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High-performance liquid chromatographic computer simulation based on a restricted multi-parameter approach

II. Applications

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ABSTRACT

A computer program (DryLab® MP) is described that allows restricted multi-parameter mapping for any number (or kind) of separation variables, based on only a few experiments. Multi-parameter computer simulation can be used to develop an high-performance liquid chromatographic method from the beginning, or it can be used to enhance a method developed by other means; *e.g.*, by trial-and-error, single-parameter mapping, etc. The software can also be used to evaluate (and improve) method ruggedness. Finally, various problems (column-to-column variability, change of retention with ambient temperature fluctuations, experimental errors, etc.) that are commonly encountered during routine operation can be handled in the same general way. Examples of these various applications are given.

INTRODUCTION

The preceding paper [1] describes a new approach for high-performance liquid chromatographic (HPLC) computer simulation: restricted multi-parameter mapping of separation and resolution. Software based on this procedure (DryLab MP, for use with an IBM-compatible personal computer) has been developed as an aid in (a) HPLC method development and (b) related problems that can arise in the routine use of a final method. For method development, two initial experimental runs are carried out in order to define a mobile phase composition (%B) that provides a satisfactory k' range for all sample components. Next, one or two additional separations are made for each variable that is to be varied or mapped (see Fig. 1 of Ref. 1). Finally it is possible to predict separation as a function of change in the different separation variables.

Alternatively, a method that has already been developed can be used as the starting point for similar computer simulations directed toward other goals: improvement of separation, determination (and improvement) of method ruggedness, anticipation and solution of various problems that can arise during routine use of the method. The present paper provides examples of these and other possibilities, based on restricted multi-parameter mapping.

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THEORY

Column plate number

In the preceding paper [1], the prediction of retention via multi-parameter mapping has been emphasized. However, the column plate number N also plays an important role in determining sample resolution. Previous examples of computer simulation have often assumed that N is constant for all bands in the chromatogram (for a specific set of separation conditions). Thus, a value of N obtained for any well-resolved band can be used for other bands as well. This is often not true in practice, however. The reason is that there is a general tendency for N to increase with k', and extra-column effects (which work in the same direction) are also often significant in a given HPLC system.

We have previously observed [2] (for capillary gas chromatographic separation) that this (total) increase in values of N with increasing retention can be described quantitatively in terms of a "pseudo" extra-column correction. A similar increase in N with k' was observed in the present study for the reversed-phase HPLC separation of a sample of substituted benzoic acids. Table I summarizes this effect for a given set of conditions. The approach in ref. 2 (for gas chromatography) is as follows. The "limiting" plate number N_0 for a large value of k' is defined, and this yields a corresponding bandwidth W_0 for all solutes in the chromatogram

$$W_0 = (4\, N_0^{-0.5})\, t_R \tag{1}$$

Here, t_R is the band retention time. The actual bandwidth W is then given as

$$W^2 = W_0^2 + W_{ec}^2 \tag{2}$$

TABLE I

VARIATION OF PLATE NUMBER N WITH SAMPLE RETENTION

Substituted benzoic acid sample (see Table IV in ref. 1 for band identification); pH = 2.9, 35% B, 30°C, 25 mM buffer.

Band	Plate number N		
	Experimental	Calculated[a]	Error (%)
A	11 100	9 630	−13
B	9 500	10 800	+13
C	15 000	15 600	+4
D	15 500	16 100	+4
E	17 000	16 900	0
F	17 400	18 100	+4
G	14 100	18 500	(+31)[b]
H	19 300	19 800	+3
I	23 000	20 200	−13
Average error			±6

[a] From Eqns. 1 and 2 with W_{ec} = 0.22 min, N_0 = 22 000.
[b] Out-of-line value, not included in average error.

where W_{ec} is a "pseudo extra-column" contribution to bandwidth that reflects both the dependence of N on k' and actual extra-column effects. DryLab MP requests bandwidths and retention times for an early and late band in the reference chromatogram, from which values of N_0 and W_{ec} are derived. The latter are then used to estimate N for any band as a function of its retention time.

The calculated plate numbers in Table I show an average error of only ±6%, corresponding to a 3% error in resolution. This is negligible compared to the effect of expected errors in predicted values of α; see the discussion in ref. 1.

Change in temperature. If temperature is selected as one of the variables, the plate number N (therefore values of N_0 and W_{ec}) is expected to change somewhat. However, for changes in temperature less than 10–20°C, the effect on N is predicted to be minor. One study [3] has found little change in N with temperature for a typical reversed-phase system. The present program (DryLab MP) ignores any change in N with temperature.

Column variability

A common, potentially serious problem in routine HPLC analysis is a change in sample retention when a new column (from a different lot) is used. Several studies [6–8] have noted striking differences in retention for a given sample on different columns. It would be a great convenience, if these changes in column retention (and separation) could be easily corrected by an appropriate change in one or more separation conditions. This can be done using computer simulation, if the effects of each variable on the retention of different solutes remain approximately the same from column to column. This is equivalent to requiring that the chromatographic parameters S, $\bar{B}$, K_a, k°, $k^\pm$, and D (see the preceding paper [1]) be largely independent of the column.

There is some evidence that these latter chromatographic parameters tend to remain roughly the same from column to column. We will consider this question for each parameter.

Effect of %B: values of S. The origin of solute S values has been discussed by various workers. One hypothesis [9] is that these S values represent the ratio of molecular sizes of the solute (A_s) and strong solvent B (A_b): $S = A_s/A_b$[a]. If this is true, then the column would appear to be of little importance in determining values of S. This is confirmed by several reversed-phase HPLC studies in the literature, which are discussed below.

