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Choosing an equivalent replacement column for a reversed-phase liquid chromatographic assay procedure

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Abstract

The column selectivity parameters (**H**, **S***, **A**, **B** and **C**) described in the preceding paper [L.R. Snyder, A. Maule, A. Heebsch, R. Cuellar, S. Paulson, J. Carrano, L. Wrisley C.C. Chan, N. Pearson, J.W. Dolan, J.J. Gilroy, J. Chromatogr. A 1057 (2004) 49–57] can be used to compare columns in terms of selectivity. A detailed procedure for such column comparisons is presented here, and evaluated by its use in finding suitable replacement columns for 12 different routine separations performed in five different pharmaceutical analysis laboratories.

Keywords: Column selectivity; Selectivity parameters; Hydrophobicity; Steric resistance; Hydrogen-bond acidity; Hydrogen-bond basicity; Cation-exchange capacity

1. Introduction

Routine assay procedures by means of reversed-phase HPLC (RP-LC) are often carried out over periods of months or years, as well as in different laboratories. Therefore during the application of such a procedure, several "equivalent" columns may be required in order to obtain a satisfactory separation in each run (in this paper, by "equivalent" columns we mean columns that will be accepted as interchangeable for a routine separation by the responsible party). Nominally equivalent replacement columns from the original supplier (i.e., with the same name or designation) *should* provide identical separation, but sometimes there are significant batch-to-batch changes in column selectivity, or the sale of a column may be discontinued. At such times, as well as for other reasons, it may be necessary to use a different column (i.e., different designation) in order to obtain a satisfactory separation without changing experimental conditions. It is also recommended during the development of a RP-LC procedure that one or more backup columns be identified; i.e., columns with a different designation (from the same or other source) that can provide equivalent results in a routine procedure.

The trial-and-error search for a suitable replacement (or backup) column can be tedious and is often unproductive, suggesting a need for an alternative approach. Column selectivity is a primary concern and can be characterized by five, measurable characteristics of the column [1–3]: hydrophobicity **H**, steric resistance **S***, hydrogen-bond acidity **A** and basicity **B**, and cation-exchange activity **C**. If two columns have "sufficiently" similar values of these five quantities (**H**, **S***, etc.), the columns should provide equivalent separations

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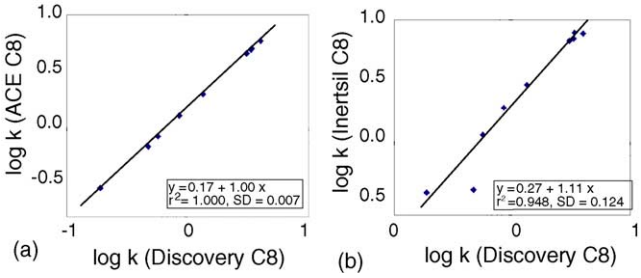

Fig. 1. Comparing column selectivity and equivalency for a given sample and RP-LC procedure by means of log–log plots of k for one column vs. the other. Sample: (1) *N*,*N*-diethylacetamide; (2) nortriptyline; (3) 5,5-diphenylhydantoin; (4) benzonitrile; (5) anisole; (6) toluene; (7) *cis*-chalcone; (8) *trans*-chalcone; (9) mefenamic acid. Columns identified in the figure; see Fig. 2 for chromatograms. Experimental conditions: 50% acetonitrile/pH 2.8 buffer, 35 °C, 2.0 mL/min. Data for separations described in [2].

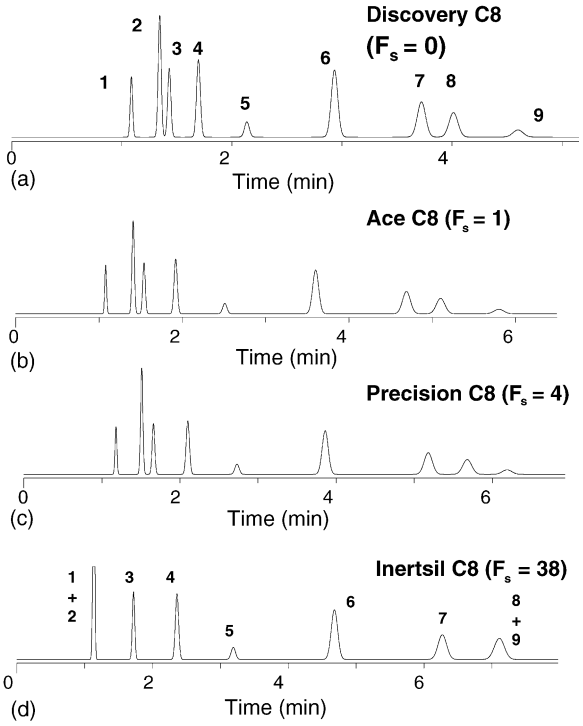

Fig. 2. Comparisons of column selectivity for a given sample and RP-LC procedure. Same sample and separation conditions as in Fig. 1.Computer simulations adapted from [2], based on compounds and experimental conditions that were also used to measure values of **H**, **S***, etc.

for any sample or separation conditions. In the present paper, column selectivity as defined by values of **H**, **S***, etc. is examined as a basis for selecting equivalent columns.

Previous comparisons of separation on "equivalent" columns in this way [2] have involved the same samples and experimental conditions that were used to derive values of **H**, **S***, etc. for the columns compared. Circular comparisons of this kind are inherently suspect as a means of verifying the present approach for selecting equivalent columns when different samples and conditions are involved (the usual case in practice). In the present study, columns predicted to be similar (on the basis of values of **H**, **S***, etc.) were compared for the separation of several samples and experimental conditions that were completely different from those used in the procedure for measuring values of **H**, **S***, etc.

2. Background and theory

2.1. Comparing columns in terms of selectivity

Two columns can be compared in terms of selectivity for a given isocratic separation by plots of $\log k$ for one column versus the other [2]. This is illustrated in Fig. 1 for a particular sample (a mixture of nine acidic, basic or neutral compounds) and separation conditions. In Fig. 1a, separation on an ACE C8 column is compared with separation on a Discovery C8 column. In Fig. 1b, separation is compared for the same sample and conditions, using Inertsil C8 and Discovery C8 columns. If there is a linear correlation of $\log k$ values with no deviation of data points (standard deviation, S.D. = 0), the two columns are said to correlate perfectly; i.e., the two columns can be regarded as equivalent in terms of selectivity. This is never exactly the case (except for identical columns from the same production batch), yet two different columns may still be sufficiently similar to be interchangeable for a routine RP-LC assay. This is true in Fig. 1a for these two columns, as shown by the corresponding chromatograms in Fig. 2a and b. Note that the present study is concerned with selectivity (values of the separation factor α), as opposed to retention times t_R. If selectivity is comparable for two isocratic separations (as in Fig. 2a and b), but retention times differ, an adjustment of flowrate for the second column can equalize retention, usually with little effect on separation and no effect on selectivity.

The least-resolved ("critical") pair of bands in a chromatogram is usually of greatest concern, and for this band-pair a resolution of $R_s \geq 2$ is often a goal in RP-LC method development [4]. A decrease in R_s of as much as 0.5 units for this case can usually be accepted because baseline separation is still achieved, as illustrated in Fig. 3a for a band-pair with $R_s = 2$ on column 1 and $R_s = 1.5$ on column 2. Note that a change in R_s of 0.5 units corresponds to a change in the separation factor α which can be estimated [5] by the well-known relationship $R_s = (1/4)(\alpha - 1)N^{1/2}[k/(k+1)]$. Assuming a plate number $N = 5000$ (for "real" samples, not standards) and an average value of k for the two bands equal to 5, we can calculate that α equals 1.137 for an initial value of $R_s = 2$, and 1.103 for $R_s = 1.5$. The allowed change in α is then (1.137/1103) = 1.03 or ±3%. This is necessarily only a rough estimate of the allowed *average* change in α.

Screening columns for equivalent selectivity as in Fig. 2a and b is effective but not efficient, because typically it will be necessary to randomly test a large number of potential replacement columns before finding one that has equivalent selectivity. An alternative approach assumes (correctly) that

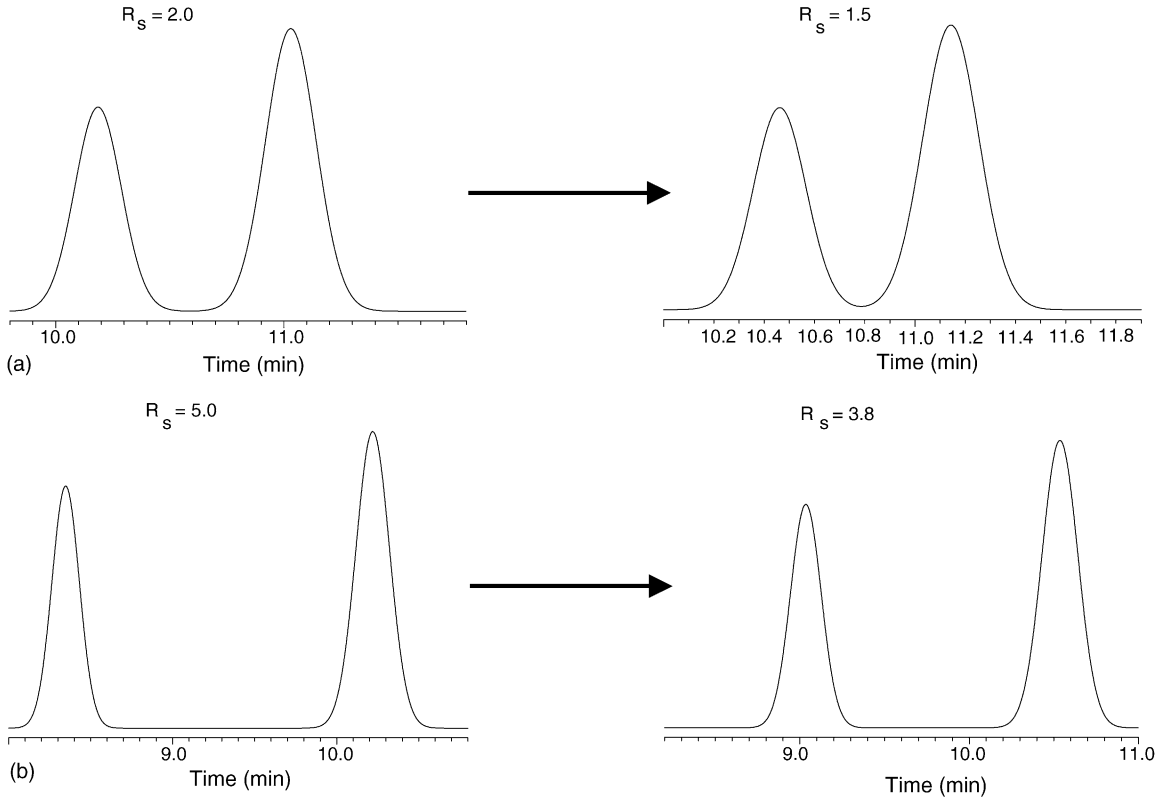

Fig. 3. Comparison of the effects of a 25% decrease in resolution R_s on the separation of two bands.

