 = propyphenazone.

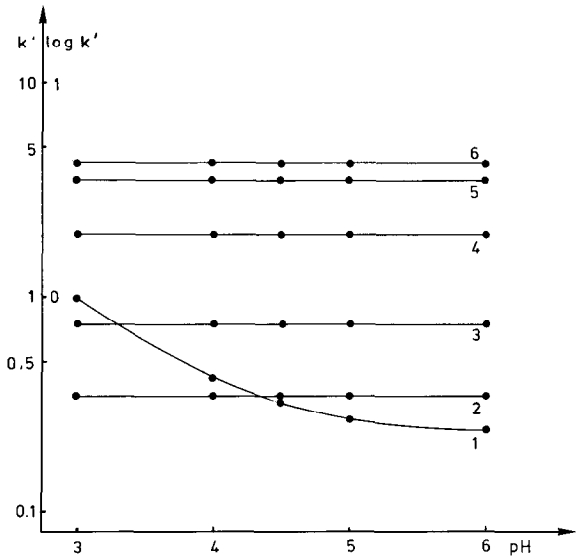

Fig. 4. Retention (log k') *vs.* the pH of the phosphate buffer. Mobile phase: methanol–phosphate buffer (55:45) (μ = 0.05). Lines as in Fig. 3.

TABLE II

FACTORIAL DESIGNS FOR A SIX-COMPONENT MIXTURE

Design	*No.*	*Variables in coded units*		*Variables in original scale*	
		X_1	X_2	*pH*	φ_{CH_3OH} ($\times 10^{-2}$)
Centred two-level design	1	−1	−1	3	45.0
	2	1	−1	6	45.0
	3	1	1	6	65.0
	4	−1	1	3	65.0
	5	0	0	4.5	55.0
Central composite design	1	1	0	6.0	55.0
	2	−1	0	3.0	55.0
	3	0	1	4.5	65.0
	4	0	−1	4.5	45.0
	5	0.707	0.707	5.6	62.0
	6	−0.707	0.707	3.4	62.0
	7	−0.707	−0.707	3.4	48.0
	8	0.707	−0.707	5.6	48.0
	9	0	0	4.5	55.0
Doehlert matrix design	1	1	0	6.0	55.0
	2	−1	0	3.0	55.0
	3	0.5	0.866	5.3	64.0
	4	−0.5	0.866	3.7	64.0
	5	0.5	−0.866	5.3	46.0
	6	−0.5	−0.866	3.7	46.0
	7	0	0	4.5	55.0

For the reasons discussed above, we prefer to use the ORM plot. Generally, the user should choose in the permissible region the higher modifier content to obtain a shorter retention time and a better peak shape.

For the first separation problem, as acetylsalicylic acid is a strong acid and therefore the pH may have a large effect, the pH was studied at five levels. Ln k' and ln $w_{1/2}$ are used as the chromatographic response. The content of methanol has a large effect on the k' of all the solutes and is in fact responsible for finding an acceptable k' range. In Fig. 3, the relationship between the k' value of these six components and methanol concentration in a buffer of pH 4.5 is shown. The effect of pH is shown in Fig. 4. One observes that, owing to its strong acidity, acetylsalicylic acid indeed shows a different behaviour to the others: as the pH value of the buffer increases, the order of elution of acetylsalicylic acid changes from third to first. To compare different designs, we also applied a centred two-level design and the central composite design. The design matrices for these three kinds of designs are listed in Table II.

To evaluate approximately the lack of fit of the model, one can compute the predicted and observed response value (here ln k'), for instance in the central point. In Table III, this is done for the Doehlert matrix design and the centred 2 × 2 design using eqn. 1. In fact, the residual estimates the sum of experimental variability and lack of fit and the analysis of variance is required to split the two. However, from our knowledge

TABLE III

COMPARISON OF DOEHLERT DESIGN (2) WITH THE CENTRED TWO LEVEL DESIGN (1) (WITH AND WITHOUT CENTRE POINT) FOR THE CENTRAL POINT OF THE MIXTURE OF SIX COMPONENTS

Methanol–phosphate buffer (55:45) (pH 4.5, μ = 0.05).

