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Selectivity differences for C_{18} and C_8 reversed-phase columns as a function of temperature and gradient steepness II. Minimizing column reproducibility problems

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Abstract

The choice of T and t_G as variables for controlling selectivity and resolution during reversed-phase liquid chromatography (RPLC) method development can be used to minimize problems caused by column batch-to-batch irreproducibility. When a new column fails to provide adequate separation of the sample, altered values of T and t_G can be predicted that will restore the separation obtained with the previous column. Alternatively, columns from different manufacturers can be tested during method development, in order to find a common set of conditions (T and t_G) that provide acceptable separation with two or more of these columns. In this way, any of several columns from different sources become usable for the method. Examples are shown of these different computer-assisted procedures for minimizing problems due to column variability.

Keywords: Stationary phases, LC; Column reproducibility; Gradient time; Temperature; Selectivity; Method development

1. Introduction

When an RPLC method has been developed and subsequently used in different laboratories for the purpose of analyzing a particular sample, it is sometimes found that columns of the same designation from the same manufacturer do not provide a similar separation. That is, batch-to-batch column reproducibility may be inadequate for a given method [1–3], an observation which is supported by various surveys [4,5]. One approach to the problem of column irreproducibility is to empirically modify the separation conditions for the new column, so as to recover the separation obtained for the original column used during initial method development. Guidelines for such method adjustment have been proposed [6,7], such that (inconvenient) revalidation of the original method can be avoided. This implicitly assumes that if differences in selectivity among different columns can be eliminated by appropriate changes in conditions, then separation on these different columns can be considered ''equivalent''. Alternatively, it has been suggested (see Fig. 11 of Ref. [8]) that data obtained from the original column

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can be used to predict alternative method conditions for a new column. For the case where temperature T and/or gradient time t_G are varied, this requires that values of S and B''/t_G for each sample compound remain relatively constant for that compound and ''similar'' columns. This was shown to be the case in the previous paper (Part I, [9]).

A final means of addressing the column reproducibility problem is to select a single set of conditions (e.g., T and t_G) that can provide adequate separation with two or more C_{18} (or C_8) columns of different type; e.g., from different manufacturers. In the latter case, if batch-to-batch reproducibility problems arise with one column, alternative columns will be available for use with the same method. Each of these approaches for dealing with column irreproducibility was investigated in the present study.

2. Experimental

Equipment, materials and procedures are described in the preceding paper (Part I, [9]). The 11-component pharmaceuticals sample (laboratory A) was used to illustrate various procedures for minimizing the effects of column variability. All examples shown are computer simulations based on initial experiments where T and t_G are varied and where $N=10\,000$.

3. Results and discussion

The preceding paper [9] has described the separation of a pharmaceutical sample on nine different C_{18} columns. For given values of T and t_G, each column will exhibit differences in relative retention and resolution; however, these differences in resolution are often small, as noted in Table 7 of Ref. [9] by strong correlations of adjacent-band resolution values ($r \geq 0.99$) between many column pairs. While certain of the latter columns appear quite similar in their retention characteristics, other columns (e.g., Eclipse vs. Inertsil C_{18} columns of Table 7 of Ref. [9]; $r=0.83$) are more different. Based on these observations, we feel that these nine columns can serve as surrogates for different *batches* of nominally identical C_{18} columns. It is likely that actual batches of columns intended to be equivalent will exhibit less overall variation in retention and selectivity than these nine columns, especially columns that have been introduced within the past decade [10–13]. Minimizing differences in column selectivity by changing (''adjusting'') separation conditions becomes easier, the more similar two columns are. Therefore, the following procedures which are intended to compensate for column-to-column variability should be even more successful when applied to different batches of nominally identical columns.

3.1. Column irreproducibility: method redevelopment

During method development, it is customary to confirm that the method works with two or more columns from different manufacturing batches, as a preliminary indication that column variability will *not* be a problem. At a later time, however, it is always possible that new columns (different batch) may perform poorly for the separation of a particular sample. When a new column is found to give an unsatisfactory separation with the same method used previously, it is usually possible to re-optimize conditions to restore the original resolution. Table 1 of Ref. [9] shows that an optimum choice of T and t_G for any of these nine C_{18} columns can provide $R_s>2$ for this sample.

3.2. Column irreproducibility: method adjustment

Assume that values of T and t_G have been determined that provide acceptable separation of the pharmaceuticals sample with the original column, using the computer-assisted procedure of Ref. [9]. For the example of Fig. 1a, involving a comparison of two similar columns [9], four initial runs with T and t_G varying were used for computer simulation, leading to $R_s=1.9$ for $T=36$°C and $t_G=46$ min (Zorbax SB C_{18} column). These are intentionally sub-optimum values of T and t_G, in order to maximize differences in resolution for the two columns of Fig. 1a and b and thereby create a clearer example. The principle is the same if we start with optimized conditions for the initial column (Zorbax SB C_{18}).