values of **H**, **S***, etc. are available for a large number of RP-LC columns, and that a comparison of these column selectivity parameters can identify columns with equivalent selectivity. A column-comparison function based on values of **H**, **S***, etc. for columns 1 and 2 has been derived [2]:

$$F_s = \{[12.5(\mathbf{H}_2 - \mathbf{H}_1)]^2 + [100\mathbf{S}_2^* - \mathbf{S}_1^*)]^2 + [30(\mathbf{A}_2 - \mathbf{A}_1)]^2 + [143(\mathbf{B}_2 - \mathbf{B}_1)]^2 + [83(\mathbf{C}_2 - \mathbf{C}_1)]^2\}^{1/2} \quad (1)$$

Here, $\mathbf{H}_1$ and $\mathbf{H}_2$ refer to values of **H** for columns 1 and 2, respectively, (and similarly for values of $\mathbf{S}_1^*$ and $\mathbf{S}_2^*$, etc.). F_s can be regarded as the distance between two columns whose values of **H**, **S***, etc. are plotted in five-dimensional space, with the weighting factors (12.5, 100, etc.) added for a sample of "average" composition. These weighting factors take into account the effect of each column parameter (**H**, **S***, etc.) on the relative retention of different compounds in this "average" sample. Specific samples may benefit from an adjustment of these weighting factors, as discussed below (Section 2.2).

It was found [2] that if $F_s \leq 3$ for two columns 1 and 2, the standard deviation (S.D.) of log *k*–log *k* plots will be ≤0.013 (or ±3% in *k*), so that the two columns are *likely* to provide equivalent selectivity and separation for different samples and conditions. That is, values of S.D. can be related to values of F_s as illustrated in Fig. 4a, where each data-point refers to a column-pair selected from nine different C_{18} columns for a sample that contains $n = 88$ solutes of varied structure (data of [6,7]; see a similar plot in Fig. 3b of [2] for $n = 67$). Note also the dashed curve of Fig. 4a for $F_s < 10$, which on the basis of additional data (see Fig. 3c of [2]) can be described by:

$$\text{S.D.} = 0.002 + 0.0036F_s \quad (2)$$

Eq. (2) yields S.D. = 0.013 for $F_s = 3$, which is the basis for the above requirement that $F_s \leq 3$ for two columns that will *probably* be interchangeable for a given separation. For $F_s < 10$, Eq. (2) is more accurate than the solid, best-fit line of Fig. 4a for all the data (for which S.D. = 0.016 + 0.0020F_s). Values of $F_s < 10$ are of primary interest when trying to select two equivalent columns.

An example of the application of Eq. (1) is shown in Fig. 2, for the separation of a mixture of neutral, basic or acidic compounds on four different columns. Values of F_s from Eq. (1) are shown for the three columns of Fig. 2b–d, each of which is compared with the Discovery C8 column of Fig. 2a. The values of F_s for the Ace C8 (b) and Precision C8 (c) columns are relatively small ($F_s \leq 4$), and as expected separation on these columns is quite similar to that for the Discovery C8 column. For the Inertsil C8 column, $F_s = 38$, indicating that this column has a selectivity that is very different from that of the Discovery C8 column; e.g., note the co-elution of bands #1/2 and 8/9 in Fig. 2d.

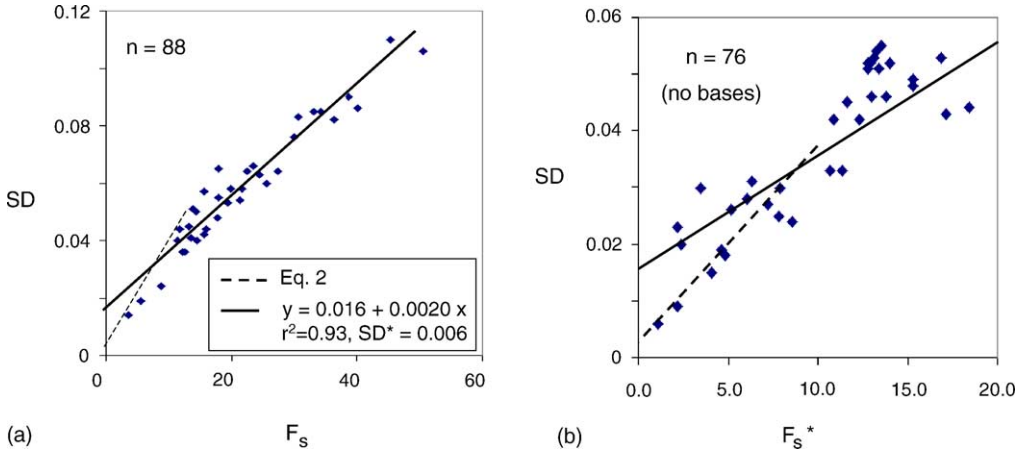

Fig. 4. The dependence of column similarity (as measured by the standard deviation S.D. of $\log k$–$\log k$ plots) on values of F_s or F_s^*: (a) separation of an 88-solute sample with various pairs of nine different columns; (b) separation of a 76-solute sample which does not contain basic solutes. All data in Fig. 4 are calculated from values of k presented in [5,6]. See text for details.

The application of Eq. (1) for the selection of equivalent columns also depends to some extent on the composition of the sample, the difficulty of the separation, and separation conditions—as discussed in the remainder of Section 2. Appendix A summarizes this treatment for the convenient use of Eq. (1) with collected values of **H**, $\mathbf{S}^*$, etc.

2.2. Column similarity as a function of the sample

Eq. (1) was derived from data for 67 test solutes (including neutral, acidic or basic compounds) on 10 different columns [2]. The weighting factors in Eq. (1) (12.5, 100, etc.) reflect the average relative importance of **H**, $\mathbf{S}^*$, etc. in affecting separation selectivity for a sample containing these compounds; "best" values of these weighting factors are expected to vary somewhat with sample composition. Specifically, the presence or absence of certain compound types leads to a reweighting of the last two terms of Eq. (1):

$$F_s^* = \{[12.5(\mathbf{H}_2 - \mathbf{H}_1)]^2 + [100(\mathbf{S}_2^* - \mathbf{S}_1^*)]^2 + [30(\mathbf{A}_2 - \mathbf{A}_1)]^2 + [143x_B(\mathbf{B}_2 - \mathbf{B}_1)]^2 + [83x_C(\mathbf{C}_2 - \mathbf{C}_1)]^2\}^{1/2} \quad (3)$$

Here, x_B and x_C (with values between 0 and 1) represent possible correction factors that depend on sample composition. For example, if bases are absent from the sample, the term $x_C \approx 0$, because values of **C** mainly affect the retention of ionized basic solutes. For similar reasons, if carboxylic acids are absent from the sample, $x_B = 0$. Note that if x_B and x_C equal one (equivalent to setting $F_s^* = F_s$), maximum values of F_s^* result, and the likelihood of finding two equivalent columns (with $F_s^* \leq 3$) is thereby reduced. The advantage of using Eq. (3) (when x_B or $x_C < 1$) is that $F_s^* < F_s$, so that the likelihood of finding an equivalent column with $F_s^* \leq 3$ is increased; i.e., the smaller the value of F_s^* for different columns, the more columns with $F_s^* < 3$. On the other hand, when Eq. (1) is used and $F_s \leq 3$, the two columns are likely to prove equivalent for any sample.

Fig. 4b illustrates the correlation of S.D. values (from plots as in Fig. 1) with F_s^* from Eq. (3) for samples which do not contain basic compounds. The remaining sample components in Fig. 4b are the same as for the sample of Fig. 4a, except that the number of solutes $n = 76$, and for this sample $x_C = 0$. Because **C** has a relatively large effect on values of F_s (due to the greater variation in values of **C** compared to other column selectivity parameters), values of F_s^* for samples without bases (i.e., $x_C = 0$) are always much smaller than values of F_s, as seen by comparing the x-axes of Fig. 4a and b, where $2 \leq F_s \leq 50$ and $1 \leq F_s^* \leq 20$, respectively. This also means that there are more equivalent columns (with $F_s^* \leq 3$) for the sample of Fig. 4b (no basic solutes; four column-pairs with $F_s^* \leq 3$) than for the sample of Fig. 4a (acidic, basic and neutral solutes; no column-pairs with $F_s^* \leq 3$). Eq. (2) can be extended to the function F_s^* of Eq. (3) by replacing F_s by F_s^* in Eq. (2).

2.3. Column similarity as a function of the separation

We have defined a maximum allowable value of $F_s^* \leq 3$ on the basis of an allowable loss in R_s of ≤ 0.5 units (Fig. 3a, assuming a minimum starting resolution of $R_s = 2$; i.e., an allowable loss in resolution of 25%). When the critical resolution of the sample is $R_s > 2$, it seems reasonable to accept larger possible changes in R_s for the separation on column 2 versus column 1. For example, in Fig. 3b the resolution of these two bands is $R_s = 5$. If a 25% decrease in R_s is allowed (as in Fig. 3a), so that $R_s = 3.8$ in Fig. 3b, the two separations of Fig. 3b would still be considered as equivalent by many workers. We therefore propose to define a maximum allowable value of F_s^* ($\equiv Q$) as a function of the critical resolution $R_s(1)$ for column 1:

$$Q = \tfrac{3}{2} R_s(1) \quad (4)$$

Therefore, for equivalent columns,

$$F_s^* \leq Q \quad (4a)$$

If the *only* requirement is separation of the critical band-pair with a resolution of $R_s(1) \geq 2$, even larger values of F_s^* may be acceptable. (A reviewer has questioned the assumptions upon which Eq. (4) is based as being arbitrary. The criterion of Eq. (4) for equivalent columns [and to some extent, the original assumptions that resulted in a maximum value of $F_s^* = 3$] *is* subjective and therefore can be changed according to the preference and experience of the individual user.)

