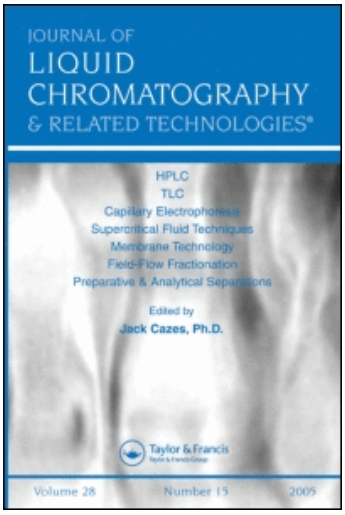


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Optimization of Mobile Phase Composition in Liquid Chromatography—A Survey of Most Commonly Used Chemometric Procedures

Chérie E. Goewie[a]
[a] Laboratory of Organic Chemistry, National Institute of Health and Environmental Hygiene, Bilthoven, BA, The Netherlands

To cite this Article Goewie, Chérie E.(1986) 'Optimization of Mobile Phase Composition in Liquid Chromatography—A Survey of Most Commonly Used Chemometric Procedures', Journal of Liquid Chromatography & Related Technologies, 9: 7, 1431 — 1461
To link to this Article: DOI: 10.1080/01483918608076695
URL: http://dx.doi.org/10.1080/01483918608076695

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(7), 1431-1461 (1986)

OPTIMIZATION OF MOBILE PHASE COMPOSITION IN LIQUID CHROMATOGRAPHY— A SURVEY OF MOST COMMONLY USED CHEMOMETRIC PROCEDURES

Chérie E. Goewie

Laboratory of Organic Chemistry
National Institute of Health and Environmental Hygiene
P. O. Box 1
3720 BA Bilthoven
The Netherlands

ABSTRACT

Chemometrics offers techniques to reduce the number of experiments necessary for obtaining reliable predictions about the optimum conditions for liquid chromatographic separations. This article describes the different chemometric procedures that are currently used for mobile phase optimization. These procedures can be divided in three stages: the selection of the optimization criteria, the choise of the experimental set-up (design) and the evaluation and interpretation of the results. The optimization criteria usually involve resolution (either expressed as α, Rs or P), often analysis time and sometimes column length. The experimental set-up can be either sequential (e.g. simplex algorithm) or simultaneous (e.g. factorial designs). Data can be evaluated either graphically or by mathematical methods. The applicability of the different methods in general and for specific problems is discussed, using examples from the literature.

 0148-3919/86/0907-1431$3.50/0

INTRODUCTION

Every chromatographer frequently encounters the necessity to optimize separations. On the basis of theoretical knowledge and experience a chromatographic system, either normal (NP) or reversed phase (RPLC) ion-pair (IP), ion-exchange (IE) or gelpermeation chromatography (GPC) is selected. How does one proceed? Roughly, the influence of mobile-phase changes can be predicted from the rules given by Snyder and Kirkland (1).

But what if very complicated separations have to be effected; if selectivity has to be changed slightly in order to separate a difficult-to-resolve peak pair or if the mobile phase gets very complicated? Only in a few special cases theory or empirical knowledge are sound enough to enable accurate prediction of retention and selectivity. These problems can be solved either by carrying out a large number of experiments and simply investigating every possible condition, until the optimum is located or by systematically investigating the response at certain conditions and interpolate or extrapolate the optimum values from them. Chemometric techniques can aid in systematically solving optimization problems. During the last decade several different procedures have been developed to attack the mobile-phase optimization problem in liquid chromatography. Some of them have even been incorporated as software into LC equipment and often allow unattended optimization to be carried out. Most of these procedures are based on chemometric optimization strategies.

The strategies used in the different published procedures are outlined in this paper, together with an evaluation of their merits and drawbacks.

Chemometric techniques can be used to optimize systems that are complete 'black boxes' but also to fit data in some theoretical model. Both methods are used in LC optimization. Another important differentiation can be made according to the planning of the experiments used for acquiring the data which are needed to base the predic-

tion of the optimal conditions upon. These experiments can be run either sequentially or simultaneously. Which means that planning of the number of experiments and their conditions is done resp. either during the course of the experimental work or according to a preplanned scheme. In both cases, the experimental set-up is determined by stringent rules. This constitutes the difference between chemometric optimization and trial-and-error.
Although the latter, in combination with theoretical insight can be succesful, for more complex separations or systems, adequate chemometric procedures will be more efficient.

Such procedures can be divided in three stages:

1a. Determination of the optimization criterium.
What has to be optimized? Usually this is selectivity (α or Rs) or separation factor (P), sometimes also analysis time.

1b. Which parameters play a role? In this paper we will restrict ourselves to mobile phase optimization. Factors such as type and number and percentage of modifier, pH, concentration of ionpairing reagent etc. have to be considered.

2. Selection of the experimental design. Should one use a sequential or simultaneous design? The choise depends on the number and nature of the parameters involved, the theoretical knowledge of the system and the degree of need of detailed knowledge of the behaviour of the compounds in the system.

3. Selection of the evaluation method. Depending on the above mentioned selected features, the complexity of the solved problem and personal taste, one can choose between representation of the result of the optimization as a single figure, a mathematical function and/or a graphical representation.

The most important literature procedures are given in Table I, together with a classification of each of their (three) stages.

OPTIMIZATION CRITERIA

Generally, the parameter to be optimized is resolution. Resolution can either be defined as the selectivity factor, α:

$$\alpha = k_2/k_1 \qquad [1]$$

with k_i= capacity factor ($k = (Vr-Vo)/Vo$) for compound i, or as

$$Rs = \frac{1}{4}\,\frac{(\alpha-1)}{\alpha}\,(\sqrt{N})\,\left(\frac{k}{1+k}\right) \qquad [2]$$

The selectivity factor, α, in itself is not meaningful without knowledge of the column efficiency. Rs, in contrast, gives an immediate indication about the performance of the actual system.
However, one may argue that the column plate number may easily be adapted afterwards, if this should be necessary to improve the separation.
A major disadvantage of α is that, in contrast to Rs, it does not take into account the value of k. Equal values of α have different meaning in terms of resolution for different k values. The resolution, Rs, is therefore to be preferred.

A comparable case exists with the use of another measure for the separation efficiency, P. The peak separation factor, P, is defined as

$$P = f/g \qquad [3]$$

where f an g are defined as indicated in Fig. 1. This parameter is, contrary to the situation with α and Rs, dependent on the relative heights of the adjacent peaks and also on peak shape. P deteriorates quickly when $Rs < 1.0$ and when the relative peak heights ratio becomes > 10 (2).
P-values reach the value zero much quicker than Rs, ($P = 0$ for $Rs < 0.4$) and are therefore useful in a more limited range.
Further, P-values are only valid for the specific column on which they are measured.