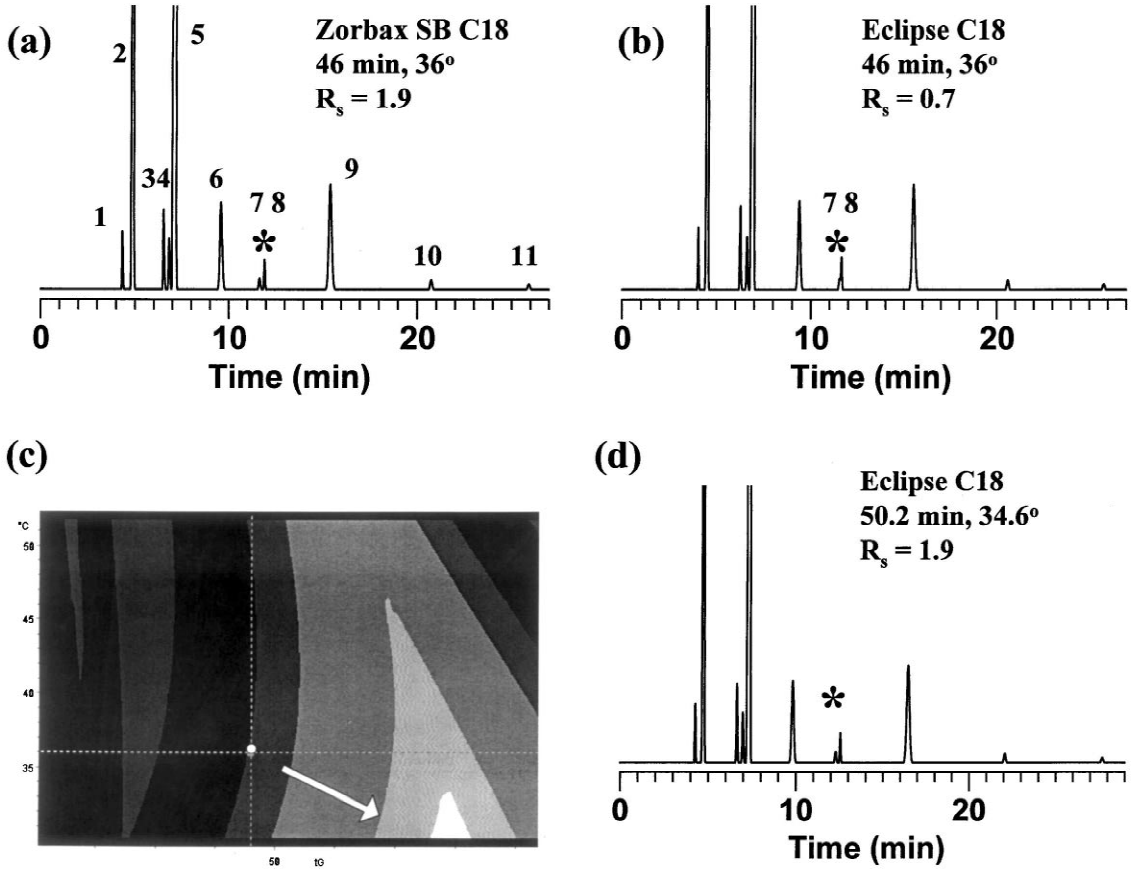

Fig. 1. Separation of pharmaceuticals sample on two different columns. (a) Separation on Zorbax SB C_{18} column; $t_G = 46$ min, $T = 36$°C; (b) separation on Eclipse C_{18} column, same conditions; (c) resolution map for separation of bands 7 and 8 only; (d) separation on Eclipse column; $t_G = 50.2$ min, $T = 34.6$°C. The arrow in (c) indicates changes in T and t_G which lead to increased resolution for bands 7 and 8, starting with $t_G = 46$ min, $T = 36$°C (circle); *, designates critical band-pair for each separation.

Next, assume that a new column gives an unsatisfactory separation, due to a change in column selectivity. This is illustrated in Fig. 1b for an Eclipse column ($R_s = 0.7$, same experimental conditions) in place of the original Zorbax SB column. It is possible to use the original computer-simulation input runs to estimate a change in conditions to restore the original separation on the new column. Fig. 1c shows a resolution map (Zorbax SB column) for just the two compounds (7 and 8) that overlap in Fig. 1b. Increasing resolution is predicted for a decrease in T and especially an increase in t_G (see arrow), which could guide a trial and error adjustment in T and t_G for the Eclipse C_{18} column (note separation of Fig. 1d).

The latter approach for adjusting experimental conditions in order to minimize differences in separation on two different columns can be made predictable, and therefore more efficient. If changes in resolution with T and t_G are similar for the two columns (similar values of S and B''/t_G, see discussion of Ref. [9]), it is possible to make a quantitative prediction of the required values of T and t_G for similar separation on the second column. The approach is similar to a ''reflection'' procedure described in Ref. [14] for the correction of computer predictions when unacceptable errors are encountered. A modified version of this procedure will next be described, using the separations of Fig. 1 as example.

3.2.1. Predictable choice of new values of T and t_G for column 2

Our first step is to predict changes in T and t_G (δT and δt_G) for the initial column (Zorbax SB C_{18} in Fig. 1) that will duplicate the (inadequate) separation on the second column (Fig. 1b, Eclipse C_{18} column). Reversing these changes ($-\delta T$ and $-\delta t_G$) for the second column should then give the desired separation (as in Fig. 1a) on the second column. This is equivalent to assuming that the differences in the two separations (Fig. 1a and b) are equivalent to an ''error'' in values of T and t_G for the separation of Fig. 1b (see further discussion of Ref. [14]). It is assumed that computer simulation was used to determine desired values of T and t_G for the initial column, which also allows prediction of changes in resolution, R_s, for each band-pair as a function of T and t_G: dR_s/dT and dR_s/dt_G. On the basis of data presented in Ref. [9], which show similar values of S and B''/t_G for the different components of the pharmaceuticals sample and the present nine C_{18} columns, we can anticipate similar values of dR_s/dT and dR_s/dt_G for these same columns.

Given the separations of Fig. 1a and b (runs 1 and 2, respectively), the value of R_s for each band-pair in each chromatogram will be known. The corresponding change in R_s for each band-pair i in run 2 vs. run 1 can be calculated: $(\delta R_s)_i=(R_{si})_2-(R_{si})_1$. Our goal is to select new values of T and t_G for the separation on column 2 such that values of $(\delta R_s)_i$ will be minimized for all band-pairs. This can be accomplished by carrying out a least-squares regression of values of $(\delta R_s)_i$ vs. values of dR_s/dT and dR_s/dt_G:

$$(\delta R_s)_i = a(dR_s/dT) + b(dR_s/dt_G) \quad (1)$$

The resulting coefficients a and b are equal, respectively, to the changes in T and t_G that will bring the separation of Fig. 1b into closest-possible agreement with that of Fig. 1a. While the proposed relationship (Eq. (1)) would be appropriate if an exact fit to experimental data were possible, this will rarely be the case in practice. Therefore, we must recognize that differences in R_s [''errors'' in $(R_s)_i$] will be more significant for ''critical'' band-pairs which are least resolved. This practical consideration can be addressed by replacing values of δR_s in Eq. (1) by $(\delta R_s/R_s)$, where R_s refers to separation on the original column (presumably values of $R_s>1$). The latter substitution ($\delta R_s/R_s$ for R_s in Eq. 1) is assumed throughout the following discussion.

