Journal of Chromatography, 389 (1987) 351–367
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 219

APPLICATION OF A MIXTURE DESIGN TECHNIQUE TO THE SIMULTANEOUS OPTIMIZATION OF ANALYSIS TIME AND RESOLUTION IN REVERSED-PHASE ION-PAIR LIQUID CHROMATOGRAPHY USING A MINIMAL RESOLUTION PLOT

P. M. J. COENEGRACHT*, NGUYEN VAN TUYEN, H. J. METTING and P. J. M. COENEGRACHT-LAMERS

Research Group Chemometrics, Pharmaceutical Laboratories, Ant. Deusinglaan 2, 9713 AW Groningen (The Netherlands)

(First received January 14th, 1986; revised manuscript received October 30th, 1986)

SUMMARY

Based on a simple model, the influence of the experimental parameters on solvent strength and selectivity in reversed-phase ion-pair high-performance liquid chromatography is discussed. For optimization of the separation, a mixture design is proposed in which the pH, the content of organic modifier and the concentration of the pairing ion in the mobile phase vary. This design was applied to the separation of two mixtures of four pharmaceutically important amines (water-soluble vitamins and local anaesthetics). By a suitable choice of the three (pseudo) components, which are mixed to compose the mobile phase, it is possible to optimize the resolution of the worst separated pair of peaks and to locate in the optimal region the chromatogram with the minimal analysis time.

THEORY

Reversed-phase ion-pair chromatography

A unified theory for reversed-phase ion-pair chromatography (RP-IPC) has not yet been described. Different models currently proposed for a quantitative description of the mechanisms were reviewed recently[1]. In this paper, we adopt the dynamic ion-exchange model, which provides a sound basis for method development[2] and describes adequately the influence of the experimental variables on the retention of organic amines in RP-IPC. The main equilibria considered to be important[1,3,4] are depicted in Fig. 1 and are described by the following expressions:

$$P^- + M^+ + L = P^-M^+L \quad (1)$$

for sorption of the ion-pair reagent P^- and counter ion M^+ on the hydrophobic surface, L, of the column;

$$XH^+ + P^-M^+L = P^-XH^+L + M^+ \quad (2)$$

0021-9673/87/$03.50

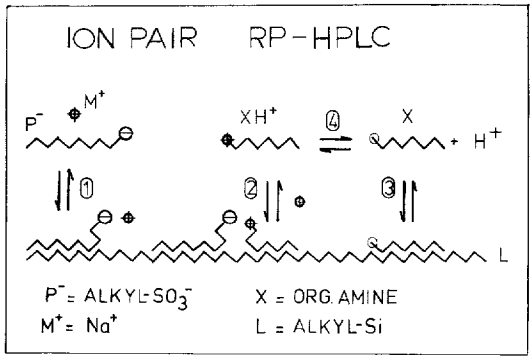

Fig. 1. Schematic presentation of equilibria in the reversed-phase ion-pair liquid chromatography of a cationic solute X^+ and an anionic pairing reagent P^-.

for exchange of the solute ion XH^+ with the counter ion M^+;

$$X + L = XL \tag{3}$$

for sorption of the solute molecule X on the hydrophobic surface of the column; and

$$XH^+ = X + H^+ \tag{4}$$

for dissociation of the solute XH^+.

For simplicity we shall limit the discussion to a positively charged solute (amine) and a negatively charged pairing ion.

According to eqn. 2, the retention of a positively charged solute ion, XH^+, depends on the concentration of the negatively charged pairing ion, P^-, at the hydrophobic surface of the stationary phase, L[3–5]. Higher surface concentrations of P^- lead to longer retention times of XH^+. The surface concentration of P^- increases with increasing mobile phase concentration of P^- (eqn. 1) and with higher hydrophobicity of P^- and the stationary phase L.

Increasing the content of the organic modifier at a constant mobile phase concentration of P^- lowers the surface concentration of P^- and reduces the capacity factor of XH^+[6–9]. A higher concentration of the counter ion, M^+, in the mobile phase increases the surface concentration of P^- (eqn. 1), but reduces the retention of XH^+ according to eqn. 2. The net result of these two counteracting effects is a reduction of the retention of XH^+[3,10,11].

Retention of ionic solutes is governed mainly by ionic interactions (eqn. 2), but by changing the pH of the mobile phase it is possible to bring the solute into a non-ionized form (eqn. 4). Then the interaction between the non-polar moieties of the solute and the non-polar carbon chain of the stationary phase can contribute more to the retention (eqn. 3), and the contribution of ionic interactions to the retention of the solute diminishes. The influence of the pH depends on the pK_a of the individual solute, so the pH of the eluent is the primary factor for controlling the selectivity[12].

Given a constant pH, the type of ion-pairing reagent and the chain length of the stationary phase material, variation of the mobile phase concentration of ion-

pairing reagent, P^-, and of the counter ion introduces only minor selectivity effects for the separation of equally charged amines[3,8,10]; secondary selectivity effects should be invoked by changing the type and/or concentration of the organic modifier[8,9]. Addition of methanol not only lowers the surface concentration of the ion-pair reagent (eqn. 1), but also influences the electrostatic (eqn. 2) and hydrophobic interactions (eqn. 3) in a selective way.

Eqns. 1–4 give a limited description of the phase distribution processes in reversed-phase ion-pair liquid chromatography. It has been shown that the stationary phase contains residual silanol groups[3] with the ability to retain ammonium compounds[13,14], possibly by hydrogen bonding and ion exchange[11]. The silanophilic interactions can contribute to the selectivity of the separation, but can also cause unwanted peak tailing. Therefore, "competing" amines are sometimes added to reduce tailing.

Generalizing, it can be stated that the retention of ionic solutes depends mainly on ionic interactions with adsorbed pairing ions. Because the mobile phase concentration of the pairing ion and the organic modifier both affect the surface concentration of the pairing ion, these variables should have the greatest effect on the capacity factor of the solute ion[15], and should be used for adjusting the mobile phase strength. Selectivity effects should be controlled by varying firstly the pH and secondly the organic modifier content of the eluent.

