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Selectivity differences for C₁₈ and C₈ reversed-phase columns as a function of temperature and gradient steepness

I. Optimizing selectivity and resolution

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Abstract

Four experimental runs where temperature T and gradient time t_G are varied allow the computer-prediction of reversed-phase liquid chromatographic (RPLC) separation for different combinations of temperature and gradient time. This in turn can provide significant changes in selectivity and a resulting optimization of separation. If this procedure is repeated for different columns, additional control over selectivity and resolution becomes possible. The simultaneous variation of T and t_G for columns from different sources was studied for two samples, as a means of evaluating the general advantage of this approach for RPLC method development. Changes in relative retention with T were found to be approximately constant for different values of t_G and for different RPLC columns; similarly, changes in relative retention with t_G were roughly independent of changes in temperature or the column. The latter relationships can be useful in matching (“tracking”) peaks between runs during method development based on the present approach, as well as for other applications discussed in here and in Part II. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; Temperature; Gradient time; Selectivity; Optimization

1. Introduction

Simultaneous changes in temperature T and gradient time t_G have been shown useful in controlling separation selectivity and maximizing sample resolution for separations by reversed-phase liquid chromatography (RPLC) [1–7]. In some cases, however, the variation of T and t_G alone has proven insufficient to achieve adequate resolution [3]. For

these situations, a change in some other condition (pH, column type, etc.) followed by re-optimization of T and t_G is sometimes successful [4]. In the present study, we have combined T and t_G optimization with the use of different C₈ and C₁₈ columns for the separation of two, moderately challenging samples, as a means of assessing the usefulness of this approach.

In the following paper (Part II) [8], differences in column selectivity as a function of T and t_G are examined from a different standpoint: choosing values of T and t_G so as to *minimize* differences in column selectivity and thereby allow the use of

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different C₈ and C₁₈ columns for the same RPLC assay procedure. In this way, it is possible to anticipate and alleviate problems caused by column irreproducibility.

2. Theory

2.1. Computer simulation

Solute retention in RPLC can be described as a function of T and t_G by the following, well known relationships [9,10]:

$$\log k = \log k_w - S\phi \quad (1)$$

$$\log k = A + B/T_K \quad (2)$$

where k is the retention factor, k_w is the (extrapolated) value of k for $\phi=0$, ϕ is the volume fraction of organic in the organic–water mobile phase (equal to 0.01% B), T_K is the temperature in K, and S , A and B are constants for a given solute and other conditions constant (only ϕ varies in Eq. (1), only T_K varies in Eq. (2)). For gradient elution as in the present study, it has been found [11] that retention time t_R can be described by the semi-empirical relationship:

$$t_R = A'' - B''T \quad (3)$$

Here, A'' and B'' are constants for a given solute with only T (°C) varying. Based on four isocratic separations with two different values of ϕ and T , the combination of Eqs. (1) and (2) allows prediction of t_R as a function of T and ϕ . Similarly, the use of Eqs. (1) and (3) allows accurate predictions of t_R in gradient elution [12]. For predictions of either isocratic or gradient separation, it should be noted values of k_w and S vary with T .

2.2. Separation parameters S and B''

Further insight into the effects of T and t_G on RPLC separation can be obtained from a study of the parameters S and B'' . A knowledge of how these parameters vary with the column can also be of value in the use of computer simulation, some examples of which are given in the present and following papers.

Values of S in Eq. (1) usually decrease for higher T , while B tends to decrease for larger ϕ [9,13]. That is, S and B are often smaller for conditions that result in smaller values of k . In gradient elution, however, solutes have an average retention k^* which does not vary with T [14]. As a result, values of S measured from gradient experiments are approximately constant for different temperatures T [15], as further illustrated by the data of the present study.

2.2.1. B'' as a function of t_G

Concerning the temperature dependence of retention (values of B'' in Eq. (3)), the dependence of B'' on gradient time t_G can be important, for the same reasons that the approximate constancy of S at different temperatures (see above) was of interest. Gradient retention time t_R for initially well-retained bands is given by [14]:

$$t_R = (t_0/b) \cdot \log(2.3k_0b) + t_0 + t_D \quad (4)$$

where

$$b = t_0 \Delta\phi S / t_G \quad (5)$$

The quantities k_0 , t_0 , t_D , and $\Delta\phi$ refer, respectively, to the value of k at the start of the gradient, the column dead time, the equipment dwell volume, and the change in ϕ during the gradient. Combining Eqs. (4) and (5) for retention times t_{R1} and t_{R2} at temperatures T_1 and T_2 :

$$t_{R2} - t_{R1} = (t_G / \Delta\phi S) \cdot \log(k_{02}/k_{01}) \quad (6)$$

(because S is approximately the same at the two temperatures, and t_G is the same, b can also be considered constant). Eq. (3) can be rewritten:

$$t_{R2} - t_{R1} = B''(T_1 - T_2) \quad (7)$$

and combining Eqs. (3) and (6) then yields:

$$B'' = (1/[T_2 - T_1]) \cdot (t_G / \Delta\phi S) \cdot \log(k_{02}/k_{01}) \quad (7a)$$

The use of computer simulation to predict separation as a function of T and t_G relies on four initial runs where T_1 , T_2 and $\Delta\phi$ are fixed; for a given solute, values of S and k_{02}/k_{01} can be assumed constant for these conditions. For this case, therefore, $B'' = (\text{constant}) \cdot t_G$ or:

$$B''/t_G = \text{constant} \quad (8)$$

Eq. (8) assumes that values of k_0 are large (e.g., $k_0 > 20$). Thus, for isocratic elution of compounds prior to the gradient ($t_R < t_0 + t_D$), B'' will be the same regardless of t_G , and B''/t_G will be inversely proportional to t_G . For compounds with small k_0 , but which elute after $t_0 + t_D$, values of B''/t_G will not be constant as t_G is varied.

Computer simulation as in the present study does not assume constant values of S or B''/t_G . However, the approximate constancy of these quantities when T or t_G are varied can be useful for other purposes: column characterization and peak tracking during method development (discussed later in this paper) and other applications examined in the following paper [8].