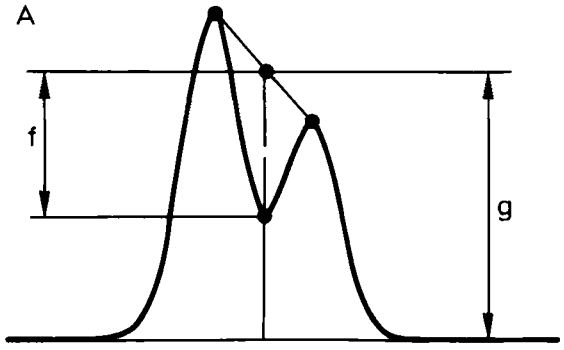


Fig. 1. Definition of the peak separation factor P = f/g. Taken from Ref. 2.

Since P also reflects detectability, this criterium is an excellent choice for systems where the peak ratios are relatively constant. Such situations are encountered in quality control and in trace-level analysis when a relatively constant minor amount of analyte has to be separated from bulk components. For most general cases, however, the use of Rs is to be preferred.

The simplest method to determine optimal LC conditions, which, for clarity, is applicable to systems with preferably a small number of solutes and variables, is the graphical window-diagram method (WD), introduced by Laub and Purnell (3). The method can be applied to systems where (approximately) linear relationships between retention data and mobile phase parameter(s) exist. An example is given in Fig. 2. Here, from plots of lnk vs ϕ, plots of α vs ϕ are calculated by linear interpolation of a limited number of measured k-values.
The α vs ϕ plots can either be calculated manually or by computer. Then the minimal allowable value for α (or Rs) is established.
In the ln k vs α (or Rs) plots, the regions of α (Rs)-values which are attainable in practice are indicated (shaded "windows"). The window with the highest value of α (Rs) (and also the most favorable range of capacity factors), is now selected as the optimum. In Fig. 2 this condition is fulfilled for ϕ = 0.12.

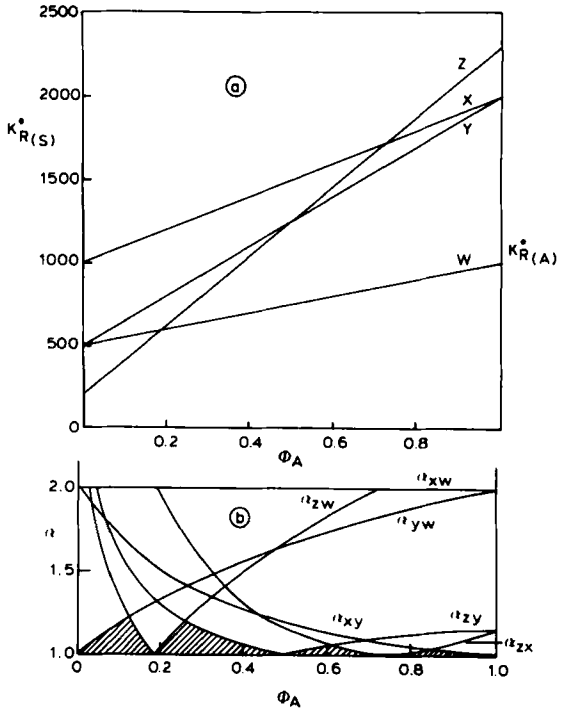
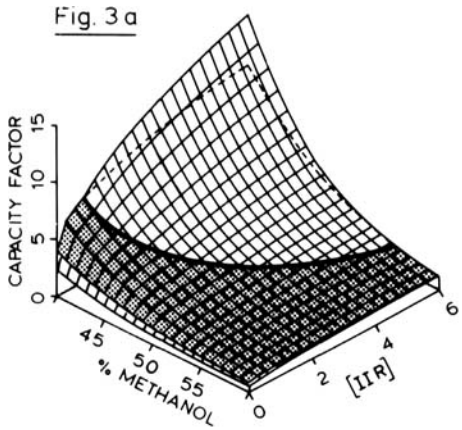


Fig. 2a. Graphical presentation of the relationship between the capacity factors (k) for four hypothetical solutes W, X, Y and Z and the mobile phase composition, ϕ_a.

2b. Plots of α, calculated from fig. 2a, vs. ϕ_a. The minimum α-values for each peak pair are indicated as shaded windows. The highest window, at $\phi_a = 0.12$, represents the optimum. Taken from Ref. 3.
Courtesy of Friedr. Vieweg & Sohn, Wiesbaden.

Minimum α (or Rs) plots can also be used in multifactor optimization problems, such as ion-pair chromatography.
This method is outlined by Sachok et al. (4). From plots of k vs % modifier and ionic strength (IIR) for all solutes (Fig. 3a), the least separated pair at each mobile phase composition is determined. From these data a pseudo three-dimensional minimum α plot is created, as demonstrated in Fig. 3b.



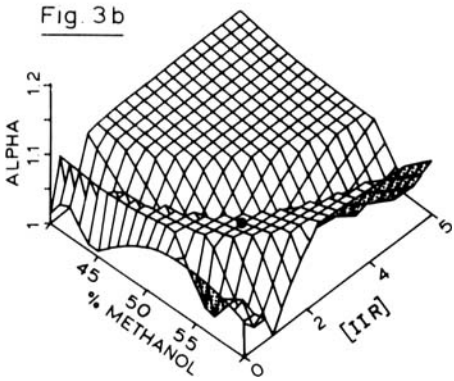


Fig. 3a. Two-dimensional plot of the capacity factors for two analytes (shaded and non-shaded regions) vs. two mobile phase components, % methanol and concentration ion interaction reagent (IIR).

3b. Minimal plot for 5 analytes (substituted anilines) amongst which the two analytes from Fig. 3a, against two mobile phase components. The MAP shows the worst separated pair of peaks at each composition. Valley: complete peak overlap for one pair of analytes. Dot: highest point of the surface and therefore optimal mobile phase composition.
Taken from Ref. 4.
Courtesy of the American Chemical Society, Washington.

The method of minimum α or resolution plots is a fast and simple one, for which, in the two-dimensional case, no micro-computer is needed.

The fact that only the least-resolved peak-pair is considered, however, is a disadvantage, since the overall chromatogram may look just as bad, while other conditions may be possible where one peak-pair is only partly resolved but all others are separated well.