It should be noted that our goal in the procedure of Figs. 1 and 2 is *not* the selection of conditions (T and t_G) that will provide maximum resolution of the ''critical'' or least-resolved band pair on column 2. Rather, we are trying to minimize *differences* in resolution (and selectivity) for the two columns.

3.2.2. Application of Eq. (1) to several representative examples

The application of Eq. (1) to the examples of Fig. 1a and b leads to values of $a=1.4$°C and $b=-4.2$ min, or $T=36-1.4=34.6$°C and $t_G=46+4.2=50.2$ min. The resulting separation on column 2 for these conditions is shown in Fig. 1d. There is an obvious improvement in the separation, and the critical resolution $R_s=1.9$ is now identical to that in Fig. 1a for column 1. While an adequate separation of the sample, as represented by R_s for the critical band-pair, is important, an additional goal of the present study was to minimize differences in selectivity (or R_s) for *all* band-pairs in the two separations (column 1 vs. 2). *To the extent that we succeed, and especially for small changes in conditions δT and δ t_G, it becomes more difficult to argue that the separation with column 2 (with adjusted values of T and t_G) is sufficiently different from that with column 1 to require revalidation of the RPLC method.* Once the coefficients a and b of Eq. (1) have been used to adjust values of T and t_G for column 2, separation on column 2 with these new conditions can be compared with that on column 1 in terms of differences ($\delta' R_s$) in R_s for each band pair in the chromatogram. These residual differences $|\delta' R_s|$ can be summarized in terms of an average value and a standard deviation. For the example of Fig. 1, $|\delta' R_s|=0.10\pm0.08$. If we take the sum of the average and standard deviation values ($0.10+0.08=0.18$) we obtain a single number, ϑ, that can be used to characterize how closely the two separations agree in terms of selectivity and resolution. On average, five out of every six band-pairs should have $|\delta' R_s|<\vartheta$, and this was confirmed for the different examples of Table 1.

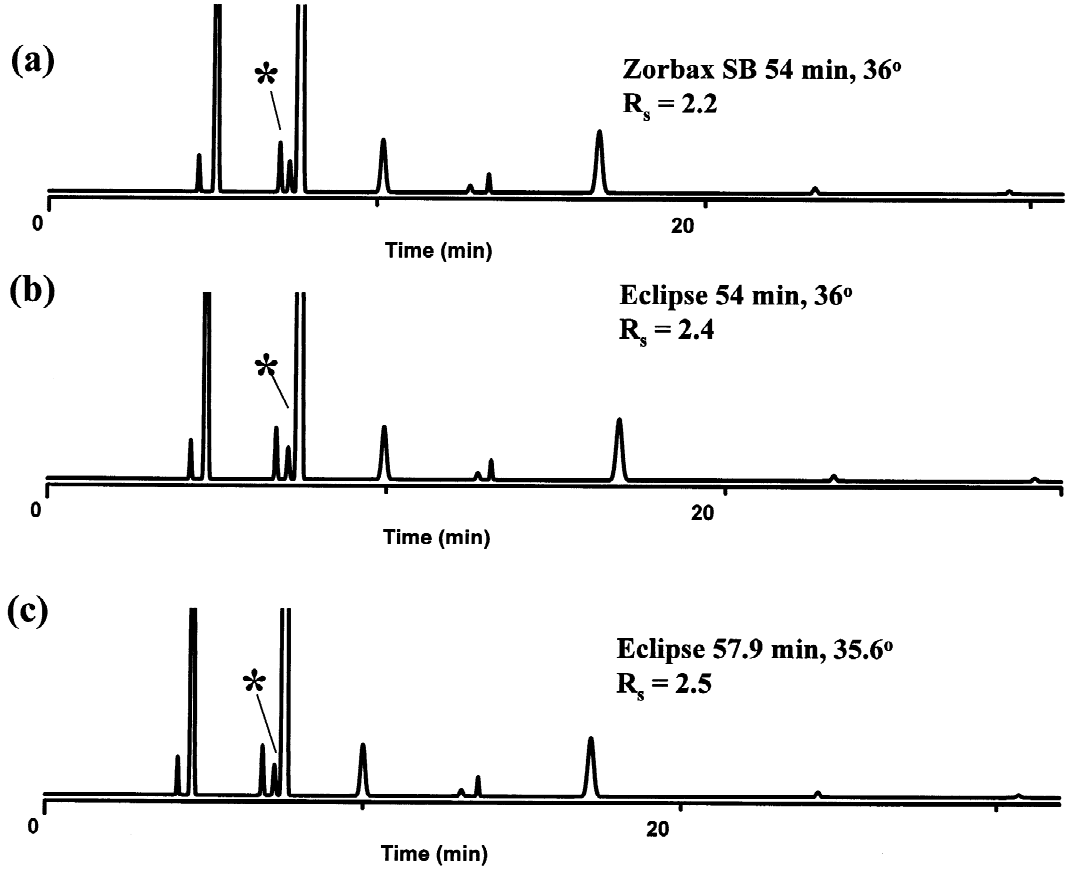

Fig. 2. Separation of pharmaceuticals sample on two different columns. (a) Separation on Zorbax SB C_{18} column; $t_G = 54$ min, $T = 36$°C; (b) separation on Eclipse C_{18} column, same conditions; (c) separation on Eclipse column; $t_G = 57.9$ min, $T = 35.6$°C. *, Designates critical band-pair for each separation.