3. Experimental

3.1. Laboratory A (T.B.)

3.1.1. Equipment

Two different high-performance liquid chromatography (HPLC) systems were used. System 1 was a Model 2690 Separations Module with a Model 996 photodiode array UV detector (Waters Associates).

System 2 was a Model P4000 pump with vacuum degasser, a Model AS3500 autosampler, and a Model UV3000 UV detector (Thermo Separations Products). The data system was a Chemstation version A.03.04 data system (Hewlett-Packard).

3.1.2. Experimental conditions

Ten different 25×0.46 cm columns were studied: (a) Zorbax Eclipse C_{18} and (b) Zorbax SB C_{18} (Hewlett-Packard), (c) Discovery C_{18} and (d) LC-18 (Supelco), (e) Symmetry C_{18} and (f) Symmetry Shield C_8 (Waters), (g) YMC-ODS-AM (YMC), (h) Inertsil C_{18} (GL Sciences), (i) Alltima C_{18} (Alltech), and (j) Luna C_{18} (Phenomenex). The same conditions were used for each column: 0–100% acetonitrile–buffer gradient; 1.5 ml/min. Four different experiments were carried out for each column: $t_G = 20$ and 60 min, $T = 32$ and 50°C . For further details, see Ref. [16].

3.1.3. Sample

The sample is a mixture of 11 components: a drug

substance and eight impurities or degradation products expected to be present in mixtures containing the drug substance, plus two internal standards (benzyl alcohol and *m*-cresol). The drug substance is a proprietary derivative of 9-(2'-deoxy-b-D-ribofuranosyl) purine.

3.2. Laboratory B (L.V.H.)

3.2.1. Equipment and materials

These are described in Ref. [17].

3.2.2. Experimental conditions

Three different columns were used: Hypersil C_8 (Phenomenex), Luna C_8 (Phenomenex) and YMC C_{18} (YMC). Conditions varied somewhat in terms of column length, flow-rate and particle size, as described in Ref. [17]. By means of computer simulation (Drylab, LC Resources), conditions were adjusted to be the same for all three columns: 71.5–100% methanol–water, 1.0 ml/min, 25×0.46 cm column, plate number $N = 16\,000$. Conditions for the four experiments used for computer simulation are given in Table 1. For further details, see Ref. [17].

3.2.3. Sample

The sample is an arbitrary mixture of 12 plant pigments: chlorophyll c1, peridinin, BOF, fucoxanthin, neoxanthin, HOF, diadinoxanthin, alloxanthin, diatoxanthin, lutein, zeaxanthin, canthaxanthin.

3.3. Computer simulation

Computer simulations were carried out using DryLab for Windows, version 2.0 software (LC Resources) [3–7,12]. All chromatograms shown are computer simulations based on the four experimental runs used as input for computer simulation. Numerous comparisons of predicted vs. actual separations confirm the accuracy of these simulations [12,14].

Table 1
Experimental conditions used by laboratory B

Column	Temperature (T , $^\circ\text{C}$)	Gradient time (t_G , min)
Hypersil C_8	40, 60	20, 60
Luna C_8	40, 60	15, 45
YMC C_{18}	40, 60	20, 60

Because the present investigation emphasizes selectivity as opposed to column efficiency, differences in column plate number were eliminated as a variable by assuming $N=10\,000$ for the simulations of data from laboratory A (15-cm column), and $N=16\,000$ for the simulations for laboratory B (25-cm column). Thus, *changes* in resolution, R_s , reported here as a function of conditions (T , t_G) or column are not affected by actual differences in N for these various columns.

4. Results and discussion

4.1. Optimizing selectivity for maximum resolution

Four experiments with T and t_G varying allow the prediction of separation as a function of T and t_G . This is illustrated in Fig. 1a by a resolution map (DryLab) for the pharmaceutical mixture and Zorbax Eclipse column. Maximum resolution $R_s=2.6$ is found for $T=35^\circ\text{C}$ and $t_G=57$ min (circle, arrow). The corresponding separation is shown in Fig. 1b. If resolution $R_s>2.0$ (baseline separation) is adequate, Fig. 1a (square, arrow) shows that t_G can be reduced to 27 min ($T=32^\circ\text{C}$), with $R_s=2.3$ (Fig. 1c). This would result in a reduction in run time from 26 min (Fig. 1b) to 18 min (Fig. 1c), assuming that the gradient is terminated at the time the last peak leaves the column. Note also the reversal of peak 7 and 8 in the separations of Figs. 1b vs. c.

4.1.1. Pharmaceutical mixture

Resolution maps were constructed for the remaining nine columns in order to determine conditions for maximum R_s in each case. We can summarize these results (Table 2) as follows. First, with the exception of the Symmetry Shield C_8 column, there is not much difference in maximum resolution when T and t_G have been optimized ($2.1\leq R_s\leq 2.7$); i.e., for this sample, little increase in resolution is possible by changing from one C_{18} column to another, followed by re-optimization of T and t_G . The one exception (Symmetry Shield C_8 , $R_s=3.3$) differs from the other columns in (a) being a C_8 rather than C_{18} phase and (b) in having an embedded polar group in the alkyl-silica bonded phase. Other studies [18] have confirmed that the latter changes in the bonded phase

can result in larger changes in column selectivity than are observed among different C_{18} columns that lack an embedded polar group. For all columns, maximum resolution occurs within a similar range of conditions: $45\leq t_G\leq 70$ min, $32\leq T\leq 50^\circ\text{C}$.

Columns from the same source (Zorbax Eclipse and SB; Discovery and Supelco LC-18) exhibit remarkably similar resolution maps, as illustrated in Fig. 2a–d. Presumably, this reflects some common feature in the manufacturing process within a given company (e.g., the silica particles) that applies to different columns. However, a comparison of the two Waters columns (Symmetry C_{18} , Symmetry Shield C_8 ; Fig. 2e and f) presents a quite different picture, presumably because of major differences in the Symmetry Shield bonded phase (C_8 , embedded polar group) noted above. If changes in column selectivity are desired, the examples of Fig. 2 suggest that C_{18} columns from different manufacturers are more likely to prove useful than are columns from the same manufacturer, but bonded phases of different type (C_8 vs. C_{18} ; with or without embedded polar groups) may exhibit larger differences in selectivity. Resolution maps for the remaining columns are shown as Fig. 2g–j.