This disadvantage is partly overcome with response functions. A response function reflects the sum or product of all resolutions or separation factors considered in one chromatogram. The simplest response function is:

$$CRF_1 = \sum_{i=1}^{k} \ln P_i = \ln \prod_{i} P_i \qquad [4]$$

This is the multicomponent extension of the peak separation number, P. The latter was defined by Kaiser (5) in analogy to the informing power in spectroscopy, which also contains a resolution factor, and is based on information theory. Just as is the situation in the two component case, different optimization criteria can be used for the multicomponent cases. Most response functions used in optimization procedures are either based on the peak separation factor, P, or the resolution, Rs. Glajch et al. (6), for instance, use a so called 'chromatographic optimization function', which is based on the sum of ln Rs-values (see Table I). The use of sums or products of resolutions (functions of Rs or P) means the reduction of a chromatogram to a single figure. Such a figure is certainly practical, but does not always give an adequate picture of the actual separation.

The same figure may result from many possible different peak distributions over the chromatogram.

To overcome this problem, Drouen et al. defined the relative resolution product, r, as optimization criterium (7):

$$r = \prod_{i=1}^{n-1} Rs_{i+1,i} / \left(\sum_{i=1}^{n-1} Rs_{i+1,i}\right)/(n-1)^{n-1} \qquad [5]$$

where $Rs_{i+1,i}$ = resolution between peak i and its next neighbour and n = number of peaks.
Their criterium aims at an even distribution of all peaks over the chromatogram, i.e., in terms of information theory, the situation with equal information for each analyte (8).

Wegscheider et al. optimize information rate by incorporating time in criterium [4] :

$$CRF_2 = 1/t_{95} \prod_{i=1}^{m=1} f_i/(g_i + 2n_i) \qquad [6]$$

with: m = total number of analytes, t_{95} = total analysis time and n = noise level (= 2 x amplitude of noise) (9).
This criterium is especially developed to take the detectability of the peaks into account.

Optimization of analysis time should however not take place together with optimization of resolution, as the analysis time can be influenced by other factors than mobile phase composition. These changes - i.e. changing column length, flow rate or particle size - can and should be carried out after optimization of the mobile phase composition.

Optimization criteria such as proposed by Watson and Carr (10), Berridge (11) and Glajch (6) of the general form:

$$CRF_3 = \Sigma f (S_i, S_o) + g (T_1, T_m) \qquad [7]$$

with S_o = minimal allowable peak separation (P_o), or resolution (Rs_o), T_m and T_1 = resp. maximal allowable and actually measured analysis time and f and g = arbitrarily chosen weighing factors, should not be applied.
Apart from the fact that this subjective 'response function' has no information-theoretical meaning, due to the incorporation of analysis time as optimization criterium, it often leads to erroneous results. This is caused by the contradiction of the two incorporated criteria of analysis time and resolution. (Resolution namely, is proportional to

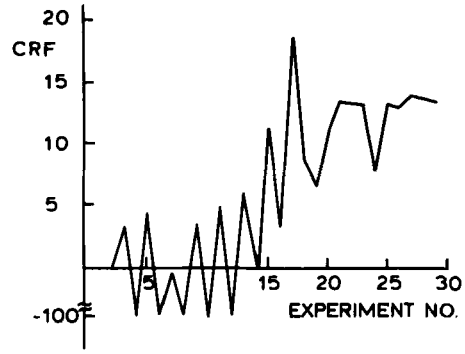


Fig. 4. Progression of a Simplex procedure with CRF2 [7] as response function. The two contradicting boundaries of CRF2, analysis time and peak separation, result in an unnecessarily high number of experiments.
Taken from Ref. 11. Courtesy of Elsevier, Amsterdam.

$\sqrt{N}$ and so to $\sqrt{(L/H)}$, while analysis time is inversely proportional to column length, L.) This contradiction could have easily been overcome by leaving parameters, such as column length, out of the optimization procedure. If criteria such as [7] are used together with sequential optimization methods, an unnecessarily high number of experiments may be the result, as is illustrated in Fig. 4. This figure shows the proceeding of response function [7], used in a 'simplex' optimization procedure. The oscillating figure with high amplitude is caused by repetitive violation of the boundaries set by the criterium, one of them being a too long analysis time.

More response functions than the ones mentioned can be read from Table I and Ref. 11. The serious drawback of all response functions is the loss of individual peak information.
Response functions are generally used in combination with sequential optimization procedures (see next section). This is done because the evaluation of the resulting data, being single figures, can easily be carried out automatically by a microcomputer. In order to overcome

Table I. Classification of some representative mobile phase optimization procedures for liquid chromatography, described in literature.

Optimization criterium	Experimental design	Evaluation method	Application HPLC technique*	Type of analytes	Parameters optimized* Number	Type	First author	Ref.nr.
Minimum α	(3^2) factorial (4^2) factorial	Fitting of theor. model for k + 2 dim. WD	RPIP	2,6-disubst. anilines, amines org. acids	2	% org., [counter-ion]	Sachok	4 41 42
Min. rel. ret. time ratio	Simultaneous	Fitting of theor. model for t_r + WD	RPIS	Weak org. acids	1	pH	Deming	32
Min α	(2^2) factorial	WD	IE	Inorg. anions	2	pH, [eluent]	Jenke	33
Min ret. time ratio	Sequential	WD	RP (tern.)	Polycycl. Aromatics	1	% org.	Issaq	34
Min $S^{1)}$	Simultaneous	WD	RP (tern.)	Arom. acids	1	pH	Jones	35
Min	Univariate	2 dim. WD	RPIP	Phencyclidines	4	Bonded phase, type + % org., type + [counter-ion]	Jones	36
$P_{inf}{}^{2)}$	Seq. Simplex	Iso-response lines	IE	Inorg. cations	2	% org., [counter-ion]	Smits	15
CRF_2	Seq. Simplex	3 dim. response surface	RPIP	Iodinated amino-acids + dipeptides	3	pH, % org., [buffer]	Wegscheider	9
Separation time & column length	Seq. Simplex vs. factorial	3 dim. response surface		Nucleotides	3	% org., pH, [buffer]	Svoboda	25

(continued)

Table I.

Continued.

