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PRACTICAL OPTIMIZATION OF SOLVENT SELECTIVITY IN LIQUID–SOLID CHROMATOGRAPHY USING A MIXTURE–DESIGN STATISTICAL TECHNIQUE

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SUMMARY

A systematic approach is described for the optimization of solvent selectivity in liquid–solid chromatography (LSC), with emphasis on changes in selectivity as a result of variation of mobile phase composition. Major contributions to selectivity are provided by solvent–solute localization and solvent-specific localization. Exploitation of these effects is achieved by the use of a mixture-design statistical technique to minimize the number of experiments to find an optimum solvent mixture for LSC separation. Quaternary-solvent mobile phases are required for difficult separations to invoke the full range of selectivity effects possible for LSC separation. The four preferred solvents for LSC optimization based on localization effects are hexane, methylene chloride, methyl *tert.*-butyl ether and acetonitrile. In the optimization process retention data are required for only seven mobile-phase systems, and an overlapping resolution mapping (ORM) technique of data analysis is used to establish the optimum solvent mixture for the highest resolution of all adjacent bands in the chromatogram.

INTRODUCTION

As the application of liquid chromatography (LC) has become more widespread, increasing interest has developed in practical procedures for optimizing separations. Such separations are increasingly used in areas such as quality control, process control and clinical analysis, where large numbers of samples have to be analysed each day. Such applications place special emphasis on the complete separation of samples of interest in a minimum time per sample. Adequate separation can be measured in terms of the usual resolution function R_s[1], where R_s can be related to other separation variables by

$$R_s = 1/4\,(\alpha - 1)\,(N^{1/2})\left(\frac{k'}{1 + k'}\right) \quad (1)$$

where k' is the average value of the capacity factors k_1 and k_2 of two adjacent bands 1 and 2, α is the separation factor (k_2/k_1), and N is the column plate number. To a first approximation, these three terms of eqn. 1 are independent of each other and can be separately optimized.

Several workers[1-4] have discussed the optimization of plate number N in LC. In previous papers[5,6] we discussed the prediction of solvent strength in liquid–solid chromatographic (LSC) separation, which in turn determines values of k'; optimum values of k' generally lie in the range $1 < k' < 10$. Finally, values of α in LSC can be systematically varied by changing the composition of the mobile phase[7].

It has been demonstrated for reversed-phase LC separations[8] that large changes in values of α are possible as a result of change in the mobile phase organic modifier. For example, a change from methanol–water to acetonitrile–water or tetrahydrofuran–water can greatly influence the α values or the selectivities among solutes. Often, however, various binary solvent mixtures are incapable of separating all compounds in a given mixture. In such cases, it has been found[9-12] that the use of ternary-solvent mobile phases is generally advantageous. However, for these more complex mobile phases, the systematic optimization of values for every pair of adjacent bands in the chromatogram becomes more complicated.

Recently, we have introduced a mixture-design statistical approach for the efficient optimization of selectivity in reversed-phase LC[13]. The potential of this scheme for retention optimization using quaternary-solvent mobile phases has been demonstrated in the successful separation of complex mixtures of both substituted naphthalenes[13] and phenylthiohydantoin (PTH) amino acids[14] using reversed-phase LC. The latter optimization procedure is based on the solvent-triangle classification of solvents according to their separation selectivity[15,16]. For bonded-phase LC, solvents can be categorized according to their proton donor, proton acceptor or dipole interactions. With the three "extreme" solvents from the corners of the solvent triangle (methanol, acetonitrile, tetrahydrofuran) plus water as carrier, the mixture-design procedure then requires a continuous variation of α values for all peak pairs in the chromatogram. The approaches which have been described for reversed-phase LC should also be applicable to liquid–solid chromatography (LSC). We have recently reported[7] on the theoretical background necessary for the optimization of the mobile phase in LSC. We report here experimental results in support of this theory and describe approaches for the systematic optimization of mobile phases in LSC.

EXPERIMENTAL

The apparatus, materials and procedures used in this study have been described previously. In this work, three 15 × 0.46 cm I.D. columns of Zorbax®-SIL chromatographic packings (Du Pont Analytical Instruments Division, Wilmington, DE, U.S.A.) from the same lot were used. Mobile-phase solvents were 50% water-saturated[1]. Solvents were vacuum degassed individually and then mixed before water saturation. Solvents with 50% water-saturation were obtained by adding equal volumes of water-saturated solvent (obtained using 30% water in silica gel as in ref. 1) and water-free solvent.

