Journal of Chromatography, 485 (1989) 541–556
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 005

SOLVENT MODULATION IN LIQUID CHROMATOGRAPHY: OPTIMIZATION STRATEGIES

JON H. WAHL and VICTORIA L. McGUFFIN*
Department of Chemistry, Michigan State University, East Lansing, MI 48824 (U.S.A.)

SUMMARY

In solvent modulation, individual solvent zones are introduced onto the liquid chromatographic column in either a repeating or a random sequence. Because the solvent zones are spatially and temporally separated, solute retention is controlled independently within each zone. Therefore, the overall retention of the solute is a simple time-weighted average of its capacity factor in the individual solvent zones. This technique provides a versatile alternative to premixed mobile phases for the rapid optimization of separations with a minimum number of preliminary experiments. The separation of a complex mixture of fourteen saturated and unsaturated fatty acids was optimized utilizing the solvent modulation technique. Two different optimization strategies were explored: (1) the complete resolution response surface was systematically calculated for a two-dimensional solvent modulation system and the optimum was determined by visual inspection, and (2) an iterative searching method was used to locate rapidly the optimum response for a two-dimensional or higher order resolution response surface. Excellent agreement was observed between the predicted retention and that measured experimentally, with a relative error of less than 4.5% for all fatty acid standards. The optimum separation using solvent modulation was comparable to that obtained using premixed solvent systems, yet was achieved with only two initial experiments.

INTRODUCTION

In liquid chromatography, a variety of computational methods are utilized to predict the optimum conditions for a separation[1–3]. Generally, two different types of optimization strategies can be distinguished. In the first type, all experimental retention or resolution data are acquired prior to the evaluation. These data are fitted to a predefined mathematical function by either a linear or non-linear regression technique. The optimum conditions are then deduced by either interpolation or extrapolation of the regression analysis. Many popular optimization techniques make use of regression analysis, including the window-diagram approach[4], computer simulation programs such as DryLab[5] and many others[6,7]. The second type of optimization strategy is an iterative search, such as the simplex method[8], in which the results of one experiment are used to predict the most desirable conditions for the next

0021-9673/89/$03.50

experiment. This successive process is repeated, directing the search until the optimum chromatographic conditions are determined[9–12]. An advantage of the iterative search methods over regression techniques is that no assumptions are necessary concerning the mathematical form or function of the retention data. Separations requiring multiple experimental variables may be more readily optimized, even if such variables are not completely independent. Finally, fewer experiments may be required to locate the optimum conditions, since only the most promising regions of the response surface are examined in detail.

When these optimization methods are used in liquid chromatography, solute retention is most commonly controlled by modification of the mobile phase. It is generally assumed in theoretically based models that solute retention is a linear function of the mobile-phase composition[13]. This assumption implies that molecular interactions are ideal, so that solute interactions with each solvent are completely independent in mixed solvent systems. However, if solvent–solvent interactions vary in strength or selectivity as a function of composition, the corresponding solute–solvent interations must be concurrently and inversely altered. Such deviations from ideal behavior are frequently observed for polar, highly interacting solvents such as those used in reversed-phase liquid chromatography[10,11,14]. Whereas these non-ideal solvent–solvent interactions are often intuitively recognizable and justifiable, they cannot be predicted even qualitatively by present solution theories[15,16]. To overcome this limitation of theoretical models, general-purpose parametric equations are commonly used to characterize the retention data. For example, a linear, quadratic or other simple mathematical relationship with several adjustable parameters is used to correlate the logarithm of the capacity factor and the mobile-phase composition. Whereas such empirical methods are generally believed to be sufficiently accurate[1–3], substantial deviations of the capacity factors measured experimentally and those predicted from the presumed mathematical relationship may be observed[10,11,14]. Thus, accurate modelling of solute retention, which is necessary to predict optimum chromatographic conditions, may not be possible in premixed mobile phases owing to non-ideal solvent–solvent interactions.

Recently, the solvent modulation technique was introduced and was demonstrated to be a practical alternative to premixed mobile phases[17,18]. In solvent modulation, individual solvent zones are introduced on to the chromatographic column in a repeating or random sequence. Because the solvent zones are separated from one another spatially and temporally, non-ideal solvent–solvent interactions are effectively eliminated. Therefore, solute retention is controlled independently within each zone and the overall retention is simply a time-weighted average of that in the individual solvents.

THEORY

The concept and theory of solvent modulation were discussed in previous publications[17,18]. The overall retention of a solute (i) under the conditions of solvent modulation is given by the capacity factor (k_i):

$$k_i = \frac{\sum_{j=0}^{n} x_j \left(\frac{1 + k_{ij}}{k_{ij}} \right)}{L} - 1 \qquad (1)$$

Thus, solute retention is controlled by two factors, the capacity factor (k_{ij}) of that solute in each individual solvent (j) and the length of each solvent zone (x_j). The magnitude of the capacity factors in the individual solvents may be varied by compositional modification. Typically, the individual solvent systems are chosen to provide a large difference in either solvent strength or selectivity. Overall solute retention may then be controlled within the limits of the capacity factors in the individual solvents by varying the solvent zone length.

There are two parameters which define the physical length of the solvent zones. The proportion (Fig. 1, left) defines the fractional length of each solvent within one cycle of the modulation sequence and is analogous to the compositional ratio in a premixed solvent system. For a two-solvent system, a proportion of 25% A–75% B is defined when the zone length of solvent A is 25% of the modulation cycle while that for solvent B is 75%. The repetition rate (Fig. 1, right) represents the number of modulation cycles which comprise a single chromatographic column length. Again, for a two-solvent system, a repetition rate of 3.0 is defined when exactly three cycles of the 25% A–75% B sequence encompass the column length. In the limit of infinite repetition rate, the solvent-modulated mobile phase becomes conceptually analogous to an ideal, premixed mobile phase of the same nominal composition. It is apparent from Fig. 1 that the repetition rate is inversely related to solvent zone length, whereas the proportion is directly related. The interdependence of these variables and their influence on the individual zone lengths are shown in Fig. 2 for a two-solvent system. Thus, for any given modulation cycle with zones of length x_A and x_B, the sequence is defined by a single proportion and repetition rate. While the examples shown here are limited to two-solvent systems with a single column, the concept and theory of solvent