Optimization criterium	Experimental design	Evaluation method	Application HPLC technique*	Application Type of analytes	Parameters optimized* Number	Parameters optimized* Type	First author	Ref.nr.
P	(6x3x2) factorial	Fitting of theor. model + response surface + computer. grid search	RPIP	Dipeptides	3	pH, % org., [buffer]	Otto	23
F_{obj}[3])	Seq. Simplex	Highest F_{obj} value	NP	Cartenoids	3	%'s org.	Kester	37
CRF_3	Seq. Simplex	Highest CRF value	RP (isocr. + gradient)	PTH amino acids, antioxidants, phenols	5 3	Type + % org., pH, gradient shape % org.	Watson Berridge	10 11
CRF_4[4])	Seq. Simplex	Highest CRF value	RP (bin. & tern.)	Subst. pyridines	3	% org., flow	Berridge	11
Ks, r	Sequential, based on semi-empirical relationships	Rs or r value	RP (tern.)	PTH amino acids	2	% + type org.	Schoenmakers Drouen	14 7
			RPIP	Org. acids; Cat. amines	2	% + type org., pH	Haddad; Billiet	15, 18
			IE	Inorg. anions	1	[eluent]	Haddad	38
Rs	Sequential, based on semi-empirical relationships	Graphical procedure (ORM)	RP (tern.)	Aromatics	2	% + type org.	Colin	19
COF	Simplex lattice	1) highest COF value	RP (tern.)	Subst. naphtalenes	2	% + type org.	Glajch	6
		2) ORM	NP (tern.)	Steroids	2	% + type org.		39
			IP	Drugs, anilines	4	% + type org., pH, [counter-ion]	Goldberg	40

Rs	Scouting seq. Simplex + Simplex lattice	Fitting of linear regression model + computerized non-linear programming	RP (tern.)	Saccharin, caffein + benzoic acid	2	% + type org.	Weyland	30
k, Rs	(4^2) factorial	Fitting of quadratic regression model + response surface contour plots + ORM	RPIP	Alkaloids	4	% org., pH, [buffer], [counter-ion]	Lindberg	22
COC[5])	Simplex lattice	Fitting of 6^{th} degree/polynome	RP (quatern & gradient NP	Chatechol oestrogens, corticosteroids	4	% + type org.	D'Agostino	43

* Abbreviations: RP = reversed phase, IP = ion-pair, IS = ion suppression, IE = ion exchange, NP = normal phase, bin = binary, tern = ternary mobile phase system, isocr. = isocratic elution, org. = organic modifier, [x] = concentration of x.

1) $S = 2Rs/\sqrt{n}$, with N = plate number of the column.

2) $P_{inf} = 1/t_{95} \sum_{i}^{2} \log \left[1/(\Omega_{i-1} + \Omega_{i+1})\right]$ with Ω = relative peakoverlap and t_{95} = analysis time.

3) $F_{obj} = \sum_{i=1}^{n-1} \left[10\ (1.5-Rs)\right]^2$.

4) $CRF_4 = \sum_{i=1}^{1} Rs_i + L^x - a/T_m - T_1/ - b\ (T_o - T_1)$

with x, a and b = arbitrary weighing factors, T_o = min. and T_m = max. anal. time and T_1 = elution time of last peak.

5) $COC = \sum_{i=1}^{n} i \neq j \sum_{j=1}^{n-1} A_i \cdot A_j \cdot \ln \left(\frac{Rs_{i,j}}{Rs_{i,jd}}\right) + \sum_{j=1}^{n} B \cdot \sqrt{A_i} \cdot \frac{T_m - T_i}{T_m}$

mathematical or graphical impossibilities and inconveniences, which may occur with all response functions when peaks show strong overlap or become more than baseline-resolved, constraints or corrections have to be incorporated into the computerprogram.
In a comparitive study, Debets et al. tested the criteria from Table I plus some additional, rarely used, ones (12).
They concluded that all these response functions lead to similar results. These authors point out two serious drawbacks of the use of reponse functions in automated sequential optimization. First, the responses change sharply when the number of detected peak maxima change and secondly, without prior information on the number of peaks most criteria do not give an optimal response when all peaks are baseline-resolved. Finally, all criteria give intractable responses when the elution order of peaks in a chromatogram changes. The latter problem can only be overcome by using additional information, e.g. from diode-array UV detectors, on peak identity (13).

EXPERIMENTAL DESIGN AND DATA EVALUATION

Two types of formal experimental design can be distinguished: sequential and simultaneous design. Theoretically the nature of the design, optimization criterium and the type of data evaluation are isolated entities. In practice, however, certain combinations are more efficaceous than others.
In mobile phase optimization, the following combinations are most frequently encountered: sequential design + response functions and simultaneous design + window diagrams (minimum resolution plots) or simultaneous design + response surfaces followed by either graphical or mathematical search of the optimum on the response surface (see also Table I).
Because of the existing connections between certain designs and data evaluation methods, they are treated together in this section.

The most popular sequential technique is Simplex design (14). This design is applicable to processes which are influenced by continuous variables.

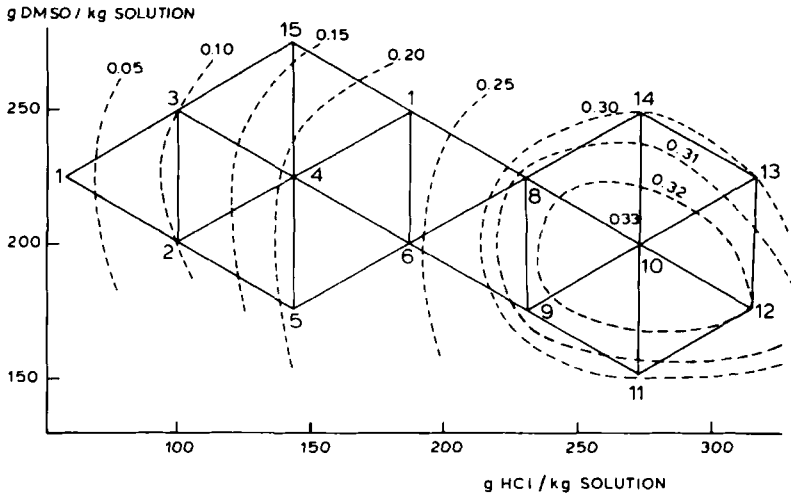


Fig. 5. Progression of a Simplex procedure. 1-9: Experiment number; dotted lines: iso-response curves. The indicated numbers 0.05, 0.30 etc. are response values. Further explanation: see text. Taken from Ref. 15. Courtesy of Springer Verlag, Berlin.

The starting conditions and parameters involved are selected from theoretical considerations and/or preliminary experiments. Further, the system to be optimized is treated as a black box. A 'Simplex' is a geometrical figure which is defined by the number of parameters (dimensions) involved. A two-dimensional Simplex is a triangle, a three-dimensional one a tetrahedron, etc. Fig. 5 gives an example of a two-dimensional Simplex. The example is taken from Ref. 15 and concerns the cation exchange separation of some inorganic ions.
Two mobile phase parameters, concentration DMSO and HCl, are being varied. The degree of separation is being optimized expressed as peak overlap, using response function P_{inf} (see Table I).
Identical values of P_{inf} in space, are indicated by dotted (iso-response) lines. They are unknown at the beginning of the procedure. The procedure is started with three experiments, arranged in a trian-

gle with starting points 1, 2 and 3. The responses of these measurements are evaluated and a 4th experiment is carried out with conditions determined by inflecting the coordinates of the point in the initial triangle with lowest response (here: point 1).