Thus, for the separation of various ribosomal proteins on four C_3 or C_4 columns (three different suppliers), it was found [12] that retention varied considerably for these compounds, but values of S were quite similar. The average variation of S from column to column was only ±3.6 units, for $31 < S < 66$. For an even larger change in column composition, another study [13] reported S values for several digitalis derivatives on a C_{18} *vs.* a cyano column. Values of S for the cyano column equalled 79±6% of the value for the C_{18} column. In still another study [14] it was

[a] More accurately, S is believed to reflect the number of solvent molecules B displaced by a solute molecule upon retention in reversed-phase, ion-exchange or normal-phase [10] HPLC. Constancy in S (for different columns) would also be expected if the mobile phase (solution activity coefficients) dominates the separation [11].

found for C_1–C_{18}, cyano, phenyl and fluoroalkyl columns that relative values of S were also similar. Finally, the preceding paper [1] has reported S values for various alkyl phthalates on two different C_8 columns (Table VIII of ref. 1) and found approximately equal values for a given solute and each column.

It can be concluded on the basis of these findings that values of S should be similar for different columns, particularly those of the same type (*e.g.*, C_8 and C_{18}).

Effect of temperature: values of $\bar{B}$. Similar studies of values of $\bar{B}$ for various solutes on different columns have not been reported. However the factors that contribute to the enthalpy of retention (and $\bar{B}$) are expected to be the same for different reversed-phase columns of the same type. This should in turn lead to similar relative values of $\bar{B}$ for the same solute on different columns.

Effect of pH; values of K_a, k° and $k^\pm$. The value of K_a is a property of the solute and mobile phase, and should be the same for different columns. The ratio of values of $k^\circ/k^\pm$ tends to be similar for different systems, and in any case $k^\pm$ is usually small. On this basis, it can be argued that the effect of a change in pH should be similar for different columns of the same general type (*e.g.*, reversed phase).

EXPERIMENTAL

Equipment and materials

See the preceding paper [1].

Software

All computer simulations described in this paper (except where noted otherwise) were carried out with a prototype version of software that is still under development (DryLab MP, LC Resources).

RESULTS AND DISCUSSION

A nine-component sample of substituted benzoic acids (see Part I [1]) was used in most of the following examples. Two initial exploratory separations were carried out to define conditions for a reasonable k' range for the sample. These starting conditions ("reference" conditions) were: pH = 2.9, 40% methanol, 35°C, 25 mM acetate buffer. The resulting separation is shown in Fig. 1A and compared with the corresponding computer simulation (DryLab MP) in Fig. 1B[a]. The range of k' values is $1.3 < k' < 5.8$.

Restricted multi-parameter mapping requires one or two additional experimental runs for each variable to be studied. In the present case, pH, %B, temperature and buffer concentration were selected. The conditions for these additional five runs are given in Table II. The bandwidth data in Table I were also used in further computer simulations. The resulting retention times for each experimental run are summarized in Table IV of ref. 1.

[a] The chromatogram of Fig. 1B is based on the experimental run of Fig. 1A as input; therefore, the retention times in these two chromatograms should be (and are) exactly the same. The chromatogram of Fig. 1B does provide a test of the ability of DryLab MP to predict column plate number and bandwidth as a function of k', however.

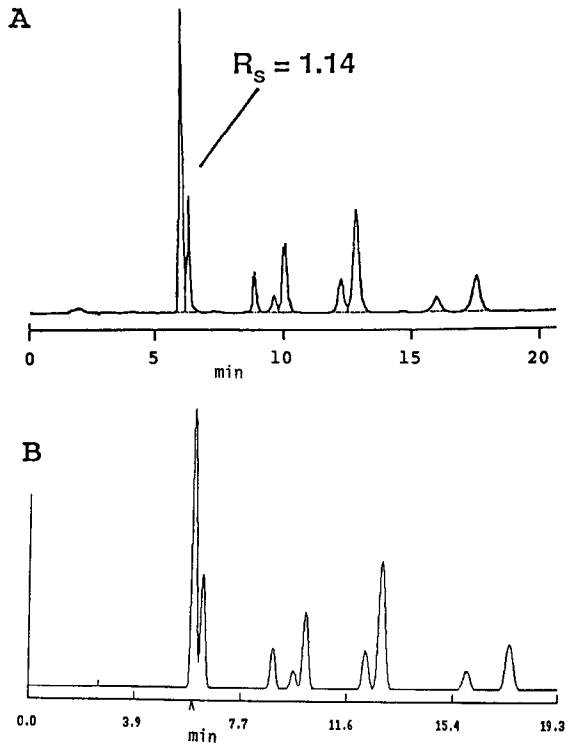

Fig. 1. Chromatograms for reference conditions in Table II. (A) Experimental; (B) computer simulation.

Method development

Beginning with the separation in Fig. 1 (the reference run), a good approach in multi-parameter mapping is to examine resolution maps for each variable (single-parameter mapping). Resolution maps for the four variables of Table II are shown in Fig. 2. The pH map in Fig. 2A exhibits a maximum resolution of 1.2 for a pH of 2.91, which is essentially the same resolution as is exhibited by the reference run (pH 2.90). Therefore, a change in pH does not appear useful as a means of improving resolution. The %B map (Fig. 2B) indicates that resolution can be improved significantly by lowering %B from 40% (reference run) to a value of about 36%; R_s = 1.8 *vs.* 1.2 for the reference run. Similar, but smaller, increases in resolution are possible by varying temperature (Fig. 2C) or buffer concentration (Fig. 2D). Our first choice is therefore to optimize solvent strength (%B).

The resolution map in Fig. 2B shows a flattening for mobile phases of < 38%B, meaning that further increase in resolution for lower %B values is more costly in terms of increase in run time (which increases as %B decreases). This suggests an optimum mobile phase composition of about 37%B[a]. With this initial choice of

[a] The choice of 37%B *vs.* 36 or 38%B is somewhat arbitrary, and is in any case not an important distinction.

TABLE II

EXPERIMENTAL SEPARATIONS CARRIED OUT PRIOR TO MULTI-PARAMETER MAPPING

Substituted benzoic acid sample; see Table IV in ref. 1 for retention times for each run.