Optimization strategy

Optimization strategies aim at reaching the global maximum critical value by performing a minimal number of experiments. Optimization methods for high-performance liquid chromatography (HPLC) have been reviewed recently[16–18]. We decided to use a simultaneous optimization technique in which the retention behaviour of the solutes is modelled as a function of the experimental variables. Factorial experimental designs can be used[12,15], but mixture designs[19] are more convenient to use with dependent variables such as the constituents of the mobile phase. Glajch *et al.*[20] introduced in reversed-phase chromatography a ternary mixture design in which the three components at the corners of the triangle are not pure solvents but pseudo-components. The binary pseudo-components are made up of a "selectivity adjusting solvent" (an organic modifier) plus a "strength adjusting solvent" (water) in such a way that all three binary pseudo-components have an identical elution strength. The selectivity is finely tuned by mixing the three pseudo-components. In this approach the analysis time is determined by the elution strength of the pseudo-components and is fixed before the optimization of the separation begins. Goldberg *et al.*[21] applied this strategy to reversed phase ion-pair chromatography. The solvent strength of the pseudo-components was adjusted with methanol and the three "selectivity adjusting solvents" were citric acid buffers of pH 2.5, 7.5 and 5.4. To the last buffer hexanesulphonic acid was added. All buffers also contained triethylamine to suppress peak tailing.

In their approach, the solvent strength of the pseudo-components is adjusted by varying the methanol content and the selectivity by varying the pH and the concentration of the ion-pair reagent. In our opinion, the eluent strength in reversed phase ion-pair chromatography is determined not only by the content of the organic modifier but also by the concentration of ion-pair reagent, P^- (ref. 15).

TABLE I

MOBILE PHASE COMPONENTS

Symbol	*Components*
x_1	Methanol
x_2	Citrate buffer (0.1 *M*, pH 6.5)–sodium octanesulphonate (5 m*M*)
x_3	Citrate buffer (0.1 *M*, pH 3.5)–sodium octanesulphonate (5 m*M*)

As mentioned in the theoretical section, the pH is the primary factor for controlling the selectivity; the type and the concentration of the organic modifier also have a clear significance in this respect, but the concentration of the ion-pair reagent is less important for the separation of closely related solutes with similar charges[8]. The selection of variables is a critical part of an optimization procedure and therefore we propose the use of a ternary mixture design with different (pseudo)components as the basis of a new strategy, which should allow one to adjust the selectivity and the solvent strength of the mobile phase and to optimize simultaneously the separation and the analysis time.

In optimizing the mobile phase composition in reversed-phase ion-pair chromatography, we consider three parameters to be the most important: the concentration of the ion-pairing reagent, the concentration of the organic modifier and the pH. We decided to model the dependence of the retention time on three interdependent mobile phase components, which allow the variation of these parameters simultaneously. The compositions of the three mobile phase components are given in Table I.

The addition of methanol (component x_1) to both pseudo-components introduces selectivity effects by altering the content of the organic modifier, but with the proposed selection of the components the most important effect is a reduction of the retention time of the last peak, t_{max}. The addition of methanol reduces the retention time of every peak by two effects: first by lowering the adsorption of the ion-pair

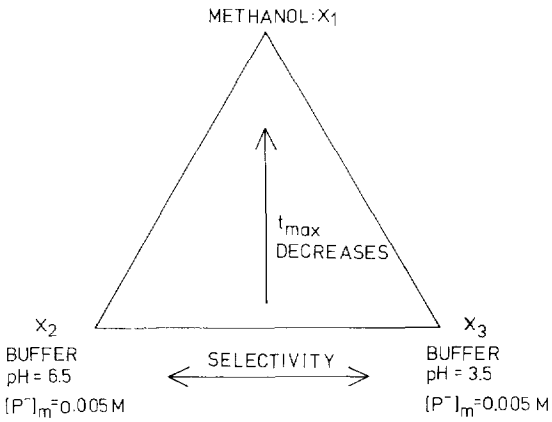

Fig. 2. Mixture design showing the direction in which solvent strength and selectivity vary. $[P^-]_m$ = Mobile phase concentration of sodium octylsulphonate; t_{max} = retention time of the last peak.

reagent, P^-, at the hydrophobic surface, L, and secondly by diminishing the mobile phase concentration of the ion-pair reagent at the same time (eqn. 2). The reduction of the retention time is counteracted by the accompanying decrease in the counter ion, M^+, concentration, but the overall effect should be an increase in solvent strength into the direction of the top of the triangle (Fig. 2).

Mixing pseudo-component x_2 with pseudo-component x_3 provides an approximately linear variation of the pH of the mobile phase[21] and a strong selectivity effect can be expected when the eluent composition changes parallel to the base of the triangle (Fig. 2). The proposed choice of the three (pseudo)components offers the advantage that (from the minimal resolution plot) one can select an acceptable separation that has also the shortest analysis time: optimal resolution is indicated by the plot and the retention time of all components decreases in the direction of the top of the solvent triangle. The reversed-phase ion-pair chromatographic separation of four structurally different amines (water-soluble vitamins) and four structurally similar amines (local anaesthetics) will be used as examples to demonstrate the proposed strategy.

Optimization procedure

The optimization procedure of Goldberg and Nowakowska[21] was based on the mixture design approach described by Glajch *et al.*[20]. Their paper, however, is not very explicit in the description of the procedure and the mathematical model is not given. Moreover, because we planned to present the data in a different way, an outline of our approach is presented below.

We decided to use a special cubic model[22] to fit the logarithm of the capacity factor:

$$\ln k = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3 \quad (5)$$

where x_1, x_2 and x_3 are the volume fractions of the components of the mobile phase.

The proposed optimization procedure encompasses the following steps. Firstly, the feasible region of the factor space is established by preliminary experiments. The feasible region is limited by the minimal and maximal analysis times which one choses to accept. Within the feasible region the retention times, t_{ij}, of every solute i ($i = 1, \ldots, n$) are measured at nine solvent compositions, j (Fig. 3). Nine experiments were performed to make the validation of the model possible. From these measurements an estimate of the coefficients in eqn. 5 is obtained by linear multiple regression. The resulting n equations describe the retention time of every solute as a function of the eluent composition and allow the prediction of all retention times, t_{ij}, at every solvent composition, j, within the feasible region of the factor space. These retention times are predicted at all solvent compositions necessary to obtain a grid with a 1% interval in x_2 and x_3 and a 2% interval in x_1. Next, the resolutions, R_s, are calculated for all adjacent peaks at all points of the grid, where

$$R_s = \sqrt{N}(k_2 - k_1)/2(k_2 + k_1 + 2) \quad (6)$$

where N is the plate number and k_2 and k_1 are the capacity factors of the adjacent

peaks. The minimal value of the $n - 1$ resolutions obtained at every point of the grid, R_s(min), is stored to be plotted after scaling. To obtain a suitable plot of all R_s(min) in the feasible region of the solvent triangle, all values of R_s(min) smaller than a chosen minimal value are represented by a dot; all values between the minimal value and 5 are scaled by the characters *a* to *z* and every R_s(min) greater than 5 is also represented by the *z* character. This representation facilitates a quick and easy location of the optimal region in the plot. The optimal solvent composition corresponds to the point in this region that is closest to the top of the solvent triangle; at this point all resolutions of the corresponding chromatogram are equal to or greater than the maximal R_s(min) and the analysis time, given that maximal R_s(min), is the shortest.

