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OPTIMIZATION OF SOLVENT STRENGTH AND SELECTIVITY FOR REVERSED-PHASE LIQUID CHROMATOGRAPHY USING AN INTERACTIVE MIXTURE-DESIGN STATISTICAL TECHNIQUE

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SUMMARY

A general scheme combines the Snyder solvent selectivity-triangle concept with a mixture-design statistical technique to optimize the strength and selectivity of mobile phase solvents for reversed-phase liquid chromatography (LC) separations. In particular, a new method of data analysis called overlapping resolution mapping (ORM) shows advantages over previous chromatographic optimization schemes. The approach can be used to achieve a minimum resolution of all components of a mixture or, alternatively, a single pair or several different pairs of compounds within the mixture. In reversed-phase separations of nine naphthalene compounds substituted with different functional groups, tests with mixtures of methanol–, acetonitrile– and tetrahydrofuran–water that have significant selectivity differences revealed that no single organic modifier in water could separate all components. However, when data from seven separation experiments were analyzed with the interactive computer routine, an optimum solvent mixture was predicted that subsequently gave complete isocratic separation of all components. While anticipated selectivity changes were found with aqueous mixtures of single organic solvents, aqueous binary or ternary mixtures of these organic solvents exhibited anomalous behavior toward certain solutes. In addition, individual solvent strengths were sometimes different from those predicted by previous studies.

Tests on a literature LC data set using simulated solvent mixtures with fifteen compounds, some exhibiting peak crossovers with different solvent mobile phases, clearly demonstrated the advantages of the mixture-design ORM method over other chromatographic optimization techniques.

INTRODUCTION

In many analytical chemistry laboratories major effort is directed toward the development of optimum methods of analysis. Although most modern analytical

techniques have a sound theoretical background, many workers do not use state-of-the-art technology to optimize analysis methods efficiently. In addition, the complex interaction of various effects can lead to a situation where even the most knowledgeable expert cannot easily comprehend or visualize which parameters are really the most important ones for an optimum analysis. In these cases, the proper use of a computer and automated analytical instrumentation can be invaluable.

Morgan and Deming[1] have reviewed many of the methods which have been used for optimization of chromatographic systems. The chromatographic response function (CRF) as a measure of system performance has found uses in both gas chromatography[2] (GC) and liquid chromatography[3] (LC). The CRF has the general form[2]:

$$\mathrm{CRF} = \sum_{t=1}^{k} \ln (P_t) \tag{1}$$

where P_t is a measure of separation between adjacent peaks in a chromatogram for k peak pairs, where k is one less than the total number of peaks. Various types of optimization techniques have used the CRF as a judgement of analysis quality, with the largest CRF value usually indicating the best separation of a multicomponent mixture.

Laub and co-workers[4–7] have used a "window diagram" approach for optimizing mixed stationary phases in GC. This method relies on the ability to predict an appropriate binary mixture of stationary phases which will give the best separation for the most poorly resolved pair of peaks in the chromatogram.

The systematic optimization of the mobile phase for selectivity in LC is a relatively new development and is not yet widely practiced. Many LC separations are developed by a random process of selecting various mobile phase solvent mixtures with little guidance from well-established theoretical considerations. A more efficient and effective procedure has the potential for both saving considerable development time and significantly increasing the information content of the final result.

A systematic method for improving resolution in LC has been summarized by Snyder and Kirkland[8]. This method uses an empirical but quantitative relationship for solvent strength and selectivity developed by Rohrschneider[9] and Snyder[10]. We have incorporated these procedures into a mixture-design statistical technique for optimizing the mobile phase composition in reversed-phase LC. A new method of measuring the quality of a separation called overlapping resolution mapping (ORM) shows distinct advantages over other measures of chromatographic separation "goodness". In particular, the best mobile phase composition can be predicted which will give a specified, minimum resolution of all peak pairs.

EXPERIMENTAL

All separations were carried out on a DuPont Model 850 liquid chromatograph (DuPont, Wilmington, DE, U.S.A.), equipped with both ultraviolet (UV) absorbance and refractive index (RI) detectors and a 6-port air-actuated Valco microsampling valve equipped with a 25-μl loop. A reversed-phase system using water as the base solvent was chosen for study, since most current LC analyses are performed

in this mode. A single 15 × 0.46 cm column of DuPont Zorbax*-C_8 was used during the study. This C_8 column showed excellent performance throughout the studies with little loss of efficiency (<15%) over the course of more than 2000 separations involving many solvent changes. Stability of the packing in this column was enhanced by using a short (5 cm) precolumn of the C_8 bonded-phase packing inserted prior to the sampling valve.

Methanol (MeOH), acetonitrile (ACN) and tetrahydrofuran (THF) were used as the mobile phase modifying solvents. Isopropanol (IPA) and hexafluoroisopropanol (HFIP) were also tested, but not used in the final optimization. Samples were dissolved in the mobile phase of interest.

Solvents were thoroughly degassed individually before use and the organic modifier(s) was mixed with Milli-Q-treated water (Millipore, Bedford, MA, U.S.A.) by volume percent outside the instrument. The notation $\overline{\text{solvent}}$ will be used to indicate a defined mixture of an organic modifier in the base solvent water. For example, $\overline{\text{MeOH}}$ denotes methanol in water for a reversed-phase system. All of the runs were performed at 40°C unless otherwise noted.

To obtain meaningful results, mobile phase flow-rate and the t_0 value of an unretained peak for the separation must be carefully measured. The flow-rate was set at 2.0 ml/min and confirmed volumetrically by collecting the column effluent after passing through the detector. Values of t_0 were obtained by injecting a small volume of solvent with a slightly weaker composition than the actual mobile phase. For example, if the mobile phase was 55% MeOH in water, an appropriate volume of 52% MeOH in water was injected. The RI detector was used to monitor the first peak resulting from this injection (avoiding the total exclusion peak) to determine the t_0 value.

A statistical simplex design (*not* the same as the widely used simplex algorithm) was used when acquiring the experimental data. These data were then fitted to a quadratic model to generate a response surface as described by Snee[11]. An optimal locator algorithm was designed to find the best solvent mixture for up to eight mixture variables, although only three solvent variables were used in the present study. The chromatographic data were collected and analyzed on a PDP-10 computer[12] and calculations were performed on a PDP 11/40 minicomputer (Digital Equipment, Maynard, MA, U.S.A.) programmed in FORTRAN. A Tektronix (Beaverton, OR, U.S.A.) Model 4014-1 Graphics Terminal was used for plotting results.

RESULTS

A major problem that has to be addressed before an optimization method can be developed is to devise some way of measuring the quality of an analysis as well as criteria to compare one method with another. The exact definition of quality depends on the particular analytical technique and on the operating parameters which are to be investigated. For example, in atomic absorption spectroscopy the sensitivities toward various metals might be more important than the separation of individual elemental lines. In LC, however, the major problem is usually not sensitivity, but

* DuPont trademark for LC columns and packings.

rather the separation of two or more components, usually measured by the resolution of peaks. Resolution in LC can be affected by three independent factors[8]:

$$R_s = \underbrace{\frac{1}{4}(\alpha - 1)}_{\text{selectivity factor}} \cdot \underbrace{(\sqrt{N})}_{\text{efficiency factor}} \cdot \underbrace{\left(\frac{k'}{1 + k'}\right)}_{\text{capacity factor}} \qquad (2)$$

where $\alpha = k_2'/k_1'$ is the selectivity factor for two peaks; N is the column plate number; and k' is the capacity factor[8]. To obtain the best resolution of two peaks in the shortest time, all three of these separation factors must be optimized. Techniques for adjusting column resolution in LC have been detailed in ref. 8. For convenience a brief summary follows, so the reader may more easily follow the optimization scheme herein proposed.