4.1.2. Mixture of plant pigments

Resolution maps for the separation of this sample on the Hypersil C_8 and YMC C_{18} columns are shown in Fig. 3a and b, with the simulated separation for maximum resolution on the Hypersil C_8 column shown in Fig. 3c. The resolution map for the Luna C_8 column is similar to that for the Hypersil C_8 column. Table 3 summarizes separations for optimized conditions on these three columns. For this sample, there is a greater difference among the three columns in terms of maximum possible resolution.

The range in maximum resolution among different columns for the same sample can be expressed as the relative standard deviation (RSD) of the maximum R_s values, which should be roughly independent of the number of columns used for each sample (10 columns for the pharmaceutical sample vs. three columns for the plant pigments) or the average value of R_s for a given sample. Table 4 summarizes these results for the two samples, confirming a much larger variation of column selectivity for the plant pigments sample. If the Symmetry Shield column is removed

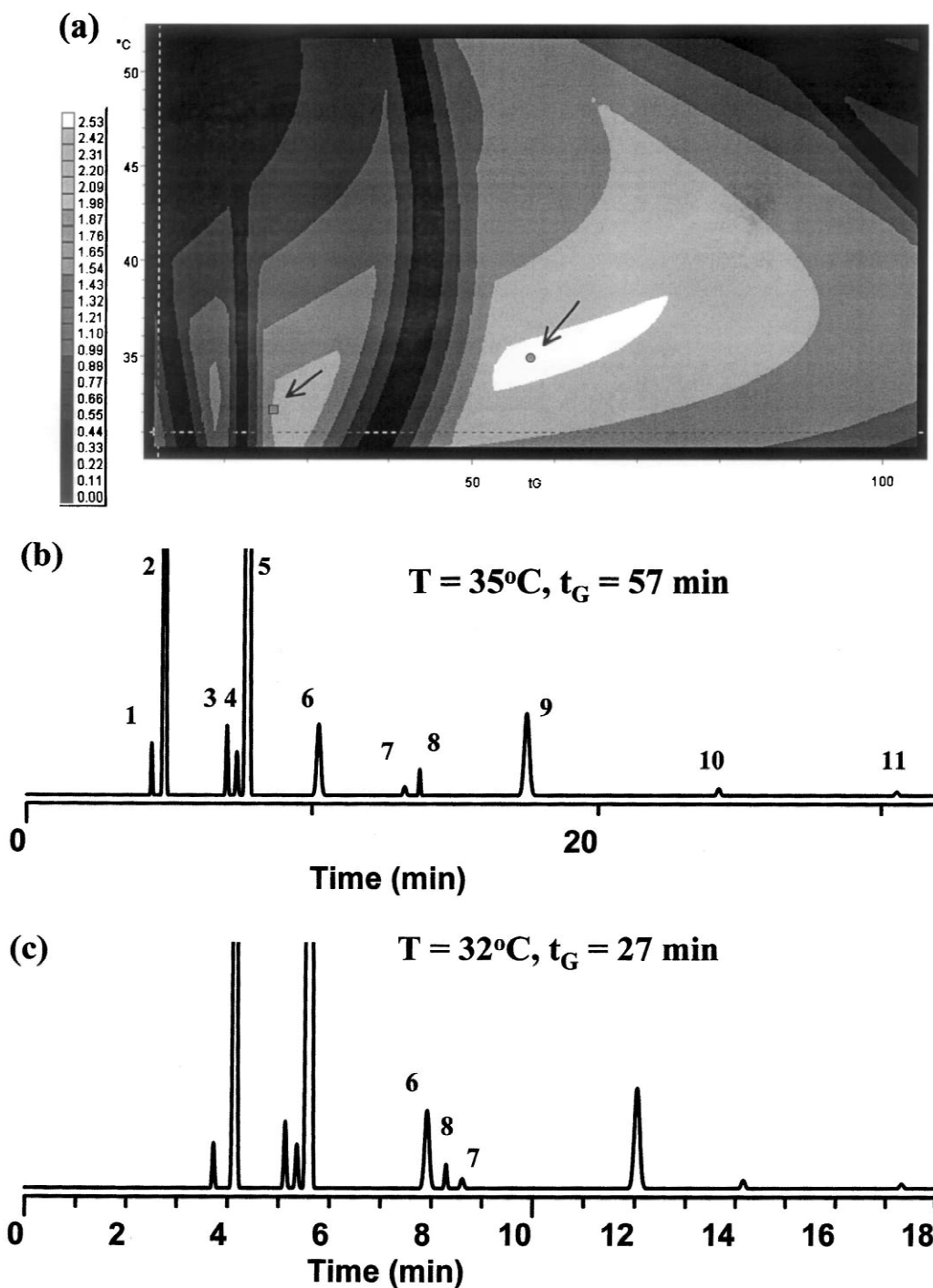


Fig. 1. Separation of pharmaceutical sample with Zorbax Eclipse C₁₈ column by simultaneous change in temperature T and gradient time t_G . (a) Resolution map; (b) separation for conditions ($T=35^\circ\text{C}$, $t_G=57$ min) of maximum resolution ($R_s=2.6$); (c) separation for conditions ($T=32^\circ\text{C}$, $t_G=27$ min) that provide $R_s>2$ in the shortest run time. Other conditions as in Experimental section.

Table 2

Summary of optimized conditions for the pharmaceutical sample and 10 different columns

Column	Optimum T ($^{\circ}\text{C}$)	Optimum t_G (min)	Maximum R_s
(a) Zorbax Eclipse	35	60	2.6
(b) Zorbax SB C_{18}	36	54	2.2
(c) Discovery C_{18}	33	51	2.1
(d) Supelco LC-18	31	54	2.1
(e) Symmetry C_{18}	36	72	2.7
(f) Symmetry Shield C_8	33	60	3.3
(g) YMC-ODS-AM	50	66	2.3
(h) Inertsil C_{18}	32	45	2.6
(i) Alltima C_{18}	35	48	2.2
(j) Luna C_{18}	39	45	2.5

from the 10 columns used for the pharmaceutical sample, there is only a $\pm 10\%$ variation (1 SD) in maximum resolution for the remaining nine columns.