Run	pH	%B	T (°C)	Buffer (mM)
Reference	2.9	40	35	25
Vary pH	2.6	40	35	25
	3.2	40	35	25
Vary %B	2.9	35	35	25
Vary T	2.9	40	30	25
Vary buffer	2.9	40	35	10

37%B (other reference conditions remaining the same), we can now examine the advantage of changes in other variables. Since a change in either temperature or buffer concentration seems equally likely, resolution maps are requested for each

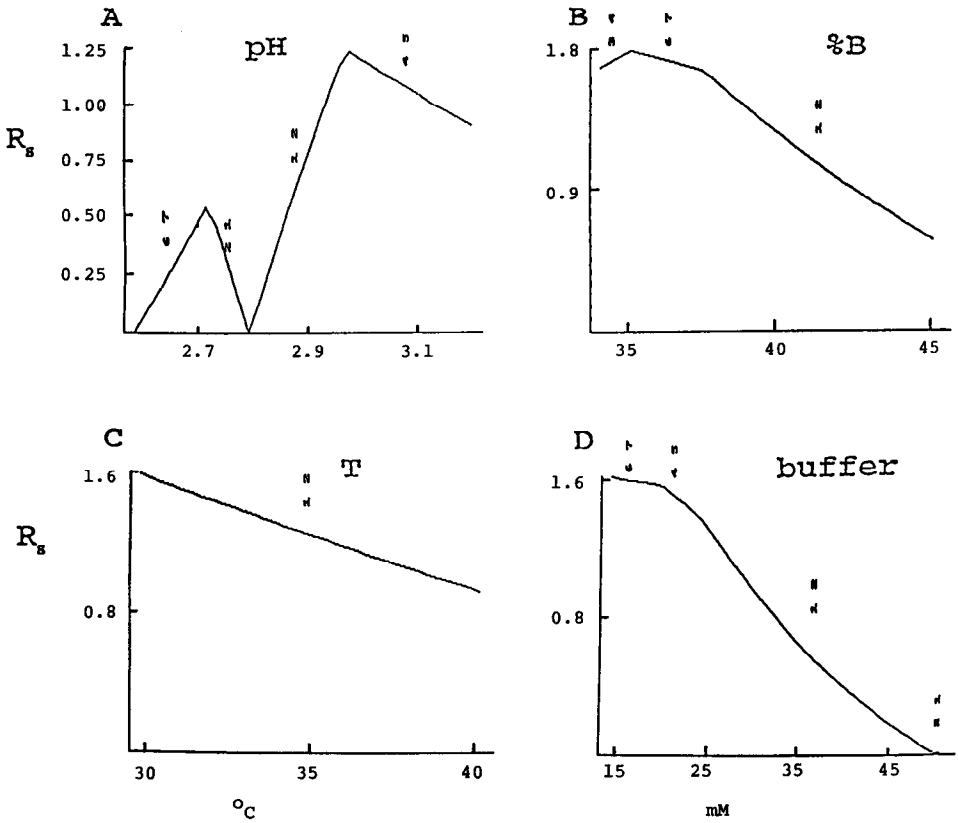

Fig. 2. Resolution maps for different variables (single-parameter changes beginning with reference conditions in Table I). (A) pH; (B) %B; (C) temperature (°C); (D) buffer concentration (mM).

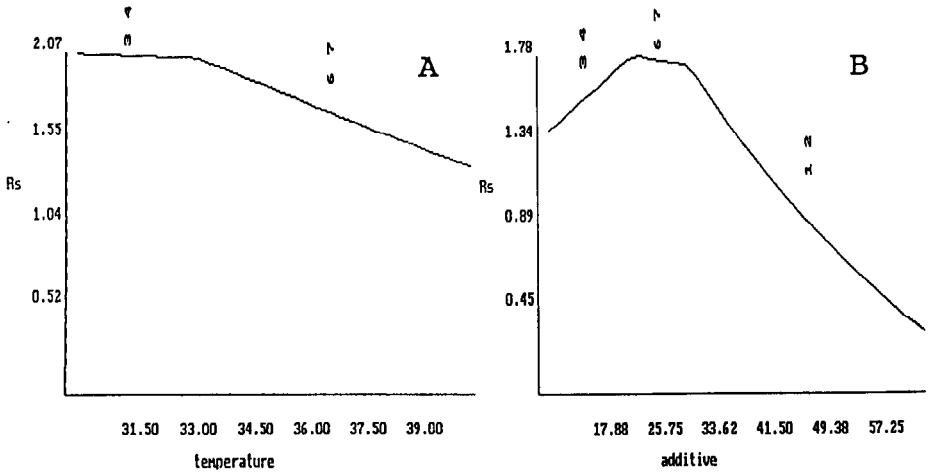

Fig. 3. Resolution maps for (A) temperature (°C) and (B) buffer concentration (m*M*) [single-parameter changes beginning with (A) 37% B, pH 2.90, 25 m*M* buffer or (B) 35°C, pH 2.90, 25 m*M*].

variable (37%B, other conditions as for the reference run). These maps are shown in Fig. 3.

Fig. 3B (buffer map) shows little advantage in varying buffer concentration; 25 m*M* (the reference value) is very close to the optimum value. A change in temperature (Fig. 3A), on the other hand, is clearly beneficial. A temperature of 32°C (*vs.* 35°C in the reference run) yields an increase in sample resolution to $R_s = 2.06$. The experimental and predicted chromatograms for these conditions (pH 2.9, 37% B, 32°C, 25 m*M*) are shown in Fig. 4A and B, respectively. There is close agreement between the two chromatograms in terms of both retention time (± 1.3 %) and resolution (± 5%), as summarized in Table III.

Further variation in conditions is possible, but resolution maps for pH and buffer concentration (for 37% B and 32°C) show no significant further improvement in resolution. Our final separation (Fig. 4A) is clearly much better than the starting in Fig. 1; resolution is almost doubled, with an increase in run time of only 20%. If desired, the run time can be reduced to the starting value (18 min) by an increase in flow-rate, as shown in the simulation of Fig. 4C (flow-rate = 1.3 ml/min, $R_s = 1.91$).