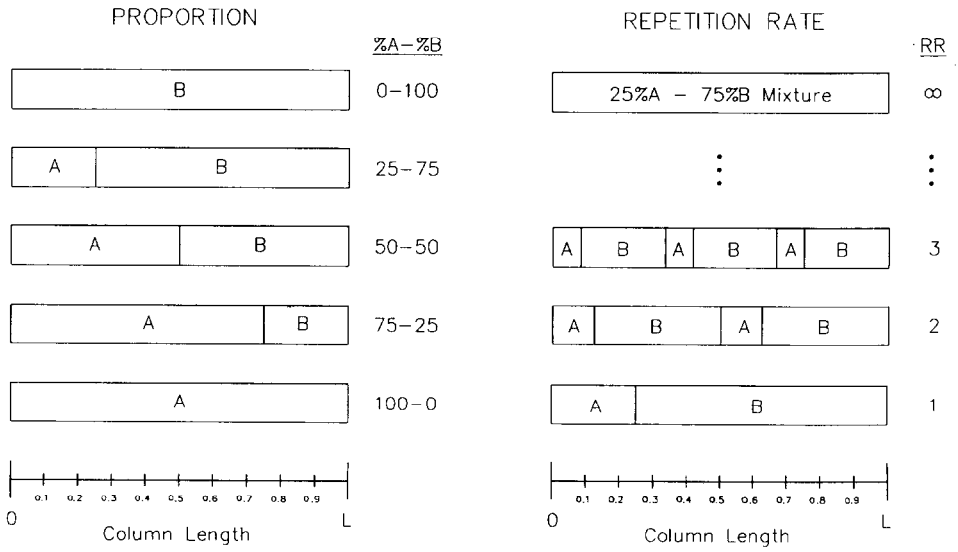

Fig. 1. Schematic representation of the proportion and repetition rate of a solvent modulation cycle. The proportion is defined as the length ratio of solvent A and B within one cycle of a modulation sequence while the repetition rate is the number of cycles which comprises the length of the chromatographic column (L).

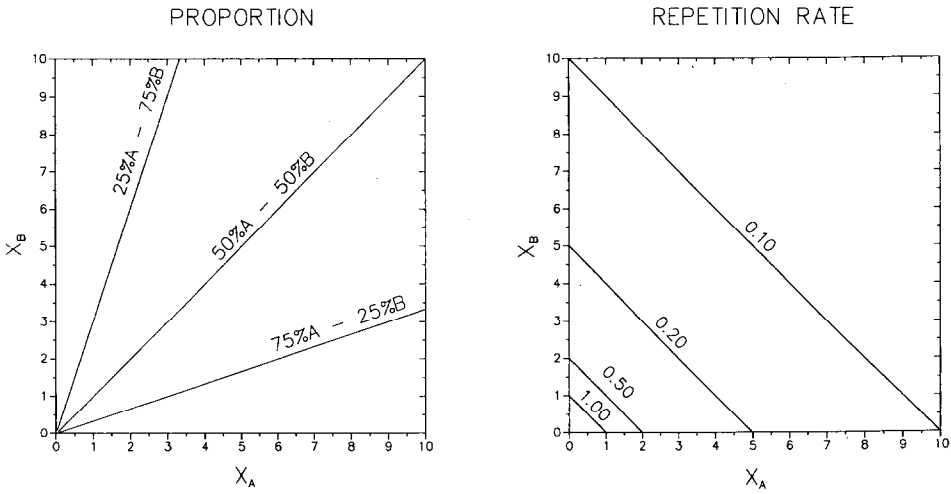

Fig. 2. Schematic representation of the interdependence of proportion and repetition rate and their relationship to the fractional lengths (x_A and x_B) for a two-solvent system. The conditions of discrete proportion and repetition rate with constant values are represented by the solid lines.

modulation can be readily extended to higher order solvent systems and to multiple column systems.

Practical application of the solvent modulation technique requires that solute retention be measured only in the individual solvent systems in order to predict retention in any modulation sequence. This implies, conversely, that the number of characterizing experiments necessary to optimize the separation is simply equal to the number of solvents employed in the modulation sequence: two preliminary experiments are needed for a two-solvent modulation sequence, three experiments for a three-solvent sequence, and so on. Consequently, the optimization of liquid chromatographic separations is inherently simpler, faster, and more accurate using solvent modulation than using premixed solvent systems.

Optimization strategies

The strategy for optimizing a solvent modulation separation utilizes eqn. 1 to predict accurately the overall capacity factor for each solute. This requires that the individual solute capacity factors (k_{ij}) in each solvent system (j) be measured prior to performing the optimization procedure. Once these capacity factors are known, the proportion and the repetition rate (which define the values of x_j) are selected and the overall capacity factor for each solute is calculated.

The extent of separation between adjacent pairs of solutes is defined by the resolution ($R_{i,i+1}$):

$$R_{i,i+1} = \frac{\sqrt{N}}{2}\left(\frac{k_{i+1} - k_i}{2 + k_i + k_{i+1}}\right) \tag{2}$$

where k_i and k_{i+1} are the overall capacity factors for adjacent solutes calculated using eqn. 1. The resolution is estimated by assuming that the plate number (N) is relatively

constant for the column under specified conditions of solvent composition, flow-rate, and temperature.

The quality of the entire separation is assessed using the multivariate function developed by Schlabach and Excoffier[19], termed the chromatographic resolution statistic (*CRS*):

$$CRS = \left[\sum_{i=1}^{m-1}\left(\frac{R_{i,i+1} - R_{opt}}{R_{i,i+1} - R_{min}}\right)^2 \frac{1}{R_{i,i+1}} + \sum_{i=1}^{m-1}\frac{R_{i,i+1}^2}{(m-1)\bar{R}^2}\right]\frac{T_f}{m} \quad (3)$$

where $R_{i,i+1}$ is the individual resolution element, R_{opt} is the optimum desired resolution, R_{min} is the minimum acceptable resolution, $\bar{R}$ is the average resolution, T_f is the total separation time and m is the number of solutes. The first term in eqn. 3 is related inversely to the individual resolution elements, weighted by the difference between each element and the defined optimum and minimum conditions. When each resolution element is equal to the optimum value, the numerator in the first term will be zero. The denominator of this term causes the *CRS* value to increase rapidly as the resolution approaches the minimum acceptable value. When each resolution element is equal to the average resolution (regardless of its value), the second term in eqn. 3 will reach a minimum value of unity, which results in uniform spacing between solutes. The third term (T_f/m) of the *CRS* value defined by Schlabach and Excoffier[19] is intended to minimize the overall analysis time. For the purposes of this study, analysis time was not considered as an optimization goal and the third term was neglected; thus, an optimum separation will have a minimum *CRS* value of 1.0.