2.4. Column similarity as a function of the mobile phase

The pH of the mobile phase also affects the relative selectivity of two columns, as measured by Eq. (1) or (3). Values of the column selectivity parameter **C** are related to the ionization of the stationary phase, which varies with pH; previous tabulations of values of **H**, **S***, etc. for almost 200 different RP-LC columns [2,3,8–10] list values of **C** for both pH 2.8 and 7.0. Given an experimental value of the pH of the mobile phase (e.g., equal to that of the buffer before addition of organic solvent), it is possible to calculate approximate values of **C** for any pH by linear interpolation of the values at pH 2.8 and 7.0 (as discussed in [11]).

Values of the weighting factor x_C can also vary with mobile phase pH, due to the effect of pH on the ionization of sample compounds. Thus, at pH 7 weak bases such as anilines and pyridines will usually be present as the non-ionized molecules, in which case solute retention will no longer be a function of **C** (i.e., $x_C = 0$, because **C** only affects the retention of ionized solutes). When the basic solutes in the sample are only partly ionized, values of $x_C \approx 0.1$ [6,7], which can be the case for anilines and pyridines at lower pH, as well as strong bases (aminoalkyl derivatives) at higher pH. See Appendix B for further details.

2.5. Gradient separation

In gradient elution, plots of retention time t_R for one column versus another can be used to compare the similarity of two columns in terms of selectivity (similar to Fig. 1 for isocratic elution). The standard deviation S.D. of such gradient plots (in units of t_R) can be related to S.D. (units of $\log k$) in Eq. (2) as:

$$\text{S.D.}(\log k) = \left(\frac{b}{t_0}\right) \text{S.D.}(t_R) \quad (5)$$

Here, t_0 is the column dead-time, and b is a gradient steepness parameter. See Appendix C for the derivation of Eq. (5) and other details. Note that column size and flowrate affect selectivity in gradient (but not isocratic) elution; therefore, when comparing two columns for gradient elution separation, column size and flowrate should be the same for the two columns. If column size differs, an appropriate adjustment of flowrate for column 2 must be made, so that the ratio (column volume)/(flowrate) is the same for both columns.

At first glance, two columns with $F_s^* \leq 3$ would appear to be equivalent for both isocratic *and* gradient separations. However, this may be only approximately the case, because small shifts in retention time t_R in gradient elution are often observed for different columns (regardless of column selectivity as measured by F_s^*), and these changes in t_R correspond to changes in the average mobile phase composition (%B) during elution – which has an additional effect on separation selectivity ("solvent strength" selectivity; see Section 6.3.2 of [4]). Also, in the case of segmented gradients, b in Eq. (5) varies from segment to segment, rendering its effective value in Eq. (5) less obvious. Finally, early eluting bands are affected by gradient delay, due to the holdup volume of the gradient equipment. All of these factors can render Eqs. (1)–(3) less reliable when applied to gradient separations—especially for the case of segmented gradients. However, since a change in flowrate in gradient elution affects sample retention in the same way as in isocratic elution (e.g., a decrease in flowrate increases sample retention), a change in flowrate for column 2 that minimizes differences in retention time between columns 1 and 2 will also minimize the effects of solvent strength selectivity.

3. Experimental

Experiments described in Section 4 were carried out in laboratories that are experienced in the RP-LC analysis of pharmaceutical products, and it can be assumed that the equipment, materials and procedures used for these assays meet the usual regulatory requirements. However, in most cases these routine RP-LC assay procedures are proprietary, which precludes our disclosure of certain details. For similar reasons, the contributing laboratory for individual separations in Tables 1 and 2 is not disclosed; the four contributing lab-

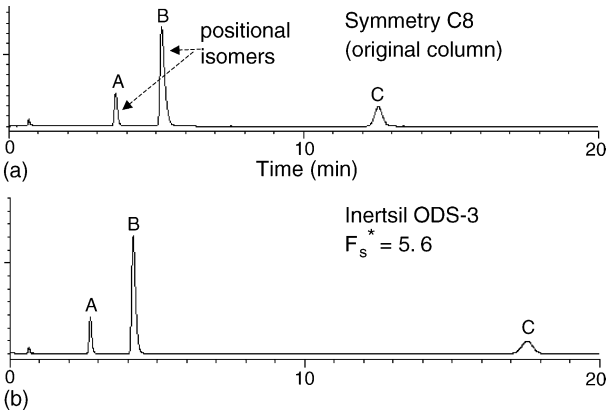

Fig. 5. Separations for example A of Table 1. See Sections 3 and 4.1 for details.

Table 1
Experimental conditions for the 12 separations of the present study

Example[a]	Solvents[b]				Gradient[c]		Column[d]	Flow[e]
	A		B		%B	Time		
	%B	pH	%B	pH				
A	36% ACN	6.5					15 × 0.46	2.0
B	28% ACN	2.0					15 × 0.46	2.0
C	5% ACN	6.8	95% ACN	7.7	0/19/25/50/100	0/5/28/40/60	15 × 0.2	0.20
D	25% ACN	5.9	95% ACN	6.8	0/10/20/20/60/60	0/25/30/45/65/80	25 × 0.46	1.1
E	35% ACN	6.0	80% ACN	6.0	0/0/100/100	0/25/45/55	15 × 0.46	1.0
F	35% ACN	2.0	90% ACN	2.0	0/100	0/50	15 × 0.46	1.2
G	0% ACN	2.7	100% ACN		10/10/22/88/88	0/5/15/25/27	15 × 0.46	1.0
H	0% ACN	2.7	100% ACN		6/6/10/10/47/47	0/9/12/17/25/28	15 × 0.46	1.0
I	0% ACN	2.8	100% ACN		10/70/70	0/13/19	15 × 0.46	2.0
J	0% ACN	2.5	100% ACN		10/70/70	0/15/20	15 × 0.46	2.0
K	0% ACN	7.0	100% ACN		5/70	0/60	15 × 0.46	1.0
L	0% ACN	7.0	100% MeOH		5/70	0/60	15 × 0.46	1.0

[a] Designation of different examples or separations; see Section 4.1 and chromatograms in Figs. 5–16.
[b] A and B: solvents for gradient elution; examples A and B are isocratic separations (no B-solvent).
[c] Gradient defined in terms of %B over some time interval; e.g., 0/19/25 %B in 0/5/28 min.
[d] Column dimensions in cm (length and internal diameter).
[e] Flow rate in mL/min.

oratories were from Wyeth Research, 3M, Eli Lilly and the University of Kansas.

Twelve different routine separations (examples A–L) from four different laboratories were studied, each of which was carried out on two or more different columns. For each example, the sample and separation conditions were the same on these two (or more) columns. Tables 1 and 2 summarize the conditions for each separation, including the general nature of the sample and the types of column used. Figs. 5–16 summarize the resulting chromatograms.

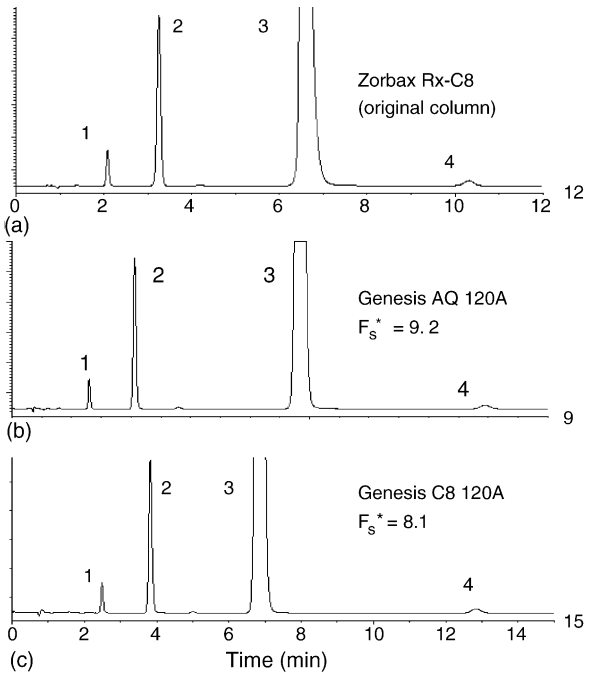

Fig. 6. Separations for example B of Table 1. See Sections 3 and 4.1 for details.

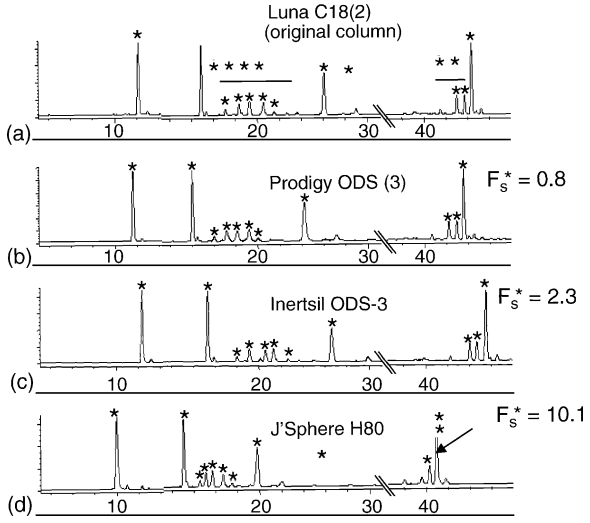

Fig. 7. Separations for example C of Table 1. See Sections 3 and 4.1 for details. Note duplication of "*" for emphasis.

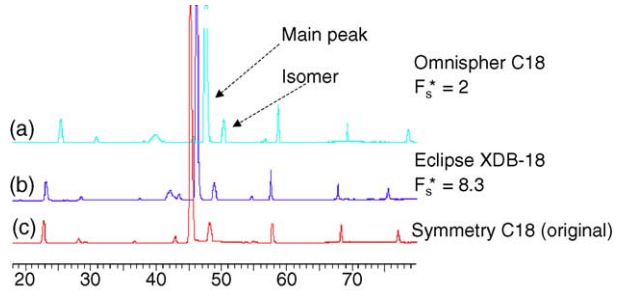

Fig. 8. Separations for example D of Table 1. See Sections 3 and 4.1 for details.