Developing an assay for selected bands. In many cases, the separation of the entire sample is not of interest; instead, the assay of only one or a few bands in the chromatogram is required. We will use the mixture of substituted benzenes (Table II of ref. 1) as an example, inasmuch as data have been reported [15] which allow us to map %B and temperature for this sample. Fig. 5 shows reconstructed chromatograms (from the data of ref. 6) for this sample[a] (25 × 0.46 cm I.D., 5-μm column, 1 ml/min) for reference conditions (Fig. 5A; 50% methanol, 30°C), and for runs where

[a] Anisole has been omitted from this sample for better illustration; the revised sample contains eight components.

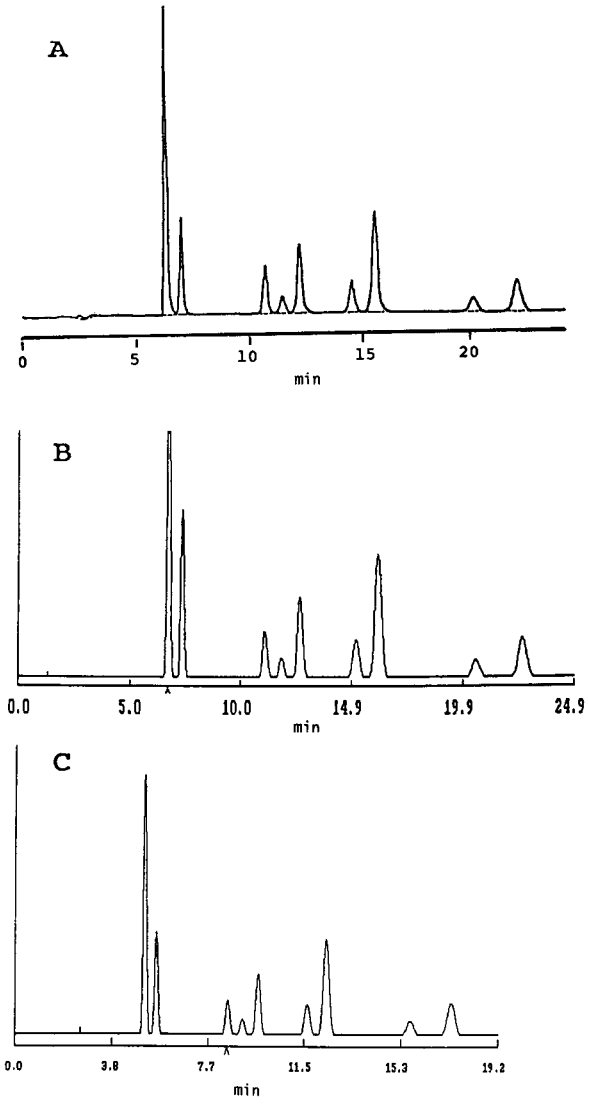

Fig. 4. Separation of substituted benzoic acids for optimized conditions; pH 2.90, 37% methanol, 32°C, 25 m*M* buffer. (A) Experimental chromatogram, 1 ml/min; (B) predicted chromatogram, 1 ml/min; (C) predicted chromatogram, 1.3 ml/min.

%B (Fig. 5B; 60% B, 30°C) and temperature (Fig. 5C; 50% B, 41°C) are varied. These data allow us to carry out multi-parameter mapping using DryLab MP.

The resolution maps for the entire sample (as a function of %B and *T*) are shown in Fig. 6A and B. These suggest marginal separation, although $R_s = 1.7$ is possible for a temperature of about 20°C). However, this involves a long run time, and temperature control will be a problem (resolution is seen to vary markedly with temperature, and most temperature controllers cannot be used near room temper-

TABLE III

COMPARISON OF EXPERIMENTAL *VS.* PREDICTED SEPARATION OF SUBSTITUTED BENZOIC ACIDS FOR OPTIMIZED CONDITIONS

pH 2.9, 37% methanol, 32°C, 25 m*M* buffer.

Band	Retention times (min)		Resolution R_s	
	Experimental	Calculated	Experimental	Calculated
A	6.4	6.6	2.4	2.1
B	7.1	7.2	11.3	11.3
C	10.8	10.9	2.1	2.0
D	11.6	11.7	2.0	2.1
E	12.3	12.5	6.5	6.0
F	14.7	15.1	2.0	2.1
G	15.8	16.0	8.6	8.2
H	20.3	20.4	3.3	3.5
I	22.3	22.5	3.3	3.5
Average error	±0.2 (1.3%)		±0.2 (5%)	

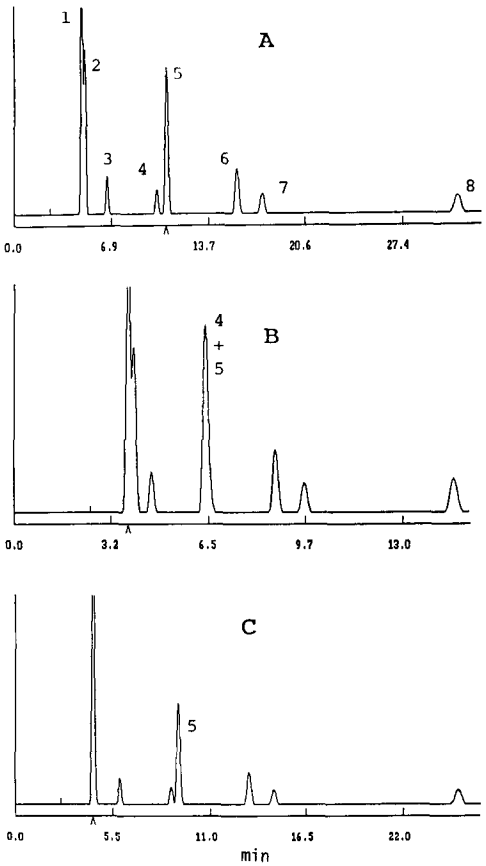

Fig. 5. Reconstructed chromatograms for the separation of the substituted-benzene sample in ref. 6 (anisole band excluded). (A) Reference run: 50% methanol, 30°C, 25 × 0.46 cm I.D., 5-μm C_8 column; (B) same as A, except 60% B; (C) same as A, except 41°C.