The optimum conditions for a solvent modulation experiment are determined systematically by first calculating the overall capacity factor for each solute using eqn. 1 for a defined proportion and repetition rate. Next, the resolution between adjacent solute pairs is calculated from eqn. 2. Then, the *CRS* value is calculated using eqn. 3 in order to evaluate the overall quality of the separation. The minimum *CRS* value may be determined in two ways: (1) by varying the fractional lengths of the solvents zones systematically to produce a complete *CRS* response surface from which the optimum is determined by visual inspection, or (2) by using an iterative search routine such as the simplex method. These optimization strategies are demonstrated by application of the solvent modulation technique to the separation of biologically important fatty acids by reversed-phase liquid chromatography.

EXPERIMENTAL

Materials

Saturated fatty acid standards were obtained from Sigma (St. Louis, MO, U.S.A.) and unsaturated fatty acids from Nu Chek Prep (Elysian, MN, U.S.A.) or Cayman Chemical (Ann Arbor, MI, U.S.A.). A 10^{-3} *M* stock solution of five saturated and nine unsaturated fatty acids in dry acetone was prepared. An aliquot of this solution was evaporated to dryness with nitrogen and reconstituted in 600 μl of dry acetone. A powdered, anhydrous mixture (1:1) of sodium sulfate and potassium hydrogencarbonate (5 mg) was added, together with the complexing agent dibenzo-18-crown-6 (3.6 mg, 10^{-5} mol). The fluorescent reagent 4-bromomethyl-7-methoxy-

coumarin (2.7 mg, 10^{-5} mol) was added, and the derivatization was allowed to proceed in the dark at 37°C for 5 h with periodic stirring[20,21].

Organic solvents were of high-purity, distilled-in-glass grade (Baxter Healthcare, Burdick and Jackson Division, Muskegon, MI, U.S.A.). Water was deionized and doubly distilled in glass (Mega-Pure System, Corning Glass Works, Corning, NY, U.S.A.).

Chromatographic system

The chromatographic system utilized is shown schematically in Fig. 3. A dual syringe pump (Model MPLC-MG; Applied Biosystems, San Jose, CA, U.S.A.), used in the constant-flow mode, is programmed to deliver the solvents (A or B) for specified time durations. Sample introduction is achieved using a 1.0-μl injection valve (Model ECI4W1; Valco Instruments, Houston, TX, U.S.A.), after which the effluent stream is split (120:1) and applied to the chromatographic column. The microcolumn was prepared from fused-silica capillary tubing (140 cm × 200 μm I.D.; Hewlett-Packard, Avondale, PA, U.S.A.), which was packed with an octadecylsilica material (Micro-Pak-SP, 3 μm, Varian Associates, Walnut Creek, CA, U.S.A.), as described previously[22].

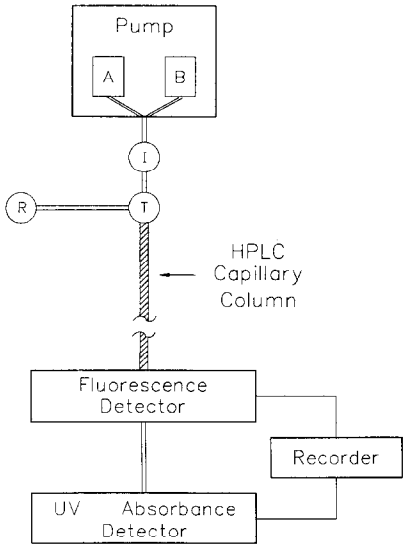

Fig. 3. Schematic diagram of the experimental system. (A) 90% methanol–10% water; (B) 100% methanol. I = Sample injection valve; T = effluent splitter tee; R = restrictor.

Laser fluorescence detector

A helium–cadmium laser (Model 3112-10S; Omnichrome, Chino, CA, U.S.A.), with continuous-wave output at 325 nm and approximately 10 mW power, is used as the excitation source. The laser radiation is focused using a fused-silica lens (2.8 cm diameter, 10.0 cm focal length, A612810; Esco Products, Oak Ridge, NJ, U.S.A.) directly on a portion of the fused-silica microcolumn from which the protective

polyimide coating has been removed. The fluorescence emission is collected perpendicular and coplanar to the excitation beam with another fused-silica lens (2.54 cm diameter, 2.54 cm focal length, A110010; Esco Products), spectrally isolated at 420 nm by using bandpass interference filters (FS-204-F; Corion, Holliston, MA, U.S.A.), and focused onto a photomultiplier tube (Model Q4249B; Centronic, Bailey Instruments, Saddle Brook, NJ, U.S.A.). The photocurrent is amplified and converted to voltage by a picoammeter (Model 480; Keithley Instruments, Cleveland, OH, U.S.A.) and finally is displayed on a chart recorder (Model 585; Linear Instruments, Reno, NV, U.S.A.).

UV absorbance detector

A commercial variable-wavelength UV absorbance detector (Model Uvidec 100-V; Jasco, Tokyo, Japan) was modified to contain a 10-nl fused-silica capillary flow cell. The light throughput from the deuterium lamp source is controlled by means of razor-blade slits mounted parallel to the flow cell.

Computer simulation

The computer simulation and optimization programs are written in FORTRAN 77 language and executed on a VAXstation 3200 computer (Digital Equipment, Maynard, MA, U.S.A.).