Table 2
Summary of column comparisons

Columns #1 and #2[a]	Sample[b], pH	x_B	x_C	F_s^*	S.D. (log k)		R_s[e]	Q[f]
					Experimental[c]	Calculated[d]		
(A) Symmetry C8, Inertsil ODS-3	SB, 6.5	0	0.1	5.6	0.032	0.022	8.7	13.0
(B) Zorbax Rx C8, Genesis C8	WB, 2.0	0	0.1	8.1	0.046	0.031	11	16.5
Zorbax Rx C8, Genesis AQ				9.2	0.025	0.035		
Genesis C8, Genesis AQ				3.7	0.062	0.015		
(C) Luna C18(2), Prodigy ODS(3)	CA, 6.8	1	0	0.8	0.025	0.005	2	3.0
Luna C18(2), InertsilODS-3				2.3	0.020	0.010		
Luna C18(2), J'Sphere H80				10.1	0.081	0.038		
Prodigy ODS(3), InertsilODS-3				1.6	0.035	0.008		
Prodigy ODS(3), J'Sphere H80				9.4	0.056	0.036		
InertsilODS-3, J'Sphere H80				8.6	0.081	0.033		
(D) Symmetry C18, Eclipse XDB-18	5.9	0	0	8.3	0.010	0.032	4	6.0
Symmetry C18, Omnisphere C18				2	0.013	0.009		
Eclipse XDB-18, Omnisphere C18				6.4	0.013	0.025		
(E) YMC Pro C18, Synergi Max-RP	CA, 2.0	1	0	3.8	0.023	0.016	2	3.0
(F) Symmetry C18, Prodigy ODS(3)	WB, 5.8	0	0	5.9	0.031	0.023	1.3	2.0
Symmetry C18, Luna C18(2)				5.8	0.027	0.023		
Prodigy ODS(3), Luna C18(2)				0.4	0.019	0.003		
(G) Ace C8, Discovery C8	CA, SB, 2.7	1	1	1.3	0.081	0.007	1.5	2.3
Ace C8, Kromasil C8				4.7	0.049	0.019		
Ace C8, Bonus RP				248	0.848	0.896		
Discovery C8, Kromasil C8				5.7	0.097	0.022		
Discovery C8, Bonus RP				249	0.850	0.899		
Kromasil C8, Bonus RP				244	0.865	0.880		
(H) Ace C8, Discovery C8	CA, SB, 2.7	1	1	1.3	0.031	0.007	2.0	3.0
Ace C8, Kromasil C8				4.7	0.018	0.019		
Ace C8, Bonus RP				248	0.358	0.896		
Discovery C8, Kromasil C8				5.7	0.033	0.022		
Discovery C8, Bonus RP				249	0.348	0.899		
Kromasil C8, Bonus RP				244	0.368	0.880		
(I) Zorbax C8/Zorbax Rx-C8	CA, 2.8	1	0	23.5	0.099	0.087	0.8	1.2
Zorbax C8/Genesis C8120A				24.6	0.093	0.091		
Zorbax C8/Genesis AQ 120A				26.5	0.124	0.097		
Zorbax Rx-C8/Genesis C8120A				8.1	0.025	0.031		
Zorbax Rx-C8/Genesis AQ 120A				9.2	0.031	0.035		
Genesis C8120A/Genesis AQ 120A				3.7	0.029	0.015		
(J) Zorbax Rx-C8/Genesis C8120A	CA, WB, 2.5	1	0.1	8.1	0.072	0.031	1.5	2.3
Zorbax Rx-C8/Genesis AQ 120A				9.2	0.076	0.035		
Genesis C8 120A/Genesis AQ 120A				3.7	0.015	0.015		
(K) See Fig. 14; ACN solvent	WB, 7.0	0	0				1.5	2.3
(L) See Fig. 15; MeOH solvent	WB, 7.0	0	0				1.5	2.3

See text for details.

[a] Columns compared; e.g., for sample A, Symmetry C8 is column #1 (original column) and Inertsil ODS-3 is column #2 (replacement column); for each example (A, B, etc.), the original column is listed first; e.g., for example "B", the original column is Zorbax Rx C8.

[b] Contains strong bases (SB), weak bases (WB), carboxylic acids (CA) as indicated; mobile phase pH indicated; if no acids or bases indicated, these are absent from the sample (as in example D).

[c] Standard deviation (S.D.) of plots of log k for column 1 vs. log k for column 2 in isocratic elution; for gradient elution, values shown here are calculated from Eq. (5), using S.D. values from plots of t_R for one column vs. the other.

[d] S.D. values calculated from Eq. (2), using F_s^* values from Eq. (3).

[e] Critical resolution of original separation.

[f] Value of maximum allowable F_s^* (= Q) from Eq. (4).

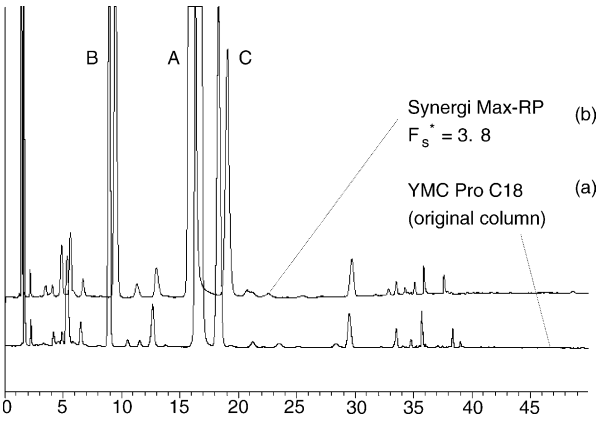

Fig. 9. Separations for example E of Table 1. See Sections 3 and 4.1 for details.

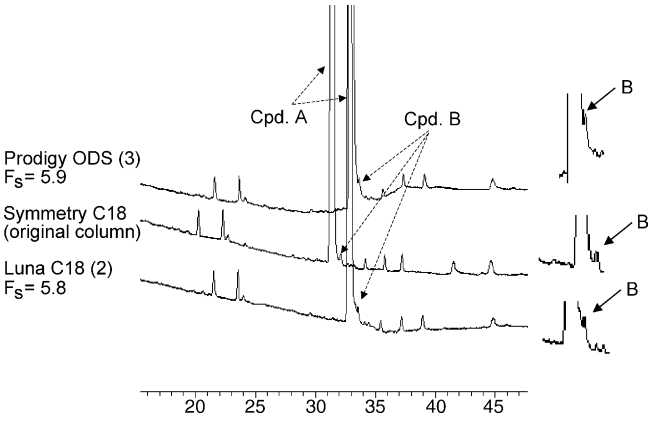

Fig. 10. Separations for example F of Table 1. See Sections 3 and 4.1 for details.

3.1. Calculations

The calculations described in this paper (see Appendix A) are moderately tedious, but commercial software is available that eliminates any inconvenience, as well as provides values of **H**, **S***, etc. for more than 300 different columns (Column Match®; Rheodyne LLC; Rohnert Park, CA).

4. Results and discussion

The validity of the column-comparison procedure described in Section 2 and summarized in Appendix A can be evaluated both practically and theoretically. Our practical goal is to select a column that can serve as a replacement for an original column with minimum experimental effort. If this goal is achieved for a given separation, then our procedure can be judged a success (Section 4.1). A statistical evaluation (Section 4.2) can probe more deeply, by comparing S.D. values for log–log plots of k in isocratic separation (or retention time plots in gradient elution) with corresponding values of F_s^* (as in Fig. 4).

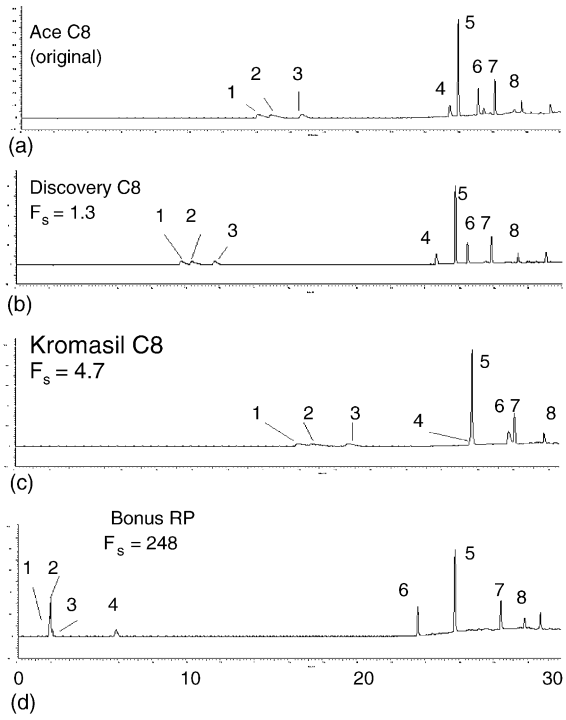

Fig. 11. Separations for example G of Table 1. See Sections 3 and 4.1 for details.

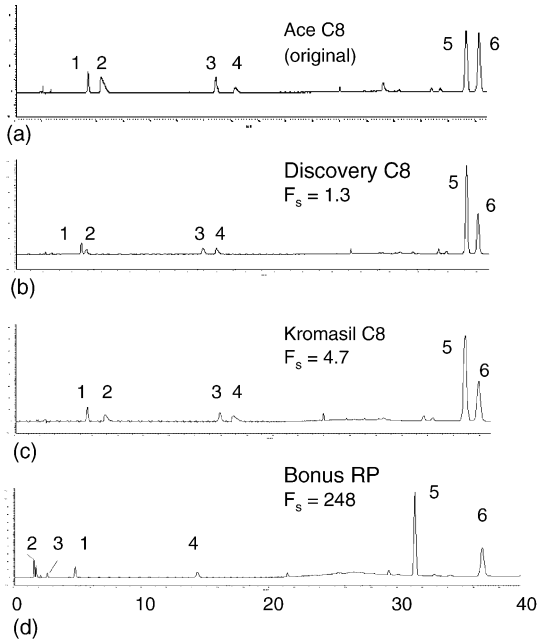

Fig. 12. Separations for example H of Table 1. See Sections 3 and 4.1 for details.