This suggests that changing from one C_{18} column to another, while optimizing t_G and T , is not an attractive option – at least not for the present

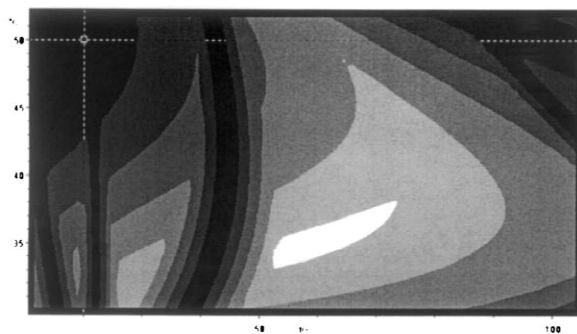
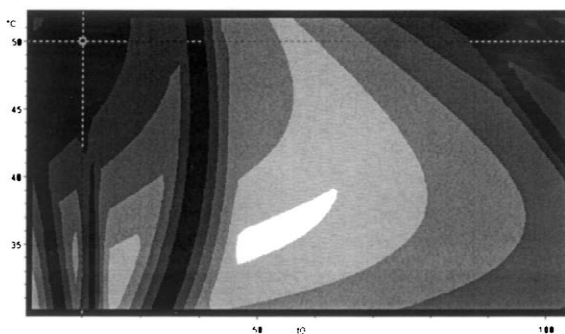
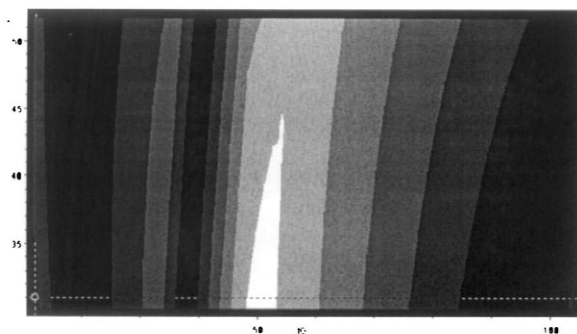
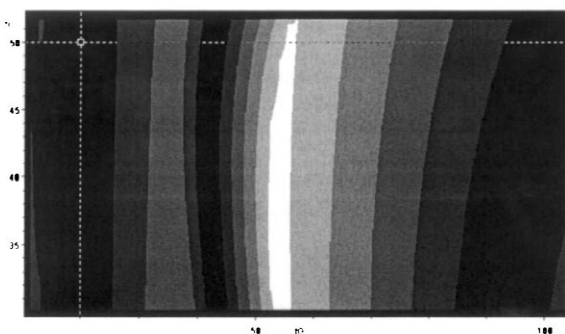
(a) Zorbax Eclipse**(b) Zorbax SB****(c) Discovery****(d) Supelco LC-C18**

Fig. 2. Resolution maps for pharmaceutical sample and other columns. Other conditions as in Experimental section.

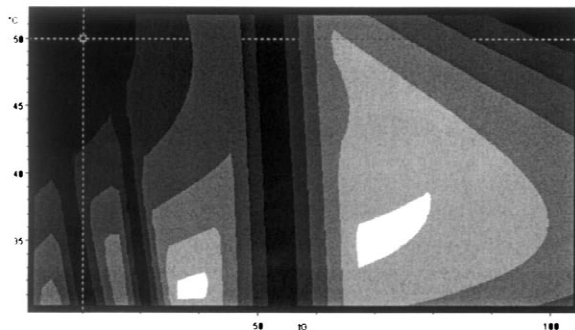
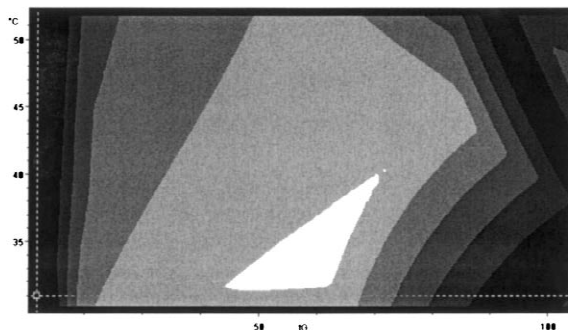
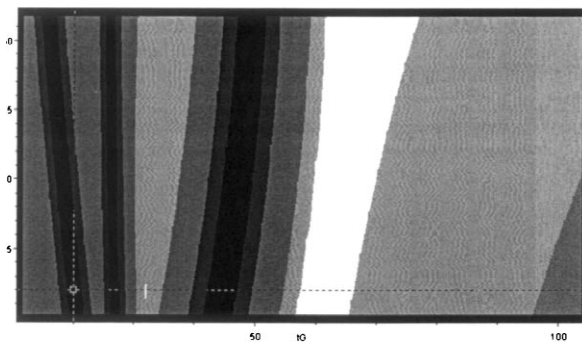
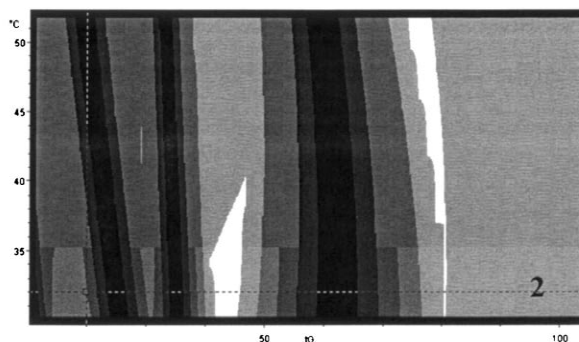
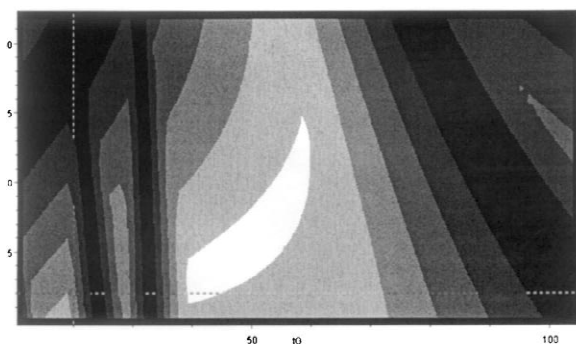
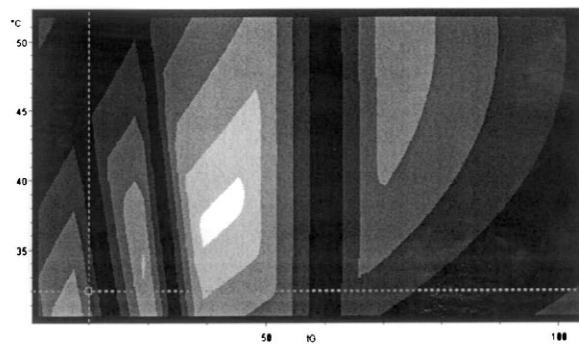
(e) Symmetry C18**(f) Symmetry Shield C8****(g) YMC-ODS-AM****(h) Inertsil C18****(i) Alltima C18****(j) Luna C18**