Adjustment of column resolution

The normal order in which the three terms of the resolution expression should be experimentally determined is k', α and N, respectively. Clearly, from previous discussions[8], changes in the capacity factor, k', will result in the largest effect on R_s with minimal effort; therefore, adjustment of k' is usually the initial step in optimizing the LC separation. The k' value is easily changed for a peak (or pair of closely related peaks) by changing mobile phase solvent strength. However, there is a practical limit in changing k' to increase resolution, as is evident in an examination of eqn. 2. It can be shown that R_s is proportional to $k'/(1 + k')$, therefore, increases in $k' > 10$ will have a very small effect on the resolution *versus* those increases in the range of 1–10. Therefore, a range of $1 \leqslant k' \leqslant 10$ is generally accepted to be optimum[8].

The second term that should be optimized for a LC separation is α. Once again, the mobile phase has the greatest effect on α for most systems; however, in optimizing α, solvent composition is more important than solvent strength.

Rohrschneider[9] and Snyder[10] have developed empirical quantitative relationships for the strength and selectivity of 84 solvents. The solvent strength (sometimes called "polarity") scale composed of P' values is an approximate measure of solvent strength and can be used as a first-order prediction for optimizing the capacity factor. The relative ability of each solvent to interact as a proton-donor, proton-acceptor, or dipole is measured by x_d, x_e and x_n values, respectively. These values are useful for selecting solvents that will exhibit the different chemical interactions with sample compounds of interest. Solvents with the widest potential differences in interactions are those most likely to affect selectivity changes leading to better separation of sample component pairs. Table I lists solvent strength P' and selectivity (x) values for some solvents commonly used in LC.

The relative selectivity of LC solvents has been conveniently grouped into definitive solvent selectivity classification groups by Snyder[10], as exemplified in the last column of Table I. Fig. 1 shows a plot of these solvent selectivity groups with trilinear coordinates of proton-donor (x_d), proton-acceptor (x_e) and dipole–dipole (x_n). Each circle on this plot represents a group of solvents with similar characteristics and generally the same functionality, as shown by the listing in Table II.

TABLE I

SUGGESTED SOLVENTS FOR LC

P' = Solvent polarity; x_e = proton acceptor contribution; x_d = proton donor contribution; x_n = strong dipole contribution. $x_e + x_d + x_n = 1.00$.

	P'	x_e	x_d	x_n	*Solvent* group*
Normal phase					
Ethyl ether**	2.8	0.53	0.13	0.34	I
Chloroform	4.1	0.25	0.41	0.33	VIII
Methylene chloride	3.1	0.29	0.18	0.53	V
n-Hexane (carrier)	0.1	—	—	—	—
Reversed phase					
Methanol	5.1	0.48	0.22	0.31	II
Acetonitrile	5.8	0.31	0.27	0.42	VIb
Tetrahydrofuran	4.0	0.38	0.20	0.42	III
Water (carrier)	10.2	—	—	—	—

* Solvent classification group, see Fig. 1 and Table II.
** More practical alternative: methyl *tert.*-butyl ether.

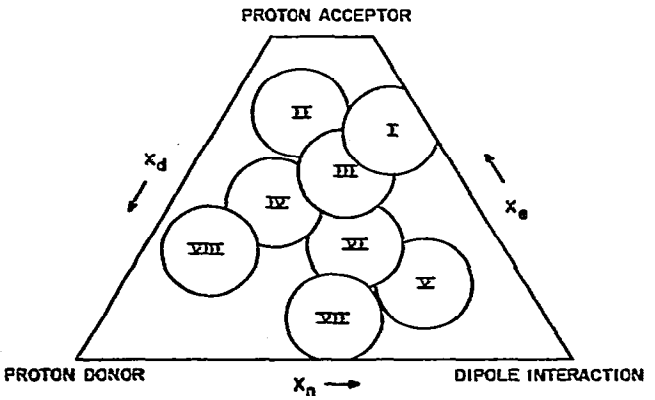


Fig. 1. Selectivity triangle for solvents of Table II. See ref. 10.

TABLE II

CLASSIFICATION OF SOLVENT SELECTIVITY

Group	*Solvents*
I	Aliphatic ethers, tetramethylguanidine, hexamethyl phosphoric acid amide, (trialkyl amines)
II	Aliphatic alcohols
III	Pyridine derivatives, tetrahydrofuran, amides (except formamide), glycol ethers, sulfoxides
IV	Glycols, benzyl alcohol, acetic acid, formamide
V	Methylene chloride, ethylene chloride
VI	(a) Tricresyl phosphate, aliphatic ketones and esters, polyethers, dioxane (b) Sulfones, nitriles, propylene carbonate
VII	Aromatic hydrocarbons, halo-substituted aromatic hydrocarbons, nitro compounds, aromatic ethers
VIII	Fluoroalkanols, *m*-cresol, water, (chloroform)

To affect a change in selectivity (α values) in LC, the strategy is to investigate solvents from the apices of the solvent selectivity triangle, since from these solvents, the greatest differences in chemical selectivity can be expected. Fig. 2 shows solvent interaction triangles of convenient solvents for both normal- and reversed-phase LC. As given in Table I, these solvents are ethyl ether (or, more preferably, methyl *tert.*-butyl ether), methylene chloride and chloroform at appropriate concentrations in *n*-hexane for normal-phase chromatography; and methanol, tetrahydrofuran and acetonitrile in water for reversed-phase chromatography.

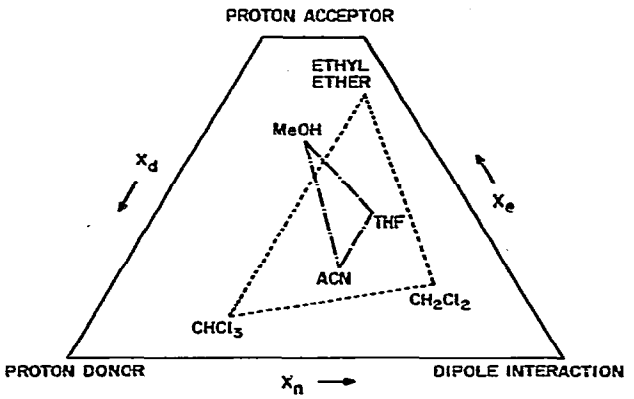


Fig. 2. Selectivity triangles for preferred solvents in reversed-phase and normal-phase chromatography. · – · – ·, reversed phase; – – – –, normal phase.

As a starting point in all LC separations, a convenient method is first selected and an appropriate solvent system is chosen, for example, water–methanol in reversed-phase LC. The concentration of the organic modifier in the carrier solvent is adjusted to place the solute peaks of interest in the preferred k' range. An example of the change in k' with solvent strength can be seen in one system[13] with phenol and acetophenone, which have $k' = 0.7$ and 1.2, respectively with methanol–water (60:40), and 1.6 to 2.7 with the weaker solvent, methanol–water (50:50) as the mobile phase.