Topographic method. The response surface is created by first systematically incrementing the fractional lengths of the two solvent zones and calculating the capacity factor for each solute using eqn. 1. From these values, the resolution of each solute pair and the overall quality of the separation (*CRS* value) can be determined using eqns. 2 and 3, respectively. By graphing the *CRS* value as a function of the solvent zone length, a complete three-dimensional response surface is constructed. The minimum *CRS* value is located by visual inspection of this response surface. Graphical determination of the optimum *CRS* is restricted to two-solvent systems, because of the difficulty in representing and interpreting systems of higher dimensionality.

Simplex search method. The optimum conditions may be determined more rapidly by an iterative search method than by topographic methods, as the search is directed to the most promising regions of the *CRS* response surface. In this study, the modified simplex routine developed by Nelder and Mead[23] was utilized to determine the minimum *CRS* value. This algorithm permits the expansion and contraction of the simplex during the determination and will converge at the optimum position.

A flow chart of the computer program is shown in Fig. 4. This program requires as input parameters the number and sequence of solvents and the solute capacity factors in each solvent. The computational algorithms are divided into two subroutines. In the first subroutine, the overall capacity factors are calculated using eqn. 1, from which the resolutions are calculated using eqn. 2, and the corresponding *CRS* value from eqn. 3. This *CRS* value is then transferred to the simplex subroutine[24], where it is evaluated and used to predict a new, more promising, set of fractional solvent zone lengths. This process is repeated until the minimum *CRS* value is located under specified tolerance conditions.

In order to increase the probability of locating the global, rather than local, optimum of the *CRS* response surface, both the starting size and position of the initial simplex and the limits of the searching range are varied systematically. For a typical two-solvent modulation sequence, 200 different initial simplex conditions are exam-

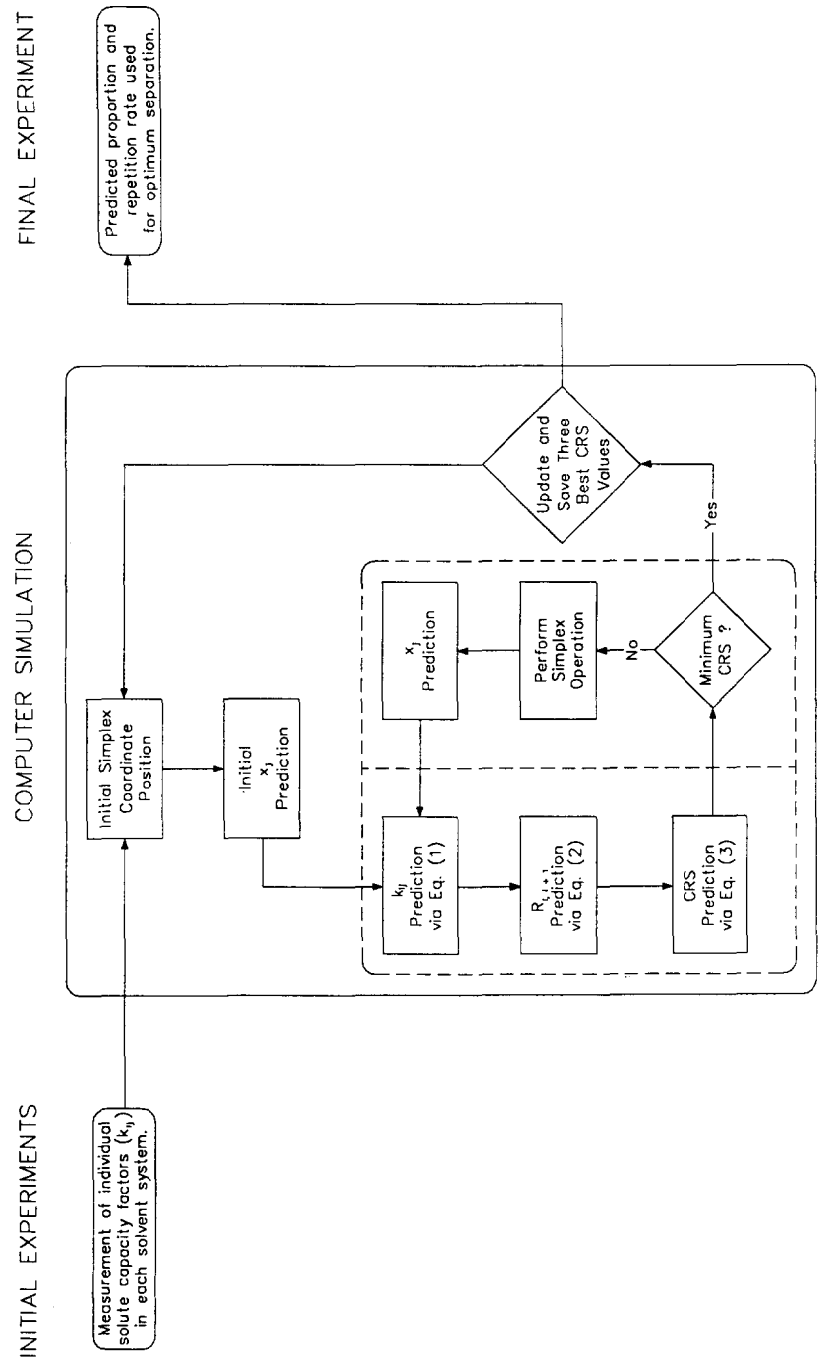

Fig. 4. Flow chart of the computer program used to locate the optimum separation conditions by the simplex method.

ined automatically with over 10 000 simulated solvent modulation experiments for a single set of solutes.

During the computer experiments, the three best *CRS* values are continuously updated and stored in a file. This file contains the proportion and repetition rate for the solvent modulation sequence, the solute elution order, the predicted capacity factors, the predicted resolutions, and the corresponding *CRS* value for the separation.