Fig. 2. (continued).

pharmaceutical sample. However, changing from a traditional C_{18} column to one with an embedded polar group and a shorter alkyl chain (C_8) provides a

considerable increase in maximum resolution (39% for the Symmetry Shield C_8 column vs. the average value of $R_s=2.37$ for the nine C_{18} columns).

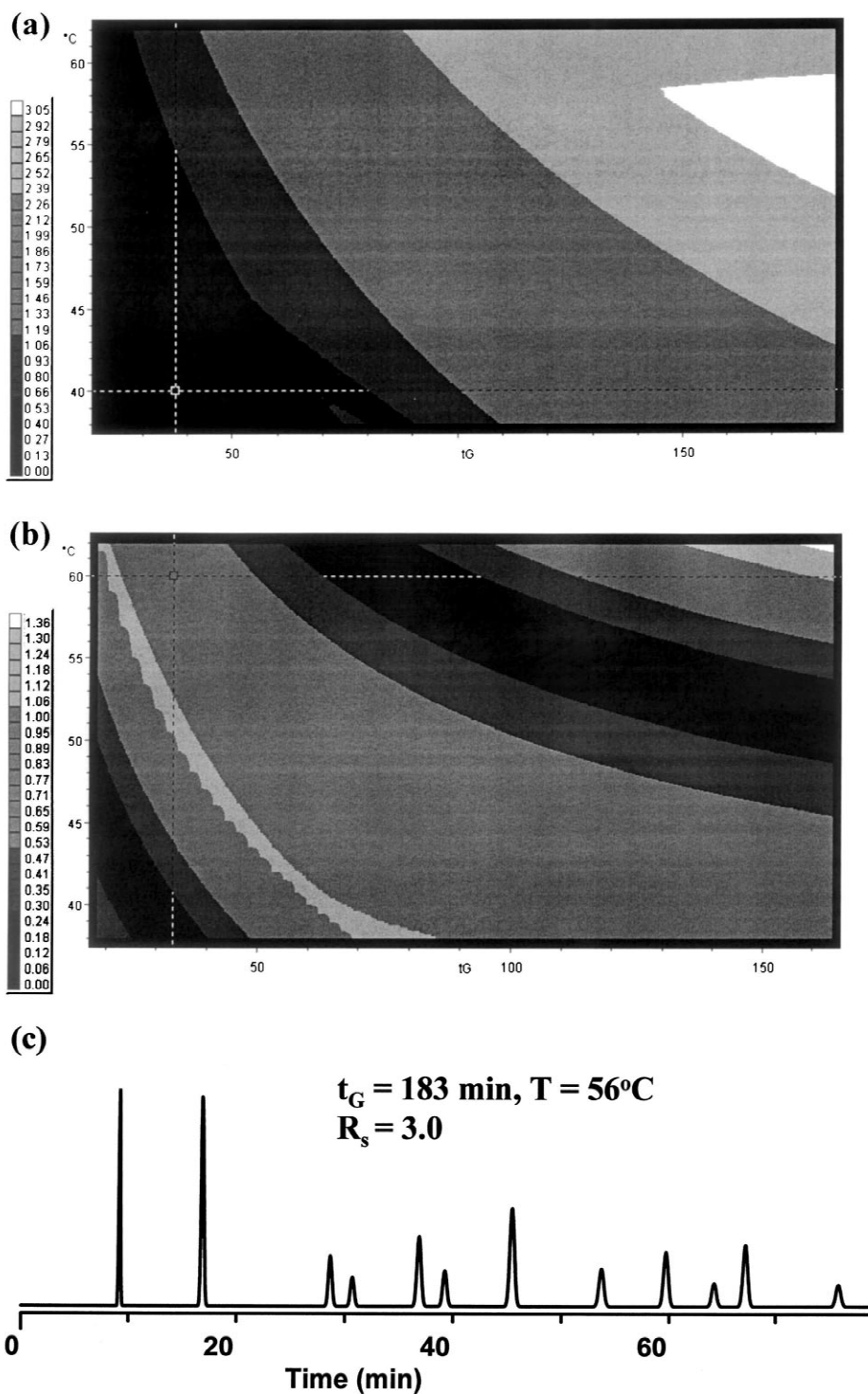


Fig. 3. Separation of plant pigments sample. (a) Resolution map for Hypersil C_8 column; (b) resolution map for YMC C_{18} column; (c) maximum-resolution separation with Hypersil C_8 column. Other conditions as in Experimental section.

Table 3
Summary of optimized conditions for the mixture of plant pigments and three different columns

Column	Optimum T (°C)	Optimum t_G (min)	Maximum R_s
Hypersil C_8	56	183	3.1
Luna C_8	62	155	1.8
YMC C_{18}	62	164	1.4

The separation of certain sample types (polynuclear aromatic hydrocarbons, unsaturated fatty acids, carotenoids) has been found to be especially responsive to the type of alkyl-silica column used. For the latter compound types, “polymeric” or long-chain (e.g., C_{22} , C_{30}) column packings have been found to give generally superior separations vs. those obtained on “monomeric” packings [4,19–21]. The sample from laboratory B is comprised mainly of carotenoids, which may explain the greater range in maximum resolution among the three columns of Table 4. The use of both C_8 and C_{18} columns may also be significant in this regard.