Using the relationships in eqns. 3–5, other mobile phase compositions of different selectivity which have equal elution strength can be predicted, using *n*-hexane as the base solvent for normal-phase and water for reversed-phase chromatography[10]. These base solvents should have little or no influence on the selectivity of the separating system.

$$\frac{k_2}{k_1} = 10^{(P_1 - P_2)/2} \text{ normal phase} \tag{3}$$

$$\frac{k_2}{k_1} = 10^{(P_2 - P_1)/2} \text{ reversed phase} \tag{4}$$

$$P' = \varphi_a P_a + \varphi_b P_b \tag{5}$$

Here, k_1 and k_2 are the k' values for a sample compound in pure solvents with P' values of P_1 and P_2, φ_a and φ_b are the volume fractions of solvent A and B, and P_a and P_b are the P' values of the pure solvents. For example, in a normal-phase system, 52% ethyl ether in *n*-hexane, 35% chloroform in *n*-hexane and 47% methylene chloride in hexane would all correpond to nearly equivalent solvent strengths. A similar situation would result in the reversed-phase case with 45% methanol in water, 52% acetonitrile in water and 37% tetrahydrofuran in water. On the other hand the relative contributions of proton-donor (x_d), proton-acceptor (x_e), and dipole (x_n) effect for each solvent would be different, resulting in potentially different α values for pairs within the same k' range.

The third term of the resolution expression in eqn. 2 to be optimized is N. This value is related to column efficiency and depends on column length, particle size and mobile phase velocity, among other factors. Unlike changes in k' and α which must be experimentally determined, the effect of operating parameters on N can be quantitatively predicted using the Knox equation and basic principles described elsewhere[14]. Therefore, optimization of N has not been included in our present method.

Thus in summary, the basic separation strategy is to choose solvents from the apices of the solvent triangle in Fig. 2 for maximum selectivity differences in the desired separation. The composition of each mobile phase is adjusted to give similar k' range (equal solvent strengths) for the compounds of interest, and an attempt is made to determine the optimum composition mixture of the solvents that will result in the best overall selectivity. If the resolution of any pair(s) is still insufficient, N values must be appropriately increased, or other parameter changes (pH, temperature, etc.) must be considered, or alternatively, another LC method used.

Evaluation of column resolution: single value approaches

Despite the ability to change selectivity and resolution by altering LC operating conditions, the major problem experienced in optimizing the chromatographic system is the difficulty in distinguishing a good separation from a poor one. The resolution concept is only valid for a pair of peaks, but often the analyst is interested in a number of peaks simultaneously. If the "best" separation of all peaks is desired, there must be a way to relate the importance of each pair of peaks to other pairs, even if the resolution of all peaks is equally important. A number of methods for measuring the performance of a multicomponent system have been proposed[1], most of which involve the linear combination of the measurements for particular pairs to give a single value or number related to separation goodness under a single set of conditions. This single value concept is important, since it is quite convenient in an optimization scheme to have one numerical value per experiment.

Chromatographic response function

One of the most useful of the reported functions to judge separation quality is the CRF[2,3]:

$$\mathrm{CRF} = \sum_{i=1}^{k} \ln (P_i) \qquad (6)$$

where P_i is a measure of the peak separation of the ith pair of peaks in a system with k total pairs of interest. Peak separation, P_i, is simply defined

$$P_i = \frac{f}{g} \tag{7}$$

where f is the depth of the valley below a straight line connecting two adjacent peak maxima and g is the height of the straight line above the baseline at the valley, as illustrated in Fig. 3.

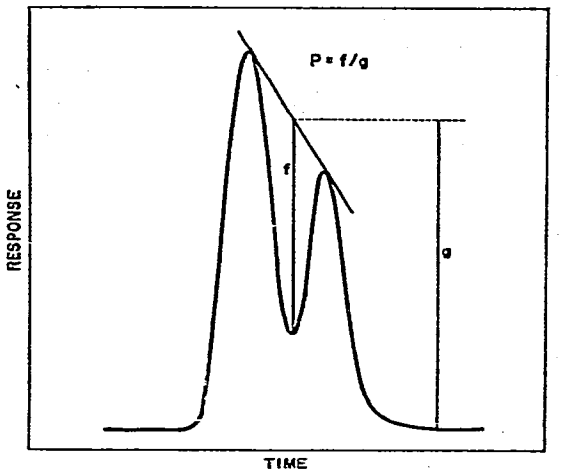


Fig. 3. Illustration of the measurement of the peak separation factor, P_i. See eqn. 7; figure adapted from ref. 3.

The CRF of eqn. 6 is a useful method for relating the resolution for pairs of peaks in a chromatogram. This approach has proven to be convenient for certain types of separations where components are present in relatively equal amounts and when separation of all components is equally desirable. However, in our work a more general method was needed for analyzing a chromatogram in terms of both relative compound concentrations and separation importance. Watson and Carr[3] have proposed the following version of a general CRF:

$$\text{CRF} = \sum_{i=1}^{k} \ln \frac{P_i}{P_0} + \alpha(t_M - t_L) \tag{8}$$

where k is the number of adjacent pairs of peaks, P_0 is the desired peak separation, P_i is the peak separation for the ith pair of peaks, t_M is the maximum acceptable analysis time, t_L is the experimental time and α is an arbitrary weighting factor. This function is constrained so that if $P_i > P_0$, P_i is set equal to P_0, and if $t_M > t_L$, t_M is set equal to t_L. This arrangement has the effect of neglecting those pairs whose resolution is sufficient ($\ln P_i/P_0 = \ln 1 = 0$) and those chromatograms where the analysis time is less than that maximum allowable.

Chromatographic optimization function

A revised measurement of chromatographic performance, the chromatographic optimization function (COF), is proposed, which incorporates two modifications to make this approach more general. First, peak resolution instead of peak separation is used in the first term of the function. Resolution is a more familiar chromatographic measure than peak separation, and Watson and Carr have already discussed the disadvantages of the peak separation function P_t when the peak height ratio of adjacent peaks approaches 10:1. In addition, the peak separation function deteriorates when $R_s < 1.0$ ($P < 0.75$). Contrary to previously expressed opinion[3], we feel this limitation can be quite restrictive when very difficult separations are to be optimized.

The second improvement incorporated into the COF method was to include a coefficient, A_t, for each pair of peaks of interest. By including this coefficient, each pair of peaks can be weighted differently if one or more separations of peak pairs is more important than the others. The COF is defined as:

$$\text{COF} = \sum_{t=1}^{t} A_t \ln \frac{R_t}{R_{td}} + B(t_M - t_L) \qquad (9)$$

where R_t is the resolution of the ith pair, B is an arbitrary weighing factor and R_{td} is the desired resolution for that pair. Of course, if all A_t values and all R_{td} values are equal for all pairs of any index i, the equation converges to a form closely resembling that of the CRF. In any case, the COF reduces data from each chromatogram to a single number which can be used in the optimization procedure, and this value approaches zero for an optimum separation. Thus chromatograms with good peak resolutions produce small negative COF values (or preferably zero), and those with poor separations result in large negative values.

With such modifications, the COF becomes a much improved method, but still has limitations in analyzing chromatograms for separation quality. The COF works satisfactorily if all the peaks have the same relative order of retention in all solvents. In this case, components need not be explicitly identified in chromatograms, and the COF can be used with unknown mixtures. However, if the relative order of retention changes with solvent composition (peak "crossover"), the COF value may not reflect this change. Also, what is actually desired in a separation cannot be conveniently related to the numerical COF value. Even though the COF value uses the definitive resolution term in the calculations, a certain amount of qualitative judgement is still needed to adequately relate individual resolutions to the final COF value.