This minimal resolution plot differs from the overlapping resolution map. The purpose of the ORM technique is to establish an area within which all resolutions of all pairs of peaks are greater than one specified value. Because the minimal resolution plot shows the value of the minimal resolution at all compositions of the mobile phase, this plot provides more information than the overlapping resolution map, if a good separation is the only objective and therefore only the worst separated pair of peaks is of interest.

EXPERIMENTAL

Chemicals

For the mobile phase, mixtures of buffers prepared with deionized water from analytical-reagent grade reagents (Merck, Darmstadt, F.R.G.) and nitrogen flushed methanol (Janssen, Beerse, Belgium) were used. The 0.1 *M* citric acid buffers of pH 3.5 and 6.5 were prepared from citric acid. Sodium octanesulphonate was dissolved in the buffer solutions to a concentration of 5 m*M*. The pH was adjusted with sodium hydroxide solution (after the addition of 20 m*M* triethylamine).

Stock solutions of four vitamins were prepared using degassed buffer of pH 6.5 as the solvent and stored in a refrigerator. Standard solutions were prepared immediately before use and contained 10 μg/ml of thiamine hydrochloride (vitamin B_1), riboflavin (vitamin B_2) and nicotinamide (vitamin B_3) and 30 μg/ml of pyridoxine hydrochloride (vitamin B_6). A standard mixture was prepared by mixing equal amounts of the standard solutions.

Stock solutions of four local anaesthetics were prepared in methanol and stored in a refrigerator. Standard solutions were prepared immediately before use by dilution with the mobile phase and contained 40 μg/ml of procaine hydrochloride, procaineamide hydrochloride and ethyl aminobenzoate (benzocaine) and 136 μg/ml of butacaine hydrochloride. A standard mixture was prepared by mixing equal amounts of standard solutions.

Vitamins, sodium octanesulphonate and triethylamine (TEA) were obtained from Sigma (St. Louis, MO, U.S.A.) and were used as received. The local anaesthetics were of pharmacopoeial grade.

Instrumentation

The HPLC apparatus was assembled from a Model SP740 pump (Spectra-Physics) with a Model 740C pump control and a Model 714 pressure monitor, a

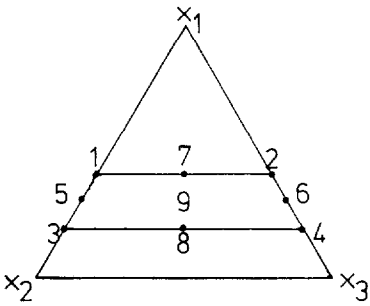

Fig. 3. Mixture design for the separation of vitamins in which the feasible region and measuring points 1–9 in Table II are indicated.

Valco 7000 p.s.i. injection valve, fitted with a 25 μl sample loop, a Model 9202 dual-channel fixed-wavelength UV absorbance detector (254 nm) (Kipp Analytica) and a Model B40 recorder (Kipp Analytica). The column used in the analysis of the vitamins was 15.0 cm × 4.6 mm I.D. stainless-steel tube packed with Nucleosil RP-8, particle size 5 μm; for the analysis of the local anaesthetics a column of the same dimensions was packed with Nucleosil RP-18, particle size 5 μm (see below).

Measurements or retention times were made using a flow-rate of 1.0 ml/min. Calculations were performed on a Apple IIe microcomputer (CP/M) equipped with an Epson FX 80 printer using programs written in Fortran IV.

RESULTS AND DISCUSSION

The mobile phase compositions at which the capacity factors of the vitamins were measured are shown in Fig. 3 and given in Table II together with the capacity factors. For the local anaesthetics a similar design was used for which the nine mobile phase compositions are given in Tables III and IV, again with the capacity factors.

TABLE II

CAPACITY FACTORS OF FOUR VITAMINS MEASURED AT NINE MOBILE PHASE COMPOSITIONS

Mobile phase composition					*Capacity factors of vitamins**			
No.	x_1	: x_2	:	x_3	*A*	*B*	*C*	*D*
1	0.4	: 0.6	:	0.0	1.11	0.89	0.56	0.39
2	0.4	: 0.0	:	0.6	0.49	0.76	0.49	0.35
3	0.2	: 0.8	:	0.0	5.81	5.10	1.30	1.70
4	0.2	: 0.0	:	0.8	5.59	5.05	1.35	1.70
5	0.3	: 0.7	:	0.0	2.00	1.64	0.84	0.46
6	0.3	: 0.0	:	0.7	1.42	1.78	0.81	0.75
7	0.4	: 0.3	:	0.3	0.57	0.86	0.57	0.32
8	0.2	: 0.4	:	0.4	4.80	6.02	1.65	1.09
9	0.3	: 0.35	:	0.35	1.78	2.39	1.00	0.61

* A = vitamin B_1; B = vitamin B_2; C = vitamin B_3; D = vitamin B_6.

TABLE III

CAPACITY FACTORS OF FOUR LOCAL ANAESTHETICS MEASURED AT NINE MOBILE PHASE COMPOSITIONS

Mobile phase composition					*Capacity factors of anaesthetics**			
No.	x_1	: x_2	:	x_3	*A*	*B*	*C*	*D*
1	0.55	: 0.45	:	0.00	1.15	2.16	2.46	8.00
2	0.50	: 0.00	:	0.50	0.62	0.69	2.75	2.73
3	0.45	: 0.55	:	0.00	1.43	2.85	4.07	14.87
4	0.40	: 0.00	:	0.60	1.48	1.97	6.17	11.75
5	0.50	: 0.50	:	0.00	1.07	2.05	2.79	9.61
6	0.45	: 0.00	:	0.55	0.91	1.21	4.29	6.93
7	0.525	: 0.237	:	0.237	0.35	0.44	1.95	1.62
8	0.45	: 0.275	:	0.275	0.57	0.80	3.26	3.30
9	0.50	: 0.25	:	0.25	0.47	0.58	2.35	2.22

* A = procaineamide hydrochloride; B = procaine hydrochloride; C = ethyl aminobenzoate; D = butacaine hydrochloride.