RESULTS

The basis of selectivity and the variation of α values with change in mobile phase composition is substantially different in LSC than in other LC methods. In bonded-phase LC methods, interactions between solvent and solute molecules in the mobile phase are of primary importance, and the solvent triangle serves as a useful guide for selecting extreme solvents of very different selectivity. However, for most LSC separations, we have shown[7] that localization effects in the stationary phase are of greater importance. These localization effects, which involve competition between mobile phase and sample molecules for a position directly over adsorption sites on the surface of the stationary phase (*e.g.*, silanol groups in the case of silica), can be subdivided into solvent–solute localization and solvent-specific localization. The degree of solvent localization can be measured by a mobile phase parameter m, which increases as the concentration of some polar solvent in the mobile phase increases. We have shown previously[7] that methyl *tert.*-butyl ether (MTBE) and acetonitrile (ACN) are solvents that give large values of m for mobile phases which contain these solvents.

Solvent-specific localization appears to involve direct hydrogen bonding of a basic, polar solvent with surface sites on the adsorbent. Thus, basic polar solvents such as MTBE yield additional selectivity effects as opposed to less basic polar solvents such as ACN. Therefore, values of α can be further varied by changing the ratio of concentrations of MTBE to ACN while holding m constant.

These considerations have led us to the design of an LSC selectivity triangle (Fig. 1) based on solvent localization for use in the present optimization scheme. This selectivity triangle is analogous to that used previously for optimization in reversed-phase LC[13]. In Fig. 1 it can be seen that the corners of the triangle, corresponding to solvents of extreme selectivity, consist of (1) a non-localizing solvent (methylene

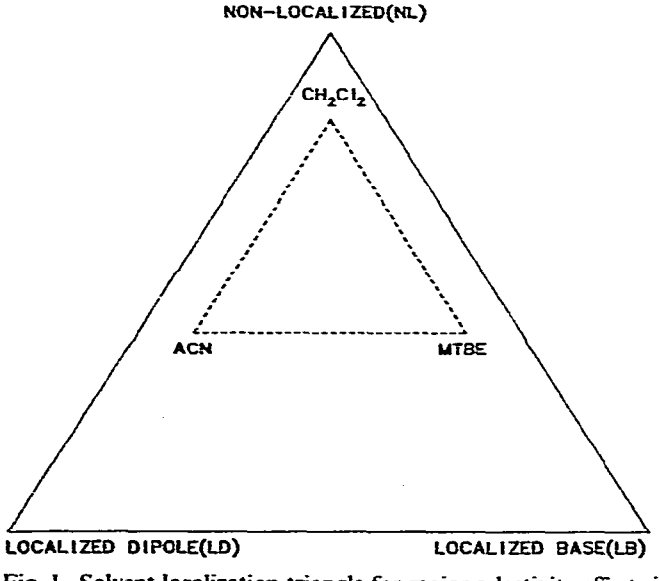

Fig. 1. Solvent localization triangle for major selectivity effects in LSC.

chloride, MC), (2) a basic, localizing solvent (MTBE) and (3) a non-basic localizing solvent (ACN). Adjustment of the ratios of these three solvents in the final mobile phase allows the systematic, continuous variation of α values in LSC over very wide limits. A fourth "inert" solvent such as hexane (HEX) or Freon®-113[17] is the carrier used to adjust the solvent strength of the mobile phase. The four solvents for LSC in Fig. 1 may be compared with the use of methanol, acetonitrile and tetrahydrofuran plus water in reversed-phase LC[13].

Once the extreme selectivity solvents of Fig. 1 have been selected for optimization studies, a strategy for the development of a given separation by LSC can be planned. As with other LC methods, the first step is the selection of a column, flow-rate and temperature for the sample of interest. These variables will be held constant while retention is optimized by a change in mobile phase composition. These initial choices largely determine the plate number N of the column. The optimum solvent strength for the given separation can next be determined by trial-and-error, using the binary-solvent mobile phase MC–hexane and varying the concentration of MC.