Finally, considerable information is discarded if the COF or similar functions where peak pairs are combined into a single number are used to evaluate the separation in a chromatogram. While this approach is necessary for optimization in the manner described above, it results in the serious loss of information of individual pairs of peaks. In addition, the COF approach can have disadvantages, like that illustrated in Fig. 4. Both of the chromatograms in this figure have the same COF value, but the second separation, which shows five peaks rather than four, clearly is preferred. Despite reservations just discussed, the COF value was chosen as the starting point for the present LC optimization study.

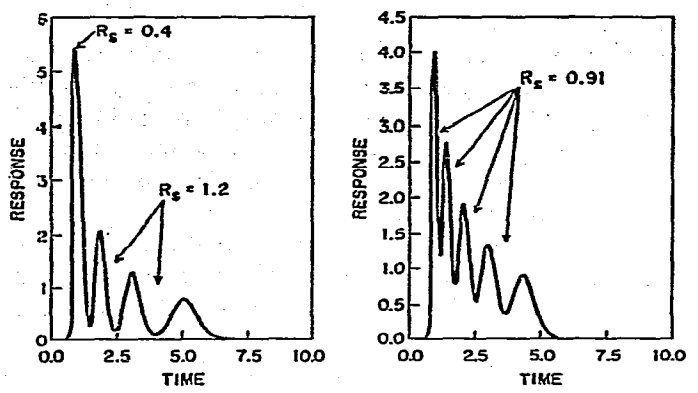


Fig. 4. Comparison of simulated separations with the same COF value, but different R_s values.

Statistical design and optimization

Application of the new statistical simplex system is illustrated in Fig. 5 for selecting an optimum mixture of three solvents: A, B and C. A ten-run design is used in this case. Runs 1–7 are used to estimate the coefficients in the quadratic equation that describes the surface formed by the resulting resolution data, and runs 8–10 are used to check the precision of the predicted optimum solvent composition[11]. This ten-run design has three major advantages in systems such as LC solvent optimization: (1) reasonably accurate estimates are obtained for the coefficients in the quadratic equation (*i.e.*, a good model of the surface); (2) an estimate of experimental error is provided and (3) a measure of the lack of fit of the assumed model is obtained. It is important to note that the second point offers a distinct advantage over a simple simplex algorithm which assumes no experimental error.

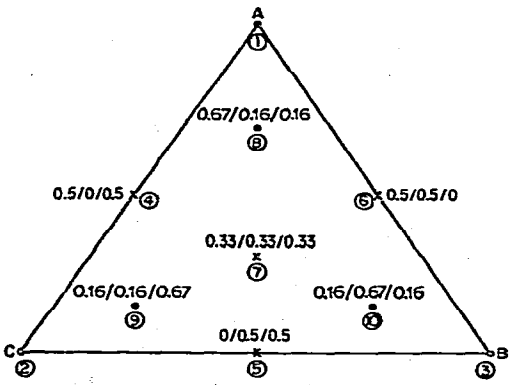


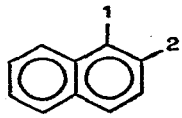
Fig. 5. Simplex design for three solvents (A, B and C) and mixtures. Values for each point are trilinear coordinates of A/B/C.

Substituted naphthalenes

A mixture of nine substituted naphthalenes with different functionalities (Table III) was chosen as the initial test for the optimization technique. The diversity

TABLE III

TEST COMPOUNDS



Substituted naphthalenes.

Compound No.	*Place of substituent*	*Substituent*
1	1	(N-1)
2	2	SO_2CH_3
3	2	OH
4	1	
5	1	NO_2
6	2	OCH_3
7	—	Naphthalene (unsubstituted)
8	1	SCH_3
9	1	Cl

of the substituent groups provided the potential for different selectivity characteristics in proton-acceptor, proton-donor and dipole solvents.

The first step in optimizing mobile phase composition was to adjust the solvent strength of a modifier in water to produce a k' range of about 1–10 for all of the solutes. Once the necessary composition had been experimentally determined for one of the solvents, the compositions for the other solvents can be predicted, based on known empirical relationships (ref. 8, Ch. 6), as described in the Introduction. These empirical solvent strength relationships are approximate, so minor adjustments in the final solvent concentrations were often needed to produce equivalent k' ranges for a particular sample mixture.

Data in Table IV show the calculated and experimental solvent mixtures which produced the indicated k' ranges for the substituted naphthalene mixture on a 15 × 0.46 cm I.D. column of Zorbax-C_8. Small differences were apparent from the empirical predictions, especially for methanol–water mixtures, but these were not considered unusual. The solvent strength relationships used for the calculations depend somewhat on the solutes as well as the solvents used. The three solvents used for the selectivity study, MeOH, ACN and THF, are not widely separated in the solvent triangle diagram of Fig. 2, however, they still showed significant selectivity differences for the substituted naphthalenes, suggesting better separations with certain solvent mixtures.

Following the statistical mixture-design approach previously described, experiments were performed in four other mixed solvent systems. The results of all seven runs are listed in Table V and shown diagrammatically in Fig. 6. In addition to the

TABLE IV

SOLVENT COMPOSITION FOR ZORBAX-C_8 COLUMN

Solvent	*% Organic (in water)*		
	*Predicted**	*Experimental*	*Approximate k′ range*
ACN	52	52	0.7–8
MeOH	45	63	0.6–8
THF	37	39	0.6–7

* Based on 52% ACN in water.

expected k' changes with the ternary and quaternary solvents, some interesting anomalies appeared for certain solutes. For example, naphthalene showed $k' = 4.04$ in 63% MeOH, and $k' = 5.20$ in 39% THF. However, in a 50:50 mixture of these two solvents (31.5% MeOH, 19.5% THF, 49% water), $k' = 6.00$. This result is contrary to intuition, where the k' in the 50:50 mixture might be expected to fall between the k' values ($k' = 4.04$ and 5.20) for each individual solvent. This unexpected experimental observation was reproducible, and a similar effect was seen with other solvent mixtures and other solutes. Additional studies are needed to determine the origin of this effect, but we believe that it may be a function of changes in the aqueous base solvent. Apparently, water is not acting as an inert carrier; addition of miscible organic solvents to water may be changing its normal bulk properties.

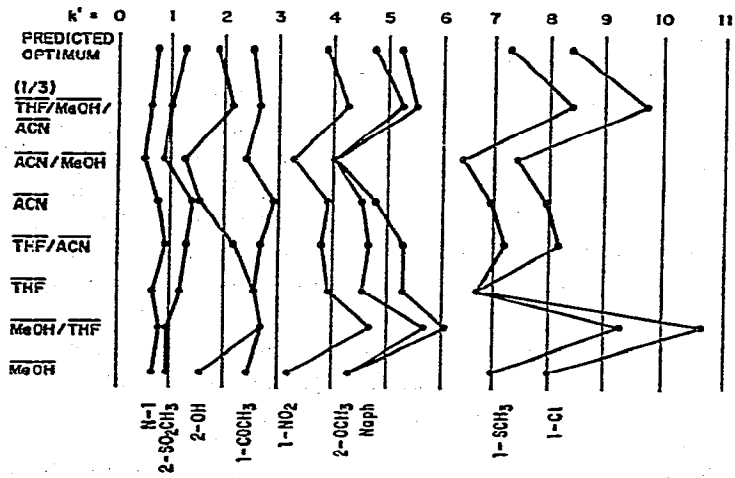


Fig. 6. Solvent selectivity data for eight mobile phases. Column, 15 × 0.46 cm Zorbax-C_8; flow-rate, 2.0 ml/min; temperature, 40°C; detector, UV photometer, 254 nm; sample compounds as listed in Table III.