The feasible region of the factor space (Fig. 3) was determined by preliminary experiments with binary mixtures of methanol (x_1) and the pseudo-components x_2 and x_3. The lower boundary of the feasible region (points 3, 8 and 4) was chosen in such a way that the analysis time, which is defined by the retention time of the last eluting peak, was about 15–20 min. This should correspond to a maximum capacity factor of about 10. The upper boundary (points 1, 7 and 2) was determined by the fact that the first eluted solute should not coincide with the solvent front.

The dead time as measured by the solvent front was about 1.8 min for the RP-8 column and about 1.5 min for the RP-18 column. The dead time varied by at most 10% over the feasible region of the factor space. In one design it was established that run-to-run variations in the dead time could be attributed to the variation of

TABLE IV

CAPACITY FACTORS OF FOUR LOCAL ANAESTHETICS MEASURED AT NINE MOBILE PHASE COMPOSITIONS WITH TEA

Mobile phase composition					*Capacity factors of anaesthetics**			
No.	x_1	: x_2	:	x_3	*A*	*B*	*C*	*D*
1	0.55	: 0.45	:	0.00	0.65	1.19	1.95	4.86
2	0.50	: 0.00	:	0.50	0.62	0.75	2.88	2.88
3	0.45	: 0.55	:	0.00	0.99	1.89	4.43	12.72
4	0.40	: 0.00	:	0.60	1.47	1.97	6.74	12.79
5	0.50	: 0.50	:	0.00	0.77	1.47	2.72	7.39
6	0.45	: 0.00	:	0.55	0.92	1.16	4.25	5.50
7	0.525	: 0.237	:	0.237	0.39	0.50	2.21	1.87
8	0.425	: 0.287	:	0.287	0.83	1.13	4.92	6.55
9	0.475	: 0.262	:	0.262	0.50	0.65	3.02	3.02

* Anaesthetics as in Table III.

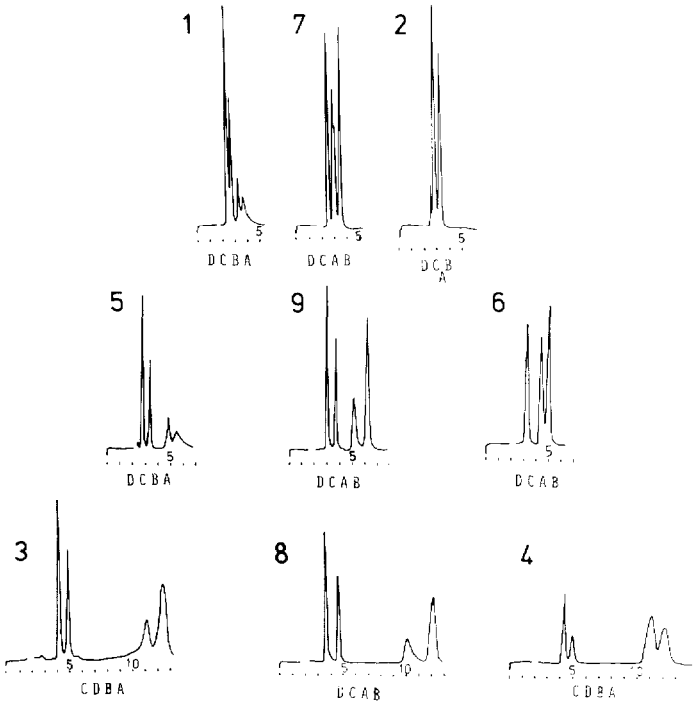

Fig. 4. Chromatograms of vitamins obtained at the measuring points 1–9 in Fig. 3. A = vitamin B_1; B = vitamin B_2; C = vitamin B_3; D = vitamin B_6. Retention time in min.

the mobile phase velocity. Therefore, the capacity factor was used as a measure of retention.

Polynomial regression was performed on the data in Tables II, III and IV according to eqn. 5 with a program for multiple regression that first normalizes the data to minimize rounding-off errors[23]. Subsequently the capacity factors were predicted and from the predicted capacity factors the resolutions were calculated using eqn. 6. The plotted minimal resolutions, R_s(min), are shown in Figs. 5, 8 and 11.

Vitamins

The chromatograms obtained with the nine mobile phase compositions in Fig. 3 are shown in Fig. 4. The mobile phase compositions and the capacity factors of the solutes are given in Table II. Standard solutions of the individual vitamins had to be injected in order to determine the identities of the peaks because of the eight peak cross-overs that occurred within the feasible region. This indicates the presence of important selectivity effects. Fig. 4 also shows that the analysis the time decreases in the direction of the top of the solvent triangle.

The models of the capacity factors of the four vitamins fit well; the mean relative difference between the measured values (Table II) and the predicted values of the capacity factors was approximately 5.5%. The minimal resolution plot in Fig. 5 corresponds well with the chromatograms in Fig. 4; the region of the peak cross-overs between chromatograms 1 and 7, 5 and 9, and 3 and 8, where R_s(min) passes