After the appropriate concentration of MC in the mobile phase has been determined, the value of ε, the mobile phase strength, can be calculated[5]. This, in turn, defines the various mobile phase compositions in the selectivity triangle of Fig. 1 for that value of ε as calculated by the procedure in ref. 6. Corresponding values of m for this particular selectivity triangle can also be calculated from the various mobile phase compositions represented in the triangle (as in ref. 7). Thus, points within the triangle represent mobile phase compositions that can be described as follows: (1) all compositions have the same value of ε; (2) values of m for these compositions vary linearly from a maximum and equal value for the bottom corners (MTBE and ACN) of the triangle to a minimum value for the top corner (MC); (3) the concentration ratio [MTBE]/([MTBE] + [ACN]) varies linearly along any horizontal line of the

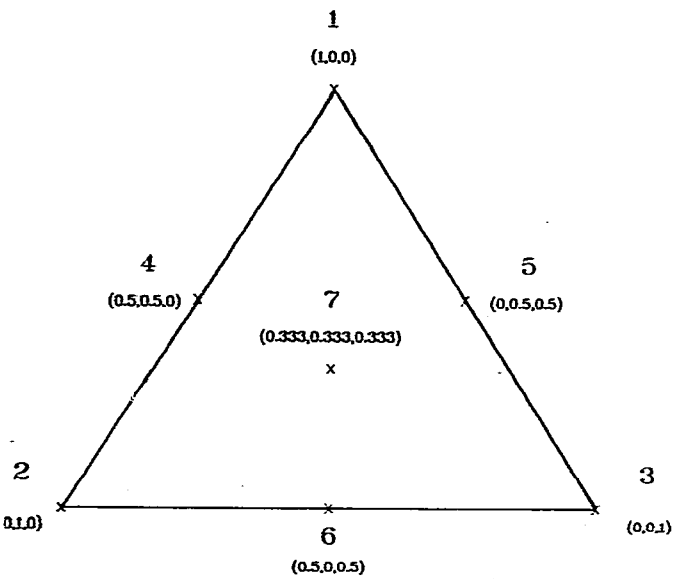

Fig. 2. Simplex design for three solvents (A, B and C) and mixtures. Values for each point are trilinear coordinates of A/B/C.

triangle; (4) all solvent compositions are miscible (note that this particular system requires addition of MC as co-solvent for some mixtures of hexane and ACN; with Freon-113 carrier[17], no MC co-solvent is required). Fig. 2 also classifies the seven standard solvents for the solvent selectivity triangle in terms of values of m and R = [MTBE]/([MTBE] + [ACN]). Here the relative value of m is arbitrarily set equal to 0 for the MC corner and 1 for the MTBE/ACN corners.

The statistical approach used here represents an efficient procedure for identifying the point or points in the selectivity triangle which will optimize the resolution of the sample, *i.e.*, yield the largest value of α and R_s for the most poorly resolved band-pair in the chromatogram. Fig. 2 illustrates the application of the statistical approach in terms of the use of the seven prescribed mobile phases from the selectivity triangle. These seven mobile phases are selected for approximately equal spacing of values of log k' for any solute pair within the sample of interest. For example, solvent 7 in Fig. 2 is a quaternary mixture of the four standard solvents in Fig. 1. The composition of the quaternary mixture is chosen to give approximately equal contributions from the three corner-solvents (Fig. 1) to log k'. Thus, the value of m for this composition is the average of the m for MC, MTBE and ACN or 0.67. The value of [MTBE]/([MTBE] + [ACN]) is 0.5.

After the compositions of the seven reference mobile phases in Fig. 2 have been calculated according to the above principles, the sample is then separated in each of

TABLE I

k' DATA FOR SEVEN MOBILE PHASES IN FIG. 2 AND OPTIMUM PREDICTED BY PROGRAM

Zorbax-SIL 15 × 0.46 cm I.D. column; 35°C; 2.0 ml/min.