Results for the example of Fig. 1 are summarized in Table 1 as example 1. Note for this example that the value of ϑ is lowered from 0.32 (Fig. 1b) to 0.18 (Fig. 1d) after adjustment of T and t_G for column 2. Values of $\vartheta < 0.3$ generally correspond to acceptable agreement between the separations on two columns, either before or after adjustment of T and t_G. In a second example (2 of Table 1), the separation on column 1 (Zorbax SB) was optimized to give a maximum value of $R_s = 2.2$ (Fig. 2a). Repeating this separation (same conditions) on column 2 (Eclipse) gives a very similar separation (Fig. 2b), as noted also by a value of $\vartheta = 0.21$ in Table 1 (example 2, same conditions for both columns). This result is not unexpected, as in the region of maximum resolution (see R_s maps of Fig. 2a and b of Part I [9]) these two columns both exhibit similar values of R_s over a comparable range in T and t_G (± 2°C and ± 10 min). The application of Eq. (1) to this example (Fig. 2) results in the separation of Fig. 2c, which is little different from the separations of Fig. 2a and b ($\vartheta = 0.16$ vs. 0.21 originally). That is, when separation is quite similar for two different columns, there is only a limited possibility of minimizing differences in resolution by a change in conditions.

The adjustment of conditions in this way in order to minimize differences in resolution will often result in small changes in absolute retention time t_R, as

Table 1
Summary of the application of Eq. (1) for the adjustment of conditions (T and t_G) that will minimize differences in resolution for the separation of the pharmaceuticals sample on two different columns

Column 1	Column 2	t_{Ga}, T_a	t_{Gb}, T_b	$(R_j/R_i)-1$		r^c
				Original[a]	Corrected[b]	
1. Zorbax SB	Eclipse	46 min, 36°C	50.2 min, 34.6°C	0.14±0.18	0.10±0.08	0.81
2. Zorbax SB	Eclipse	54 min, 36°C	57.9 min, 35.6°C	0.11±0.10	0.09±0.07	0.58
3. Eclipse	Zorbax SB	35 min, 35°C	32.1 min, 37.1°C	0.13±0.20	0.11±0.08	0.76
4. Eclipse	Zorbax SB	19 min, 34°C	19.1 min, 35.4°C	0.08±0.10	0.10±0.08	0.45
5. Zorbax SB	Eclipse	21 min, 45°C	21.4 min, 45.5°C	0.10±0.09	0.06±0.12	0.63
			Average ϑ	0.25	0.18	
6. Zorbax SB	Inertsil	54 min, 36°C	76.4 min, 40.9°C	0.36±0.36	0.14±0.12	0.98
7. Zorbax SB	Symmetry	54 min, 36°C	70.8 min, 38.1°C	0.28±0.31	0.12±0.08	0.92
8. Symmetry	Zorbax SB	39 min, 32°C	27.1 min, 33.1°C	0.26±0.30	0.18±0.10	0.91
			Average ϑ	0.62	0.24	
9. Zorbax SB	SymmShield	54 min, 36°C	30.9 min, 41.5°C	0.61±0.61	0.52±0.38	0.59
			Average ϑ	1.22	0.90	

[a] R_s for column j divided by R_s for column i, minus 1; this represents the relative difference in R_s between the two runs for the same original conditions (54 min, 36°C in example 1); average values are reported with their standard deviation; where $R_s<1$, the error in R_s is substituted for (R_j/R_i) [based on absolute values of $(R_j/R_i)-1$].

[b] Same as ''a'', but column j is for corrected conditions (57.9 min and 35.6°C in example 1), while column i is for original conditions (54 min, 36°C) in the first example.

[c] Correlation coefficient for Eq. (1) applied to original separations on columns 1 and 2 (same values of T and t_G).

seen by comparing the separations of Fig. 1a vs. d or Fig. 2a vs. c. Such differences in t_R can require changes in the data system parameters that are used for identifying different compounds in the sample. Such differences in t_R values can be minimized in most cases by further, simultaneous adjustments in flow-rate and gradient time so as to maintain (t_G/F) constant. This allows a controlled shift in values of t_R for all peaks, without appreciably affecting resolution.

Some additional examples (3–5) involving these same two columns (Zorbax SB and Eclipse C_{18}) of similar selectivity are summarized in Table 1, with average values of ϑ for the same conditions (and these two columns) compared with separations with adjusted conditions: 0.25 and 0.18, respectively. That is, the agreement of the initial separations on columns 1 and 2 (same conditions) is rather good in terms of overall resolution, and it can be improved somewhat by adjusting the conditions for column 2. In some cases, as in the example of Fig. 1, the improvement in *critical* resolution is more dramatic when conditions for column 2 are adjusted, which reflects the presumed normal distribution of differences in resolution $\delta' R_s$ for different band-pairs in the chromatogram.

A second group of examples is summarized in Table 1 (6–8), involving C_{18} columns that are more different in selectivity (see Ref. [9]). The initial differences in resolution (same conditions) are greater for these columns ($\vartheta=0.62$), but a change in conditions reduces these differences to a value ($\vartheta=0.24$) that is similar for the columns of examples 1–5 ($\vartheta=0.18$), which are more similar in terms of selectivity. The final example of Table 1 (9) is for two columns which are quite different in selectivity [9]: the Symmetry Shield RP8 column has a C_8 group with an embedded polar group, in contrast to the Zorbax SB C_{18} column with no embedded group. The initial value of $\vartheta=1.09$ is quite large and is not much improved by adjusting conditions for column 2 via Eq. (1) ($\vartheta=0.71$). That is, when column selectivity is sufficiently different for two columns, Eq. (1) becomes less useful as a means of minimizing these selectivity differences. Note also in Table 1 that the change in conditions suggested by Eq. (1) is generally larger for columns that are more different (examples 6–9). It has been suggested [6,7] that

Table 2
Critical resolution values compared for separation of the pharmaceutical sample on two different columns (same examples as in Table 1)