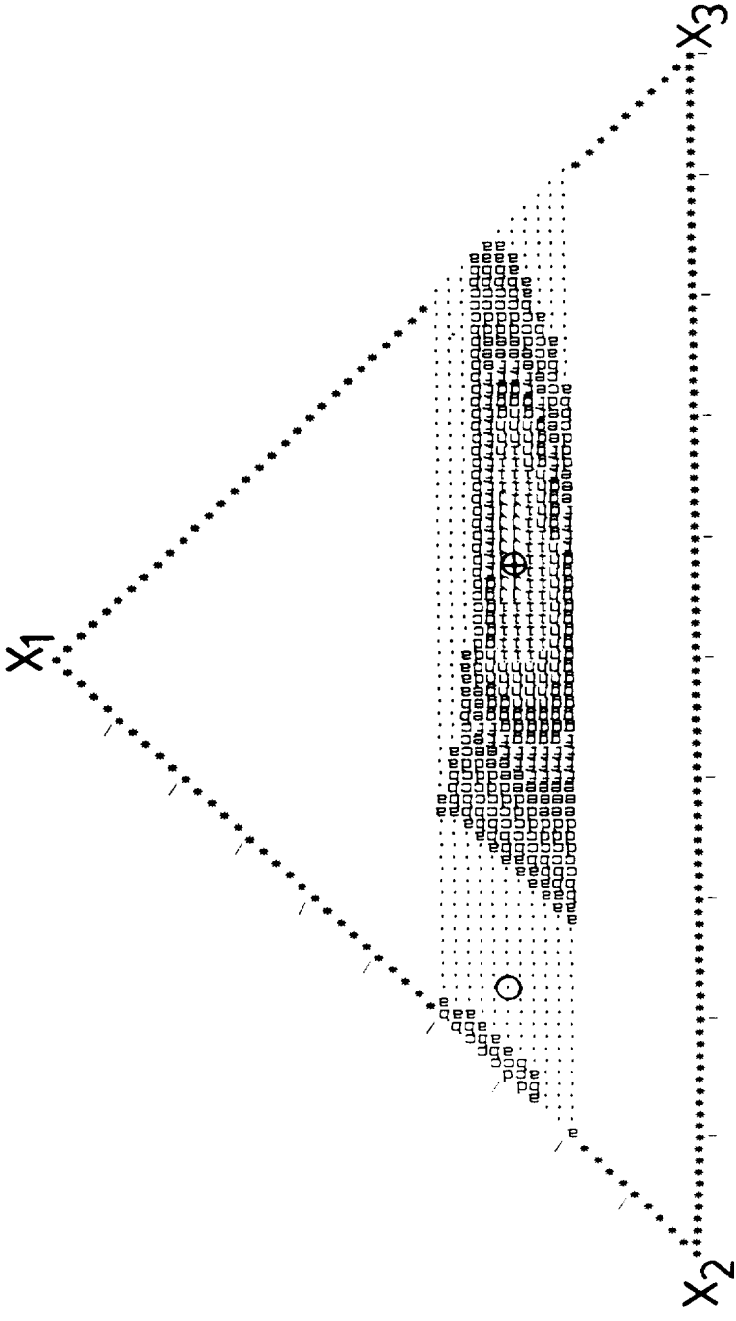

Fig. 5. Minimal resolution plot for the separation of vitamins obtained with the data in Table II. A poor separation is indicated by ⊙ and the optimal mobile phase composition by ⊕. R_s(min) < 1.0 is indicated by a dot; R_s(min): a = 1.00; b = 1.16; c = 1.32; ..... z ⩾ 5.00.

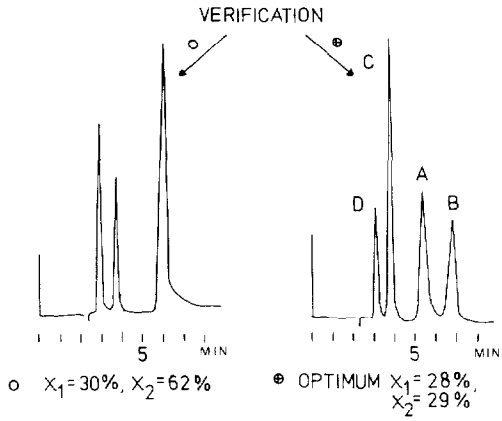

Fig. 6. Chromatograms obtained at the points indicated in Fig. 5. ○ = Fig. 6A; ⊕ = Fig. 6B. A = vitamin B_1; B = vitamin B_2; C = vitamin B_3; D = vitamin B_6.

through zero, is indicated by dots in Fig. 5. The peak cross-overs between chromatograms 3 and 5 and 4 and 6 are also represented by dots. For $N = 2000$ the values of R_s(min) calculated with eqn. 6 from the measured capacity factors of the

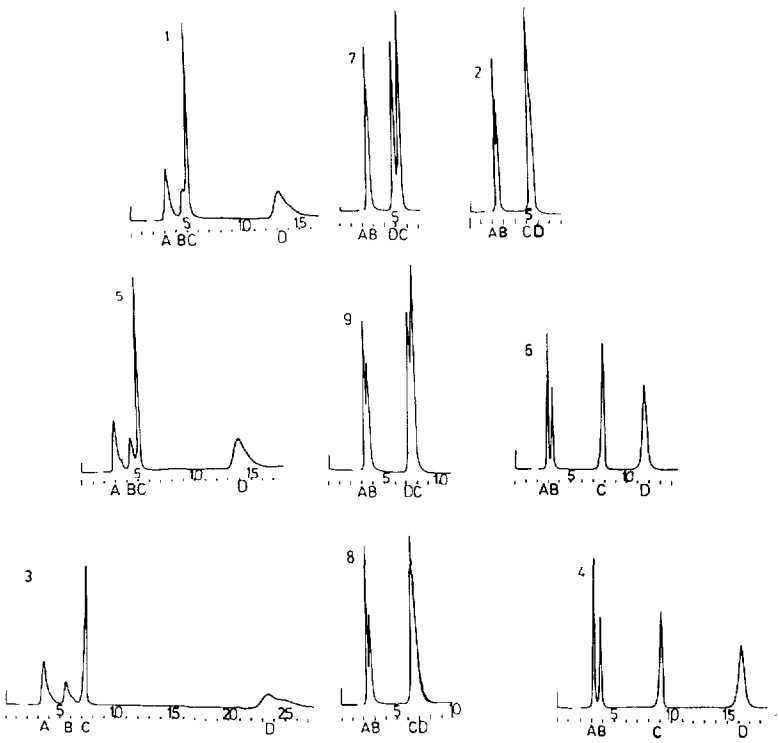

Fig. 7. Chromatograms of local anaesthetics obtained at the measuring points 1–9 in Table III. A–D as in Table III. Retention times in min.