*Molar fractions**	*Mobile phase No.*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8 (Opt.)*
N_A	0.422	0.870	0.958	0.686	0.920	0.768	0.887	0.913
N_B	0.578	0.100	0.000	0.300	0.048	0.220	0.090	0.060
N_C	0.000	0.030	0.000	0.014	0.016	0.000	0.012	0.020
N_D	0.000	0.000	0.042	0.000	0.016	0.012	0.011	0.007
*Solutes***								
2-OCH_3	0.58	0.57	0.59	0.65	0.67	0.54	0.67	0.70
1-NO_2	0.86	1.20	1.62	1.10	1.36	0.90	1.30	1.48
1,2-$(OCH_3)_2$	1.15	0.82	1.00	1.02	0.95	0.91	1.02	1.09
1,5-$(NO_2)_2$	2.37	3.27	3.70	2.98	3.62	2.63	3.63	4.13
1-CHO	2.75	1.69	2.45	2.22	2.11	2.27	2.33	2.20
2-CO_2CH_3	3.29	2.49	2.83	3.00	2.99	2.78	3.25	3.23
1-CO_2CH_3	3.31	2.71	3.07	3.13	3.33	2.85	3.57	3.67
2-CHO	3.97	2.22	3.25	3.19	2.83	3.12	3.17	2.97
1-CH_2CN	4.06	4.73	7.23	4.86	6.09	4.83	6.30	6.55
1-OH	4.44	8.17	6.65	6.77	7.14	6.27	8.00	8.96
1-$COCH_3$	5.17	2.58	3.54	3.71	3.25	3.72	3.72	3.42
2-$COCH_3$	7.33	3.33	4.76	5.14	4.39	5.16	5.10	4.67
2-OH	7.98	11.86	11.35	10.69	11.58	11.57	13.42	14.19

* Molar fractions: N_A = hexane; N_B = methylene chloride; N_C = acetonitrile; N_D = methyl *tert.*-butyl ether.

** Substituted naphthalenes.

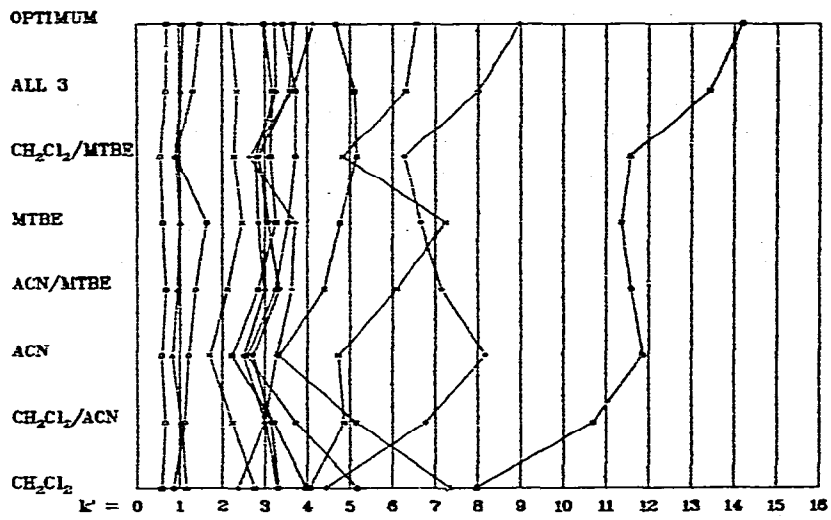

Fig. 3. k' maps for each solute in eight different mobile phases. Each line represents one solute (data in Table I). Mobile phases: optimum, 8; all, 3, 7; CH_2Cl_2–MTBE, 6; MTBE, 3; ACN–MTBE, 5; ACN, 2; CH_2Cl_2–ACN, 3; CH_2Cl_2, 1. Solutes (in order for CH_2Cl_2): □, 2-OCH_3; ○, 1-NO_2; △, 1,2-$(OCH_3)_2$; +, 1,5-$(NO_2)_2$; ×, 1-CHO; ◇, 2-CO_2CH_3; ▽, 1-CO_2CH_3; ■, 2-CHO; ✳, 1-CH_2CN; ◆, 1-OH; ●, 1-$COCH_3$; ⧗, 2-$COCH_3$; ⊠, 2-OH.

these mobile phases and values of k' for each solute in each mobile phase are measured. Where overlapping bands prevent the accurate determination of k' values, individual solutes must be re-run separately.

The above procedure for optimizing the mobile phase solvent was applied to the mixture of thirteen substituted naphthalenes listed in Table I. The optimum value of ε was determined as 0.23 for separating the substituted naphthalene mixture, and the mobile phase compositions for the seven standard solvents of this strength are shown separately in Table I. Two conclusions can immediately be drawn from the data in Table I: (1) none of the seven standard mobile phases alone provides complete separation of the sample components; (2) there are large changes in α values among the various mobile phases. These changes are evident when examining the k' values for the solute bands, which are plotted in Fig. 3.