Component	*Design*	*Capacity factor (k')*				
		Calc.		*Exptl.*	*Error*	
		Without centre point	*With centre point*		*Without centre point*	*With centre point*
Paracetamol	1	0.28	0.35	0.33	−0.05	0.02
	2	0.28	0.35		−0.05	0.02
Acetylsalicylic acid	1	0.49	0.46	0.35	0.14	0.11
	2	0.65	0.37		0.30	0.02
Caffeine	1	0.50	0.75	0.76	−0.26	−0.01
	2	0.73	0.76		−0.03	0.00
Benzocaine	1	0.85	1.78	1.92	−1.07	−0.14
	2	2.25	1.92		0.33	0.00
Carbamazepine	1	1.31	3.31	3.58	−2.27	−0.27
	2	4.30	3.59		0.72	0.01
Propyphenazone	1	1.51	3.85	4.19	−2.68	−0.34
	2	13.31	4.20		9.12	0.01
Average absolute error	1				±1.08	±0.15
	2				±1.76	±0.01

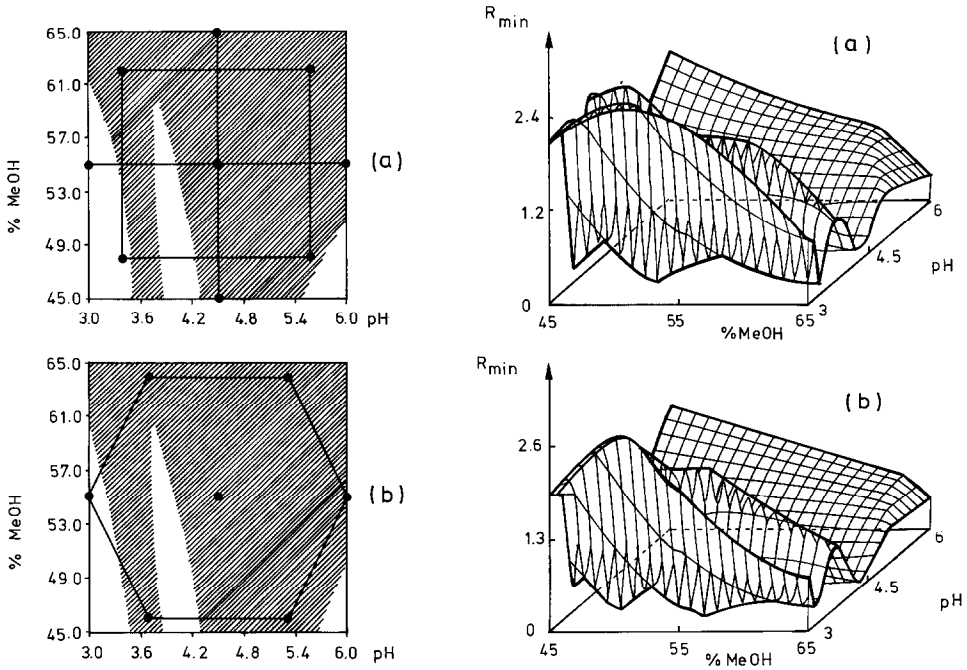

Fig. 5. Overlapping resolution maps for the sample described in Figs. 3 and 4. (a) The central composite design; (b) the Doehlert matrix design.

Fig. 6. Minimum resolution plot for the same sample as in Fig. 5. (a) Data based on the central composite design; (b) data based on the Doehlert matrix design.

of the variability in this system, we can safely accept that the larger residuals are due to lack of fit. Better results are obtained with the Doehlert matrix design.

The designs are also compared with and without the central point. This shows that the regression equation obtained may not fit the data well when the central point is missing, so that the central point clearly has to be included.

In Fig. 5, for both designs, there are three similar unshaded regions obtained by ORM where the R_{min} is larger than the required value (here 1.5). From the unshaded area on the ORM, a methanol content of 50% and pH 4.0, and a methanol content of 50% and pH 3.0 are chosen as the optimum conditions. The minimum resolution plots from the Doehlert design and the central composite design for this mixture are shown in Fig. 6. They show that the response surfaces of the R_{min} obtained from the central composite design and the Doehlert matrix design are very similar.

In Table IV, a comparison between the predicted and experimental results is carried out for the central composite and the Doehlert design in the two chosen mobile phases. The observed retention and predicted retention are very similar. The predicted minimum resolutions of these two designs are also close to the experimental value. It must be noted that $w_{1/2}$ measured to determine the resolution is also modelled with the regression eqns. 1–3. This value is less stable; the repeatability is not as good as for the retention time, so that the prediction error is much larger than for the retention.