4.1. Practical evaluation of the present column-comparison procedure

Column equivalency, based on the similarity of corresponding separations, is usually a subjective judgment, although quantitative criteria may apply in some cases; e.g., system suitability requirements (Chapter 15 of [4]). In the

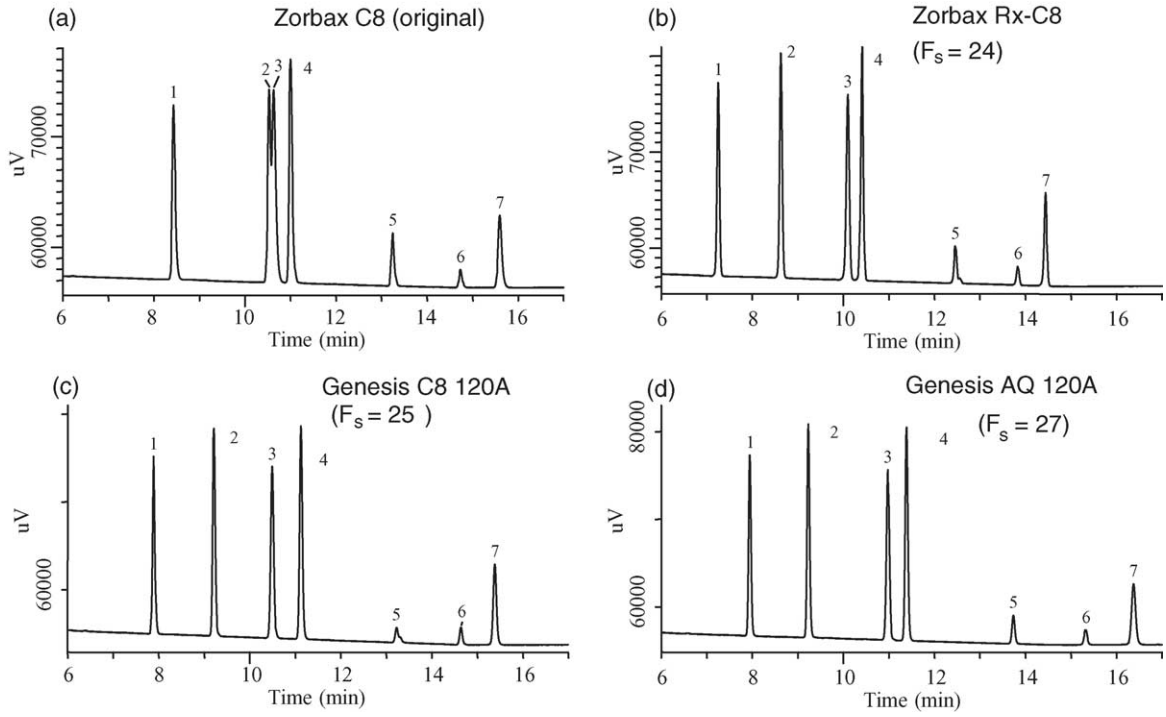

Fig. 13. Separations for example I of Table 1. See Sections 3 and 4.1 for details.

following comparisons, we regard two columns as equivalent when (a) critical resolution does not decrease by more than ≈25%; (b) major changes in the resolution of other peaks are not observed; and (c) no peak reversals result. Section 4.2 provides a less subjective and more detailed evaluation of column equivalency in terms of values of S.D. for log–log plots of k (isocratic) or corresponding plots of t_R for gradient elution.

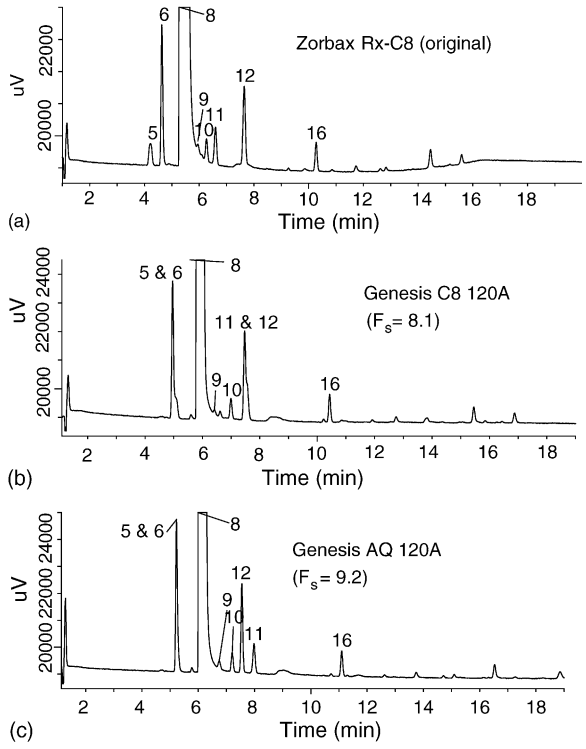

Fig. 14. Separations for example J of Table 1. See Sections 3 and 4.1 for details.

In each example of Table 2 and Figs. 5–16, separation on one or more possible replacement columns is compared with an original column. Usually the replacement-column candidates were selected from a group of readily available columns on the basis of small values of F_s^* (compared to the original column). The selection process was guided by the use of the column-comparison software cited in Section 3.

Values of F_s^* were calculated for each replacement column (see Appendix A), and compared (Eq. (4a)) with the maximum allowable value of Q (Eq. (4)) for equivalent separation. For each separation, Table 2 summarizes the columns compared (first column), the nature of the sample (acids or bases present) and mobile phase pH (column 2), values of the correction factors x_B and x_C (columns 3 and 4), values of F_s^* calculated as described in Appendix A (column 5), experimental values of S.D. from log k–log k plots for isocratic separation or derived values of S.D. (Eq. (5)) for gradient elution (column 6), calculated values of S.D. from Eq. (2) (column 7), values of the critical resolution R_s for the separation on the original column (column 8), and the calculated value of the maximum allowable value of F_s^*(= Q) in column 9. Data in columns 6 and 7 are discussed in Section 4.2.

Fig. 5 shows the isocratic separation of a three-component mixture (example "A"). The critical resolution for the original separation on a Symmetry C8 column was $R_s = 8.7$, so the maximum value of F_s^* for equivalent separation (Eq. (4)) is $Q = 13$. The value of F_s^* for the Inertsil ODS-3 column (compared to Symmetry C8) equals 5.6, which is less than

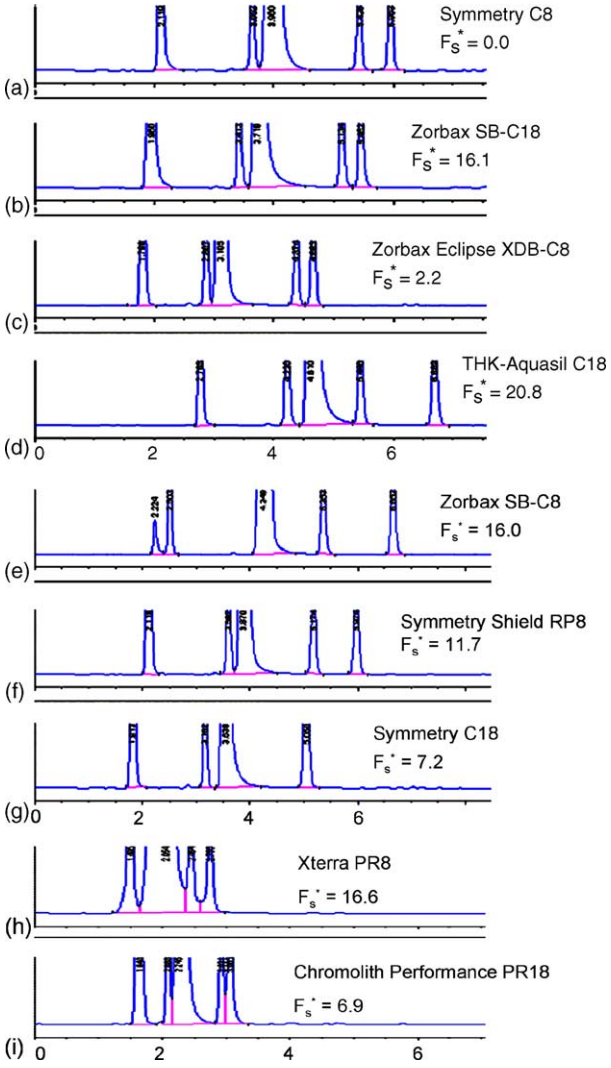

Fig. 15. Separations for example K of Table 1. See Sections 3 and 4.1 for details.

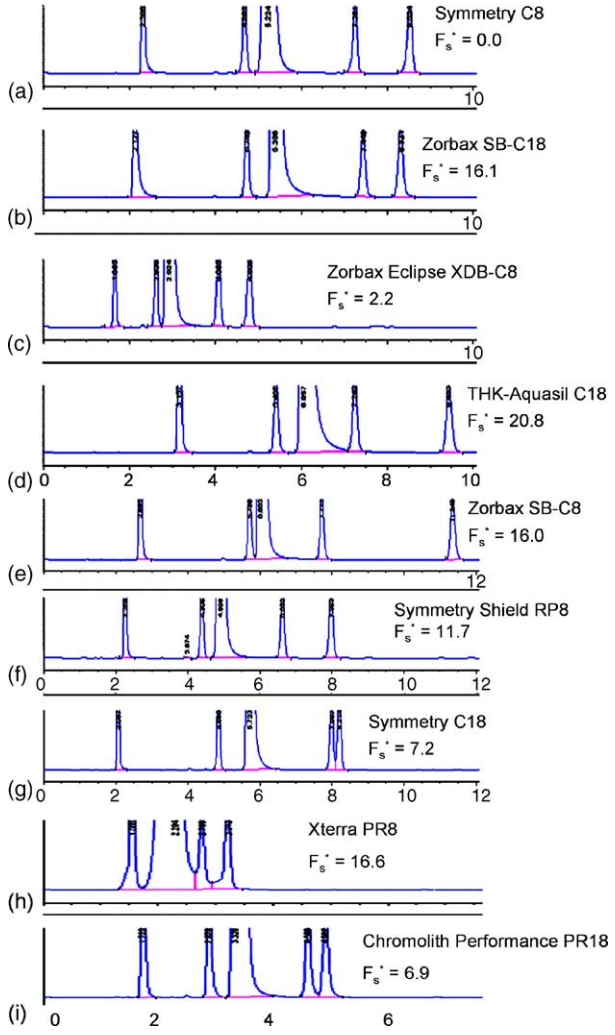

Fig. 16. Separations for example L of Table 1. See Sections 3 and 4.1 for details.