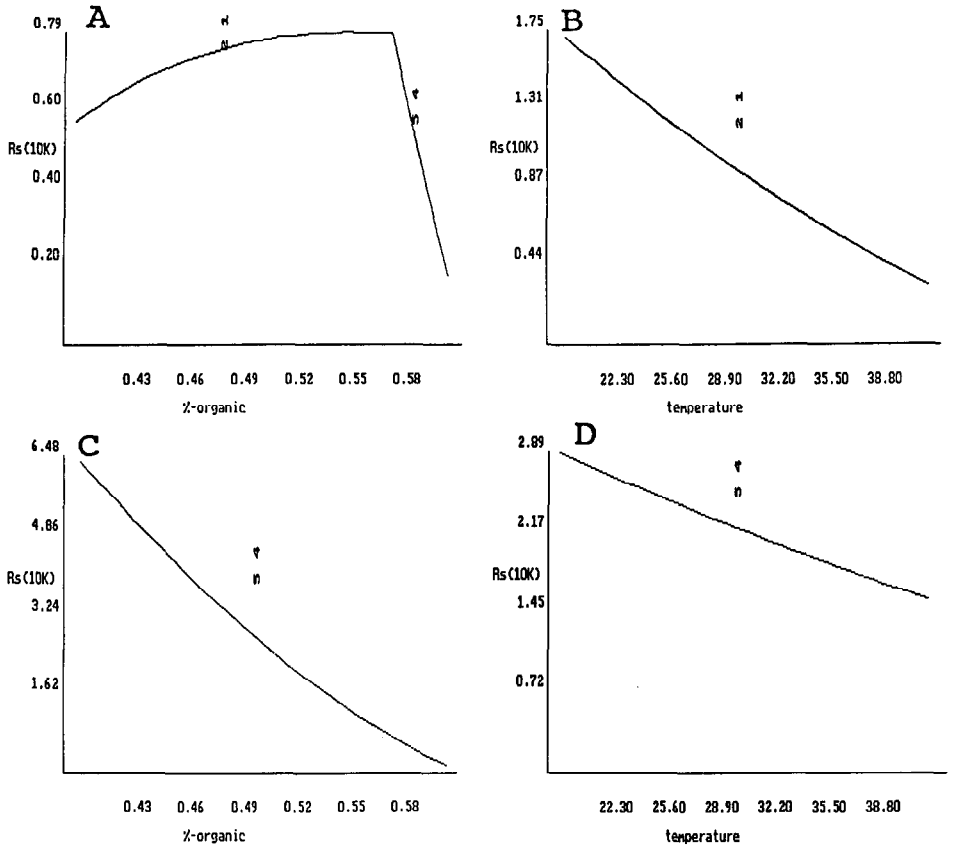

Fig. 6. Resolution maps for separations of Fig. 5. (A) Map for all bands, varying %B (T = 30°C); (B) map for all bands, varying T (50%B); (C) map for Band 5 only, varying %B (T = 30°C); (D) map for band 5 only, varying T (50% B). Temperature in °C.

ature). Let us assume, however, that only band 5 is of interest. In this case we can request *partial resolution maps* (*vs.* %B and T) for Band 5[a], ignoring the separation of other bands in the sample. Fig. 6C and D show the corresponding maps for this situation, which are more encouraging. For a temperature of 30°C, $R_s > 2$ for < 50%B. A mobile phase of 42% B provides a resolution of $R_s = 5.5$ with $k' = 22$ for the last band. This separation is shown in Fig. 7A. The run time is too long (60 min), but the excess resolution of band 5 ($R_s = 5.5$) can be traded for a shorter run time through the use of increased flow-rate and/or a shorter column. The predicted chromatogram (using computer simulation with DryLab I [4]) for a 5-cm column and 2 ml/min flow-rate is shown in Fig. 7B. The resulting run time is only 6 min, with a resolution of band 5 equal to 2.1.

[a] In a partial resolution map for Band (Fig. 6C, D), the resolution of the most poorly resolved band pair *which includes band 5* is plotted *vs.* the separation condition being varied.

Method ruggedness

Once a reasonable separation has been achieved (as in Fig. 4 or 7), the proposed method should be evaluated for ruggedness. That is, sample resolution should remain acceptable for likely variations in run conditions. Thus, a method that has been developed for ambient conditions should be able to tolerate variations in temperature of ± 5°C, since these are likely to be encountered in some laboratories. Similarly, errors in mobile phase composition of ± 2% B are not unlikely, and a change in %B of this magnitude should not compromise the separation. Possible errors in mobile phase pH are often of most concern, for two reasons. First, separations which are pH-dependent often exhibit marked changes in resolution for rather small changes in pH. Second, the control of mobile phase pH in the average laboratory is unlikely to be much better than ± 0.05 unit, and occasional errors of ± 0.10 unit should be anticipated.

The "optimized" separation of the substituted benzoic acid sample (Fig. 4) will provide an example of the potential use of multi-parameter mapping for avoiding problems that can arise from a lack of method ruggedness. If computer simulation is used initially to develop an HPLC method (as in the present case), testing for method ruggedness can be carried out (using computer simulation) without the need for further experiments. Alternatively, for methods developed in some other way, it is