4.2. Separation parameters S and B'' for the pharmaceutical mixture (laboratory A)

The significance of gradient-derived values of S and B'' has been noted earlier and will be examined further in this and the following paper [8]. Here, we are primarily interested in whether values of S and B''/t_G are constant for (a) a given column as T (for S) or t_G (for B'') are varied and/or (b) different columns. These approximate relationships (when valid) can have a number of practical applications, as we will see in the present and following papers (Parts I and II). For example, constant values of S and B''/t_G for each solute and *different* columns

imply that given values of these quantities for *one* column, separation on a second column as a function of T and t_G can be predicted from only one experiment with the second column.

A more detailed discussion of our findings concerning values of S and B'' as a function of conditions and for different columns is summarized in Appendix A.

4.2.1. Peak tracking

Method development based on the optimization of T and t_G (or % B), as in the example of Fig. 1, relies on matching peaks among the four initial experiments [22]. The present DryLab software can carry out peak tracking automatically, based on relative retention and area measurements for each peak. However, the assignments for peaks with similar areas and retention times can be in error, due to changes in relative retention for two (or more) peaks between runs being compared. This often proved to be the case for peaks 7 and 8 of the present sample (Fig. 1b), due to their frequent change in retention order and relatively small size (with concomitant errors in peak area measurements).

Errors in peak tracking can often be detected and corrected, if additional information on peak identity is available. Values of S and B''/t_G provide such additional information, as long as their variation with

Table 4
Variability of column selectivity for the pharmaceutical and plant pigment samples

Sample	Average R_s	Standard deviation	RSD (%)
Pharmaceuticals (10 columns)	2.46	0.37	14
Plant pigments (3 columns)	2.10	0.89	42
Pharmaceuticals (9 columns) ^a	2.37	0.23	10
Pharmaceuticals (Symmetry Shield)	3.30	0.93 ^b	39 ^c

^a Except Symmetry Shield column.

^b Maximum R_s for Symmetry Shield column (3.30) minus average (maximum) R_s = 2.37 for remaining nine columns (pharmaceutical sample).

^c Standard deviation (0.93) divided by average R_s for other columns (2.37) expressed as %.

conditions (e.g., T and t_G) is less than their compound-to-compound variation. The present and preceding [15] studies have confirmed that values of S show minimal variation with temperature ($<2\%$ for peaks that do not elute early). Values of B''/t_G are likewise independent of t_G , although there is greater random variation ($\pm 10\text{--}12\%$ for the present 20°C range in T). For peak tracking to fail for a pair of peaks such as 7 and 8 of Fig. 1b, it is necessary that their relative retention change as T or t_G is varied, which in turn requires significant differences in the values of S and/or B''/t_G for the two compounds. However, it is just this situation (significantly different values of S or B''/t_G for two adjacent bands) that can result in changes in retention order and possible problems in peak tracking.

4.3. Column selectivity

The relative selectivity of the 10 columns for the pharmaceutical sample can be determined by inter-column comparisons of t_R or R_s for the 10 compound pairs in each sample. If the Eclipse C_{18} column is chosen as reference, with a standard set of conditions ($T=41^\circ\text{C}$, $t_G=40$ min), values of R_s for column i can be correlated with values for the Eclipse C_{18} column by least-squares regression. Columns whose selectivity is more similar to that of the Eclipse C_{18} column should then exhibit values of the correlation coefficient r closer to 1.0. Table 5 summarizes such

an inter-column comparison, with the columns arranged in order of decreasing r (and increasing difference in selectivity). Equal values of r do not in themselves ensure similar column selectivity, unless the reference column (Eclipse C_{18}) represents one extreme in selectivity. That this was the case can be validated by values of r for adjacent columns in the final series of Table 5 (values in parentheses). These values in parentheses, which refer to the inter-column correlation of adjacent columns in this series, should generally be small – as observed for all but the very different Symmetry Shield C_8 column. Because of similar values of S and B''/t_G for a given compound on the various columns of Table 5, the same order of column selectivity should be maintained for other conditions of T and t_G . That is, changes in R_s for each band-pair will undergo similar shifts on each column when T and/or t_G is changed by the same amount. Table 5 also lists average values of S and B''/t_G for these 10 columns (see Appendix A for details).

5. Conclusions

The separation of two samples was studied on different C_8 or C_{18} columns as a function of temperature T and gradient time t_G . For one sample (a pharmaceutical plus eight related compounds and

Table 5

Ranking according to selectivity of the 10 columns used for the pharmaceutical sample; inter-column correlation of R_s values for $t_G=40$ min, $T=41^\circ\text{C}$, plus average values of S and B''/t_G

Column	r for correlation of R_s vs. Eclipse column ^a	Average S (7–11) ^b	Average B''/t_G (7–11) ^c
(a) Eclipse C_{18}	1.00	5.54	1.02
(b) Zorbax SB C_{18}	0.99 (0.993)	5.46	1.07
(c) Discovery C_{18}	0.99 (0.994)	5.23	0.99
(d) Supelco LC-18	0.99 (0.994)	5.32	0.95
(g) YMC-ODS-AM	0.95 (0.980)	4.86	0.95
(e) Symmetry C_{18}	0.95 (0.993)	5.28	1.01
(j) Luna C_{18}	0.90 (0.989)	4.90	1.02
(i) Alltima C_{18}	0.86 (0.995)	4.46	1.10
(h) Inertsil C_{18}	0.83 (0.997)	4.64	0.97
(f) Symmetry Shield C_8	0.72 (0.577)	4.38	1.040

^a Values of R_s for each column were correlated vs. values of R_s for the Eclipse C_{18} column; values in parentheses are similar correlation of column i with preceding column ($i-1$).

^b Values from Table 6.