The anomalous effects on k' resulting from mixing organic solvents with water did not hamper efforts to determine an optimum solvent composition for separation. The proposed statistical method performs satisfactorily since resolution between individual peaks is the primary interest. As long as resolution is a continuous mathe-

TABLE V

k' and (σ) DATA FOR SEVEN SOLVENTS WITH ZORBAX-C_8 COLUMN

Key: $\overline{\text{MeOH}}$ = methanol–water (63:37); $\overline{\text{THF}}$ = tetrahydrofuran–water (39:61); $\overline{\text{ACN}}$ = acetonitrile–water (52:48).

*Compound No.**	*Solvent k'/(σ) ml*						
	$\overline{MeOH}$	$\overline{MeOH}$–$\overline{THF}$ (50:50)	$\overline{THF}$	$\overline{THF}$–$\overline{ACN}$ (50:50)	$\overline{ACN}$	$\overline{ACN}$–$\overline{MeOH}$ (50:50)	$\overline{MeOH}$–$\overline{ACN}$–$\overline{THF}$ (33:33:33)
1	0.65 (0.045)	0.74 (0.043)	0.57 (0.043)	0.69 (0.041)	0.69 (0.040)	0.52 (0.044)	0.73 (0.043)
2	0.78 (0.047)	0.88 (0.047)	0.98 (0.047)	1.08 (0.045)	1.28 (0.042)	0.88 (0.049)	1.01 (0.047)
3	1.22 (0.054)	2.55 (0.075)	2.46 (0.118)	2.02 (0.061)	1.35 (0.042)	1.13 (0.053)	2.07 (0.064)
4	2.26 (0.072)	2.55 (0.075)	2.46 (0.118)	2.53 (0.070)	2.79 (0.067)	2.25 (0.071)	2.61 (0.071)
5	3.02 (0.086)	4.60 (0.109)	3.85 (0.101)	3.84 (0.085)	3.79 (0.078)	3.14 (0.091)	4.16 (0.097)
6	4.04 (0.101)	5.56 (0.123)	4.63 (0.117)	4.62 (0.103)	4.56 (0.083)	3.87 (0.115)	5.44 (0.120)
7	4.04 (0.101)	6.00 (0.132)	5.20 (0.131)	5.08 (0.106)	4.72 (0.091)	3.87 (0.115)	5.44 (0.120)
8	6.67 (0.154)	9.16 (0.192)	6.73 (0.163)	7.05 (0.139)	6.93 (0.128)	6.40 (0.161)	8.32 (0.167)
9	7.77 (0.164)	10.36 (0.225)	6.73 (0.163)	8.09 (0.178)	7.88 (0.145)	7.32 (0.178)	9.71 (0.188)

* See Table III for compounds.

matical function of composition, optimization of the solvents by obtaining the surface contour of the data will be successful; the function does not have to be monotonic.

Optimization of solvent composition by COF values

With eqn. 9, the data in Table V were used to generate COF values for each of the seven chromatograms, corresponding to different solvent mixtures (Fig. 5). The time variable B in the COF was not considered to be crucial in these initial experiments, so B was set equal to zero and the second term of the equation was eliminated. We also arbitrarily decided that the separation of all nine components was equally desirable, so all A_i values were set equal to unity, reducing eqn. 9 to:

$$\text{COF} = \sum_{i=1}^{k} \ln \frac{R_i}{R_d} \tag{10}$$

where R_i is the resolution of the adjacent pair of peaks i, $i + 1$, R_d is the desired resolution for every pair, and k is the number of peak pairs of interest, in this case eight.

To acquire meaningful results and to compare the effect of the optimal locator, three values of R_d were arbitrarily chosen: 1.2, 1.8 and 2.4. The resulting COF values for all seven chromatograms are shown in Table VI. These data indicate that with $R_d = 1.2$, two solvent systems produce the target value of COF = 0.0. To provide more discrimination for the optimal locator, more critical values of $R_d = 1.8$ and 2.4 were used in the final optimization procedure and found to produce the same final optimal solvent mixture location.

TABLE VI

COF VALUES FOR SUBSTITUTED NAPHTHALENES ON ZORBAX-C_8 COLUMN

COF values calculated using eqn. 10. Key: $\overline{\text{MeOH}}$ = methanol–water (63:37); $\overline{\text{ACN}}$ = acetonitrile–water (52:48); $\overline{\text{THF}}$ = tetrahydrofuran–water (39:61).

Solvent mixture	*COF values*		
	$R_d = 1.2$	*1.8*	*2.4*
$\overline{\text{MeOH}}$	−2.48	−3.21	−3.79
$\overline{\text{MeOH}}$–$\overline{\text{ACN}}$ (50:50)	−2.48	−2.89	−3.18
$\overline{\text{ACN}}$	−0.99	−1.80	−2.38
$\overline{\text{ACN}}$–$\overline{\text{THF}}$ (50:50)	0.00	−0.02	−0.30
$\overline{\text{THF}}$	−4.97	−5.78	−6.53
$\overline{\text{THF}}$–$\overline{\text{MeOH}}$ (50:50)	−2.48	−3.41	−4.27
$\overline{\text{MeOH}}$–$\overline{\text{ACN}}$–$\overline{\text{THF}}$ (33:33:33)	0.00	−0.38	−0.67
Optimum (61% $\overline{\text{ACN}}$–39% $\overline{\text{THF}}$)	0.00	−0.07	−0.36

A contour COF diagram for the naphthalene mixture over the entire surface of the solvent triangle is shown in Fig. 7. The area bounded by 0.0 represents the range of solvent compositions which produce resolutions which satisfy the target, in this case $R_d = 1.8$. The optimum solvent composition was indicated to be $\overline{\text{ACN}}$–$\overline{\text{THF}}$ (61:39) (ACN–THF–water, 32:15:53). This solvent was investigated experi-

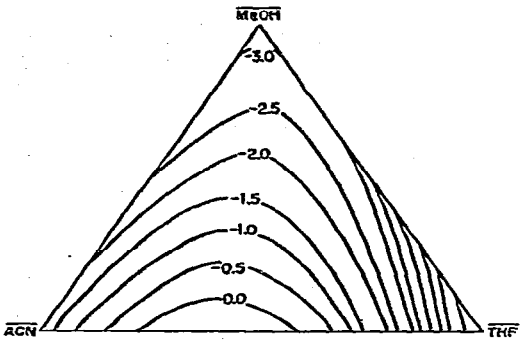


Fig. 7. COF map for substituted naphthalenes. $R_d = 1.8$. Numbers are COF values. Chromatographic conditions as for Fig. 6.

mentally and the resulting chromatogram is shown in Fig. 8. The COF for this chromatogram was −0.07 with $R_d = 1.8$, which, within experimental error, was equivalent or superior to any other solvent system tested.

Although the COF method with mixture-design statistical techniques predicted a useful optimum in the case of the nine substituted naphthalenes, a number of apparent disadvantages became obvious. Attempts to extend this method to more complex samples revealed that a COF-type optimization was cumbersome when the relative peak order in a chromatogram changed with a variation in solvent composition. In addition, the inability of the user to easily relate the COF to chromatographic terms made this approach inconvenient. But most importantly, the loss of individual peak information when using the COF suggested that a better data analysis method should be sought.