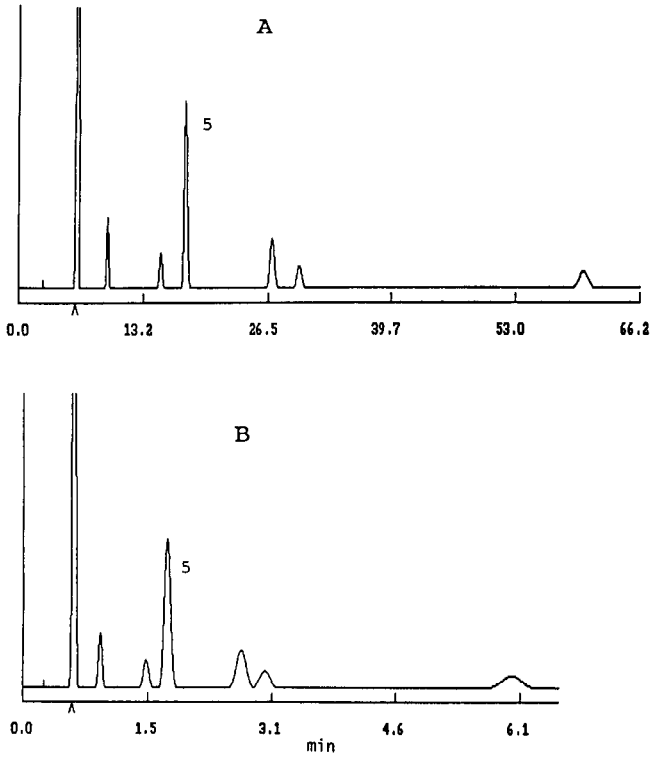

Fig. 7. Predicted separation of sample in Fig. 5 for optimized conditions (42% B, 30°C); other conditions as in Fig. 5. (A) 25-cm column, 1 ml/min (DryLab MP); (B) 5-cm column, 2 ml/min (DryLab I).

necessary to enter in run conditions and retention times for (a) the routine method and (b) additional runs where one experimental parameter is changed at a time.

In the present case, we will first evaluate the method of Fig. 4 (pH 2.9) for its sensitivity to inadvertent changes in pH; *e.g.*, ±0.1 unit. This is easily done by examining chromatograms for pH 2.8 and 3.0, as seen in Fig. 8 and summarized in Table IV. It is seen that an error in mobile phase pH of −0.10 unit (pH = 2.8) results in an unacceptable decrease in resolution (R_s = 1.16 *vs.* 2.05 expected). This result could have been anticipated from the resolution map in Fig. 2A, which shows a sharp drop in R_s for a decrease in pH below 2.9. The map in Fig. 2A also shows that the sensitivity of resolution to pH is less when pH > 2.9, suggesting that an increase in pH of the routine method might serve to diminish the sensitivity of R_s to a change in pH. This is confirmed by the data in Table IV for a target mobile-phase pH of 2.95 (designated by[b]). Now, a change of ±0.10 unit in pH does not reduce resolution below R_s = 1.66 (*vs.* 1.16 for pH 2.90).

We can further improve the latter method (pH 2.95, other conditions remaining the same as in Fig. 4) by noting that the critical band-pairs are 1/2 and 4/5. It is useful in cases such as this to request information on the response of these band pairs to a change in each of the separation variables: %B, T, pH and buffer concentration. Fig. 9 shows the resulting computer display.

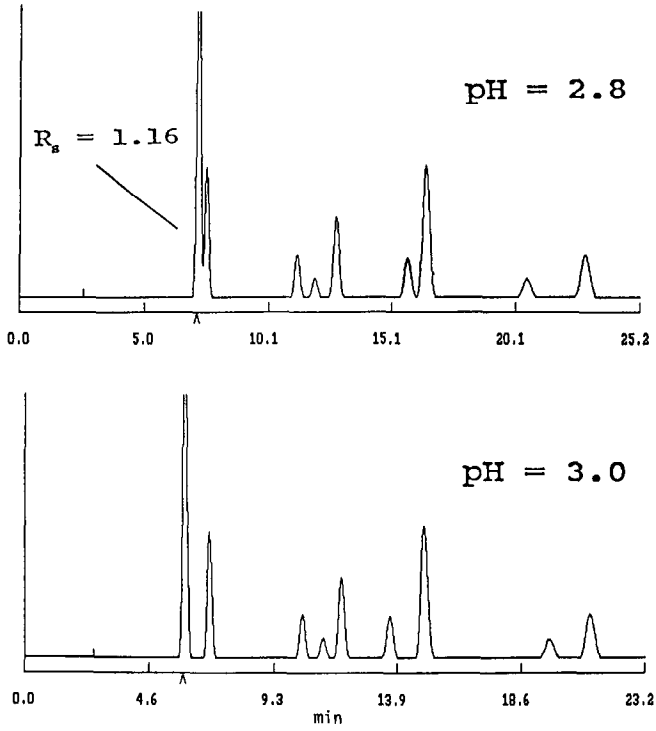

Fig. 8. Simulated chromatograms for the effect of a change in pH (by ±0.10 unit) on the "optimized" separation of Fig. 4.

TABLE IV

CHANGE OF SEPARATION WITH INADVERTENT CHANGE IN pH

"Optimized" separations of substituted benzoic acids.

Conditions	pH	Resolution	
		R_s	Bands[a]
37% B, 32°C, 25 m*M*	2.80	1.16	1/2
	2.90[b]	2.05	4/5
	3.00	1.89	4/5
Same	2.85	1.66	1/2
	2.95[b]	2.00	4/5
	3.05	1.77	4/5
36% B, 32°C, 25 m*M*[c]	2.85	1.84	3/4
	2.95[b]	1.99	3/4
	3.05	1.97	4/5

[a] Critical band pair.
[b] Optimized pH.
[c] Chromatograms in Fig. 10

What is desired is a change in one (or more) of these variables so as to improve the resolution of both critical band pairs (1/2, 4/5). Thus, it is seen that an increase in pH favors the separation of bands 1/2, but worsens the separation of bands 4/5. Likewise a change in temperature or buffer concentration has little effect on the separation of bands 4/5. A decrease in %B, on the other hand, leads to improved resolution for both band pairs. Thus the computer display in Fig. 9 suggests that the value of %B should be decreased.

Trial-and-error changes in %B were carried out by computer simulation, with examination of the effect of a ± 0.10 unit change in pH for each 1% decrease in %B. On this basis, it was found that 36% B gave a significant improvement in method ruggedness with regard to pH. These results are summarized in Table IV and Fig. 10. Now a change in pH of ± 0.10 unit has only a minor effect on separation: $1.84 < R_s < 1.99$. Note also that the resolution for the correct pH mobile phase ($R_s = 1.9$, pH 2.95) is little different from the original method (37% B, pH 2.90, $R_s = 2.06$), which was optimized for resolution alone (ruggedness ignored).