^c Values from Table 7.

two internal standards) and nine different C_{18} columns, selecting values of T and t_G for maximum (critical-pair) resolution R_s on each column gave similar resolution: $R_s = 2.5 \pm 0.4$. That is, there was little advantage of one C_{18} column over another, as long as T and t_G were optimized for a given column. The nine columns could be ranked in terms of differences in selectivity for this particular sample, although it is likely that a somewhat different column ranking would result for other samples and/or conditions. These nine C_{18} columns in each case possessed a similar bonding chemistry. For an additional column (C_8 with an embedded polar group in the ligand; Symmetry Shield C_8), column selectivity for the pharmaceutical sample was more different, and a significant improvement in maximum resolution was possible: $R_s = 3.3$. This is in agreement with other studies [20], which have found quite different column selectivity for either a change in ligand length ($C_8 \rightarrow C_{18}$) or the addition of a polar embedded group.

A similar separation of a second sample (plant pigments) by a C_{18} and two C_8 columns showed a wider variation in maximum resolution after optimizing T and t_G for each column: $R_s = 2.1 \pm 0.9$ (1 SD). In this case, selectivity varied with the column to a greater extent than for the pharmaceutical sample. This greater difference in column selectivity may be attributable to the fact that the components of this particular sample possess pronounced differences in shape, and different alkyl-silica columns are known to exhibit varying degrees of shape selectivity.

Changes in relative retention as a result of change in T or t_G were observed to be similar for a given compound on different columns. As a result, it is possible to use such changes in retention (characterized by the solute parameters S and B''/t_G) to aid in the peak matching that is usually required during LC method development. Values of S and B''/t_G for each component in a sample are also likely to be similar for two columns of "similar" selectivity; e.g., different batches of nominally equivalent columns. In this case, values of S and B''/t_G for a first column can be used to predict changes in separation for a second column, as a result of changes in temperature and either t_G (gradient) or % B (isocratic). This in turn can be useful for correcting batch-to-batch differences in column selectivity by small adjustments in T

and/or t_G for the second column, as discussed in the following paper [8].

6. Nomenclature

a, b	Coefficients in Eq. (1) of Part II [8]; equal to negative change in T and t_G which will bring resolution for column 2 into closer agreement with that of column 1
A, B	Constants (Eq. (2))
A'', B''	Constants (Eq. (3))
b	Gradient steepness parameter (Eq. (5))
$dR_s/dT, dR_s/dt_G$	Change in R_s for a given band-pair on column 1 as a result of change in T or t_G
F	Flow-rate (ml/min)
k	Retention factor
k_0	Value of k at start of a gradient (for initial mobile phase)
k_{01}, k_{02}	Values of k_0 for temperatures T_1 and T_2
k_w	Value of k for water as mobile phase (extrapolated, Eq. (1))
q	An arbitrary constant in Eq. (2) of Part II [8]
RPLC	Reversed-phase liquid chromatography
R_s	Baseline resolution for two adjacent bands; also, resolution of poorest-resolved ("critical") band-pair for an entire sample
$(R_{si})_1, (R_{si})_2$	Value of R_s for a given band-pair i on columns 1 and 2, respectively
S	$d(\log k)/d\phi$ (Eq. (1))
t_D	Hold-up (dwell) time for a gradient system (min)
t_G	Gradient time (min)
t_0	Column dead-time (min)
t_R	Retention time (min)
t_{R1}, t_{R2}	Values of t_R at temperatures T_1 and T_2
T	Temperature ($^{\circ}C$)
T_1, T_2	Temperatures T_1 and T_2

T_K	Temperature (K)
V_m	Column dead-volume (ml)
δR_s	A difference in R_s for a given band-pair for the separation on column 2 vs. column 1 (same conditions)
$\delta'R_s$	A difference in R_s for a given band-pair for the separation on column 2 vs. column 1 (adjusted conditions for column 2 to reduce differences in separation vs. column 1)
$(\delta R_s)_i$	Difference in R_s for two adjacent bands on column 2 vs. 1: equal $(R_{si})_2 - (R_{si})_1$ (Part II)
$\delta T, \delta t_G$	A change in T or t_G
$\Delta\phi$	Change in ϕ during the gradient
ϕ	Volume fraction of B solvent (organic) in RPLC mobile phase
ϑ	A measure of the similarity of separations on columns 1 vs. 2; equal to the average value plus standard deviation of $ \delta'R_s $ for the two separations; given a value of ϑ , only 1/6 of the band-pairs should show differences in R_s between the two separations such that $\delta R_s/R_s$ is larger than ϑ

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Appendix A. Values of S and B'' as a function of experimental conditions (including the column)

A.1. Values of S

Values of S for the pharmaceutical sample were determined for each solute and column at two temperatures: 32 and 50°C. As observed previously for the RPLC separation of other samples [15], values of S from these gradient experiments do not vary with temperature. Thus, for compounds 2–11, the average relative standard deviation for these two determinations of S (at 32 and 50°C) was only $\pm 4.5\%$. If bands that elute early in the gradient are excluded, the agreement improves to $\pm 1.4\%$. Note that values of S become less reliable when significant pre-elution of the band occurs, due to the dwell volume V_D of the equipment; e.g., in the case of band 1, RSD = 16%.

Table 6
Average values of S for the pharmaceutical sample

Compound	Average S for indicated columns ^a									
	Column a	Column b	Column c	Column d	Column e	Column f	Column g	Column h	Column i	Column j
1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	18.5	15.8	n.d.	19.8
2	n.d.	n.d.	13.4	13.9	n.d.	6.83	10.5	9.01	7.61	10.8
3	18.9	16.9	14.6	15.4	18.4	n.d.	12.	11.8	9.98	13.5
4	n.d.	n.d.	9.53	9.95	n.d.	7.56	10.9	9.97	n.d.	9.34
5	14.2	13.4	11.8	12.35	13.9	7.56	10.7	9.84	8.60	10.6
6	n.d.	n.d.	16.5	15.1	19.8	11.4	14.9	13.6	9.83	15.3
7	8.16	8.10	7.53	7.80	7.79	6.15	7.27	6.6	6.73	7.07
8	4.22	4.14	4.15	4.22	3.97	2.79	3.71	3.53	3.29	3.65
9	3.36	3.42	3.40	3.44	3.24	2.66	3.19	3.07	2.91	3.22
10	6.29	6.13	5.84	5.92	5.92	5.24	5.34	5.21	4.88	5.54
11	5.67	5.50	5.22	5.22	5.5	5.05	4.81	4.73	4.48	5.05
Average 7–11	5.54	5.46	5.23	5.32	5.28	4.38	4.86	4.64	4.46	4.90

^a Column designations (a, b, ...) in Experimental section; average of values for 32 and 50°C.