Column 1	Column 2	t_{Ga}, T_a Original	t_{Gb}, T_b Adjusted	Critical resolution R_s		
				Column 1	Column 2[a]	
					Original	Adjusted
1. Zorbax SB	Eclipse	46 min, 36°C	50.2 min, 34.6°C	1.9	0.7	1.9
2. Zorbax SB	Eclipse	54 min, 36°C	57.9 min, 35.6°C	2.2	2.4	2.5
3. Eclipse	Zorbax SB	35 min, 35°C	32.1 min, 37.1°C	1.8	0.5	1.5
4. Eclipse	Zorbax SB	19 min, 34°C	19.1 min, 35.4°C	1.6	1.4	1.3
5. Zorbax SB	Eclipse	21 min, 45°C	21.4 min, 45.5°C	0.2	0.0	0.3
6. Zorbax SB	Inertsil	54 min, 36°C	76.4 min, 40.9°C	2.2	1.3	2.2
7. Zorbax SB	Symmetry	54 min, 36°C	70.8 min, 38.1°C	2.2	0.1	2.5
8. Symmetry	Zorbax SB	39 min, 32°C	27.1 min, 33.1°C	2.7	0.9	2.0
9. Zorbax SB	SymmShield	54 min, 36°C	30.9 min, 41.5°C	2.2	3.0	2.3

minor changes (''adjustment'') in RPLC method conditions for the purpose of minimizing the effects of a change in column selectivity should be allowable, without requiring revalidation of the method. This is equivalent to a requirement that the selectivity of the new column be similar to that of the original column, which should generally be the case for different batches of columns that are nominally similar.

Table 2 summarizes critical resolution values for the various separations of Table 1. The initial separation on column 2 (same T and t_G) often results in a value of R_s which is unacceptably low, compared to that on column 1. However, in every case the value of R_s for the adjusted separation on column 2 is in reasonable agreement with that for column 1 (± 0.2 R_s units, 1 SD). This is true even for the two very different columns (example 9 of Table 2), which is the result of the greater emphasis given to small values of R_s in the present procedure. That is, if the goal of adjusting conditions is simply to achieve acceptable separation (e.g., $R_s > 1.5$) for all pairs of bands, this is generally not difficult.

3.2.3. Generalization of Eq. (1) for the use of additional separation variables

Results summarized in Tables 1 and 2 for the present sample and columns suggest that a similar approach may be successful for other samples, columns and experimental conditions. However, this seems unlikely to be the case for every such situation, where samples of any composition are possible. On the other hand, if Eq. (1) can be extended to include terms for additional variables j, k, ...:

$$[(\delta R_s)_i / R_s] = a(dR_s/dT) + b(dR_s/dt_G) + c(dR_s/dj) + d(dR_s/dk) + \ldots \quad (2)$$

the likelihood of a successful adjustment of separation using column 2 seems more probable. Work aimed at exploring this possibility is currently underway in the laboratory of two of the authors (J.W.D., L.R.S.).

3.3. Column irreproducibility: use of the same conditions for two or more columns

An alternative solution to the problem of column variability is to select experimental conditions that will give acceptable separation on two or more *different* columns (i.e., not different *batches* of the same column designation). This is conceptually similar to the concept of ''generic'' columns, which are assumed to be interchangeable for a given RPLC method. Once resolution maps have been obtained for several columns, an examination of these maps may result in the identification of values of T and t_G that provide acceptable resolution for two or more columns. For example, in the case of the Zorbax SB and Eclipse C_{18} columns (Fig. 2a and b of Ref. [9]),

maximum and similar resolution ($2.2 \leq R_s \leq 2.6$) is found for each column in a diagonal region extending from 50 to 65 min, and 35 to 38°C. This procedure can be made more efficient (as well as adaptable to columns whose resolution maps are less similar) by the use of ''black & white'' resolution maps, where white represents acceptable resolution; e.g., $R_s > 2$. This is illustrated in Fig. 3 for each of the 10 columns used for the pharmaceutical sample.

3.3.1. Pharmaceutical sample

If the maps of Fig. 3 are transferred to transparencies and overlapped, any white (i.e., clear) region(s) common to all columns will be obvious; i.e., light will be able to pass through each of the overlapped maps in that region of T and t_G. Any value of T and t_G within the clear region(s) common to each column should then provide acceptable resolution of the sample. The maps of Fig. 3 can also be overlapped by manipulation within a software program such as Microsoft PowerPoint (this procedure was used to prepare the overlapped maps of Figs. 4 and 6).

Examination of the individual maps of Fig. 3 shows wide regions of acceptable resolution for some columns (a, e, f, g, h, i) but not for others (c,d). When all 10 maps are overlapped, no clear region common to all columns is observed. A trial-and-error selection of sub-sets of these 10 columns was tried next, in an attempt to find as many columns as possible that give acceptable resolution ($R_s > 2$) for a single set of method conditions (values of T and t_G). One such sub-set consists of columns a, b, e, f, g; the resulting overlapped resolution map for these five columns is shown in Fig. 4. Best conditions (indi-