With the retention data in Table I, sufficient information is available to carry out the data analysis to locate the mobile phase composition within the selectivity triangle of Fig. 2 that provides maximum resolution of sample components. This is accomplished by the resolution mapping procedure illustrated in Fig. 4. The resolution values of peak pairs at the seven points on the triangle (Fig. 1) are calculated from the seven chromatograms for a single peak pair. In this case, peaks 6 and 8 are illustrated. From these seven resolution values, a resolution surface of the solvent triangle can be calculated based upon fitting the data to a second-order polynomial equation[13]. The contour lines in Fig. 4 represent predicted resolutions for this peak pair within the selected solvent triangle. These results are presented in a different manner in Fig. 5. Here, the $R_s = 1.0$ contour is shown, and the shaded area of the solvent triangle indicates mobile phases for which the resolution is less than 1.0. The white area within the solvent triangle represents mobile phases for which a resolution

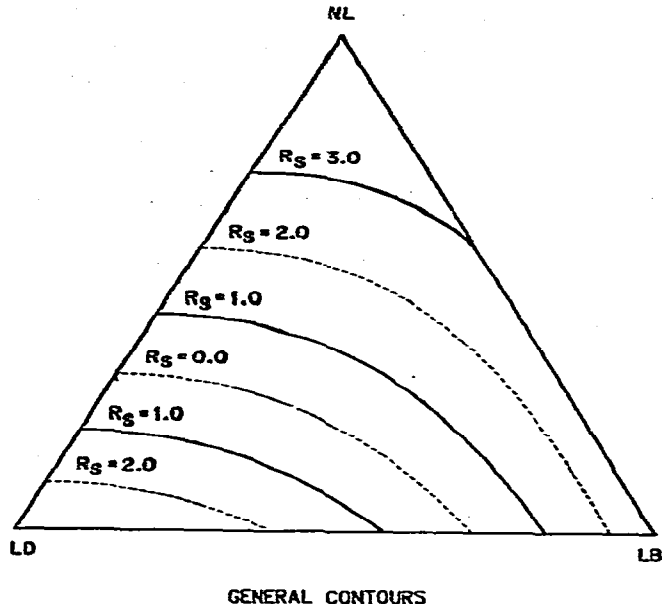

Fig. 4. Resolution map for peaks 6–8. Contours are predicted values of resolution (R_s) between peaks 6–8.

of at least 1.0 is obtained for peaks band 8. Resolution maps are prepared for all peak pairs within the chromatogram, and similar shaded areas in the solvent selectivity triangles are designated as solvents for which the derived resolution is *not* obtained. When these resolution maps for all peak pairs are manually overlayed and shaded

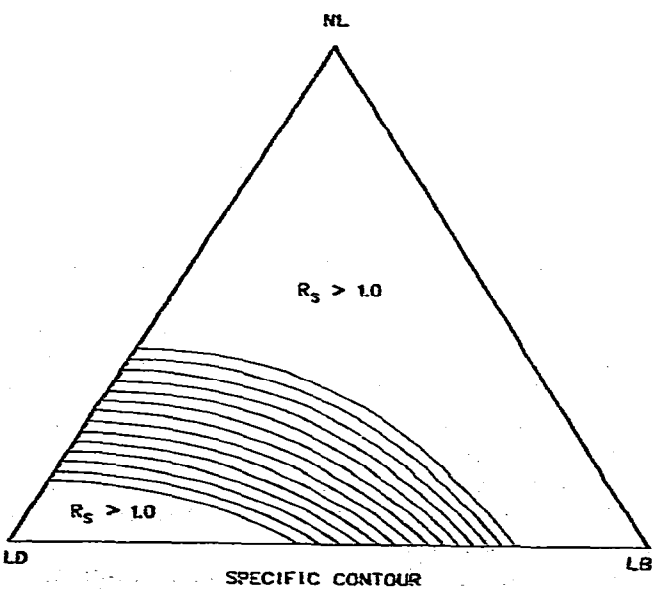

Fig. 5. Specific contour resolution map for peaks 6–8. Shaded area represents mobile phase mixtures where the resolution for peaks 6–8 is < 1.0.

areas intersected, regions which are unshaded (remain white) indicate a mobile phase which resolves *all* peak pairs to at least a resolution of 1.0. For convenience, this overlaying procedure may be carried out with a computer[13] to establish the optimum solvent region.