Table 7
Average values of B''/t_G for the pharmaceutical sample

Compound	Average B''/t_G for indicated columns ^a									
	Column a	Column b	Column c	Column d	Column e	Column f	Column g	Column h	Column i	Column j
1	0.382	0.378	0.397	0.382	0.394	0.464	0.378	0.368	0.354	0.365
2	0.418	0.410	0.429	0.400	0.434	0.465	0.413	0.370	0.382	0.392
3	0.326	0.329	0.316	0.304	0.346	n.d.	0.320	0.300	0.322	0.325
4	0.237	0.247	0.337	0.332	0.270	0.318	0.323	0.285	n.d.	0.247
5	0.306	0.328	0.296	0.311	0.337	0.329	0.321	0.300	0.308	0.307
6	0.355	0.361	0.265	0.255	0.290	0.303	0.194	0.350	0.403	0.293
7	0.262	0.268	0.242	0.153	0.298	0.289	0.290	0.105	0.156	0.281
8	0.387	0.382	0.369	0.369	0.389	0.500	0.363	0.357	0.401	0.344
9	0.280	0.316	0.333	0.320	0.277	0.350	0.326	0.336	0.377	0.316
10	0.271	0.295	0.280	0.279	0.242	0.311	0.247	0.276	0.345	0.297
11	0.272	0.294	0.293	0.311	0.292	0.310	0.258	0.304	0.329	0.297
Average 7–11	1.018	1.070	0.991	0.953	1.013	1.140	0.954	0.967	1.105	1.019

^a Column designations in Experimental section; average of values for 32 and 50°C.

Table 6 summarizes values of S for the 11 pharmaceutical solutes and 10 columns. Missing data in Table 6 (“n.d.”) reflect inaccurate values due to peak overlap, where accurate values of t_R could not be determined. Average values of S for solutes 7–11 are also shown in Table 6, and these are seen to vary

from 4.4 (column f) to 5.5 (column a). However, the ratios of values of S for adjacent bands (e.g., see 7 and 8) are more nearly constant (± 3 –6%, RSD for bands 7–11), meaning that selectivity due to a change in % B is quite similar (and therefore predictable) for all of these columns.

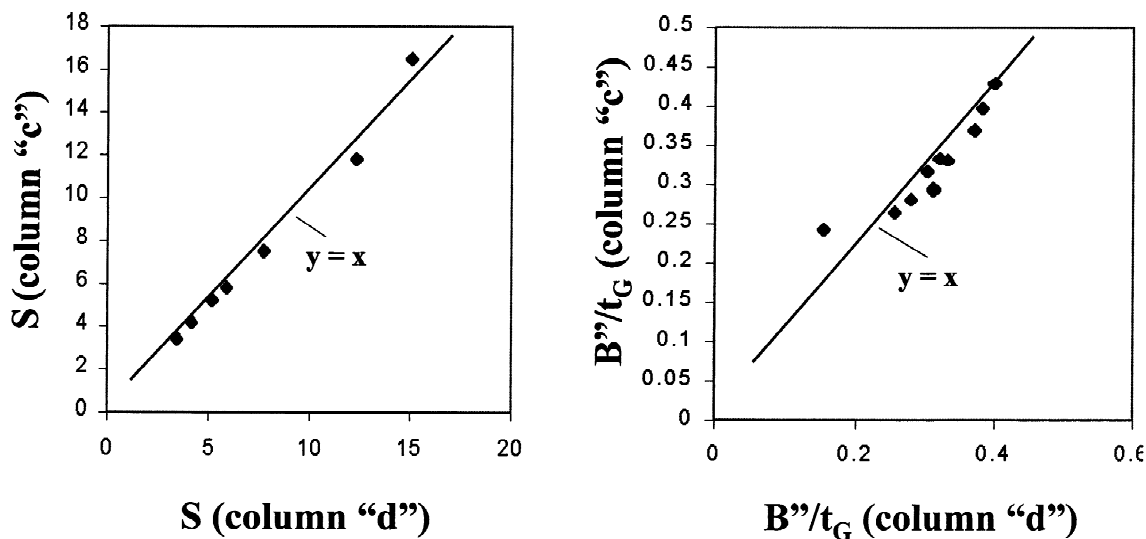


Fig. 4. Similarity of values of S and B''/t_G for “similar” C_{18} columns. Columns are Discovery C_{18} (“c”) and Supelco LC-18 (“d”); data taken from Tables 6 and 7.

A.2. Values of B''/t_G

Values of B''/t_G were determined for each compound (pharmaceutical sample) and column for t_G equal to both 20 and 60 min. For later-eluting compounds 3–11, the average ratio of B''/t_G values for the same compound and column (value for $t_G = 20$ divided by value for $t_G = 60$) was 1.02 ± 0.12 (1 SD). For early-eluting compounds 1 and 2, the average ratio was 1.33 ± 0.10 , as expected for compounds eluting before the gradient has changed by as much as 10% B (i.e., reflecting mixed isocratic-gradient separation). However, for both early and late eluting bands, *relative retention* as a result of a change in temperature is similar for both large and small values of t_G .

Average values of B''/t_G (for $t_G = 20$ and 60 min) for the pharmaceutical compounds and different columns are summarized in Table 5. Values of B''/t_G for a given solute vary somewhat from column to column, as in the case of values of S . However, the average RSD (Table 5) for values of B''/t_G for a given solute and different columns is $\pm 11.3\%$, which can be compared with the average RSD for duplicate values measured for $t_G = 20$ and 60 min ($0.12/1.02 = 12\%$).

Values of S and B''/t_G for the same solute and different columns are more nearly constant when the columns are similar in terms of selectivity (see Tables 6 and 7 and related discussion in a following section). This is illustrated in Fig. 4, where values of S and B''/t_G are compared for the similar Supelco columns: (c) Discovery and (d) LC-18. Use will be made of this observation elsewhere, as a basis for the easy adjustment of separation conditions (T and t_G or % B) in order to correct for batch-to-batch variability in column selectivity. See the further discussion of the following paper [8].

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