It is hereby assumed that the optimum will be situated in a direction opposite the point with lowest response. This procedure is repeated until an optimum is located.

Several rules are formulated for the case the direction of the Simplex movement needs adaptation and for the situation when the optimum has been passed (point 11).

For example in Simplex no. 8 (see Fig. 5) consisting of points 8, 9 and 10, 8 is rejected and replaced by 11. The latter point has the lowest P_{inf} in Simplex no. 9.

There is then no sense in replacing point 11 by its mirror image, point 8, since P_{inf} has already been determined for the latter. Instead, one rejects the second lowest point (here point 9) and takes its mirror image point (point 12) to form the new Simplex.

When these procedures have been carried out a certain number of times, it is found that one point is consistently retained. When it has been ascertained that all the points forming Simplexes around it, yield a lower response, one can conclude that the point with highest P_{inf} has been reached. Other possible modifications of the standard procedure are the contraction and expansion of the step widths.

Three major disadvantages of Simplex procedures are : 1) the relationship between the factor to be optimized and the parameters involved is seldomly revealed in detail: the procedure therefore does not lead to a better understanding of the separation process; 2) a local optimum may be found; the optimization process stops there; 3) opportunities to make clever use of previously acquired knowledge about the system involved are not seized.

Although some authors promote Simplex procedures for LC automation, their work often reflects the drawbacks of this procedure.

With Simplex many runs are often necessary and the optimum found is often not the best one possible. The procedure therefore always has to be repeated from another starting point. Simplex optimization pro-

cedures are the method of choice when many parameters have to be optimized and no theoretical or semi-empirical knowledge about the system is available. This situation is seldom encountered in chromatography. The popularity of sequential simplex procedures for chromatography is therefore hard to rationalize.

Another sequential procedure described in literature is a semi-empirical one, developed by Schoenmakers and Drouen et al. (7, 13, 14, 16-18).
Starting with a scouting gradient run comparable to the one described by Snyder and Kirkland (1), optimal binary isocratic conditions are derived, for acquiring retention within a selected range of capacity factors (usually $1 < k < 10$, as outside this range little extra resolution can be gained, due to excessive bandspreading.

From semi-empirical relationships iso-eluotropic mixtures with other modifiers are calculated. For instance, with RPLC one may apply the empirical relationships

$$\phi_{ACN} = 0.32\ \phi_M^2 + 0.57\ \phi_M \qquad [8]$$

and

$$\phi_{THF} = 0.66\ \phi_M \qquad [9]$$

in which ϕ = the mobile phase volume fraction of modifier and ACN, M and THF are respectively acetonitrile, methanol and tetrahydrofuran. If runs with those mixtures show a different elution order for some difficult to separate peak-pair, a ternary (or even quaternary) mobile phase can help to increase resolution. The composition of the ternary phase is selected by making use of the approximately lineair relationship of ln k with the ternary mobile phase composition (c.f. Fig. 6). When large deviations from linearity occur, the procedure has to be repeated in a smaller section of the parameter space. Another solution is to slightly shift the calculated composition. Both methods are illustrated in Ref. 7.

Drouen et al. use a microcomputer to calculate and visualize the reponse surface. Colin et al. (19) developed an optimization procedure

very similar to the one of Schoenmakers et al. (14), except for the evaluation of the measured data. Their procedure is a graphical one, which can easily be carried out manually.

With this method it is not necessary to plot α or Rs against the mobile phase composition, since the graphs of k vs ϕ are used directly. For a given column and peak, log α is calculated as a function of plate number, capacity factor, k, and minimally allowed resolution, Rs, via the equation:

$$\log \alpha_{min} = \log k - \log \left[k \left(1 - \frac{4Rs}{\sqrt{N}}\right) - \frac{4Rs}{\sqrt{N}} \right] \qquad [10]$$

For each k it is now known which log α is minimally necessary to obtain a certain resolution and therefore in the log k plots, "critical bands" can be drawn. Zones where critical bands are overlapping are forbidden. Outside these zones one can choose a mobile phase composition, which, e.g., gives minimal k-values. In Fig. 6 this composition is at ϕ = 0.70, being 0.30 (50% MeOH) + 0.70 (40% ACN) + rest water or MeOH : ACN : H_2O = 15 : 28 : 43.
It should be stressed that the ease of (manual) operation of this graphical procedure depends on the linearity of the interpolated log k vs ϕ plots. Colin et al. have shown that the linearity of the plots, in the case of ternary mobile phases, is dependent on the value of the column dead volume, Vo, used to calculate k. These authors recommend the use of D_2O as a dead volume marker for this purpose.

The advantage of the methods which are based on the use of isoeluotropic mobile phases is the fact that the range of capacity factors is constant and optimal. Small changes in capacity factors in the range $1 < k < 10$, result in large changes in Rs. The constantness of separation time makes it relatively easy to carry out the experiments automatically. Drouen et al. point out another important advantage of their method: compared to simplex procedures and full factorial design, the optimum is generally reached after a very small number of experiments, namely five or six. The disadvantages of both methods are the necessity of individual peak identification and the fact that a

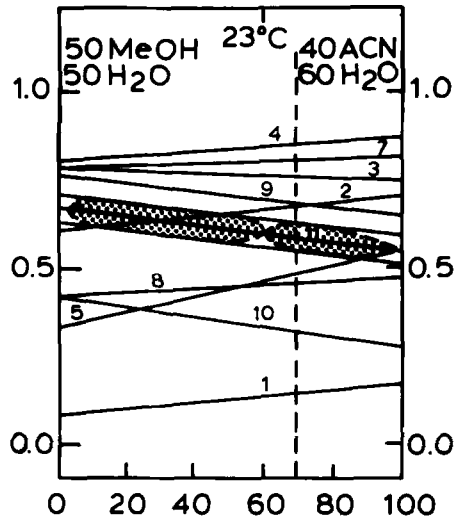


Fig. 6. Optimization diagram for a single solute (11) in a complex mixture, using a ternary mobile phase RPLC system. Starting from two approximately iso-eluotropic binary mobile phase compositions (here: methanol-water (50:50) and acetonitrile-water (40:60)) linear log k vs ϕ plots are drawn. ϕ represents the volume fraction of both binaries. Around the line indicating k_{11}, a 'critical band' is drawn (for explanation: see text). Zones where k-lines overlap the critical band are forbidden. The optimal mobile phase composition is read from the plot: $\phi = 0.70$. Taken from Ref. 19. Courtesy of Friedr. Vieweg & Sohn, Wiesbaden.

local optimum may be derived because only a part of the available parameter space is searched.