TABLE IV

COMPARISON OF CALCULATED AND OBSERVED CAPACITY FACTORS FOR THE MIXTURE OF SIX COMPONENTS USING CENTRAL COMPOSITE (1) AND DOEHLERT (2) DESIGNS

Component	*Design*	*Capacity factor (k')*					
		Mobile phase I[a]			*Mobile phase II*[b]		
		Calc.	*Exptl.*	*Error*	*Calc.*	*Exptl.*	*Error*
Paracetamol	1	0.39	0.40	−0.01	0.40	0.41	−0.01
	2	0.39		−0.01	0.39		−0.02
Acetylsalicylic acid	1	1.55	1.20	0.35	0.62	0.72	−0.10
	2	1.39		0.19	0.61		−0.11
Caffeine	1	0.87	0.89	−0.02	0.90	0.90	0.00
	2	0.87		−0.02	0.90		0.00
Benzocaine	1	2.59	2.48	0.11	2.57	2.51	0.06
	2	2.60		0.12	2.57		0.06
Carbamazepine	1	5.10	4.95	0.15	5.06	4.99	0.07
	2	5.07		0.12	5.09		0.10
Propyphenazone	1	5.96	5.74	0.22	5.98	5.80	0.18
	2	5.97		0.23	5.88		0.08
Average absolute error	1			±0.14			±0.07
	2			±0.11			±0.06
R_{min}	1	2.30	2.89	−0.59	1.99	1.94	0.05
	2	2.59		−0.30	1.93		−0.01

[a] I = methanol–phosphate buffer (50:50) (pH 3.0, μ = 0.05).
[b] II = methanol–phosphate buffer (50:50) (pH 4.0, μ = 0.05).

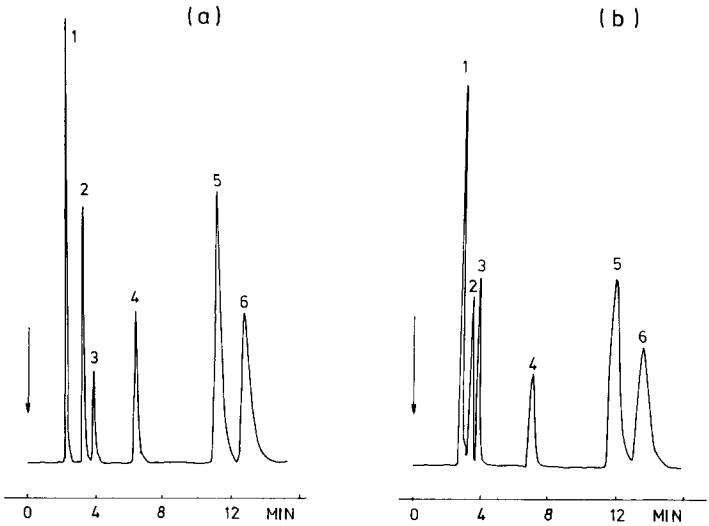

Fig. 7. Optimized chromatogram. Mobile phase: methanol–phosphate buffer (50:50) (pH 4.0, μ = 0.05). Peaks: 1 = paracetamol; 2 = acetylsalicylic acid; 3 = caffeine; 4 = benzocaine; 5 = carbamazepine; 6 = propyphenazone.

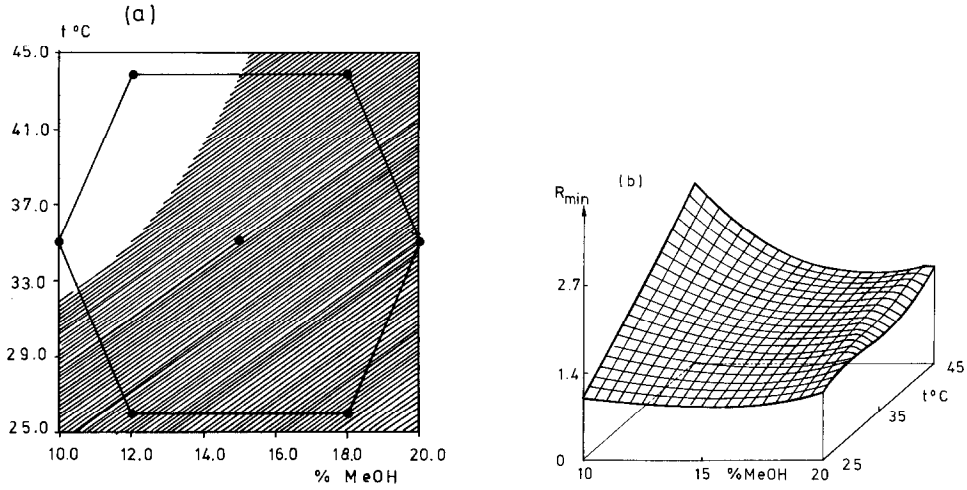

Fig. 8. Minimum resolution plot for sample II. Data based on the Doehlert matrix design. (a) ORM; (b) minimum resolution plot.