Two points should be made regarding the resolution of peak pairs by this approach. First, the required resolution for the various peak pairs of interest is arbitrarily established by the operator. Another resolution value would produce similar contour maps, but unshaded resolved areas would be more limited or nonexistent, as in this case, for $R_s = 1.2$. In practice, the computer program used for these calculations determines the maximum R_s value which still predicts some available mobile phase within the solvent triangle that satisfies the resolution requirement for all the peak pairs. This mobile phase is then the optimum for the components in the sample of interest.

The second point of interest is the number of peak pairs which must be considered in the analysis. If there is no change in peak order with changing solvent compositions, it is only necessary to examine adjacent peak pairs for the desired resolution. In most systems of interest, however, some peak crossovers will occur; that is, there will be changes in the solute retention order. This situation appears to complicate the analysis, but in fact can be handled readily by considering *all* pairs (even non-adjacent pairs) for every chromatogram obtained for the seven statistically designed experiments. Although in principle this could be a formidable task, the actual number of possible peak pairs is not overly large and can be predicted as[13]

$$\binom{n}{2} = \frac{n!}{(n-2)!\,2!}$$

where n is the number of peaks. For a thirteen-component system, this corresponds to 78 possible pairs, the data for which can be easily handled by a computer.

In the case illustrated by the data used from Table I, overlapping resolution mapping (ORM) analysis reveals that there is one region of the solvent triangle which predicts a resolution of 1.0 or more for all peak pairs, as shown in Fig. 6. The white area is the acceptable result of the ORM of all of the contour maps (similar to Fig. 5), for all peak pairs in the mixture. Any shaded region of the triangle corresponds to a solvent mixture where at least one peak pair does not have a resolution of at least 1.0. The optimum solvent for this system is indicated by $\times$ in Fig. 6, and corresponds to 0.83% ACN, 0.67% MTBE and 3.05% CH_2Cl_2 (by volume) in hexane.

A predicted resolution of 1.0, indicated for the peaks in the chromatograms shown in Fig. 7 for all three columns used in this work, is acceptable for most applications. However, the resolution could be increased, if desired, by increasing the column length by connecting columns in series. The total length of column required to achieve a certain resolution may be calculated from the discussion in ref. 1. In Fig. 7, the observed resolution of 0.9 should be increased to about 1.3 by doubling the column length (constant pressure). However, for this approach to be successful, the columns to be connected must have reproducible values of N[18], and especially values of α and k, to insure reproducible separation. Note that the observed resolution of 0.9 for this experiment is less than the predicted value of 1.0, probably owing to the approximations utilized in the optimization approach plus experimental variation.

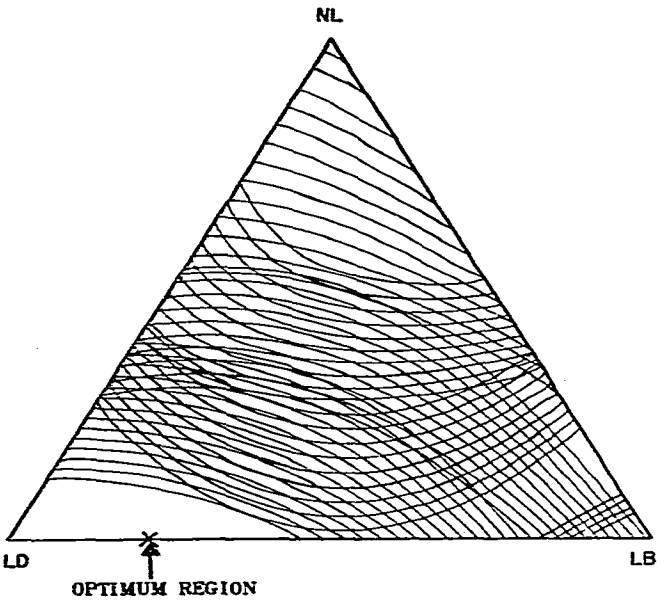

Fig. 6. Overlapping resolution map (ORM) for all peak pairs. The optimum mobile phase region ($R_s \geq 1$) is indicated in white.