13, so an "equivalent" separation on the Inertsil column is expected. Fig. 5 confirms the similarity of the two columns for this separation.

Fig. 6 summarizes the isocratic separation of a four-component sample (example "B") on the original Zorbax Rx-C8 column (a) and two possible replacement columns (b and c). The maximum value of F_s^* for this separation is $Q = 16.5$ (Table 2), which is greater than F_s^* for either the Genesis AQ (9.2) or Genesis C8 (8.1) columns. Therefore, either of the latter two columns should serve as a replacement for the original column, as observed.

Example "C" (Fig. 7) is a gradient separation of 11 components of interest (each marked by "*"; in [a] some peaks are marked twice for clarity). The maximum value of F_s^* for this separation is $Q = 3.0$, which is greater than F_s^* for either the Prodigy ODS(3) ($F_s^* = 0.8$) or Inertsil ODS-3 ($F_s^* = 2.3$) columns. Therefore, either of the latter columns should be able to serve as a replacement for the original Luna C18(2) column. An examination of Fig. 7a–c confirms this prediction. The J'Sphere H80 column of Fig. 7d has $F_s^* = 10.1$, however, and is therefore *not* expected to provide equivalent separation (note the co-elution of the last two bands of interest, indicated by an arrow).

Example "D" (Fig. 8) is a gradient separation of a "main peak" and its isomer in a sample which contains several other minor peaks. The value of $Q = 4$, so that separation on the Omnisphere C18 column ($F_s^* = 2$) is expected to be equivalent, while that on the Eclipse XDB-18 column ($F_s^* = 8.3$) may not be. In fact, equivalent separations are found for each column. The discrepancy in the case of the Eclipse XDB-18 column may be due to the molecular similarity of the two compounds (isomers), as examined further in Section 4.2. This example also emphasizes the importance of considering columns for replacement (as in Fig. 2), even when F_s^* is somewhat greater than Q.

Fig. 9 (example "E") is a gradient separation of three major bands (A–C) from each other and from several minor components of the sample. The value of $Q = 3$, while $F_s^* = 3.8$ for the Synergi Max-RP column; the two columns

might therefore be expected to be marginally equivalent. As seen in Fig. 9, the two separations would be regarded as satisfactory by most workers, and the resolution of peaks A and C by the Synergi Max-RP column is actually better than for the YMC Pro C18 column. Note that a replacement column can provide a critical resolution that is moderately better or worse than that of the original column, when $F_s^* \leq Q$; when $F_s^* > Q$, larger differences in resolution are possible (either increased or decreased R_s).

Fig. 10 (example "F") presents the gradient separation of a sample in which compounds A and B are of primary interest. The two compounds are marginally separated on the original column (Symmetry C18; $R_s \approx 1.3$), so that $Q = 2$. Values of F_s^* are noticeably larger than Q for both the Prodigy ODS(3) and Luna C18(2) columns ($F_s^* = 5.9$ and 5.8, respectively), so equivalent separations are neither expected nor found. Presumably a closer match to the original column (Symmetry C18) would have resulted if columns with $F_s^* \leq 2$ had been compared; at least three such columns exist (but were not tried): Zorbax Extend C18 ($F_s^* = 1.4$), J'Sphere H80 ($F_s^* = 1.6$), and Omnispher 5 C18 ($F_s^* = 2$).

Fig. 11 (example "G") shows the gradient separation of a mixture of eight compounds: 1, lidocaine; 2, mepivacaine; 3, prilocaine; 4, bupivacaine; 5, prednisolone; 6, amitriptyline; 7, naproxen; 8, ibuprofen, with the Ace C8 column as reference. The value of $Q = 2.3$, while $F_s^* = 1.3$ (Discovery C8), 4.7 (Kromasil C8) and 248 (Bonus RP). Therefore, the Discovery C8 and Ace C8 columns should be equivalent (they are), while the remaining columns are not expected to give comparable separations (they do not).

Fig. 12 (example "H") shows the gradient separation of a mixture of six compounds: 1, 4-hydroxyphenyl acetamide; 2, atenolol; 3, nitropyridine; 4, 4-hydroxyphenylacetic acid; 5, atenolol acid; 6, nitrendipine, with the Ace C18 column again as reference. The value of $Q = 3$, while $F_s^* = 1.3$ (Discovery C8), 4.7 (Kromasil C8) and 248 (Bonus RP). Therefore, the Discovery C8 and Ace C8 columns should be equivalent, while the remaining columns are not predicted to give comparable separations. In this case, the Kromasil C8 column appears to be an even better match for the ACE C8 column than the Discovery C8 column, while the latter is at best a marginal match to the ACE column (note the poorer separation of bands 1 and 2). This example again emphasizes the approximate nature of the relationship of column equivalency versus values of F_s^*, and the need in some cases to consider columns with $F_s^* > Q$. Of course, this is the only option when no columns with $F_s^* \leq Q$ are readily available.

Fig. 13 (example "I") is a gradient separation of a mixture of seven compounds. The original column (Zorbax C8) barely resolves peaks #2 and 3, so that $Q = 1.2$; because the Zorbax C8 column is based on type-A silica, it is less likely that an equivalent column can be found [12]. In this case, the three type-B columns compared with Zorbax C8 in Fig. 13 all have values of $F_s^* \gg 1.2$, and as expected give very different separations (actually, with much *improved* resolution). A comparison of columns c and d of Fig. 13 gives $F_s^* = 3.7$, while for these two columns $Q = 4.5$, so these two columns should be equivalent to each other. As seen in Fig. 13, the separations provided by columns c and d (Genesis C8 and AQ) are indeed similar.

Fig. 14 (example "J") shows the gradient separation of eight major peaks (#5, 6, 8, 9–12, 16). Peak #9 is barely observable for the original Zorbax Rx-C8 column, and $Q \approx 2.3$. F_s^* equals 8.1 and 9.2 for the Genesis C8 and AQ columns, so similar separation is not expected. A closer look at peaks #8–10 shows significant differences for each separation, as predicted. In this case, it might have been more appropriate to look for a very *different* column selectivity versus that of the original column (Fig. 14a), because a better separation of the sample seems to be needed. This would suggest the use of columns with much larger values of F_s^* (as in the similar case of Fig. 13a). However, while large values of F_s^* will usually lead to pronounced differences in separation, this does not necessarily mean a *better* separation. That is, it will usually be necessary to investigate more than one column with large F_s^* when an improvement in separation is needed.

Figs. 15 and 16 (examples "K" and "L") show the separation of the same sample on nine different columns. The only difference for separations K and L is the use of acetonitrile as organic solvent in Fig. 15 and methanol in Fig. 16. In each case, $Q = 2.3$. A cursory examination of Figs. 15 and 16 indicates that most of these separations are quite similar, despite values of F_s^* as large as 20. As further discussed in Section 4.2, it appears that values of F_s^* consistently overestimate differences in column selectivity for the separations of Figs. 15 and 16. This anomalous behavior likely arises from the nature of the sample (all components of possibly similar molecular structure), as discussed in Section 4.2.

To summarize the above examples, apart from examples K and L of Figs. 15 and 16, we have 10 different separations that involve a total of 22 different columns. For all but a few cases, the present column-comparison procedure was successful in predicting whether the a replacement column would or would not provide equivalent separation. In those cases where experiment disagrees with prediction, values of F_s^* and Q were usually not very different. The latter cases ($F_s^* \approx Q$) fall into a gray area, because Eqs. (1)–(3) are inexact relationships (they are based on an arbitrary, average sample). To put this another way, the *probability* of a good match of column selectivity is relatively high for $F_s^* \ll Q$, relatively poor for $F_s^* \gg Q$, and intermediate for $F_s^* \approx Q$.

We conclude that the procedure described above should be generally useful for the initial selection of potentially equivalent replacement columns, followed by a final choice or choices based on experimental runs as in Fig. 2.

4.2. Statistical evaluation of the present column-comparison procedure

The results of Section 4.1 are promising, but additional insights are possible by a further analysis of the data of Table 2. For example, our comparisons above in each case are based

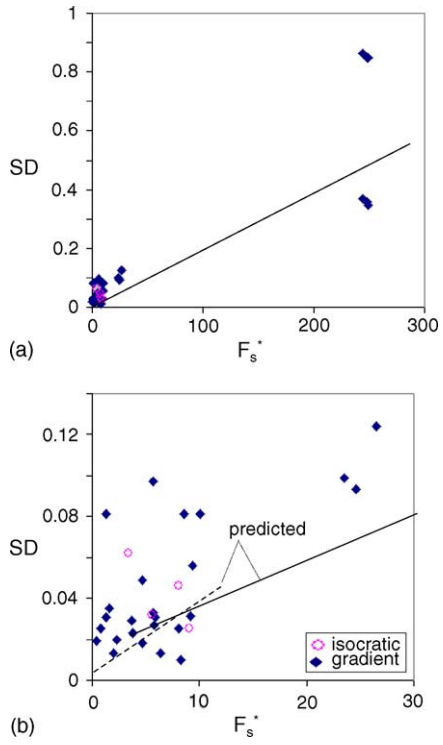

Fig. 17. Comparison of experimental values of S.D. for examples A–J with values of F_s^*. Data of Table 2. Solid and dashed curves are described in Fig. 4. See discussion of Eq. (2) in text for details.

on the original column only. However, where more than two columns are compared, additional comparisons of separation versus values of F_s^* are possible (for column-pairs which do not include the original column). The reader can examine these comparisons, using the F_s^* values for all possible column-pairs in Table 2. Experimental values of S.D. (from isocratic log–log plots of k or gradient plots of t_R) can also be compared with values of F_s^* (data of Table 2), as in Fig. 4. Note that the experimental S.D. values of Table 2 are based on retention times for most of the peaks in each chromatogram (excluding "noise" peaks), rather than just the major peaks of interest. For various reasons, the retention times of these minor peaks are usually less reliable, so that values of S.D. determined in this way may be more approximate measures of column similarity. Also, values of S.D. (like values of F_s^*) are only indirectly related to "acceptable" separation.