RESULTS AND DISCUSSION

The separation of fourteen fatty acid derivatives was chosen to demonstrate the utility of the solvent modulation technique because previous attempts using conventional optimization techniques were not entirely successful. In previous studies in our laboratory[25], this separation was optimized on a reversed-phase capillary column utilizing premixed mobile phases. The solute capacity factors were measured in aqueous mixtures of methanol and acetonitrile, with the organic component ranging from 85 to 100%. After linear regression analysis of these retention data, the optimum mobile-phase composition was predicted using a modified window-diagram method[4,26] to be 90% methanol–10% water. Owing to deviations from the expected retention behavior, however, complete resolution of all solutes was not achieved with the predicted mobile phase. Consequently, the composition was adjusted empirically until the optimum separation was obtained with 90% methanol–2% acetonitrile–8% water. Under these optimum conditions, approximately 250 000 theoretical plates were required to achieve adequate resolution of all fatty acid derivatives. The least-resolved pair of solutes was palmitoleic (16:1) and arachidonic (20:4) acids, with a resolution (R_s) of 1.39 and a separation factor (α) of 1.02. Because of this small separation factor, the capacity factors must be predicted very accurately in order for the optimization technique to be successful. However, deviations from linear retention behavior have been well documented in binary methanol–water[27,28] and acetonitrile–water[29] mixtures, and are even more prominent in ternary mixtures[10,30]. Consequently, because of non-ideal solvent–solvent interactions, an unsatisfactory separation of the fatty acid derivatives was obtained by conventional optimization techniques.

Based on the results of this previous work, a preliminary series of computer experiments was performed to determine whether this separation could be achieved by the solvent modulation technique. Two weak solvents (A), 90% methanol–10% water and 85% acetonitrile–15% water, and two strong solvents (B), 100% methanol and 100% acetonitrile, were selected for evaluation. The computer experiments examined all possible permutations for a two-solvent modulation sequence utilizing these four solvent systems. From these preliminary computer experiments, it was determined that a two-solvent system consisting of 90% methanol–10% water (A) and 100% methanol (B) achieved the best separation under the conditions of solvent modulation.

Chromatograms of the standard fatty acid derivatives obtained utilizing these two solvent systems are shown in Fig. 5. With the weak mobile phase (A), all solutes except myristic (14:0) and docosahexaenoic (22:6) acids are completely resolved and the total analysis time is approximately 10 h. With the strong mobile phase (B), the analysis time is reduced to approximately 1.5 h, but many more solutes are unresolved.

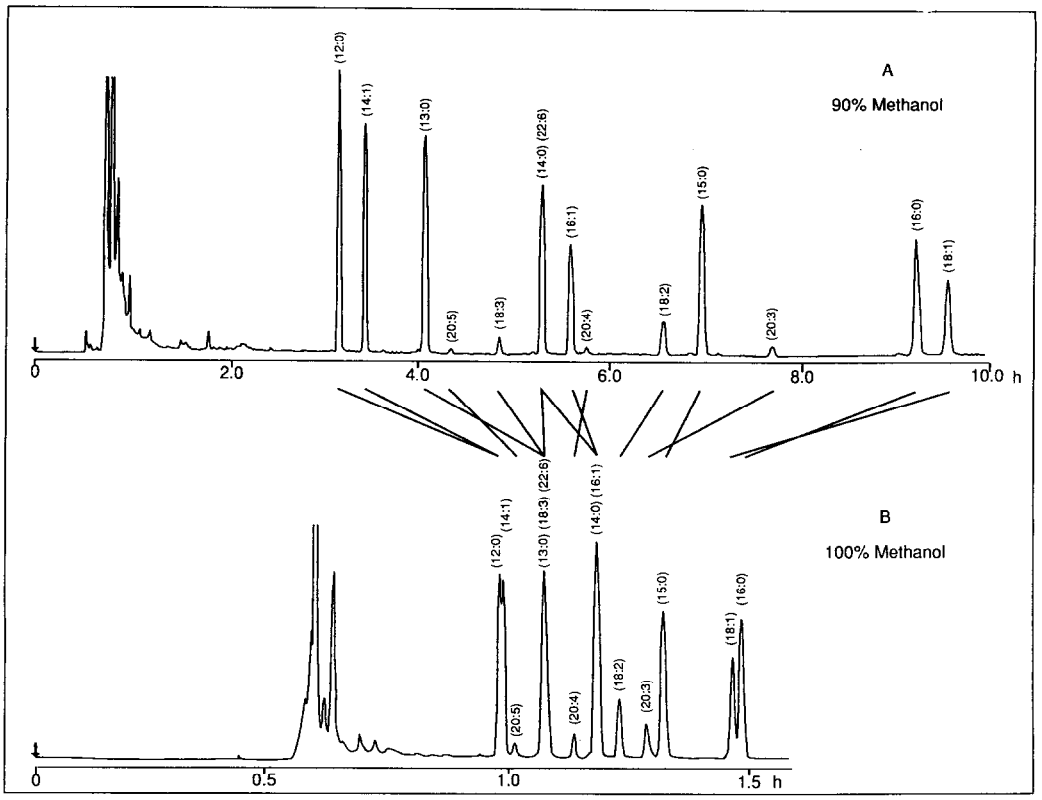

Fig. 5. Chromatograms of standard fatty acids in the individual mobile phases. Column: fused-silica capillary (140 cm × 200 μm I.D.), packed with 3-μm MicroPak-SP. Mobile phase: (A) 90% methanol–10% water; (B) 100% methanol; flow-rate, 0.7 μl/min; temperature, 30°C. Detector: laser fluorescence, λ_{ex} 325 nm, λ_{em} 420 nm, 2.0 μA full-scale. Solutes: 10^{-4} *M* fatty acid standards derivatized with 4-bromomethyl-7-methoxycoumarin; 8.3-nl injection volume. The nomenclature for the fatty acids is given as N_C:*n*, where N_C is the number of carbon atoms and *n* is the number of unsaturated sites.

In general, retention of the saturated fatty acids increases logarithmically with carbon number in both solvent systems. Because of selective interaction of the double bonds with methanol, the relative retention of the unsaturated fatty acids decreases in the strong solvent. Consequently, inversions in retention order are prevalent, especially for the highly unsaturated omega-3 fatty acids, eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids. The capacity factors for all fourteen fatty acid derivatives are summarized in Table I.