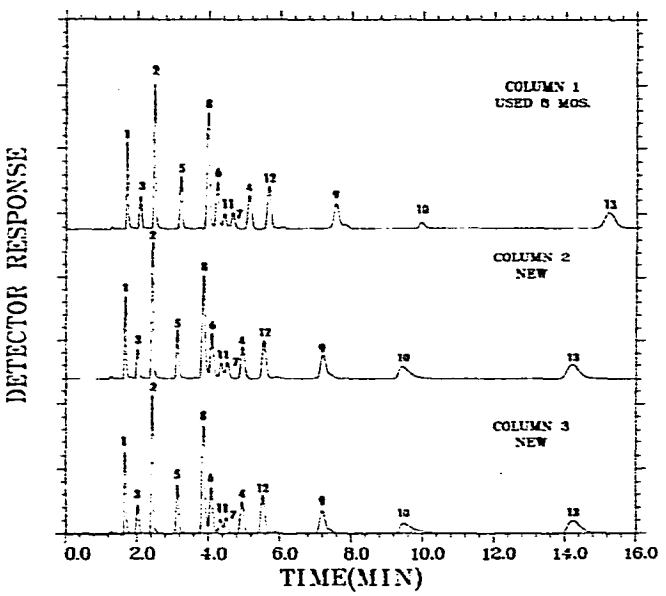

Fig. 7. Column reproducibility for three columns at the optimum mobile phase. Conditions as in Experimental. Peaks: 1 = 2-OCH_3; 2 = 1-NO_2; 3 = 1,2-$(OCH_3)_2$; 4 = 1,5-$(NO_2)_2$; 5 = 1-CHO; 6 = 2-CO_2CH_3; 7 = 1-CO_2CH_3; 8 = 2-CHO; 9 = 1-CH_2CN; 10 = 1-OH; 11 = 1-$COCH_3$; 12 = 2-$COCH_3$; 13 = 2-OH. Minor peaks after peaks 9 and 12 are unknown impurities in the substituted naphthalene standards used.

TABLE II

REPRODUCIBILITY OF k' DATA FOR THREE COLUMNS

Conditions as in Table I.

*Solute**	*Column No.*			*Relative standard deviation (%)*
	1	*2*	*3*	
2-OCH_3	0.70	0.66	0.66	3.4
1-NO_2	1.48	1.42	1.42	2.4
1,2-$(OCH_3)_2$	1.09	1.01	1.02	4.2
1,5-$(NO_2)_2$	4.13	3.95	3.94	2.7
1-CHO	2.20	2.12	2.13	2.0
2-CO_2CH_3	3.23	3.09	3.08	2.7
1-CO_2CH_3	3.67	3.52	3.50	2.6
2-CHO	2.97	2.85	2.86	2.3
1-CH_2CN	6.55	6.21	6.19	3.2
1-OH	8.96	8.42	8.45	3.5
1-$COCH_3$	3.42	3.35	3.33	1.4
2-$COCH_3$	4.67	4.55	4.52	1.7
2-OH	14.19	13.21	13.22	4.2

* Substituted naphthalene derivatives.

In this study, the three different silica columns were tested for reproducibility of k' with the optimum mobile phase predicted by ORM data analysis; Table II shows that k' data obtained for these three columns are sufficiently reproducible to permit the desired resolution increase. Using the optimum mobile phase predicted by the statistically designed experiments, the two newest of the three columns were connected to produce the chromatogram in Fig. 8. The limiting or most closely

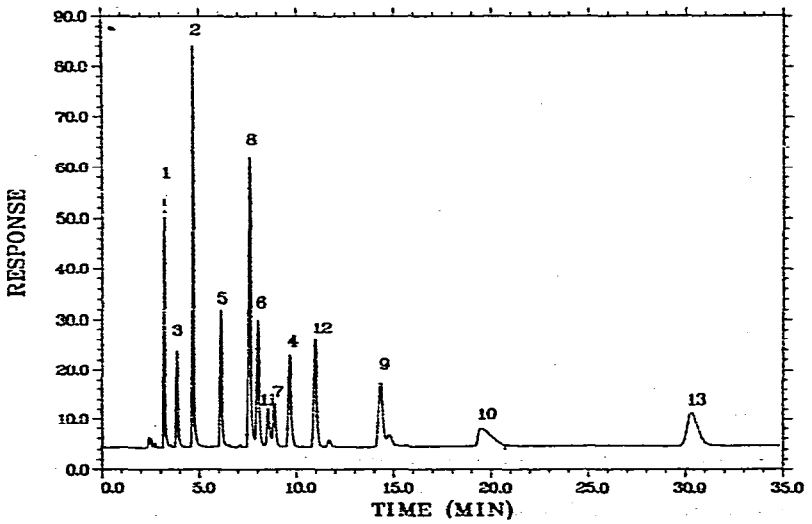

Fig. 8. Chromatogram for columns 2 and 3 in series. Solutes are naphthalene derivatives substituted as in Fig. 7.