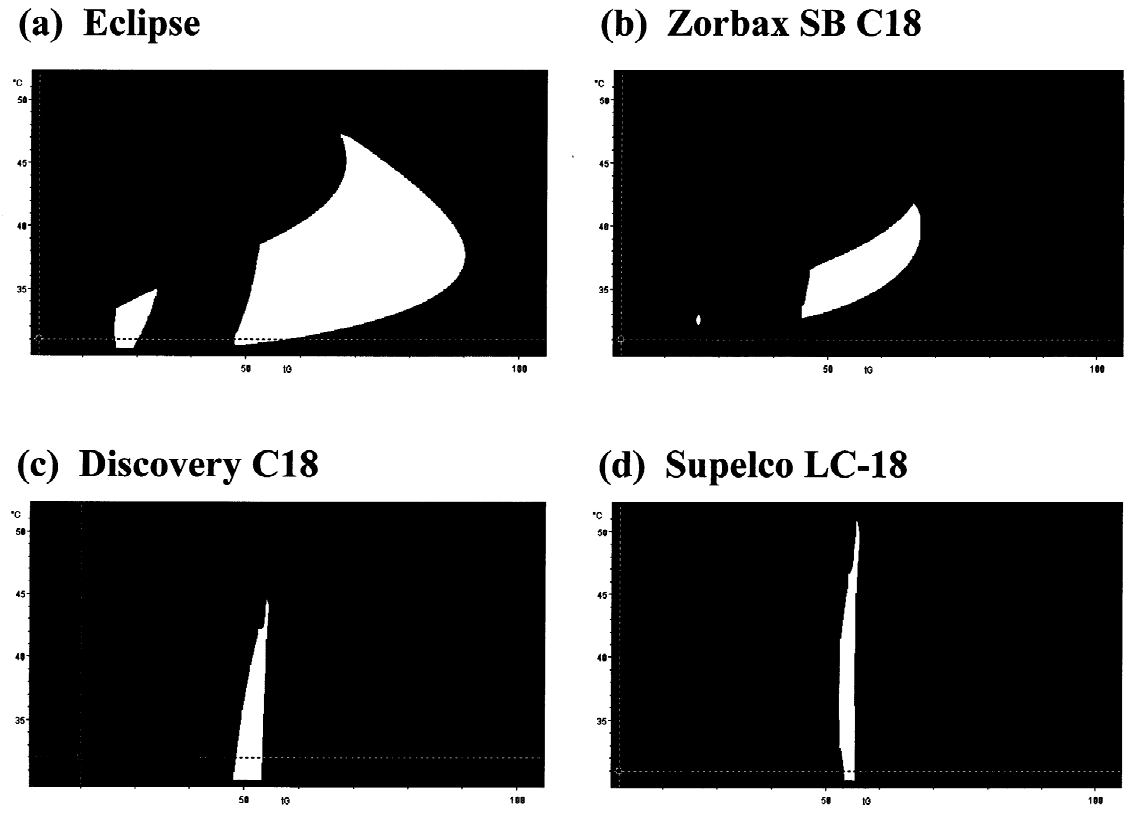

Fig. 3. ''Black/white'' resolution maps for the pharmaceutical sample and each of the 10 columns. White region indicates $R_s > 2$.

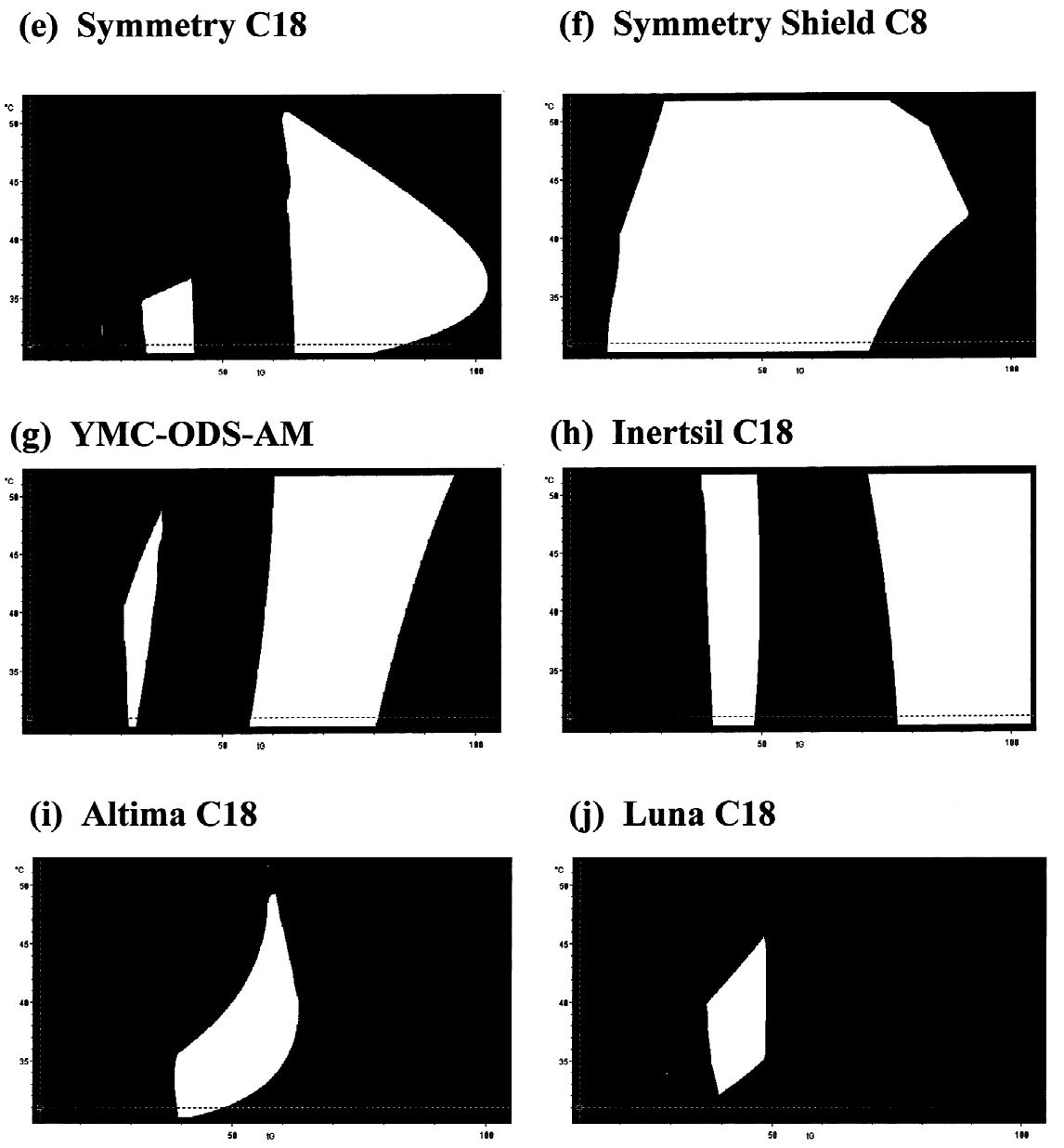

Fig. 3. (*continued*).

cated by the circle in Fig. 4) are $T=39$°C and $t_G=65$ min; Fig. 5 shows the resulting separations for these five columns and these conditions (but note the larger differences in retention, vs. the examples of Figs. 1 and 2 where conditions are adjusted).