These individual capacity factors (k_{ij}) are used in eqn. 1 to predict the overall solute capacity factors under the conditions of solvent modulation. Utilizing the predicted solute retention, a series of separations are simulated by computational experiments as described previously. These computer experiments examine the possible modulation sequences, both random and repeating, with sample injection in both solvent A and solvent B. The overall quality of the separation, represented by the *CRS* value, is shown in Fig. 6 as a function of the fractional length of solvent A and

TABLE I
RETENTION OF FATTY ACIDS IN THE INDIVIDUAL MOBILE PHASES OF 90% METHANOL–10% WATER (A) AND 100% METHANOL (B)

Solute	*Capacity factor* (k_{ij})	
	Solvent A	*Solvent B*
(12:0)	5.17	1.29
(13:0)	6.92	1.51
(14:0)	9.28	1.77
(14:1)	5.71	1.30
(15:0)	12.56	2.09
(16:0)	16.99	2.47
(16:1)	9.91	1.76
(18:1)	17.64	2.43
(18:2)	1.74	1.87
(18:3)	8.43	1.53
(20:3)	14.01	2.01
(20:4)	10.23	1.65
(20:5)	7.42	1.36
(22:6)	9.45	1.51

solvent B. In this response surface, a large plateau (*CRS* = 33 000) is observed at fractional lengths of solvent B greater than approximately 2.0. This large *CRS* value indicates that many solutes are not adequately resolved in this region. An expansion of the response surface in the region with lower *CRS* values, where the fractional length of solvent A is between 12.0 and 14.0 and solvent B is between 0.0 and 2.0, is also shown. It is apparent that there are two valleys, parallel to the axis for solvent A, in which the minimum *CRS* value may be located. By examining the topographic map of this region, shown in Fig. 7, the minimum *CRS* value (4.34) is determined to be at fractional lengths of 12.9 for solvent A and 0.83 for solvent B. Thus, the simulation predicts that the optimum solvent modulation sequence is a two-solvent system with a proportion of 96%A–4%B and a repetition rate of approximately 0.07. Sample injection is to be performed into the zone of solvent B, and all standard fatty acids will be eluted within the second zone (solvent A). A minimum resolution of 1.3 will be achieved between the solutes myristic (14:0), arachidonic (20:4), and palmitoleic (16:1) acids, which are the most difficult to resolve. The same optimum conditions are predicted by using the simplex method, which indicates that the search algorithm developed for these studies is able to identify the global optimum correctly. The simplex method will be preferred for solvent systems of higher dimensionality, which are not readily amenable to visual inspection.

The experimental separation of the fatty acid derivatives obtained under these predicted optimum conditions is shown in Fig. 8. The upper chromatogram is obtained with the UV absorbance detector, which permits direct observation of the solvent modulation sequence. The initial region of lower apparent absorbance is due to the zone of solvent B (100% methanol), where sample injection occurs, while the region of higher apparent absorbance is due to the zone of solvent A (90% methanol–10% water). The lower chromatogram is obtained from the laser fluorescence detector,

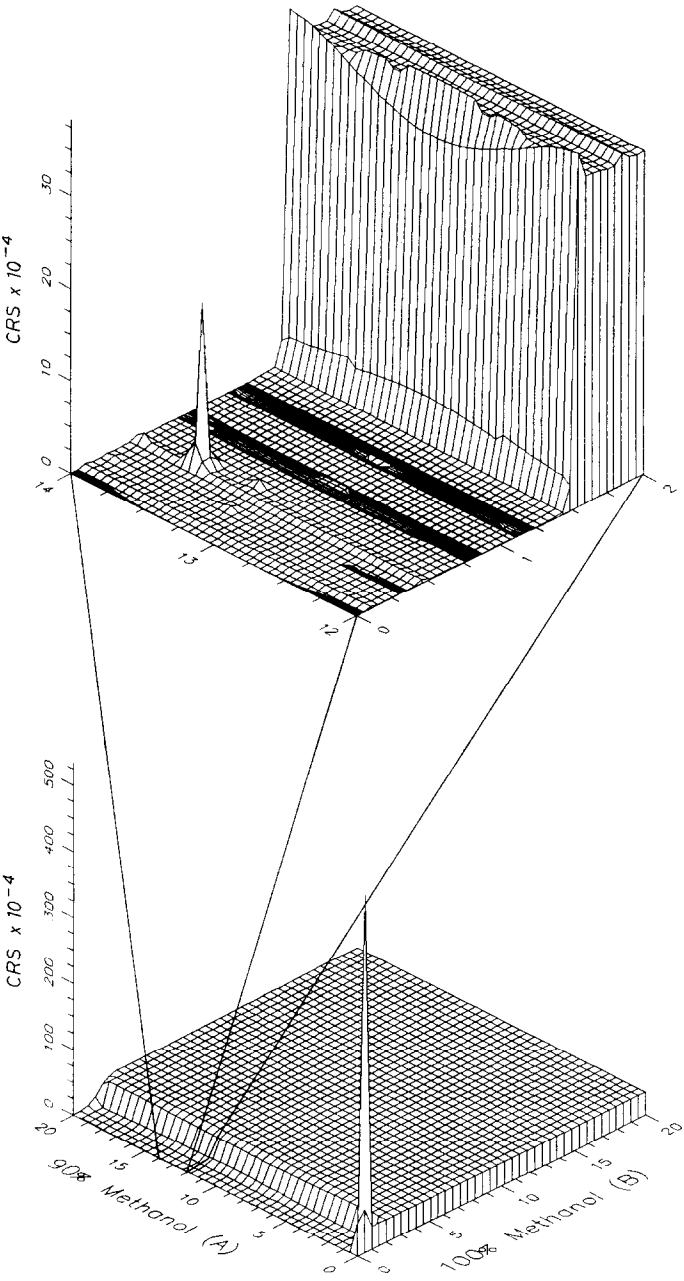

Fig. 6. Simulated response surface, with expanded view of optimum region, for the separation of the fourteen fatty acids. The *CRS* value is displayed as a function of the fractional length of solvents A and B in the modulated mobile phase.