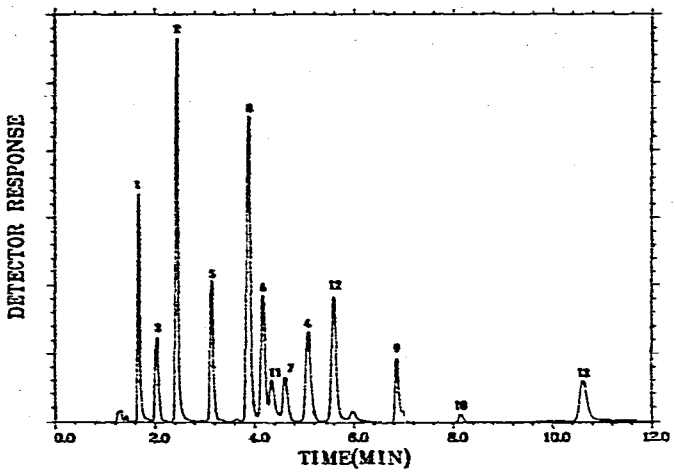

Fig. 9. Chromatogram for column 2 using step-flow programming as described in text. Solutes numbered as in Fig. 8.

spaced peaks (7, 11) now show a resolution of 1.22 compared with the initial resolution of 1.3 as predicted by theory. It should be noted, however, that this increased resolution is obtained only with longer analysis, in this case from 14 min with one column to 30 min with the two-column series. If desired, the analysis time can be decreased by doubling the column pressure to adjust the column flow-rate to 4.0 ml/min. In this case, resolution of the limiting pair 7–11 slightly decreases, but analysis time is also decreased to 15 min.

The analysis time can also be decreased by using step flow programming to increase flow-rates for peaks at the end of the chromatogram that are over-resolved. As illustrated in Fig. 9, the flow-rate is set at 2.0 ml/min to achieve optimum resolution in the early part of the chromatogram, then increased to 4.0 ml/min after 6 min to elute over-resolved later peaks at a much faster rate. In this case a resolution of at least 1.0 is maintained for all peak pairs, but the total analysis time is now 12 min instead of the 15 min in Fig. 7. This refinement for decreasing the separation time can be helpful in developing a final analysis. However, to use this approach effectively it is important that the solvent system first be optimized to achieve the best overall results.

In addition to the determination of optimum solvent composition in the preceding section by overlapping resolution mapping, it is also feasible to generate response surfaces for other separation parameters such as α and k'. The mapping of α values can also be used as a means of determining optimum solvent composition for separating a mixture; our limited experience suggests that results equivalent to resolution mapping are obtained. The mapping of k' values can be of interest in visualizing the absolute effect of various solvent modifiers on the retention of peaks of interest.

Finally, the use of the solvent optimization methods described here is not limited to silica as adsorbent and the four solvents discussed. For example, other adsorbents such as alumina should provide equally useful results with this optimization method. A more basic localizing solvent, such as triethylamine, instead of MTBE may prove to be a desirable alternative for particular mixtures. However,

based on theory and the experimental work carried out to date, we feel that methylene chloride, acetonitrile and MTBE as modifiers should prove to be optimum for most LSC separation systems. 1,1,2-Trifluorotrichloroethane (FC-113) as an attractive alternative to *n*-hexane is currently under study and will be reported shortly[17].

The optimization of mobile phase solvents is especially important in two other widely used forms of adsorption chromatography, namely, thin-layer and preparative chromatography. The present approach should be equally applicable for these areas.

CONCLUSIONS

Quaternary-solvent mobile phases can be used for the systematic optimization of α values in LSC. A mixture-design statistical technique using overlapping resolution mapping requires retention data for only seven mobile phases to predict the mobile phase composition for optimum resolution. This approach greatly reduces the number of separate experiments with mobile phases which are required to establish an acceptable separation. Prediction of α and k' values for any mobile phase is also possible.

Once the mobile phase solvent composition has been optimized, increased resolution and/or decreased analysis time can usually be obtained by increasing the column length and mobile phase flow-rate together.

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