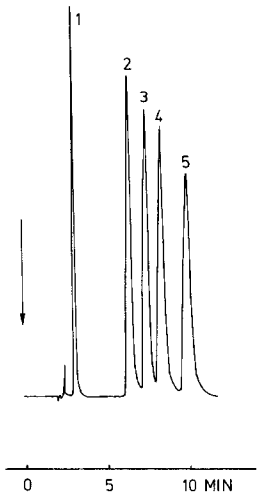

Fig. 9. Chromatogram of sample II. Optimum mobile phase: methanol–phosphate buffer (15:85) (pH 3.0, μ = 0.05). Column temperature: 45°C.

Peak No.	*Component*	*Calc.*		*Exptl.*		*Error*	
		t_R *(min)*	k'	t_R *(min)*	k'	t_R *(min)*	k'
1	Sulphanilamide	3.19	0.59	3.15	0.57	0.04	0.02
2	Sulphadiazine	6.64	2.31	6.51	2.30	0.13	0.01
3	Sulphathiazol	7.62	2.79	7.55	2.76	0.07	0.03
4	Sulphapyridine	8.59	3.27	8.47	3.21	0.12	0.06
5	Sulphamerazine	10.19	4.07	10.01	3.98	0.18	0.09
Average absolute error						±0.11	±0.04

Chromatograms under the selected conditions are shown in Fig. 7. All components are separated very well. Both the central composite design and Doehlert matrix design have therefore yielded acceptable results.

For the second separation problem (the sulphonamides), only the Doehlert matrix design was applied. The ORM is shown in Fig. 8a and the minimum resolution plot is shown in Fig. 8b. Both in fact contain the same information but, for the user, the ORM gives an easier quantitative interpretation of the response surface, whereas the pseudo-three-dimensional view allows a better qualitative appreciation. The optimum combination of factors by grid searching is 10% methanol and 45°C. However, considering the smaller measurement time and good peak shape, 15% methanol and 45°C were selected as optimum conditions. The chromatogram obtained and the comparison between the predicted and observed values are shown in Fig. 9.

CONCLUSION

Factorial designs combined with ORM can provide a means for the evaluation of the chromatographic behaviour of components and the optimization of separation in HPLC with only a few experiments. In most situations, the quadratic model is required so that the central composite designs and Doehlert matrix designs have been studied. The latter are more economical.

ACKNOWLEDGEMENTS

We thank M. De Smet and M. Moors for helpful advice, G. Wuestenberg and A. De Schrijver for technical assistance, Professor Phan Tan Luu for acquainting us with the Doehlert matrix design and FGWO for financial assistance.

REFERENCES

1 D. L. Massart, B. G. M. Vandeginste, S. N. Deming, Y. Michotte and L. Kaufman, *Chemometrics: a Textbook*, Elsevier, Amsterdam, 1988.
2 G. E. P. Box, W. G. Hunter and J. S. Hunter, *Statistics for Experimenters: an Introduction of Laboratory Methods and Analytical Procedures*, Elsevier, Amsterdam, 1978.
3 S. N. Deming and S. L. Morgan, *Experimental Design: a Chemometric Approach*, Elsevier, Amsterdam, 1987, pp. 187 and 199.
4 P. Schoenmakers, *Optimisation of Chromatographic Selectivity*, Elsevier, Amsterdam, 1986, pp. 60, 188 and 209.
5 J. C. Berridge, *Techniques for the Automated Optimisation of HPLC Separations*, Wiley, Chichester, 1985, p. 62.
6 P. Wester, J. Gottfries, K. Johansson, F. Klintebäck and B. Winblad, *J. Chromatogr.*, 415 (1987) 261.
7 D. H. Doehlert, *Appl. Statist.*, 19 (1970) 231.
8 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979, Ch. 2.
9 W. Lindberg, E. Johansson and K. Johansson, *J. Chromatogr.*, 211 (1981) 201.
10 M. Otto and W. Wegscheider, *J. Chromatogr.*, 258 (1983) 11.
11 M. L. Cotton and G. R. B. Down, *J. Chromatogr.*, 259 (1983) 17.
12 Y. Changhou, J. L. Fasching and P. R. Brown, *8th International Symposium on Column Liquid Chromatography*, New York, 1984, paper 3a19.
13 C. F. Poole and S. A. Schuette, *Contemporary Practice of Chromatography*, Elsevier, Amsterdam, 1984, p. 343.
14 J. L. Glajch, J. J. Kirkland, K. M. Squire and J. M. Minor, *J. Chromatogr.*, 199 (1980) 57.
15 A. P. Goldberg, E. Nowakowska, P. E. Antle and L. R. Snyder, *J. Chromatogr.*, 316 (1984) 241.
16 B. Sachok, R. C. Kong and S. N. Deming, *J. Chromatogr.*, 199 (1980) 317.