If the required resolution is relaxed to $R_s>1.5$ (baseline separation), then the same method conditions can be used for eight of the 10 columns, as shown by the overlapped resolution map of Fig. 6. However, because of the large difference in peak sizes, a resolution of $R_s=1.5$ may be inadequate for this sample.

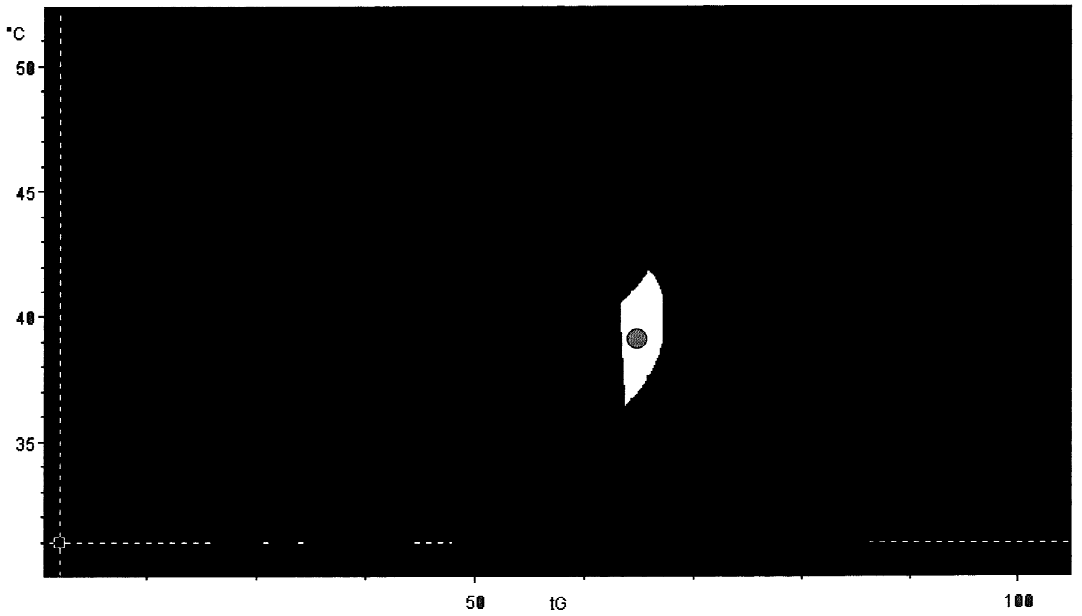

Fig. 4. Overlapped resolution map for pharmaceutical sample and columns a, b, e, f and g of Fig. 3. ''x'' marks values of $T=39$°C and $t_G=65$ min (for acceptable resolution with each of these five columns).

4. Conclusions

Given the general problem that a separation achieved on one column may not transfer to a second column (even a nominally equivalent one from a given manufacturer), two general approaches have been explored as means for addressing this problem. In each case, the goal is to achieve separations on the two columns which are as nearly identical as possible in terms of relative band spacing or selectivity. It is assumed here that column plate numbers are similar for the two columns, as will generally be the case for well-performing columns. Our primary goal was to achieve similar relative retention or equivalent selectivity for the two columns. This is the same as obtaining similar values of R_s for each band-pair in the two separations.

One procedure for adjusting selectivity and resolution is to vary the experimental conditions for the second column so as to bring the separations with columns 1 and 2 into closer agreement. This approach would be preferred for the case where a method has already been developed and all experimental conditions have been specified. Such adjustments of conditions for column 2 can be carried out empirically, but a more efficient procedure is the use of computer simulation as described here. In the present illustrative study, temperature T and gradient time t_G were initially varied to obtain a desired separation on column 1. Four initial experimental runs allow computer simulation of separation on column 1 as a function of T and t_G. Once these four runs have been used to set up computer simulation for column 1, changes in R_s for every band-pair as a function of change in T and t_G can be predicted. Since it was shown in Part I [9] that these changes in R_s with conditions are similar for all C_{18} columns (at least for the present sample), it is possible to predict values of T and t_G for column 2 that will minimize differences in separation on the two columns. A straightforward and easily automated procedure allows the selection of adjusted conditions for column 2 based on this approach. For several examples involving different C_{18} columns, the use of the same conditions for columns 1 and 2 was found to give a critical resolution that was lower on column 2 by 0.2–2.1 units, in many cases resulting in an inadequate separation on column 2