Computer simulations, such as those in Figs. 8-10 are able to both measure and improve method ruggedness for each experimental condition that can affect the separation. In some cases, it will not be possible to achieve really rugged separations for all experimental variables. Even in these cases, however, the sensitivity of the method to one or more conditions can be noted in the method procedure. That is, if the method is unduly sensitive to changes in %B, the user can be warned that greater care must be given to the formulation of the mobile phase; *e.g.*, a requirement for $\pm 1\%$ accuracy in %B.

Column variability

The problem of column variability was discussed in the Theory section. Once

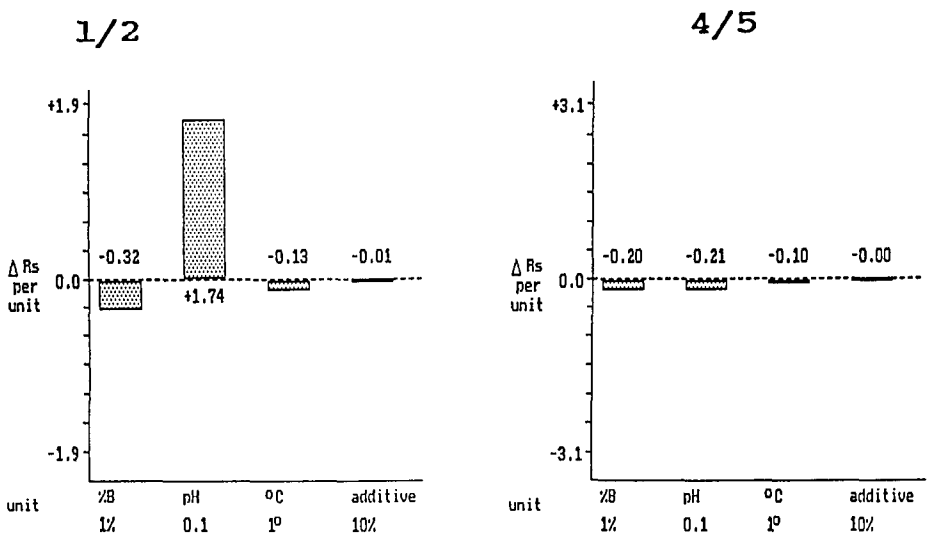

Fig. 9. DryLab MP screens, showing the effect of a change in different variables on the resolution of critical band pairs 1/2 and 4/5.

the effects of a change in different variables have been stored in DryLab MP (for the original column, as discussed above), it is possible to enter data for the separation of the same sample on a different column —presumably one that does not give the same relative retention or separation. The effect of a change in experimental conditions on the separation with the new column can then be predicted. Chromatograms or tabulated data can be used in this connection, but resolution charts as in Fig. 9 are particularly useful. Thus, if the separation in Fig. 4 or 10 were carried out on a new column, and bands 1/2 were found to merge ($R_s \approx 0$), Fig. 9 indicates that the most promising approach is to increase pH; an increase in pH of 0.1 unit should lead to an increase in the resolution of bands 1/2 by 1.74 units.

The use of computer simulation and data as in Fig. 9 in this way assumes that the dependence of sample retention on experimental conditions (values of S, B, etc.) is similar for different columns. This may not always be true, but this approach is a good way to start. Alternatively, any method can be modified for use on a new column by carrying out a few additional runs; *i.e.*, one or two runs for each experimental variable (multi-parameter mapping).

An example of correcting for differences between columns is provided by data from ref. 16, for the separation of a six-component steroid mixture on two different C_8 columns from the same supplier. In this case, it was found that the second column gave a poorer separation, as illustrated in the reconstructed chromatograms in Fig. 11. In the original paper, the mobile phase composition (%B) was modified by trial-and-error to improve the separation on the second column. We will illustrate how this same process can be facilitated with the use of computer simulation.

Fig. 11A shows the separation of this sample on the old column; bands 1/2 are the critical pair, with $R_s = 1.23$. Resolution is marginal, but this is the method that

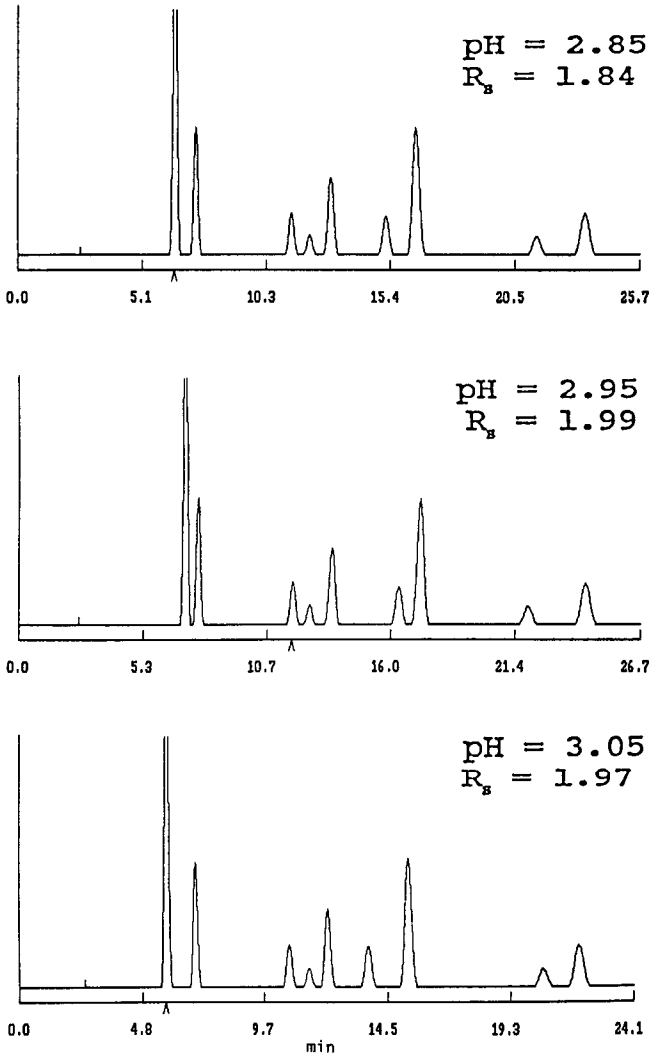

Fig. 10. Improvement in ruggedness in method of Fig. 4 (with respect to pH) for slightly different conditions: pH (intended) 2.95, 36% B, 32°C, 25 m*M* buffer.

was originally developed (49% methanol). Separation on the new column (Fig. 11B) is even worse, and the resolution of bands 4/5 (R_s = 0.89) is unacceptable for reliable quantitation. Retention times for the latter run were entered into DryLab MP, and separation was studied as a function of %B (using values of S for the old column). It was predicted that a resolution of R_s = 1.2 could be obtained for 54%B (Fig. 11C).