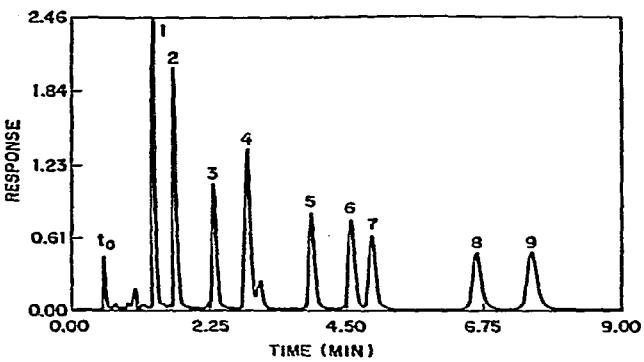


Fig. 8. Separation of substituted naphthalenes using a mobile phase with optimum selectivity. Chromatographic conditions as for Fig. 6, except: mobile phase, acetonitrile–tetrahydrofuran–water (32:15:53); compounds as identified in Table III.

Overlapping resolution maps (ORM)

A new chromatographic analysis technique was developed which relies 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 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 identified in which the desired resolution can be achieved for all component pairs. In mathematical terms, this approach comprises the intersection of Venn diagrams of the acceptable resolutions for the compounds of interest.

Basically, the new 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 are important for determining an optimum solvent. This new method does require that the peak position be mapped as solvent composition is changed, but as discussed later, the computer should be capable of performing this task. In addition, the ORM method has the potential to handle peak crossovers in contrast to the other methods discussed. We believe that this new method of LC optimization is a significant advance over COF- or CRF-type optimization methods, and that it should be applicable to optimization problems in other areas of analytical chemistry.

Analysis of substituted naphthalene data

The capability of the ORM method of optimization can be illustrated by re-examination of the data from the solvent optimization of the substituted naphthalenes discussed in an earlier section. For this test the resolution values calculated from the data in Table V were used to generate a solvent triangle contour surface of resolution for each peak pair in the chromatogram. Since there are no peak crossovers in this study, only the eight original adjacent pairs have to be considered for the optimization.

An example of the described contour diagram for one pair (peaks 8–9) by the ORM method is shown in Fig. 9. The resolution values at the vertices, midpoints, and center were obtained from the chromatograms using the corresponding solvents listed in the figure. For this mixture a resolution of 1.5 was arbitrarily set as the desired resolution for all pairs in this separation. Examination of the resulting eight contoured surfaces in Fig. 10 showed that for solvent triangles 4–5, 5–6 and 7–8, the

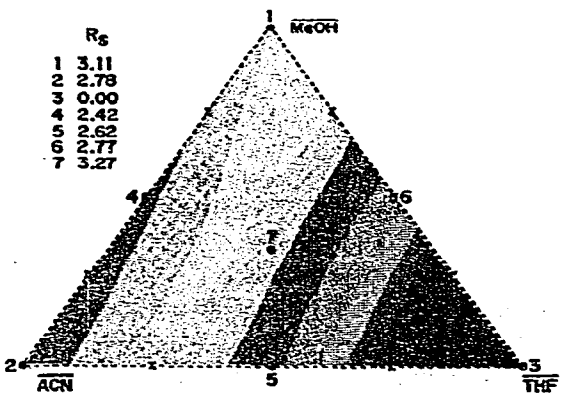


Fig. 9. Chromatographic resolution map for peaks 8–9 in Table III. Chromatographic conditions as for Fig. 6.

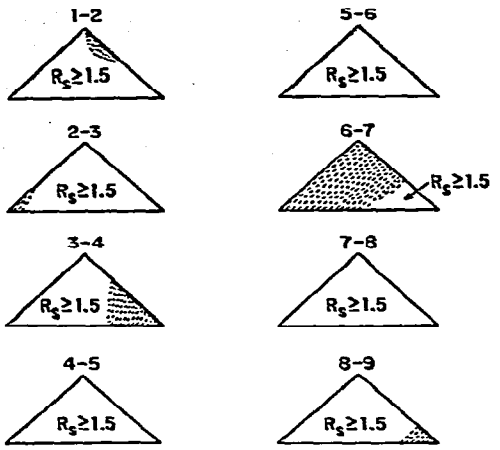


Fig. 10. Resolution maps for all eight peak pairs of substituted naphthalenes. Compounds as in Table III; chromatographic conditions as for Fig. 6.

entire surface exceeded the $R_s = 1.5$ value. This means that for the triangles associated with those peak pairs, all solvent mixtures would produce a resolution that had a value greater than 1.5. These pairs of compounds represent no problem in separation no matter which mobile phase is used; therefore, consideration of resolution for these peak pairs can now be ignored.

Fig. 11 shows the overlay intersection of the solvent-selectivity areas of interest for the other five solute pairs with more separation difficulty. The solvent region in white, A, is designated for the optimum solvent composition. Note that the COF-predicted optimum of ACN–THF (61:39), marked by an ⊗, in Fig. 11, also falls in this region.

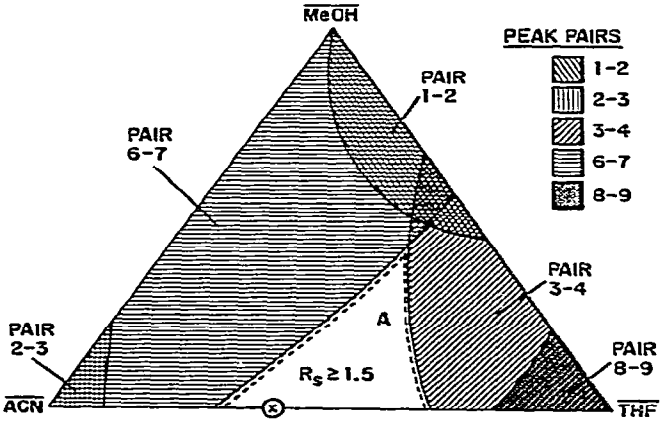


Fig. 11. Overlapping resolution map (ORM) for substituted naphthalenes. -- ----, Estimate of resolution mapping precision. Data from Fig. 10.

The intersecting maps in Fig. 11 also indicate the solute pair(s) for which resolutions are most constraining in the entire optimization procedure. For example, if peak 6 or 7 is removed from consideration, a significant broadening in the range of

optimum solvent mixture concentration occurs. This information is not available with the COF or other single number value methods, and is potentially useful in situations where separation compromises must be made. For example, an unimportant pair of peaks can be eliminated from consideration, thereby enhancing possibilities for improving the resolution of all other pairs by using a slightly different solvent.

An additional advantage of the ORM method is that a direct estimate of the experimental error of the resolution contours can be obtained. The broken dashed lines in Fig. 11 show the one sigma standard deviation for the estimated boundaries of this system to be small (equal error also above the line).

Analysis of literature separations

Although the method of ORM worked well for the substituted naphthalenes, separation of this particular mixture was fairly simple in many respects. Specifically, changing solvents produced no peak crossovers, which is a difficult problem to handle in chromatographic optimization procedures. To test the method under much more difficult circumstances, data were used from a recent publication on solvent selectivity in reversed-phase LC by Bakalyar *et al.*[13]. This study reported k' values for a range of solutes in individual aqueous solutions of MeOH, ACN and THF of equal strengths. The first 15 of a total of 30 compounds were arbitrarily selected for our study, and these are listed in Table VII. In the Bakalyar *et al.* study, solvent strengths were adjusted to produce identical retention for benzene in all three solvents ($k' = 4.7$). Unfortunately, the study did not report data on mixtures of these solvents, so theoretical k' values had to be generated in all of the solutes for the required seven mixed solvent compositions. The resulting k' values are listed in Table VIII. A linear correlation of k' with solvent concentration was assumed, although this function may not actually exist for all of the compounds in this list. However, the analysis method

TABLE VII

SYNTHETIC MIXTURE (Ref. 13)

25 cm × 0.46 cm LiChrosorb RP-8 column (10 μm particles).