Among the simultaneous designs, different types of factorial design are frequently employed in chromatography.
With full factorial design the experiments are carried out under conditions which are determined by the symmetrical arrangement of the

variables in a factor space. If M variables are involved, and each variable is given V-values, the design is an M-factor, V-level one (V^M-design).

Fig. 7 gives a schematical representation of a 2^3 full factorial orthogonal design, where the variables create a three-dimensional (cubic) space.

In this case 8 experiments are necessary to locate an optimum. Because of the generally high number of experiments necessary for full factorial design, a series of initial, scouting, experiments is generally carried out to approach the region of the optimum, prior to the use of the factorial design. For this purpose, sequential simplex is often used.

The experimental data from the factorial design are used to create a response surface, using a chromatographic theoretical model or a regression model. From visual or mathematical inspection of the response surface, the optimum is obtained. If necessary, this optimum may serve as the starting point for a new optimization scheme. More detailed information about factorial design can be found in Ref. 20 and in textbooks on chemometrics, e.g. Ref. 21.

Full factorial design has succesfully been applied, amongst others, to the optimization of reversed-phase ion-pair chromatography (4, 22).

Sachok et al. (4) have fitted the results of a 3^2 design into a semi-empirical model, describing k as a function of percentage of modifier and of the concentration of ion-interaction reagent. Non-linear least-squares techniques were used for curve-fitting. The interpretation of the resulting response surfaces, by means of two-factor minimum plots has been outlined above (see Fig. 3).

Another graphical presentation to trace the optimum is the plotting of overlapping iso-response lines. This procedure was used by Lindberg et al. (22). These authors used a factorial design, first to select the parameters which had the largest effect on the k values of their analytes and than proceeded with a reduced number of parameters, namely 2. Capacity factors were plotted, for each analyte, in a plane as iso-response curves, which were a function of both parameters. This is demonstrated in Fig. 8 for two analytes. The optimization is aimed

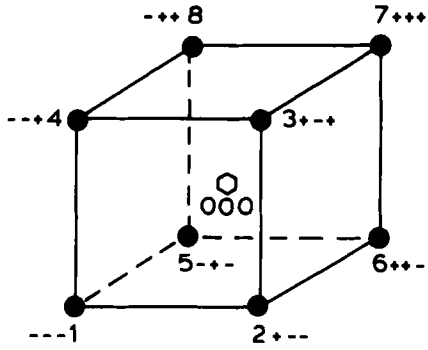


Fig. 7. Factor space of a 2^3 full factorial (orthogonal) design. The experiment has 3 factors (x_1, x_2, x_3) and two levels.

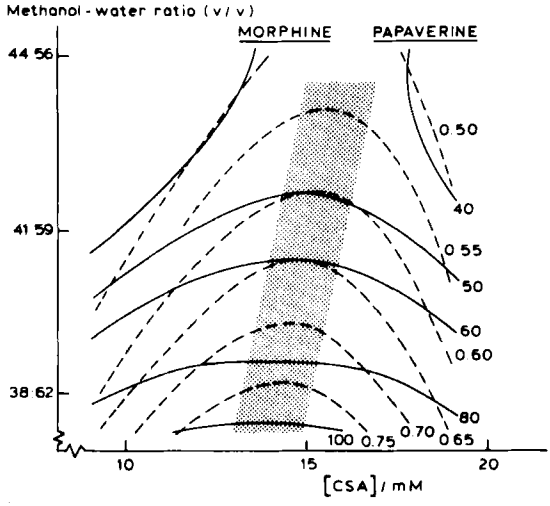


Fig. 8. Graphical representation of the iso-response surfaces created by the capacity factors for the analytes morphine and papaverine in ion-pair chromatography, as a function of the methanol-water ratio and ion-pairing reagent concentration CSA . The shaded region, with overlapping k-values, is forbidden.

Taken from Ref. 22. Courtesy of Elsevier, Amsterdam.

at low values of the response surface for the strongly retained papaverine and high values of this parameter for the weakly retained morphine. In the plot a region can be found where optimal response surfaces of the capacity factors of both analytes coincide (shaded region). In this region the optimum is located.

The optimum region consists of an upper part, where k values are low and therefore analysistime is short and a lower part, where resolution is high and accurate measurements are favoured.

This graphical procedure is especially useful if the number of analytes involved is relatively small.

Otto and Wegscheider have used the results of a 6 x 3 x 2 factorial design to obtain the coefficients of rather complicated, non-linear semi-empirical relationships, describing the retention of diprotic acids as a function of pH, organic modifier content and ionic strength (23). Visual inspection of computer-generated two-dimensional alpha plots did not reveal a global optimum for the optimization problem at hand; therefore a computerized mathematical technique, grid search, was undertaken. The latter is a standard routine for searching factor space, using least-squares fitting of arbitrary functions (24).

Svoboda (25) compared the optimization of reversed phase ion-pair chromatography of nucleotides by using factorial design and the Simplex algorithm. His experimental data were fitted into quadratic regression equations, describing k as a function of pH, volume fraction of organic modefier and ionic strength of the mobile phase.

The optimization criterium was analysis time, which was restricted by two boundary conditions: a minimum resolution of 4 and a maximally allowable column length of 1,000 mm. The author concluded that Simplex search, using 2 or more different starting conditions, was to be preferred over factorial design, as it presented the highest chance to find the global maximum in the least number of steps. This conclusion, however, is highly dependent on coincidence (i.e. the starting conditions). The observed laboriousness of full factorial design could already have been derived from theory (Table 2).

About Svoboda's criterium one can remark that, as it is reduced to a single figure, it leads to very easily interpretable response surfaces (analysis time as a function of the three variables) but, due to the

Table II. Number of experiments minimally needed for a sequential Simplex, full factorial design and simplex lattice design (SLD).

Number of variables	Minimum number of experiments		
(n)	Simplex (n+1)	Fact. design (2^n)	SLD
2	3	4	2
3	4	8	4
5	6	32	16
10	11	1024	512

boundary conditions, which often contradict the optimization criterium, the number of experiments is unnecessarily increased. This is an analogous situation to the one we encountered with the 'response functions' of Watson and Carr (10) and Berridge (11).

In order to diminish the number of experiments in full factorial design, other types of factorial design have been developed. The most useful one for liquid chromatography is Simplex Lattice Design (SLD) (which should not be confused with sequential Simplex) (6).