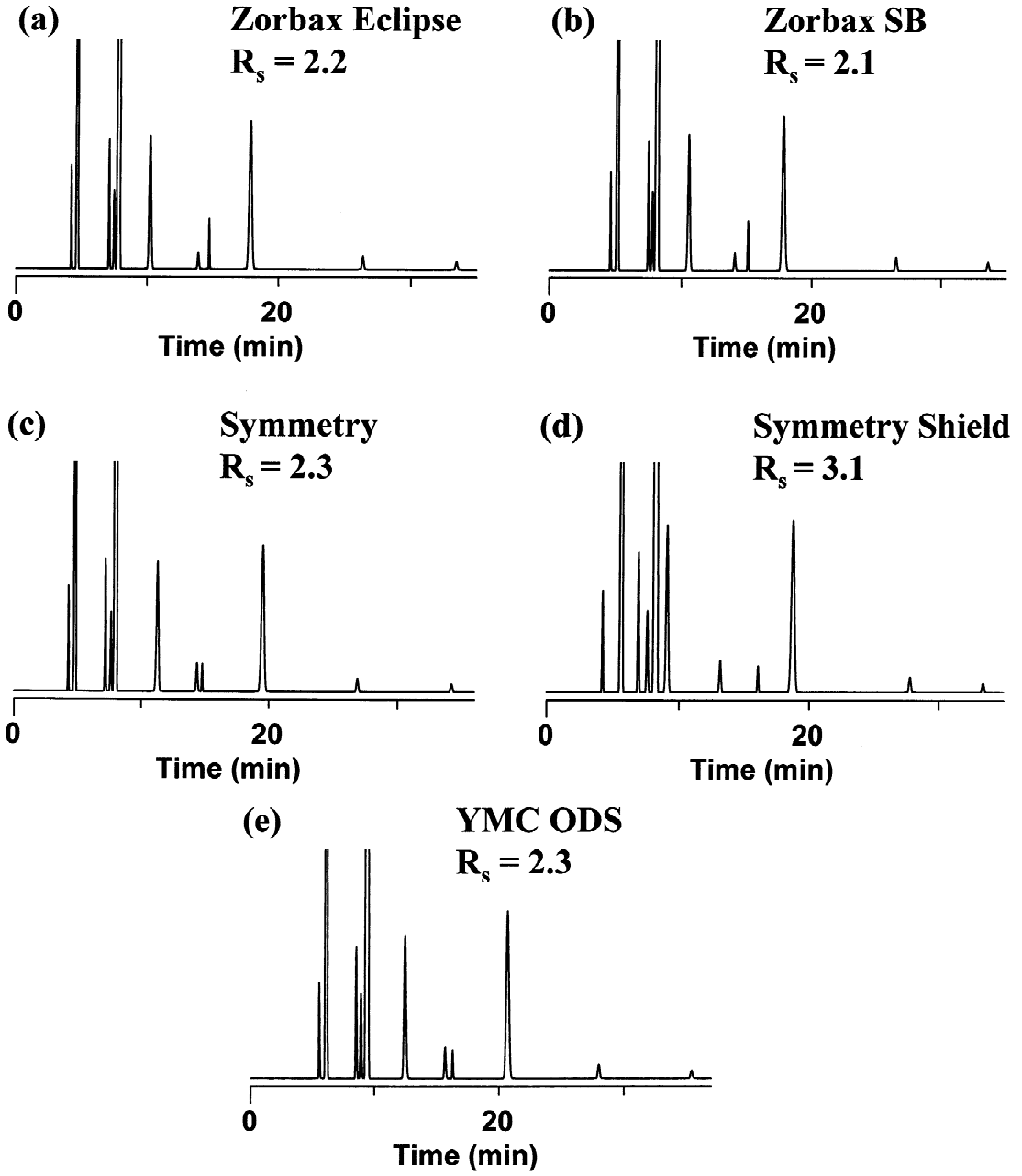

Fig. 5. Separation of pharmaceutical sample for preferred conditions (T=39°C and t_G=65 min) from Fig. 4 for these five columns.

compared to column 1. After adjusting values of T and t_G for column 2 to minimize differences in selectivity vs. column 1, the average difference in critical resolution was only ± 0.2 R_s units (1 SD). The adjustment of conditions in this way to minimize the effects of column variability will be more successful, the more similar are columns 1 and 2 in terms of selectivity (e.g., for different batches of nominally identical columns).

Column similarity has been quantified for the

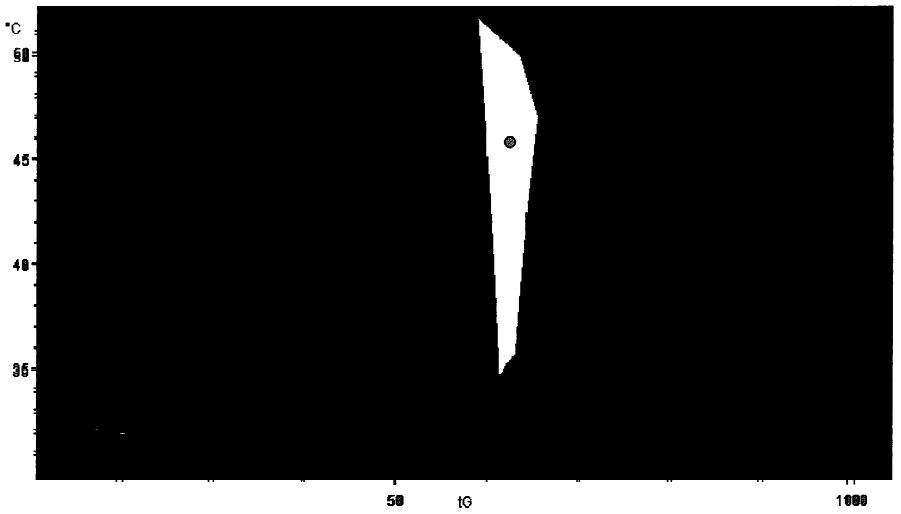

Fig. 6. Overlapped resolution map for pharmaceutical sample and columns a, b, c, d, e, f, g and i of Fig. 3. Circle marks values of $T=39$°C and $t_G=65$ min (for $R_s>1.5$ for these eight columns).

present 10 columns, in terms of one particular sample (11 pharmaceutical components). A column classification that is probably more general and therefore more useful for other samples has been reported elsewhere [15], although that classification and the one presented in Part I [9] are in general agreement. While the success we have attained for this particular sample in achieving similar column selectivity by a change in T and t_G may not be attainable for other samples, it is possible that the extension of this approach to the simultaneous variation of a larger number of experimental conditions (e.g., pH, solvent type, additive concentration, etc.) via Eq. (2) will improve its chances of success for other samples. Work in this direction is now underway.

A second approach for reducing the effects of column variability can be carried out during method development (selection of conditions for column 1). In this procedure, resolution is mapped as a function of one or more conditions for several columns from different sources. The objective is the selection of conditions that provide acceptable separation of the sample with two or more different columns. In this case, if one column proves unacceptable when a different column batch is used, one or more alternative columns are available as replacements. We have evaluated this approach where only T and t_G were varied. From the study of 10 different RPLC columns (nine C_{18} and one C_8), it was found possible to separate the present sample with $R_s>2.0$ on five of these columns, using identical separation conditions.

5. Nomenclature

See Part I [9].

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