No.	*Mixture*
1	Sodium benzene sulfonate (Na ØS)
2	Benzoic acid (CO_2H)
3	1-Naphthoic acid (CO_2H^2)
4	Benzamide (CON)
5	Aniline (N_2)
6	Phenol (OH)
7	Benzaldehyde (OA)
8	Methyl paraben (*p*-hydroxybenzoate) (PM)
9	Cyanobenzene (CN)
10	Acetophenone (OK)
11	Nitrobenzene (NO_2)
12	1-Aminonaphthalene (N_2^2)
13	Ethyl paraben (*p*-hydroxybenzoate) (PE)
14	Anisole (OM)
15	Benzene (Ø)

TABLE VIII

CALCULATED k' DATA FOR SYNTHETIC MIXTURE IN VARIOUS SOLVENTS (REF. 13)

Key: $\overline{MeOH}$ = methanol–water (50:50); $\overline{ACN}$ = acetonitrile–water (40:60); $\overline{THF}$ = tetrahydrofuran–water (37:63).

Solute	*Mobile phases*									
	$\overline{MeOH}$	$\overline{MeOH}$–$\overline{ACN}$ (50:50)	$\overline{ACN}$	$\overline{ACN}$–$\overline{THF}$ (50:50)	$\overline{THF}$	$\overline{THF}$–$\overline{MeOH}$ (50:50)	$\overline{MeOH}$–$\overline{ACN}$–$\overline{THF}$ 33:33:33	67:16:16	16:67:16	16:16:67
NaØS	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
CO_2H	4.50	4.20	3.90	4.05	4.20	4.35	4.20	4.35	4.05	4.20
CO_2H^2	5.10	4.65	4.20	4.80	5.40	5.25	4.90	5.00	4.55	5.15
CON	5.70	5.40	5.10	5.10	5.10	5.40	5.30	5.50	5.20	5.20
N_2	6.90	7.20	7.50	7.80	8.10	7.50	7.50	7.20	7.50	7.80
OH	7.80	7.50	7.20	8.55	9.90	8.85	8.30	8.05	7.75	9.10
OA	9.60	9.60	9.60	9.15	8.70	9.15	9.30	9.45	9.45	9.00
PM	9.90	8.70	7.50	8.25	9.00	9.45	8.80	9.35	8.15	8.90
CN	9.90	10.50	11.10	10.50	9.90	9.90	10.30	10.10	10.70	10.10
OK	11.10	10.05	9.00	8.85	8.70	9.90	9.60	10.35	9.30	9.15
NO_2	13.20	13.50	13.80	13.50	13.20	13.20	3.40	13.30	13.60	13.30
N_2	14.10	13.80	13.50	13.50	13.50	13.80	13.70	13.90	13.60	13.60
PE	14.40	12.00	9.60	10.50	11.40	12.90	11.80	13.10	10.70	11.60
OM	16.50	16.20	15.90	15.30	14.70	15.60	15.70	16.10	15.80	15.20
Ø	17.10	17.10	17.10	17.10	17.10	17.10	17.10	17.10	17.10	17.10

would be applicable even without such a linear correlation, albeit a different optimum might be predicted.

The mixture used by Bakalyar *et al.* is obviously much more complex than the substituted naphthalene mixture previously discussed. More importantly, a number of complicating peak crossovers occur, so the optimization analysis cannot be limited only to the 14 adjacent pairs in a chromatogram. For example, the 10th and 11th peaks in one solvent are not necessarily the same as the 10th and 11th peaks in another solvent. An indication of the extent of peak crossover can be seen in Table IX where the peaks are listed in the elution order for each solvent. The peaks in boxes were involved in peak crossover as the solvent was varied (compared to methanol-modified mobile phase). Clearly, therefore, a peak must be compared not only with its adjacent peak(s) in every solvent, but with any peak it had crossed over in any solvent. For example, because of crossover, peak 8 must be compared as a pair not only with peaks 7 and 9, but also with peaks 5, 6 and 10.

TABLE IX

PEAK ORDER IN 10 SOLVENT MIXTURES

Key: $\overline{\text{MeOH}}$ = methanol–water (50:50); $\overline{\text{ACN}}$ = acetonitrile–water (40:60); $\overline{\text{THF}}$ = tetrahydrofuran–water (37:63).

$\overline{\text{MeOH}}$	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
$\overline{\text{MeOH}}$–$\overline{\text{ACN}}$ (50:50)	1	2	3	4	5	6	8	7	10	9	13	12	11	14	15
$\overline{\text{ACN}}$	1	2	3	4	6	5	8	10	7	13	9	12	11	14	15
$\overline{\text{ACN}}$–$\overline{\text{THF}}$ (50:50)	1	2	3	4	5	8	6	10	7	9	13	11	12	14	15
$\overline{\text{THF}}$	1	2	4	3	5	7	10	8	6	9	13	11	12	14	15
$\overline{\text{THF}}$–$\overline{\text{MeOH}}$ (50:50)	1	2	3	4	5	6	7	8	9	10	13	11	12	14	15
$\overline{\text{MeOH}}$–$\overline{\text{ACN}}$–$\overline{\text{THF}}$ (33:33:33)	1	2	3	4	5	6	8	7	10	9	13	11	12	14	15
$\overline{\text{MeOH}}$–$\overline{\text{ACN}}$–$\overline{\text{THF}}$ (67:16:16)	1	2	3	4	5	6	8	7	9	10	13	11	12	14	15
$\overline{\text{MeOH}}$–$\overline{\text{ACN}}$–$\overline{\text{THF}}$ (16:67:16)	1	2	3	4	5	6	8	10	7	9	13	11	12	14	15
$\overline{\text{MeOH}}$–$\overline{\text{ACN}}$–$\overline{\text{THF}}$ (16:16:67)	1	2	3	4	5	8	7	6	10	9	13	11	12	14	15
Optimum	1	2	3	4	5	6	8	7	10	9	13	11	12	14	15

With solvent changes it is a formidable task to identify peaks after crossovers, and especially to determine which peak pairs need to be taken into account. However, the latter part of the process is greatly simplified with the computer which can sort through all of the possible pairs, eliminate from consideration those which are trivial (for example, 1–15 in this case), and focus on only important peak pairs. It can be shown that the maximum number of possible pairs for n peaks is:

$$\binom{n}{2} = \frac{n!}{(n-2)!\,2!} \tag{11}$$

In a mixture with 15 peaks, a maximum of 105 possible pairs would result. However, with the 15-component test mixture studied herein, only nine of the 14 adjacent pairs and nine of the other possible pairs were important to analyze. All other peak pairs could be ignored as unimportant because the resolution was large for any solvent mixture used.