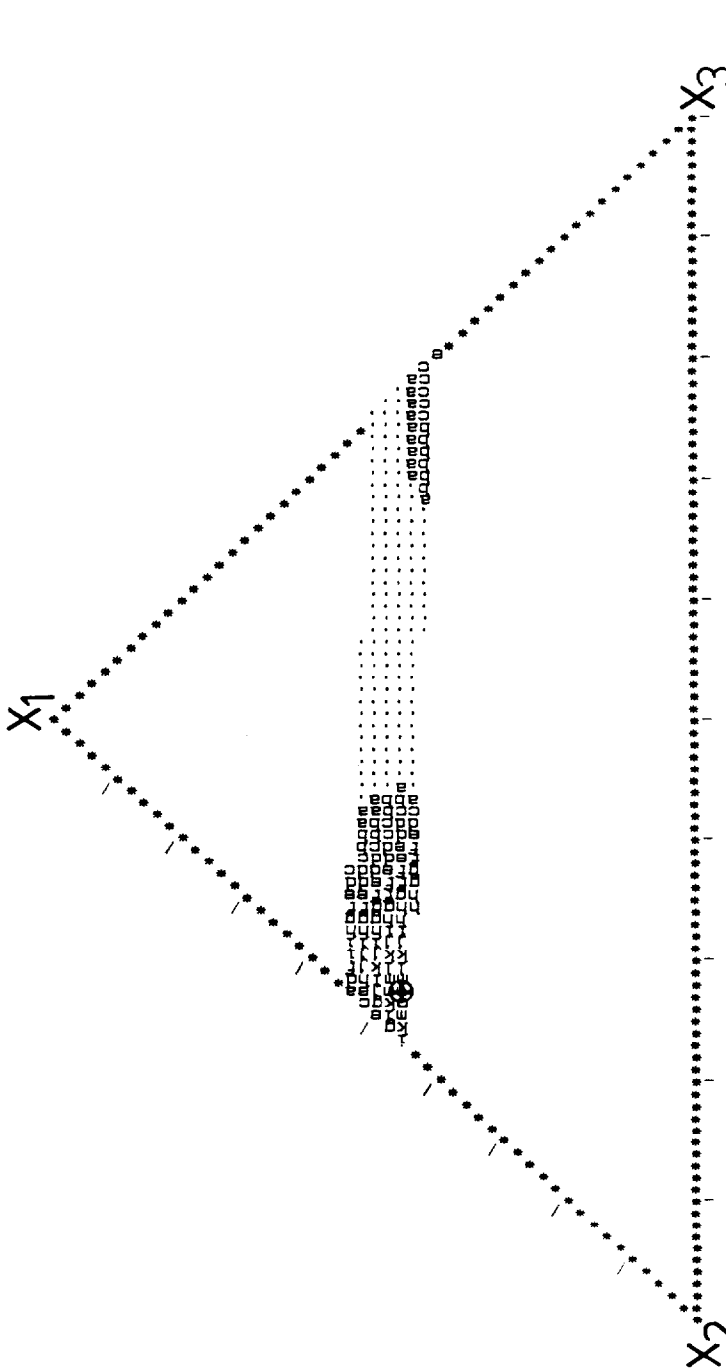

Fig. 8. Minimal resolution plot for the separation of local anaesthetics. Optimal mobile phase composition is indicated by ⊕. Symbols as in Fig. 5.

mobile phase compositions 1, 3, 5, 8 and 9 in Table II differ on the average from the estimated R_s(min) values at the corresponding points in Fig. 5 by 0.09. This is a satisfactory result considering that the difference indicated by two subsequent characters corresponds to a variation in the resolution of 0.16. Because, however, eqn. 6 is used for both the calculation and the estimation of R_s(min), the calculated and estimated values may differ from the true values if the plate number is not constant over the whole feasible region of the factor space for all components. This aspect of the procedure will be discussed in detail in the next example. The suitability of the minimal resolution plot in Fig. 5 for predicting the optimal solvent composition is confirmed by the chromatograms in Fig. 6A and B. In Fig. 6A a poor separation of only three peaks is obtained at the mobile phase composition indicated in Fig. 5 by the symbol ⊙, $x_1 = 0.30$, $x_2 = 0.62$, $x_3 = 0.08$; Fig. 6B shows the optimal separation with an analysis time of 7 min at the mobile phase composition indicated in Fig. 5 by the symbol ⊕, $x_1 = 0.28$, $x_2 = 0.29$, $x_3 = 0.43$.

Local anaesthetics

The chromatograms corresponding to the nine measurement points defined in Table III are shown in Fig. 7. This time five peak cross-overs occurred within a smaller feasible region of the factor space, which again indicates the presence of selectivity effects. The decrease in the analysis time in the direction of the top of the solvent triangle is not as clear as in the previous example. At constant pH the analysis time decreases with increasing methanol content in the mobile phase, but a strong effect of the pH on the analysis time is also apparent. The retention is shortest at intermediate values of the pH and longest at high values.

Again, good fits of the capacity factor are obtained with a mean relative difference of approximately 5% between the predicted and the measured (Table III) values of the capacity factor. The minimal resolution plot (Fig. 8) corresponds qualitatively well with the chromatograms in Fig. 7, but although the mean difference between the estimated R_s(min) values from Fig. 8 and the R_s(min) values calculated from the measured capacity factors from Table III with eqn. 6 ($N = 1200$) is only 0.18, the prediction by the minimal resolution plot is not satisfactory. The R_s(min) of chromatogram 5 in Fig. 7 is predicted to be equal to the R_s(min) of chromatogram 4 in Fig. 7, but chromatogram 4 is clearly superior to chromatogram 5 because of the better peak symmetry. The severe peak tailing in chromatogram 5 causes a large

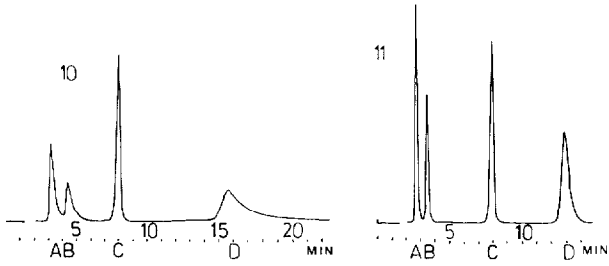

Fig. 9. Chromatogram 10 obtained at the optimum indicated in Fig. 8. Chromatogram 11 obtained with the same fractional solvent composition after the addition of 20 mM triethylamine to pseudo-components x_2 and x_3. A–D as in Table III. Retention times in min.

variation of the plate number[24] within the feasible region of the factor space. From peak D in chromatogram 4 (Fig. 7), a plate number of approximately 4600 was calculated; calculation of the plate number from the same component D in chromatogram 5 (Fig. 7) resulted in a value of about 800. The predicted value of 3.1 for the R_s(min) of chromatogram 10 (Fig. 9) at the optimal solvent composition is also too high; the resolution for peaks A and B as calculated from chromatogram 10 (Fig. 9) by dividing the distance between the peak maxima by half the sum of the peak widths is approximately 1.4.