The actual separation on the new column with 54% methanol is shown in Fig. 11D, and it is seen to differ somewhat from that predicted in Fig. 11C, due to small differences in sample *S* values on the two columns. However, now the data for the runs of Fig. 11B and D can be entered into the computer for more accurate sim-

ulations of separation on the new column, and the separation of Fig. 11E (51% methanol) represents the highest resolution that can be achieved with the new column. R_s = 1.19; *i.e.*, close to the original value of R_s = 1.23 (bands 1/2) for Fig. 11A.

Troubleshooting HPLC Problems. Changes in separation may also be observed from day to day on the same column, possibly due to errors in mobile phase composition or flow-rate settings, changes in ambient temperature, loss in column efficiency, etc. These various possibilities can be quickly checked via computer simulation. For example, if all bands leave the column with shorter retention times, it might be logical to suspect a flow-rate error or pump malfunction. This can be easily verified via computer simulation, simply by examining separation *vs.* flow-rate. If the questionable chromatogram can be reproduced with a change in flow-rate, the origin of the separation problem is then confirmed.

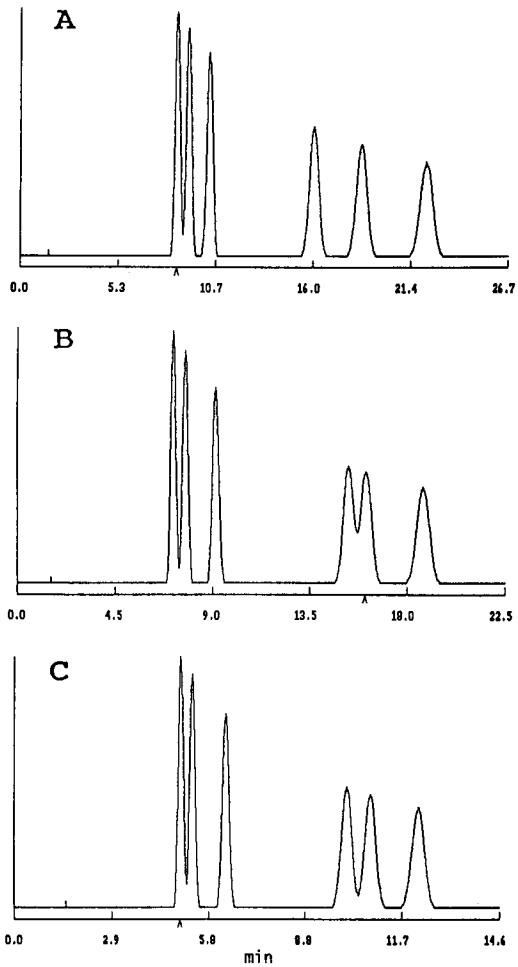

Fig. 11.

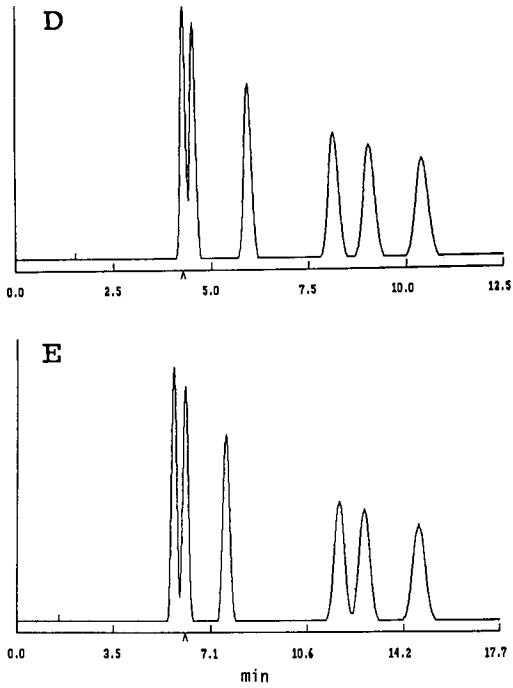

Fig. 11. Separation of six-component steroid sample in ref. 16. Conditions: 15 × 0.46 cm I.D., 5-μm Zorbax C_8 column, 1 ml/min. (A), original method and column [methanol-water (49:51)]; (B), same on new column; (C), simulated separation on new column with 54% B as mobile phase (using S values for old column); (D), actual separation on new column with 54% B; (E), separation on new column with 51% B.

A change in retention due to changes in ambient temperature can be checked in similar fashion by computer simulation, by trying different temperatures until a match with the faulty separation is achieved. Errors in the mobile phase composition (%B, pH, etc.) can be detected in the same way. This approach is especially helpful in the case of data that have already been collected, where there is no way of retrospectively checking for various errors of this kind.

CONCLUSIONS

Computer simulation based on restricted multi-parameter mapping represents a potentially powerful approach to HPLC method development and the improvement of existing methods. This procedure can also facilitate testing for method ruggedness and improving methods that are sensitive to small changes in experimental conditions. Finally, the same software can help deal with the problem of poor column-to-column reproducibility, as well as assist in the diagnosis of various errors or artifacts that cause poor separation. Only a few experimental runs (one or two per variable used in computer simulation) allow the use of computer simulation for all of these goals.

Examples that illustrate some of these capabilities are reported for several different samples: mixtures of substituted benzenes, substituted benzoic acids and steroids.

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