For the 15-component mixture an arbitrary resolution of 0.4 was used in the statistical analysis to produce a region of solvent optimization, as shown in Fig. 12. Although $R_s = 0.4$ is not a practical value for separation, analysis of the data was based on a relatively low-efficiency column of only 4000 plates. Use of a more efficient (currently commercially available) column of 16,000 plates would increase the minimum resolution for all peaks to 0.8 (see eqn. 2) —an acceptable value for many analyses. The chromatogram in Fig. 13 illustrates the simulated separation of the 15-component sample on a 16,000 plate column with the mobile phase optimized by the ORM method. This chromatogram was computer-synthesized using the k' values

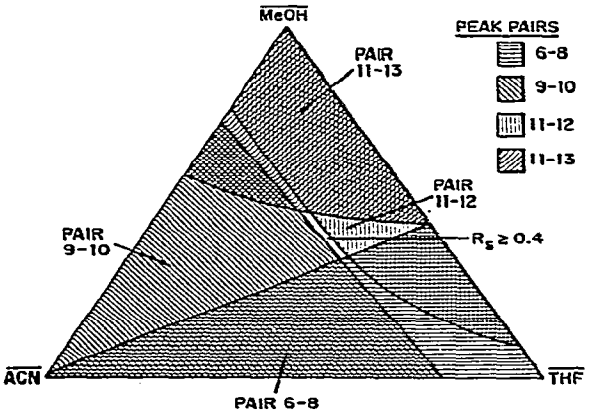


Fig. 12. Overlapping resolution map (ORM) for literature data. Data from Tables VII and VIII; shaded regions are solvent compositions that will *not* resolve the indicated pair to a resolution of 0.4. Note that only the four peak pairs with the most constraining resolution are shown.

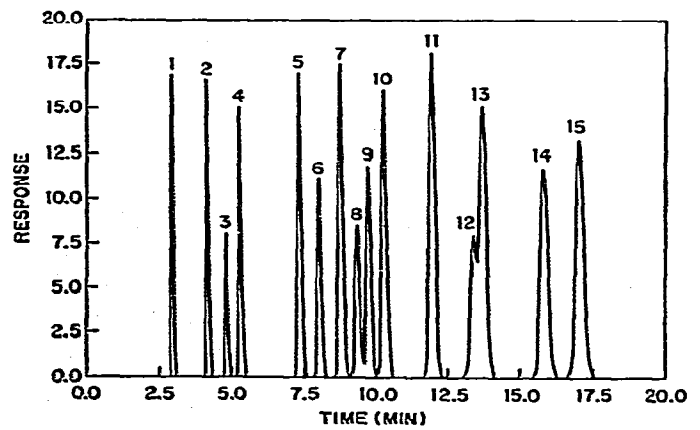


Fig. 13. Optimum separation of the 15 compounds in Table VII —simulated chromatogram.

supplied in ref. 13, appropriate peak widths for a 16,000 plate column, and arbitrary peak sizes. These results show that peaks 12–13 now are separated by the minimum predicted $R_s = 0.8$ value. However, based on the original literature k' data (given the requirement of a minimum of $R_s = 0.8$ for all of the other peak pairs in the separation), it would be predicted that a better resolution of peaks 12–13 would not be obtained with any other combination of the mobile phases used in this study. It would be possible to select other solvents that would specifically separate peaks 12–13; however, resolution of some of the other peak pairs would likely be poorer. Superior resolution might also be obtained at different operating temperatures, pH, or with an entirely different LC method.

An examination of Fig. 12 reveals the three pairs of compounds which constrain solvent optimization to the greatest extent, namely pairs 6–8, 9–10 and 11–12. If any one of these compound pairs was to be eliminated from the particular analysis, constraints in optimization of the mobile phase would be relaxed and a more effective separation for other components would result.

DISCUSSION

Important advantages are apparent for the ORM data analysis method, relative to previous techniques used in chromatographic optimization. The most important feature is that all pertinent information regarding a peak or pair of peaks is used in the optimization. In previous approaches, only one number was used to characterize an entire chromatogram —some useful information is necessarily discarded in the process of arriving at functional numerical values for optimization. The ORM method allows the operator to more easily identify and focus on those peaks which must be resolved to achieve a desired analysis.

A second advantage of the ORM method is the ability to handle peak crossovers and fused peaks more easily than functions that do not take into account relative peak positions. This important advantage makes the ORM intersecting contour method more versatile than any previously proposed chromatographic optimization function. Of course, in separation systems where no crossovers occur with solvent changes and peak merging is not a problem, optimization methods such as the COF are often satisfactory. However, many separation problems in LC are not that well-behaved and the more versatile ORM method should be preferred.

In all cases, optimized separating conditions for LC should be sought that produce symmetrical peaks[8]. However, the ORM method will often function adequately even though peak symmetry is not ideal. In this case, the deciding factor in the utility of the method is the ability to accurately measure peak resolution.

A limitation of the ORM method is that peak identity and peak positions must be determined in each solvent system to plot and contour correctly resolution as a function of solvent composition. This requirement may be tedious to accomplish in mixtures with large numbers of components (*e.g.*, > 30) or in mixtures with a large number of unknown compounds. In such instances, additional experiments would probably have to be carried out to identify certain peaks in the chromatogram. However, certain shortcuts can minimize the time and effort required. For example, several standards can be combined in a single test sample if the compounds differ widely enough in retention to be unambiguous. Alternately, the changing position of

peaks with solvent changes can also be characterized using specific detectors (*e.g.*, scanning UV absorbance) to monitor relative retention positions as well as peak size measurements. Finally, samples most likely to be encountered in optimization studies involving routine analyses often contain fewer than 10 components, and in such cases the determination of relative peak positions is often quite straightforward.

The new ORM method superficially resembles the "window diagram" optimization method developed by Laub and co-workers[4–7] for GC. However, the Laub method has two distinct disadvantages compared with the ORM. First, the "window diagram" method assumes a linear retention behavior as a function of mixing solvents (or stationary phases). This assumption does not hold in many instances, particularly in LC applications with polar mobile phases. Second, a "window diagram" for solvent optimization can only be generated with binary solvent (or stationary phase) mixtures. Therefore, in LC where at least three modifying solvents (plus the base solvent) are needed to generate the proper solvent triangle selectivity data for maximum selectivity possibility, the "window diagram" technique is at a serious disadvantage in solvent optimization studies.

CONCLUSIONS

The method for optimizing reversed-phase LC solvent composition using ORM has obvious utility in developing routine separations. In addition, the method appears to be unique for optimizing operating parameters and in general, therefore, should find wide application in a variety of analytical methods. The less versatile COF form of optimization is nevertheless satisfactory in less complex, less critical applications.

Expansion of the ORM method to other LC methods (*e.g.*, normal-phase), and to the effects of temperature, time of analysis and other parameters is feasible, and such studies are already underway. Although the optimization method currently uses an operator interface between the LC instrument and the computer, these could be directly interfaced to permit efficient, automated method development of an optimum LC separation.

REFERENCES

1 S. L. Morgan and S. N. Deming, *Sep. Purif. Methods*, 5 (1976) 333.
2 S. L. Morgan and S. N. Deming, *J. Chromatogr.*, 112 (1975) 267.
3 M. W. Watson and P. W. Carr, *Anal. Chem.*, 51 (1979) 1835.
4 R. J. Laub and J. H. Purnell, *J. Chromatogr.*, 112 (1975) 71.
5 R. J. Laub and J. H. Purnell, *Anal. Chem.*, 48 (1976) 799.
6 R. J. Laub and J. H. Purnell, *Anal. Chem.*, 48 (1976) 1720.
7 R. J. Laub, J. H. Purnell and P. S. Williams, *J. Chromatogr.*, 134 (1977) 249.
8 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979, Ch. 2.
9 L. Rohrschneider, *Anal. Chem.*, 45 (1973) 1241.
10 L. R. Snyder, *J. Chromatogr. Sci.*, 16 (1978) 223.
11 R. D. Snee, *Chemtech.*, 9 (1979) 702.
12 J. S. Fok and E. A. Abrahamson, *Amer. Lab.*, 7 (1975) 63.
13 S. R. Bakalyar, R. McIlwrick and E. Roggendorf, *J. Chromatogr.*, 142 (1977) 353.
14 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979, Ch. 5.