Fig. 17a provides a plot of values of S.D. versus F_s^* for 38 pair-wise column comparisons from Table 2 (examples A–J). Most of the values of S.D. are bunched together near the origin of Fig. 17a, with a few points for $F_s^* \approx 250$. However, Fig. 17a and b together confirm for $F_s^* > 10$ that values of S.D. are large (>0.4), meaning that the columns are quite different in terms of selectivity. Fig. 17b is an expansion of Fig. 17a for $F_s^* < 30$, allowing a comparison of experimental values of S.D. with values of F_s^* predicted by Fig. 4a (dashed curve for $F_s^* \leq 10$ and solid curve for $F_s^* > 10$). There is a rough adherence of the data of Fig. 17b to the curves of Fig. 4a, but also considerable scatter (for various reasons). Note that values of S.D. for gradient elution are expected to be less reliable (Section 2.5), especially for the use of segmented gradients; most of the examples of Fig. 17 involve segmented gradients (see Table 1). Also, we have noted above the lesser reliability of S.D. values based on both major and minor peaks (as in the present comparison). Finally, and most important, the weighting factors of Eqs. (1) and (3) were derived from an average sample, based on the 88 compounds of Fig. 4a. Any other sample, especially when the number of components n is small, will in principle require somewhat different weighting factors. If the molecular structures of the sample components are known in detail, it is in principle possible to derive improved weighting factors for all five column parameters (but only very approximately at the present time). See the further discussion of Appendix B.

4.2.1. Examples "K" and "L" and the case of samples composed of "similar" compounds

We have noted that the separations of Figs. 15 and 16 (examples K and L) are generally similar, despite large values of F_s^* for some of these columns. The composition of this sample has been *partially* revealed as follows: compound #1 is cytosine, #2–4 are derivatives of cytosine, and #5 is a cytidine analog. Thus, each compound shares a common molecular feature, and the resulting structural similarity of these five compounds *could* mean either that their solute-selectivity parameters η', σ', etc. (see Appendix B) do not vary greatly from one compound to the next, or that values of η', σ', etc. change regularly with retention time t_R. In either case, the effect of differences in the column parameters **H**, $\mathbf{S}^*$, etc. on the separation will be reduced, so that values of S.D. should then be smaller than predicted from Fig. 4a. As

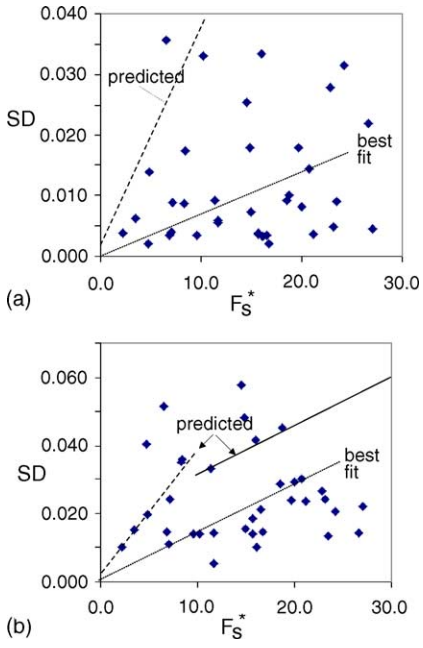

Fig. 18. Comparison of experimental values of S.D. for examples K and L with values of F_s^*. Data of Table 2; see text for details.

seen in Fig. 18 for examples K (a) and L (b), in each case values of S.D. fall mainly *below* the predicted curves, corresponding to a lesser effect of the column on separation than predicted by F_s^*; this is in contrast to the results for examples A–J in Fig. 17b. To generalize the results for these two examples, whenever the compounds in a sample are sufficiently structurally-similar (an extreme example would be a homologous series), it should be easier to find a replacement column; i.e., values of $F_s^* \gg 3$ may be acceptable in such cases.

5. Conclusions

For various reasons, a replacement column may be required for an RP-LC assay procedure. The primary requirement of the replacement column is equivalent separation selectivity (similar values of the separation factor α for all adjacent bands). For any two columns 1 and 2, a matching function F_s^* can be defined in terms of five column-selectivity properties (**H**, **S***, **A**, **B**, **C**) (Eq. (3)).

Values of **H**, **S***, etc. have been reported for >300 RP-LC columns [14], while x_B and x_C represent corrections whose values depend on whether the sample contains acids or bases (see Appendix A for details). Eq. (3) allows the calculation of F_s^* for any two columns for which values of **H**, **S***, etc. are known. Two columns can be regarded as *probably* interchangeable in a given assay procedure, when $F_s^* \leq Q$, where $Q \leq \frac{3}{2} R_s(1)$ Eq. (4). Here, $R_s(1)$ refers to the resolution of the two critical (least well separated) bands in the original separation ("column 1").

In the present study, the use of Eqs. (3) and (4) for identifying potential replacement columns has been evaluated for 12 different assay procedures which involved different samples and conditions. In most of these examples, two columns which satisfy the requirements of Eq. (4) were found to be equivalent or interchangeable. Eqs. (3) and (4) are approximate relationships, however; especially when $F_s^* \approx Q$ (e.g., for F_s^*/Q values of 0.7–1.5). Predictions of column similarity then become less certain; i.e., the two columns may or may not prove equivalent. In most cases, however, the proposed procedure for selecting equivalent columns should greatly reduce the number of columns which need to be compared experimentally.

The potential accuracy of Eqs. (3) and (4) is limited by several factors: (a) dependence of the weighting factors in Eq. (3) on sample composition, which becomes more important for samples containing a smaller number of components; (b) further approximations which are inherent for gradient separations; and (c) differences in columns of nominally the same kind from lot to lot, or as a function of column history [13]. Similar limitations apply, however, to the use of any column matching procedure. Larger values of F_s^* may be allowable in the case of separations involving compounds of similar molecular structure; e.g., isomers, homologs, or degradation products of a single compound.

The present procedure requires values of the selectivity parameters **H**, **S***, etc. for the two columns being prepared. Since it is unlikely that any two randomly selected columns will prove to be interchangeable for a given RP-LC assay, it is desirable to have values of **H**, **S***, etc. for a large number of different commercial columns. Data for more than 300 columns are listed in [13] and included as part of the software package mentioned in Section 3.

If separation with a potential replacement column is similar, but not sufficiently close, it is possible to narrow the difference between the two separations by making small changes in separation conditions such as temperature, pH, etc. (so-called *method adjustment* [14]). The combination of column matching as in the present paper with method adjustment should make it possible to replicate an original separation in almost every case.

6. Nomenclature

See Section 5 of the preceding paper published in this volume [1].

Acknowledgement

We are indebted to Astra Zeneca for the donation of atenolol acid.

Appendix A

A summary of steps for the convenient use of Eq. (3) for comparing columns in terms of selectivity.

1. Collect values of **H**, **S***, **A**, **B**, **C**(2.8) and **C**(7.0) for column 1 (original column) and column 2 (potential replacement column); data for more than 300 columns are listed in [13] and included as part of the software package mentioned in Section 3.
2. Determine the value of **C** for the pH of the mobile phase:

   $$\mathbf{C} = \mathbf{C}(2.8) + ([\mathrm{pH}] - 2.8)/[7.0 - 2.8])(\mathbf{C}[7.0] - \mathbf{C}[2.8])$$

   If pH < 2.8, assume **C** = **C**(2.8); if pH > 7.0, assume **C** = **C**(7.0).
3. Determine values of the correction factors x_B and x_C:
   a. If the sample contains strong bases (pK_a in water >9; e.g., molecules substituted by aminoalkyl groups), then $x_C = 1.0$ when pH < 6, 0.1 when 6 < pH < 10, and 0.0 when pH ≥ 10.
   b. If the sample contains weak bases (anilines, pyridines) but *not* strong bases, then $x_C = 0.1$ when pH < 5, and 0.0 when pH > 5.
   c. If the sample contains neither strong nor weak bases, $x_C = 0$.

Table A.1
An example of the procedure described in Appendix A for comparing column selectivity; example "C" of Table 2, Luna C18(2) and Prodigy ODS(3) columns

Step 1: Values of **H**, **S***, etc. for the two columns.

Column	**H**	**S***	**A**	**B**	**C**(2.8)	**C**(7.0)	**C**(6.8)
Luna C18(2)	1.003	−0.023	−0.121	−0.006	−0.269	−0.173	−0.177
Prodigy ODS(3)	1.023	−0.024	−0.129	−0.011	−0.195	−0.133	−0.136

Values of **C**(6.8) are obtained from values of **C**(2.8) and **C**(7.0) by linear interpolation vs. mobile phase pH (Step 2).

Step 2: Value of **C** for mobile phase pH = 6.8.
$\mathbf{C} = \mathbf{C}(2.8) + (1/7.0 - 2.8)([\mathrm{pH}] - 2.8)(\mathbf{C}[7.0] - \mathbf{C}[2.8])$
See values in last column of Step 1.

Step 3: The sample contains carboxylic acids, but no strong bases. Therefore, $x_B = 1.0$ and $x_C = 0.0$.

Step 4: Calculate F_s^* from Eq. (3): $F_s^* = 0.8$.

Step 5: Calculate maximum allowable F_s^* for equivalent columns. The critical resolution of the separation on the Luna C18(2) column ($R_s(1)$) is $R_s = 2$, so according to Eq. (4)
$Q = \frac{3}{2}R_s(1) = 3$

Step 6: F_s^* from Step 4 is less than Q from Step 5, so the two columns are expected to be equivalent for this separation. Comparison of the separation on the two columns (Fig. 7a and b) confirms this prediction.

d. If the sample contains carboxylic acids, $x_B = 1.0$; if not, $x_B = 0$.

The values of x_B and x_C estimated above are necessarily quite approximate, but are the best available at the present time. More precise values of x_B and x_C would require (a) a knowledge of solute pK_a values and their dependence on separation conditions (i.e., organic solvent type and concentration, buffer concentration, temperature, etc.) and (b) the quantitative dependence of values of κ' on the relative ionization of the solute.