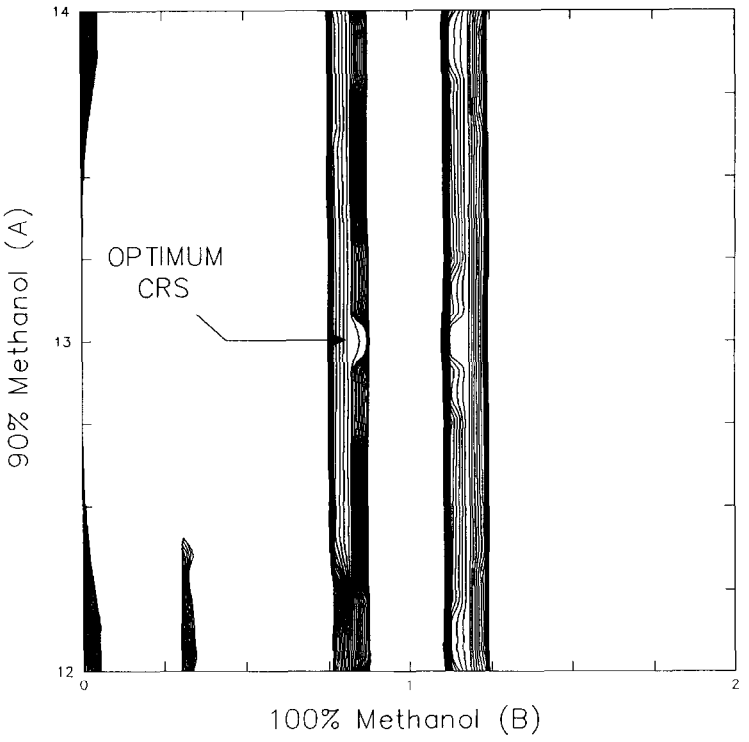

Fig. 7. Topographical representation of the response surface in Fig. 6. The optimum is located at fractional lengths of 12.9 for solvent A and 0.83 for solvent B, with a corresponding *CRS* value of 4.34.

which is sensitive to the fatty acid derivatives but unresponsive to the modulated mobile phase. In this chromatogram, all fourteen fatty acids are separated in less than 8 h with a minimum resolution of 1.24. The retention characteristics are summarized in Table II, where excellent agreement is observed between the capacity factors predicted by the simulation model and those measured experimentally. For all solutes, the relative error in capacity factor is no greater than 4.5%, which is well within the accuracy of the experimental measurements[18].

By examining the retention characteristics of the least-resolved solutes, myristic (14:0), arachidonic (20:4), and palmitoleic (16:1) acids, an understanding may be gained of the separation mechanism under the conditions of solvent modulation. As shown in Table I, the solutes (14:0) and (16:1) have approximately the same capacity factors in the strong solvent B, whereas that for (20:4) is notably smaller. In the weak solvent A, the relative magnitude of the capacity factors is inverted, so that (14:0), (16:1), and (20:4) are eluted in order of increasing retention. During solvent modulation, the solutes are exposed to these solvent systems sequentially. In the initial zone of solvent B, (20:4) can be easily separated from (14:0) and (16:1), which migrate at slower and approximately equal rates. When the solutes are subsequently exposed to solvent A, (14:0) and (16:1) can be separated from one another but now migrate faster than (20:4). Because of their differential rates of migration, the solute zones will

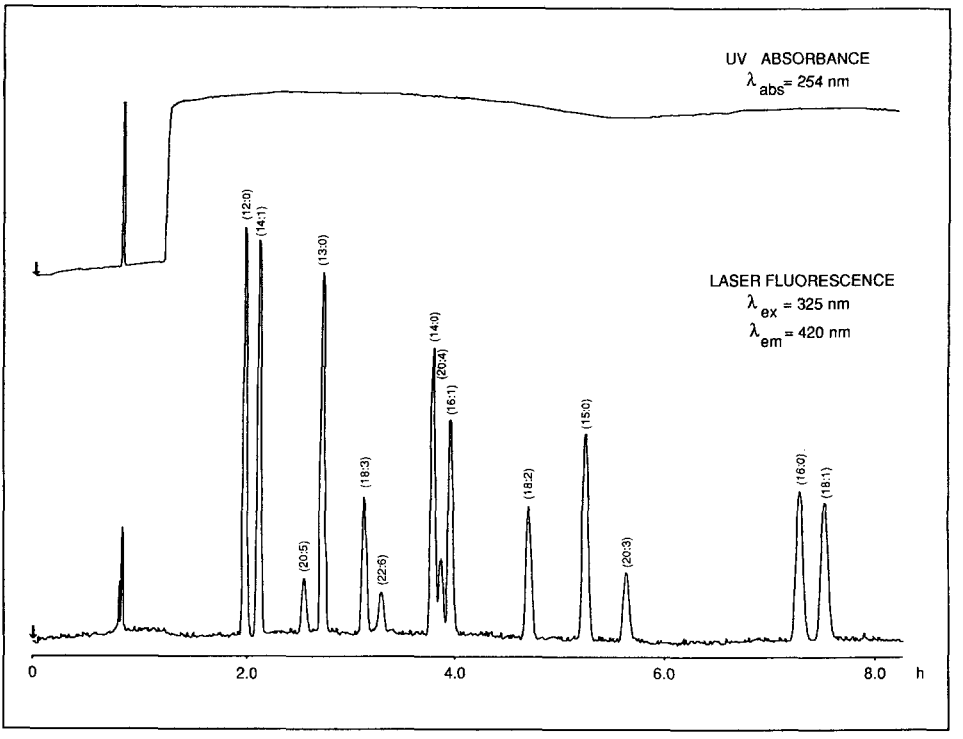

Fig. 8. Chromatogram of the standard fatty acids under the predicted optimum conditions for solvent modulation. Chromatographic conditions as in Fig. 5. Mobile phase: 96%A–4%B proportion; 0.07 repetition rate; flow-rate, 0.7 μl/min; temperature, 30°C. Detectors: UV absorbance, λ_{abs} 254 nm, 0.0125 a.u.f.s.; Laser fluorescence, λ_{ex} 325 nm, λ_{em} 420 nm, 0.5 μA full-scale.