Since for mixtures applies that the sum of compositions equals unity, the regression models used to describe the response surface can be simplified, and the number of experiments necessary to obtain estimates of the regression coefficients is drastically reduced. Compared to full factorial design, the factor space is reduced by one dimension (see Table 2). A useful design for optimization of ternary mobile phases is quadratic SLD. 6 Experiments, arranged as demonstrated in Fig. 9, are necessary to obtain the optimum, 3 extra added points are used for making an estimation of the reliability of the result. The inter-

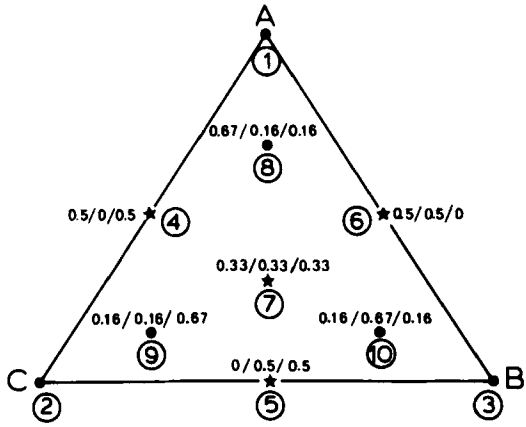


Fig. 9. Configuration of the datapoints in a special cubic Simplex lattice design for a ternary mixture composed of solvents A, B and C, with three extra added datapoints (8, 9 and 10) for estimation of the accuracy of the model.
Taken from Ref. 11. Courtesy of Elsevier, Amsterdam.

pretation of the results can be carried out either visually or by micro-computer.

SLD has been promoted by Du Pont, as it was incorporated in their Sentinel system. The theoretical background of their system is outlined in a number of papers (6, 28, 29). Like with many other currently popular solvent optimization procedures, Sentinel started with a scouting gradient from which optimal isocratic conditions were derived. Ternary mixtures were composed, if necessary, by random mixing of two iso-eluotropic binaries. This latter trial-and-error approach is the weak point in the procedure. As with the Sentinel system the emphasis was on fully automated methods development, problems arose with the interpretation of the data. Glajch has described two methods for interpretation of the data: optimization of a response function and a graphical procedure called Overlapping Resolution Mapping (ORM) (6). The development of the latter procedure stemmed from dissatisfaction with the former.

ORM is based upon measuring and comparing the resolution of every pair of peaks in the chromatogram obtained for each solvent. A resolution contour map is generated for each pair of compounds (see Fig. 10) for estimating the resolution for that pair in all solvent compositions within a selected solvent triangle. A desired resolution for each (or any) pair of compounds is then selected. Any portion of the solvent triangle that has a resolution exceeding the desired minimum value represents a region of solvents of interest for separating that particular pair.
By overlapping acceptable regions of separation for all pairs of the solvent triangle, areas identifying particular solvent mixtures can be selected In which the desired resolution can be achieved for all component pairs.

Basically, the ORM method allows the analysis of resolution for all pairs of peaks in the chromatogram, not just for adjacent pairs. However, for a system with no peak crossovers, only the resolution of adjacent pairs is important for determining an optimum solvent. This method does require that the individual peak position be mapped as solvent composition is changed.

Weyland et al. have combined Simplex lattice design (after a scouting sequential Simplex) with a boundary condition of minimal analysis time (30).
Apart from the drawbacks, of taking analysis time into account, as mentioned earlier, this article shows the applicability of the operations research technique 'linear programming' to mobile phase optimization. This, and other mathematical techniques for optimizing a constraint mixture response surface had already been proven useful in other fields of application, as reviewed by Snee (31).

CONCLUSIONS

As can be seen from Table I, many different combinations of chemometric techniques can be and have been applied for the optimization

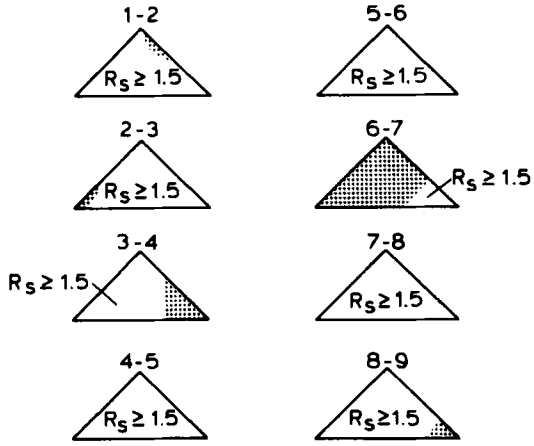


Fig. 10. Resolution maps for eight peak pairs (9 solutes) in the factor space of a Simplex lattice design. The forbidden regions where Rs is smaller than the minimally allowed value, 1.5, are shaded. Taken from Ref. 6. Courtesy of Elsevier, Amsterdam.

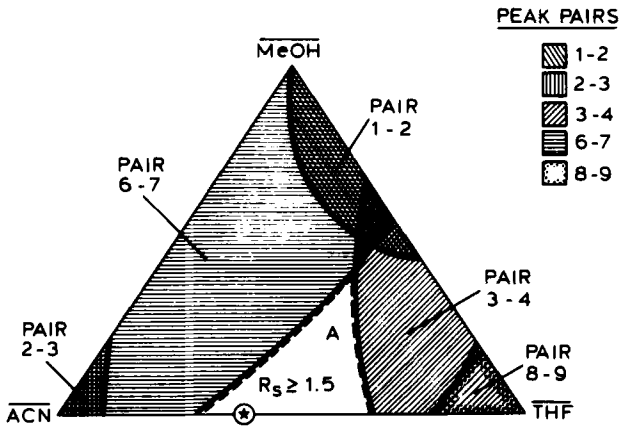


Fig. 10b. Overlapping resolution map (ORM) for all peak pairs from Fig. 10a. In all shaded areas Rs, of at least one peak pair, < 1.5. The white region indicates the optimum. The dotted line represents an estimate of ORM precision.
Taken from Ref. 6. Courtesy of Elsevier, Amsterdam.

of the mobile phase in liquid chromatography. The methods used generally depend on the preference of the researcher.

Only Wegscheider et al. (9), Debets et al. (12), Glajch et al. (6) and Svoboda (25) have made critical comparisons between two or more techniques. In refs. 9 and 12 optimization criteria are carefully tested, while ref. 25 addresses the choice between sequential Simplex and full factorial design. As for the selection of the optimization criterion, Wegscheider et al. show that criteria based on the peak separation factor, P, are the only ones that take peak detectability into account. However, for most practical problems, criteria based on Rs values are the better choice, since they are independent of the performance of the particular system and test sample composition used and thus more universally applicable.