Fig. 7 shows that the peaks of the components A, B and D, which possess a tertiary alkylamine function, become asymmetric at pH 6.5. This has been attributed to ion-exchange effects between the positively charged amine solutes and the negatively charged unprotonated silanol sites[11], which can be suppressed by the addition of triethylamine (TEA) to the mobile phase[11,21]. After the addition of approximately 10 m*M* TEA to the optimal mobile phase, chromatogram 11 (Fig. 9) was obtained, which has the same peak elution order but much better peak symmetry and shorter retention times.

Preliminary experiments performed with and without sodium octylsulphonate in the eluent, showed that the addition of 20 m*M* TEA to the pseudo-components x_2 and x_3 did not eliminate the influence of 5 m*M* sodium octylsulphonate present in x_2 and x_3. Hence the optimization procedure was repeated with the new pseudo-

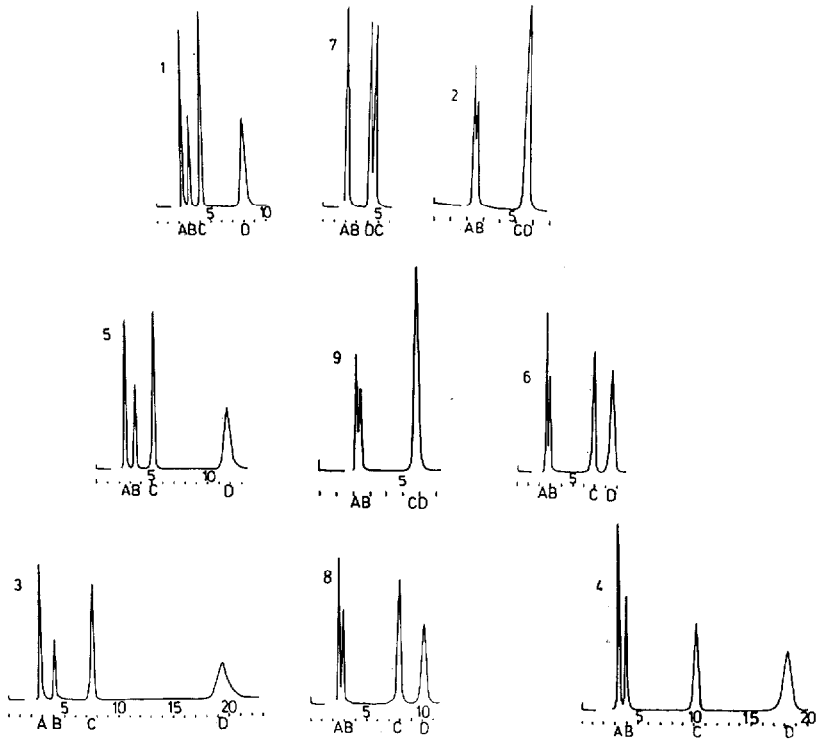

Fig. 10. Chromatograms of local anaesthetics obtained at the measuring points 1–9 in Table IV with the use of triethylamine. A–D as in Table III.

components x_2 and x_3, which contained 20 mM TEA in addition to 0.1 M citric acid buffer and 5 mM sodium octylsulfonate.

The chromatograms corresponding to the data in Table IV are shown in Fig. 10, and confirm that the influence of the pairing ion is still present, as in the Figs. 7 and 10 the peak elution orders of the corresponding chromatograms 1–8, which have equal fractional mobile phase compositions, are identical. In Fig. 10 the retention times decrease at constant pH in the direction of the top of the solvent triangle and are shortest at intermediate pH.

The quality of the models for the capacity factor was slightly better than that in the previous design without TEA; the mean relative difference between the measured (Table IV) and the estimated values of the capacity factor was approximately 1.5%. The predicted R_s(min) values from the minimal resolution plot in Fig. 11 differ on average by approximately 0.1 from the R_s(min) calculated from the capacity factors in Table IV. Moreover, the peaks are symmetrical and hence the prediction of the R_s(min) by the minimal resolution plot is better; the R_s(min) of chromatogram 5 in Fig. 10 is predicted to be higher than the R_s(min) of chromatogram 4 in Fig. 10. The resolutions for peaks A and B as calculated from retention times and peak widths from chromatograms 5 and 4 are 2.7 and 1.8, respectively, and correspond reasonably well with the predicted values of 2.8 and 1.5. Nevertheless, the quantitatively correct prediction of R_s(min) still caused problems, as again the plate number was not constant over the whole factor space, but varied with the component used for its determination. We used a plate number of 1200, which was determined from component A of chromatograms 1, 3 and 5 (Fig. 10) and was a conservative estimate of the plate number, but gave good quantitative predictions.

The minimal resolution plot (Fig. 11) indicates that R_s(min) is high for binary compositions of x_1 and x_2 and that the resolution decreases in the direction of x_1. Because for $x_1 = 0.55$ a good value for R_s(min) of 2.4 is still predicted and the analysis time indeed decreases in the direction of x_1, we consider this binary eluent composition ($x = 0.55$; $x_2 = 0.45$) to be optimal. The chromatogram that was obtained with this mobile phase composition had an analysis time of 8 min and the (minimal) resolution for the components A and B was 2.5.

CONCLUSIONS

The proposed optimization method for reversed-phase ion-pair chromatography, which uses (pseudo)components that cause the analysis time to decrease in the direction of the top of the solvent triangle, makes the simultaneous optimization of the minimal resolution and the analysis time possible.