TABLE II

COMPARISON OF THEORETICAL AND EXPERIMENTAL RETENTION OF FATTY ACIDS UNDER THE CONDITIONS OF SOLVENT MODULATION

Solute	*Solvent modulation capacity factor (k_i)*		
	Theoretical	*Experimental*	*Relative error (%)*
(12:0)	2.67	2.55	−4.49
(13:0)	3.95	3.89	−1.52
(14:0)	5.67	5.67	0.00
(14:1)	2.90	2.80	−3.45
(15:0)	8.40	8.36	−0.48
(16:0)	12.11	12.03	−0.66
(16:1)	6.07	6.07	0.00
(18:1)	12.45	12.44	0.00
(18:2)	7.36	7.39	0.41
(18:3)	4.69	4.59	−1.92
(20:3)	9.06	9.07	0.11
(20:4)	5.92	5.89	−0.51
(20:5)	3.72	3.56	−3.76
(22:6)	5.09	4.88	−4.13

eventually merge and be recombined. In order to achieve the optimum separation, the fractional lengths of solvents A and B, and thus the fractional distances travelled by the solutes, must be adjusted such that the solute zones are equally spaced when they reach the end of the chromatographic column. This goal is easily achieved by means of eqns. 1–3, together with computer optimization techniques such as topographic mapping or simplex searching methods. These strategies were selected to facilitate rapid determination of the optimum conditions, but are not necessarily the best for all applications. The optimization criteria and searching methodology can be readily modified to suit the requirements for any separation of interest. Thus, the solvent modulation technique provides a simple and versatile means to optimize the separation of complex mixtures by liquid chromatography.

CONCLUSIONS

The solvent modulation technique is a novel alternative to premixed mobile phases for liquid chromatography and permits more accurate prediction of solute retention. This technique allows the rapid optimization of separations with a minimum number of experimental measurements. In this work, the separation of fourteen fatty acid derivatives required only two preliminary experiments and a few hours of computer simulation for complete optimization by the solvent modulation technique. In contrast, conventional optimization methods with premixed mobile phases did not yield a satisfactory separation after nine preliminary experiments, requiring final adjustment of the mobile phase composition by time-consuming trial-and-error methods[25]. Therefore, solvent modulation may be considered to be the method of choice when deviation from linear retention behavior is expected, as in the highly interacting methanol, acetonitrile, tetrahydrofuran, and water mobile phases utilized for reversed-phase liquid chromatography. Although the results presented here were achieved with high-efficiency capillary columns, the solvent modulation technique can be readily adapted to conventional liquid chromatographic columns using commercially available instrumentation.

ACKNOWLEDGEMENTS

This research was supported in part by Dow Chemical and the Michigan State University Foundation. The authors are grateful to Baxter Healthcare, Burdick and Jackson Division, for the kind gift of high-purity solvents, and to Mr. James Whitford and Dr. Mack Harvey (Valco Instruments) for an injection valve and associated hardware. Finally, the authors thank Dr. Chris Enke (Michigan State University) for many illuminating discussions and Mr. Jong Shin Yoo (Michigan State University) for providing preliminary retention data for the fatty acid standards.

REFERENCES

1 J. C. Berridge, *Techniques for the Automated Optimization of HPLC Separations*, Wiley, New York, 1985.
2 S. T. Balke, *Quantitative Column Liquid Chromatography: a Survey of Chemometric Methods* (*Journal of Chromatography Library*, Vol. 29), Elsevier, Amsterdam, 1984.
3 P. J. Schoenmakers, *Optimization of Chromatographic Selectivity* (*Journal of Chromatography Library*, Vol. 35), Elsevier, Amsterdam, 1986.

4 R. J. Laub and J. H. Purnell, *J. Chromatogr.*, 112 (1975) 71.
5 J. W. Dolan, L. R. Snyder and M. A. Quarry, *Chromatographia*, 24 (1987) 261.
6 P. J. Schoenmakers, H. A. H. Billiet, R. Tijssen and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.
7 P. Jandera and J. Churáček, *Adv. Chromatogr.*, 19 (1981) 125.
8 S. N. Deming and S. L. Morgan, *Anal. Chem.*, 45 (1973) 278A.
9 V. Svoboda, *J. Chromatogr.*, 201 (1980) 241.
10 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 218 (1981) 261.
11 J. L. Glajch, J. J. Kirkland, K. M. Squire and J. M. Minor, *J. Chromatogr.*, 199 (1980) 57.
12 J. C. Berridge, *J. Chromatogr.*, 244 (1982) 1.
13 R. Kaliszan, *CRC Crit. Rev. Anal. Chem.*, 16 (1986) 323, and references cited therein.
14 H. B. Patel and T. M. Jefferies, *J. Chromatogr.*, 389 (1987) 21.
15 J. H. Hildebrand and R. L. Scott, *Regular Solutions*, Prentice-Hall, Englewood Cliffs, NJ, 1962.
16 C. Reichardt, *Solvents and Solvent Effects in Organic Chemistry*, VCH, New York, 1988.
17 J. H. Wahl, C. G. Enke and V. L. McGuffin, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 858.
18 J. H. Wahl, C. G. Enke and V. L. McGuffin, *Anal. Chem.*, submitted for publication.
19 T. D. Schlabach and J. L. Excoffier, *J. Chromatogr.*, 439 (1988) 173.
20 V. L. McGuffin and R. N. Zare, *Appl. Spectrosc.*, 39 (1985) 847.
21 W. Dünges and N. Seiler, *J. Chromatogr.*, 145 (1978) 483.
22 J. C. Gluckman, A. Hirose, V. L. McGuffin and M. Novotny, *Chromatographia*, 17 (1983) 303.
23 J. A. Nelder and R. Mead, *Comput. J.*, 7 (1965) 308.
24 W. H. Press, B. P. Flannery, S. A. Teukolsky and W. T. Vetterling, *Numerical Recipes: the Art of Scientific Computing*, Cambridge University Press, New York, 1986.
25 J. S. Yoo and V. L. McGuffin, in preparation.
26 R. J. Laub, *J. Liq. Chromatogr.*, 7 (1984) 647.
27 E. D. Katz, K. Ogan and R. P. W. Scott, *J. Chromatogr.*, 352 (1986) 67.
28 E. D. Katz, C. H. Lochmüller and R. P. W. Scott, *Anal. Chem.*, 61 (1989) 349.
29 S. M. McCown, D. Southern, B. E. Morrison and D. Garteiz, *J. Chromatogr.*, 352 (1986) 465.
30 C. H. Lochmüller, M. A. Hamzavi-Abedi and C. X. Ou, *J. Chromatogr.*, 387 (1987) 105.