Criteria which address analysis time simultaneously with resolution are to be circumvented. Analysis time should always be optimized last.

Sequential Simplex optimization methods are only useful when a large number of continuous parameters is involved and no semi-empirical or theoretical knowledge of the system to be optimized is available. This is seldom the case in chromatography. A particular, dedicated sequential design (7, 13, 16-18) and Simplex lattice design are to be preferred over sequential Simplex for application in liquid chromatography. By making use of some generally observed semi-empirical relationships, the number of experiments can usually be limited to 5-10, which is much lower than with sequential Simplex.

As for the evaluation of the measured data no general rules can be given. The use of response functions and minimal resolution plots, which express the result of the optimization procedure as a single figure or surface, lead to a gross neglection of otherwise valuable information about the individual peaks.

Overlapping resolution maps (ORM) are only useful if the number of analytes is limited, otherwise the plots become unreadable.

The best method probably is to get a quick overview of the results by looking at the response function or minimum resolution plot, followed by a closer visual inspection of the individual chromatograms. This is of course less suitable for fully automated optimization. In that case

the search of response surfaces by mathematical curve fitting procedures can be succesfully employed.
When using theoretical models and interpolation or regression techniques, to create or calculate response surfaces, one should however always bear in mind the assumptions that are made. Otherwise the results may be erroneous and useless.

Unattended solvent optimization has proven feasible, especially for not too difficult separation problems. The many pitfalls that may be encountered, however, prove that the human chromatographer has not yet become superfluous.

REFERENCES

1. L.R. Snyder and J.J. Kirkland, Introduction to Modern Liquid Chromatography, Wiley, New York, 1979.

2. W. Wegscheider, E.P. Lankmayr and M. Otto, Anal. Chim. Acta, 150, 87 (1983).

3. R.J. Laub and J.H. Purnell, J. Chromatogr. 112, 71 (1975).

4. B. Sachok, J.J. Stranahan and S.N. Denning, Anal. Chem. 53, 70 (1981).

5. H. Kaiser, Anal. Chem. 42, 24A (1970).

6. J.L. Glajch, J.J. Kirkland, K.M. Squire and J.M. Minor, J. Chromatogr. 199, 57 (1980).

7. A.C.J.H. Drouen, H.A.H. Billiet, P.J. Schoenmakers and L. de Galan, Chromatographia, 16, 48 (1982).

8. L. Brillouin, Science and Information Theory, Academic Press, New York, 1962, p. 11.

9. W. Wegscheider, E.P. Lankmayr and K.W. Budna, Chromatographia 15, 498 (1982).

10. M.W. Watson and P.W. Carr, Anal. Chem. 51, 1835 (1979).

11. J.C. Berridge, J. Chromatogr. 244, 1 (1982).

12. H.J.G. Debets, B.L. Bajema and D.A. Doornbos, Anal. Chim. Acta, 151, 131 (1983).

13. A.C.J.H. Drouen, H.A.H. Billiet and L. de Galan, Anal. Chem. 56, 971 (1984).

14. S.N. Deming and S.L. Morgan, Anal. Chem. 45, 278A (1973).

15. R. Smits, C. Vanroelen and D.L. Massart, Z. Anal. Chem. 273, 1 (1975).

16. P.J. Schoenmakers, A.C.J.H. Drouen, H.A.H. Billiet and L. de Galan, Chromatographia 15, 680 (1982).

17. P.R. Haddad, A.C.J.H. Drouen, H.A.H. Billiet and L. de Galan, J. Chromatogr. 282, 71 (1983).

18. H.A.H. Billiet, A.C.J.H. Drouen and L. de Galan, J. Chromatogr. 316 (1984) 231.

19. H. Colin, A. Krstulovic, G. Guiochou and J.P. Bounine, Chromatographia 17, 209 (1983).

20. O.L. Davies, ed., The Design and Analysis of Industrial Experiments, Oliver and Boyd, Edinburgh, 1956, Chapter 7.

21. D.L. Massart, A. Dijkstra and L. Kaufman, Evaluation and Optimization of Laboratory Methods and Analytical Procedures,

Elsevier, Amsterdam, 1978. Part II: Experimental Optimization pp. 213-300.

22. W. Lindberg, E. Johansson and K. Johansson, J. Chromatogr. 211, 201 (1981).

23. M. Otto and W. Wegscheider, J. Chromatogr. 258, 11 (1983).

24. P.R. Bevington, Data Reduction and Error Analysis for the Physical Sciences, McGraw-Hill, New York, 1969, pp. 208-215.

25. V. Svoboda, J. Chromatogr. 201, 241 (1980).

26. J.W. Gorman and J.E. Henman, Technometrics 4, 463 (1962).

27. J.L. Glajch, J.J. Kirkland and L.R. Snyder, J. Chromatogr. 238, 269 (1982).

28. L.R. Snyder and J.L. Glajch, J. Chromatogr. 214, 1 (1981).

29. J.L. Glajch and L.R. Snyder, J. Chromatogr. 214, 21 (1981).

30. J.W. Weyland, H. Rolink and D.A. Doornbos, J. Chromatogr. 247, 22 (1982).

31. R.D. Snee, Chem. Tech. 9, 702 (1979).

32. S.N. Deming and M.L.H. Turoff, Anal. Chem. 50, 546 (1978).

33. D.R. Jenke and G.K. Pagenkopf, Anal. Chem. 56, 85 (1984).

34. H.J. Issaq, G.M. Muschik and G.M. Janini, J. Liq. Chromatogr. 6, 259 (1983).

35. P. Jones and A. Wellington, J. Chromatogr. 213, 357 (1981).

36. L.A. Jones, R.W. Beaver and T.L. Schmoeger, Anal. Chem. 54, 182 (1982).

37. A.S. Kester and R.E. Thompson, J. Chromatogr. Biomed. Applic. 310, 372 (1984).

38. P.R. Haddad and C.E. Cowie, J. Chromatogr. 303, 321 (1984).

39. P.E. Antle, Chromatographia 15, 277 (1982).

40. A.P. Goldberg, E. Nowakowski, P.E. Antle and L.R. Snyder, J. Chromatogr. 316, 241 (1984).

41. R.C. Kong, B. Sachok and S.N. Deming, J. Chromatogr. 199, 307 (1980).

42. B. Sachok, R.C. Kong and S.N. Deming, J. Chromatogr. 199, 307 (1980).

43. G. D'Agostino, L. Castagnetta, F. Mitchell and M.J. O'Hare, J. Chromatogr. Biomed. Applic. 338, 1 (1985).