Quantitative prediction of the minimal resolution of the worst separated pair of peaks requires a correct estimation of the plate number for this pair. A (qualitative) prediction of the optimal solvent composition is still possible with an incorrect plate number, provided that the plate number is almost constant within the feasible region of the factor space, because the shape of the response surface does not alter with the plate number. An incorrect but constant plate number causes the predicted values of R_s(min) to be too high or too low, but a variable plate number changes the shape of the response surface. This problem, however, is inherent in every optimization procedure which uses the resolution and thus the plate number as (a factor of) the

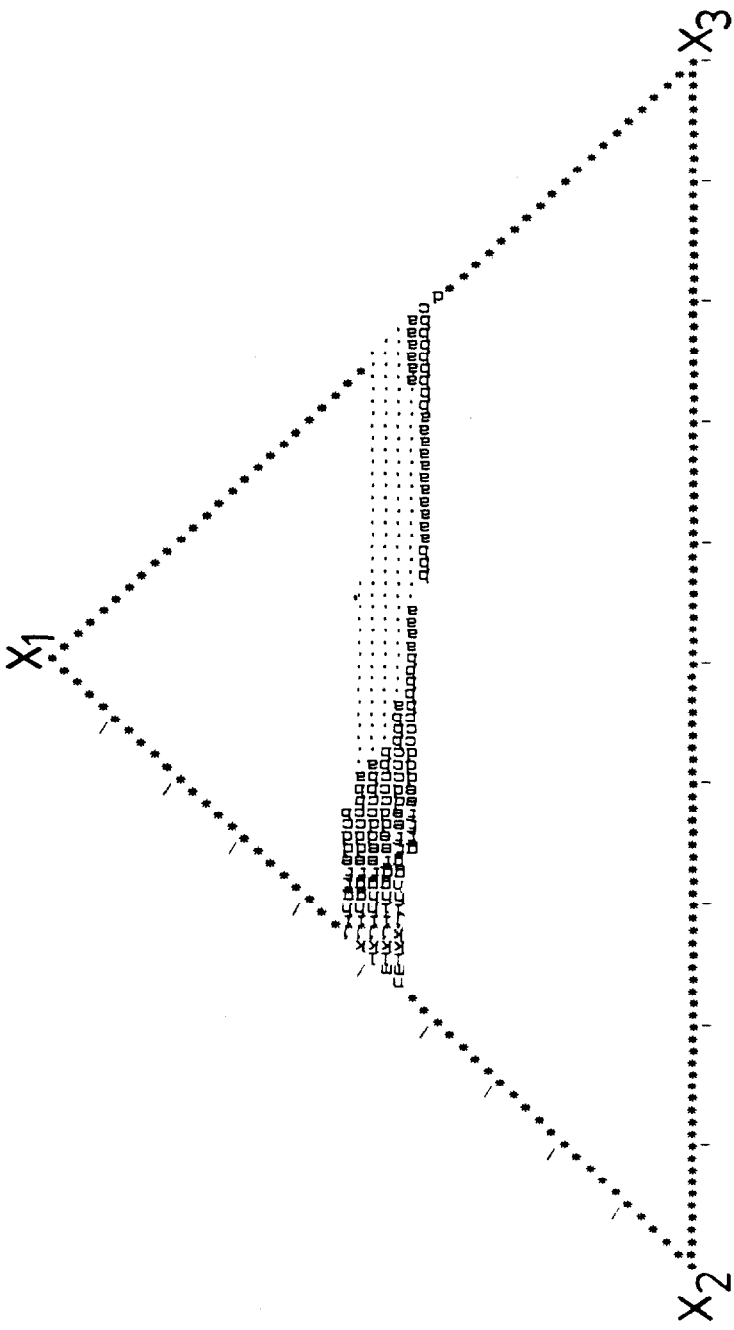

Fig. 11. Minimal resolution plot for the separation of local anaesthetics obtained with the use of triethylamine. Symbols as in Fig. 5.

optimization criterion. The quantitative impact of variation of the plate number within the feasible region on the shape of the response surface requires further investigation. The use of the minimal separation factor as an optimization criterion partly obviates this problem, but also in that instance the correct optimal mobile phase composition is predicted only if the plate number remains constant within the feasible region of the factor space.

REFERENCES

1 W. R. Melander and C. Horvath, in M. T. W. Hearn (Editor), *Ion Pair Chromatography*, Marcel Dekker, New York, 1985, Ch. 2.
2 R. E. Adams, in M. T. W. Hearn (Editor), *Ion Pair Chromatography*, Marcel Dekker, New York, 1985, p. 149.
3 J. L. M. van de Venne, *Thesis*, Eindhoven, 1979, Ch. 5.
4 J. H. Knox and R. A. Hartwick, *J. Chromatogr.*, 204 (1981) 3.
5 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 175 (1979) 51.
6 B. A. Bidlingmeyer, S. N. Deming, W. P. Price, Jr., B. Sachok and M. Petrusek, *J. Chromatogr.*, 186 (1979) 419.
7 Á. Bartha and Gy. Vigh, *J. Chromatogr.*, 265 (1983) 171.
8 Á. Bartha, Gy. Vigh, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 303 (1984) 29.
9 R. B. Taylor, R. Reid and C. T. Hung, *J. Chromatogr.*, 316 (1984) 279.
10 Á. Bartha, H. A. H. Billiet, L. de Galan and Gy. Vigh, *J. Chromatogr.*, 291 (1984) 91.
11 J. S. Kiel, S. L. Morgan and R. K. Abramson, *J. Chromatogr.*, 320 (1985) 313.
12 R. C. Kong, B. Sachok and S. N. Deming, *J. Chromatogr.*, 199 (1980) 307.
13 A. Sokolowski and K.-G. Wahlund, *J. Chromatogr.*, 189 (1980) 299.
14 W. R. Melander, J. Stoveken and C. Horváth, *J. Chromatogr.*, 185 (1979) 111.
15 W. Lindberg, E. Johansson and K. Johansson, *J. Chromatogr.*, 211 (1981) 201.
16 H. J. G. Debets, *J. Liq. Chromatogr.*, 8 (1985) 2725.
17 J. C. Berridge, *Techniques for the Automated Optimization of HPLC Separations*, Wiley, New York, 1985, Ch. 4–6.
18 P. J. Schoenmakers, *Optimization of Chromatographic Selectivity (Journal of Chromatography Library*, Vol. 35), Elsevier, Amsterdam, 1986, Ch. 4 and 5.
19 R. D. Snee, *Chemtech*, 9 (1979) 702.
20 J. L. Glajch, J. J. Kirkland, K. M. Squire and J. M. Minor, *J. Chromatogr.*, 199 (1980) 57.
21 A. P. Goldberg, E. Nowakowska, P. E. Antle and L. R. Snyder, *J. Chromatogr.*, 316 (1984) 241.
22 J. W. Weyland, C. H. P. Bruins and D. A. Doornbos, *J. Chromatogr. Sci.*, 22 (1984) 31.
23 J. C. Davis, *Statistics and Data Analysis in Geology*, Wiley, New York, 1973, pp. 423–427.
24 J. J. Kirkland, W. W. Yau, H. J. Stoklosa and C. H. Dilks, *J. Chromatogr. Sci.*, 15 (1977) 303.