4. Calculate a value of F_s^* from Eq. (3), using the above values of **H**, **S***, etc. and values of x_B and x_C.
5. Determine the critical resolution R_s of the original chromatogram; calculate a maximum value of $F_s^* = Q$ from Eq. (4).
6. Compare the above values of F_s^* and Q; if $F_s^* \leq Q$, then the two columns are likely to be equivalent. Two columns *may* be equivalent for F_s^* larger than Q.

As an example of the above procedure, consider separation "C" of Table 2, for the original column (Luna C18(2)) and a Prodigy ODS-3 column. Details are given in Table A.1.

If it is not known whether acids or bases are present in the sample, it can be assumed for purposes of calculating values of F_s^* that the latter compounds *are* present. This will result in $x_B = x_C$ and larger values of $F_s^* = F_s$, with a reduced frequency of equivalent columns. However, two columns with resulting values of $F_s \leq 3$ are still expected to be equivalent.

The above procedure is approximate, so if a column that is predicted to be equivalent to the original column proves not to be a satisfactory replacement, try another column that is also predicted to be equivalent. Similarly, if no column is predicted to be equivalent, then try the column or columns with the smallest values of F_s^*.

Correction factors x_B and x_C for Eq. 1

Correction factors (e.g., x_B and x_C) for Eq. (1) as a function of sample composition and separation conditions.

The present column characterization scheme (values of **H**, **S***, etc.) is based on a general, relationship [3]:

$$\log\left(\frac{k}{k_{EB}}\right) \equiv \log\alpha = \eta'\mathbf{H} - \sigma'\mathbf{S}^* + \beta'\mathbf{A} + \alpha'\mathbf{B} + \kappa'\mathbf{C} \quad (A.1)$$

Here, k is the retention factor of any solute, k_{EB} is the value of k for a nonpolar reference solute (ethylbenzene), and the remaining selectivity-related symbols represent empirical, eluent-dependent properties of the solute (η', σ', β' α', κ') or eluent-independent properties of the column (**H**, **S***, **A**, **B**, **C**). Values of η', σ', etc. are given for 88 solutes of widely varying structure in [7].

We see in Eq. (A.1) that the effect of each column parameter (**H**, **S***, etc.) on sample retention and column selectivity is modified by the value of the corresponding solute parameter (η', σ', etc.). Thus, if for the sample of interest the values of any one solute parameter (e.g., η') are either zero or constant for all sample components, the effect of the corresponding column parameter (e.g., **H**) on column selectivity and separation will also be zero. This will also be approximately the case for a sample whose components have values of η', σ', etc. that change regularly from one band to the next as retention increases (as for a homologous series).

Thus, for the case of a sample that contains no ionized solutes, $\kappa' \approx 0$, and the effect of **C** on the separation is negligible. This is equivalent to dropping the term $(\mathbf{C}_2 - \mathbf{C}_1)$ (as in Eq. (3) for $x_C = 0$) for samples of this kind. Returning to

Eq. (3)

$$\{[12.5x_H(\mathbf{H}_2-\mathbf{H}_1)]^2+[100x_S(\mathbf{S}^*_2-\mathbf{S}^*_1)]^2 +[30x_A(\mathbf{A}_2-\mathbf{A}_1)]^2+[143x_B(\mathbf{B}_2-\mathbf{B}_1)]^2 +[83x_C(\mathbf{C}_2-\mathbf{C}_1)]^2\}^{1/2} \quad (A.2)$$

where x_H, x_S, etc. refer to sample-related correction factors in Eq. (A.2). If maximum values of η', σ', etc. can be estimated for a given separation (dependent on sample and experimental conditions), values of x_H, x_S, etc. in Eq. (A.2) will be approximately equal to these values. For example, for fully ionized bases, maximum values of $\kappa' \approx 1$, while for partly ionized bases, $\kappa' \approx 0.1$. Therefore, values of $x_C = 1$ or 0.1 for fully or partly ionized bases, respectively, and values of x_C will vary with mobile phase pH.Because the ionization of basic compounds varies with compound acidity (pK_a), as well as with temperature and mobile phase pH, %B and buffer concentration, precise rules for estimating solute ionization and values of x_C cannot be set forth. Consequently, the estimation of values of x_C becomes less reliable for separation conditions that are more different from those used in the measurement of published values of **H**, $\mathbf{S}^*$, etc (50% organic solvent, 35 °C). Also worth noting is that acids that are substantially ionized (>30%) have significant negative values of κ', suggesting that **C** can also have a relatively larger effect on the separations of such samples.

Appendix B

Gradient separation and maximum allowable value of F^*_s.

B.1. Column comparisons in gradient versus isocratic elution

RP-LC separations based on gradient elution are most easily understood in terms of the linear-solvent-strength model [15], which assumes that isocratic elution as a function of mobile phase composition (%B) can be approximated by:

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

Here, k_w is the extrapolated value of k for water as the mobile phase (for which $\phi = 0$), ϕ is the volume-fraction of organic solvent in the mobile phase (ϕ = %B/100), and S is a constant for a given solute when only ϕ varies. It has been found that values of S do not change for different columns, especially when F_s for the two columns is small (i.e., for near-equivalent columns) [11]. For linear gradients and samples which obey Eq. (B.1), retention time t_R in gradient elution can be derived [15] as:

$$t_R = \left(\frac{t_0}{b}\right)\log[(2.3k_0b)+1]+t_0+t_D \quad (B.2)$$

$$t_R \approx \left(\frac{t_0}{b}\right)\log(2.3k_0b)+t_0+t_D \quad (B.3)$$

where t_0 is the column dead-time, k_0 is the isocratic value of k at the start of the gradient, t_D is the "dwell" time or hold-up time for the RP-LC system, and b is a gradient parameter defined by:

$$b = \frac{V_m\,\Delta\phi S}{(t_G F)} \equiv \frac{t_0\,\Delta\phi S}{t_G} \quad (B.4)$$

Here, V_m is the column dead-volume, $\Delta\phi$ is the change in ϕ during the gradient, t_G refers to gradient time, and F is the flowrate.

Consider next the effect of a change in k_0 (δk_0) on gradient retention time t_R. The resulting change in t_R (δt_R) can be related to δk_0 as:

$$\delta t_R + t_R = \left(\frac{t_0}{b}\right)\log(2.3b) + \left(\frac{t_0}{b}\right)\log(k_0+\delta k_0)+t_0+t_D \quad (B.5)$$

which with Eq. (C.3) gives

$$\delta t_R = \left(\frac{t_0}{b}\right)[\log(k_0+\delta k_0)-\log k_0] = \left(\frac{t_0}{b}\right)\log\left[1+\left(\frac{\delta k_0}{k_0}\right)\right] \quad (B.6)$$

We can approximate $\log(1+x)$ by $(1/2.3)x$ when x is small, so for $\delta k_0 \ll k_0$):

$$\delta t_R \approx \left(\frac{t_0}{b}\right)\frac{(\delta k_0/k_0)}{2.3} \quad (B.7)$$

Deviations S.D. from $\log k$–$\log k$ plots can be expressed as $\log(k_0+\delta k_0) - \log(k_0)$, which with Eq. (B.6) gives:

$$\text{S.D.} = \left(\frac{b}{t_0}\right)\text{S.D.}(\delta t_R) \quad (B.8)$$

That is, if gradient values of t_R for column-1 are correlated with values of t_R for column 2 (similar to the $\log k$–$\log k$ plots of Fig. 4a), the predicted value of S.D. for use with Eq. (3) is given by Eq. (B.8).

B.2. Column similarity in gradient versus isocratic elution

Not infrequently, isocratic separation on two columns 1 and 2 will be similar as measured by $F^*_s \le 3$, but values of t_R will differ significantly; i.e., values of k_2/k_1 for each solute will be approximately constant, but the average value of k_2/k_1 will not equal 1.0. In the case of isocratic separation, a change in flowrate for column 2 can be used to minimize differences in t_R for the two columns. When a similar situation arises for gradient elution, the result is somewhat more complicated. This can be seen from a relationship for gradient elution which is analogous to Eq. (B.1) for isocratic separation:

$$\log k^* = \log k_w - S\phi^* \quad (B.9)$$

Here, k^* can be regarded as an average or equivalent value of k for a gradient separation, and ϕ^* is equivalent to ϕ in isocratic elution. Similarly, a value of α (α^*) for compounds a and b in gradient elution can be defined:

$$\log \alpha^* = \log \left(\frac{k_{w,a}}{k_{w,b}} \right) - (S_b - S_a)\phi^* \quad \text{(B.10)}$$

which is to say that α^* varies with ϕ^*, resulting in a change in selectivity as a result of change in ϕ (or %B); i.e., so-called "solvent strength" selectivity. When the values of k in isocratic elution vary for columns 1 and 2, so will values of t_R vary for columns 1 and 2 in gradient elution, which therefore means differences in ϕ^* for the two bands on the two columns. But this corresponds to a change in α^* due to mobile phase selectivity, *whenever values of* S_b *and* S_a *are unequal* (Eq. (B.10)). Differences in S for two compounds occur fairly often, as do differences in k_2/k_1 for two columns 1 and 2 that are otherwise similar ($F_s^* \leq 3$). Because of the resulting changes in α for two columns for the latter situation (as predicted by Eq. (B.10)), a comparison of column selectivity in gradient elution will involve not only inherent column selectivity differences (as measured by F_s^*), but also differences in solvent strength selectivity as a result of differences in ϕ^*. This will have the effect of increasing observed values of S.D. in plots of t_R for one column versus another, compared to values from Eq. (2) or (3) which assume isocratic separation.

The contribution of the mobile phase (differences in ϕ^*) to separation selectivity for two columns can in principle be minimized by adjusting separation conditions so as to obtain comparable values of ϕ^* for each solute on each column. Comparable values of ϕ^* can be obtained by equalizing values of k^*, which are related to separation conditions as (Eq. (B.4)):

$$k^* = \frac{1}{1.15b} = \frac{t_G F}{1.15 V_m \delta \phi S} \quad \text{(B.11)}$$

Thus, if sample peaks elute early on column 2 (corresponding to low values of ϕ^* and high values of (k^*), a reduction of either gradient time or flowrate should result in a better match of separation selectivity (and resolution) on the two columns. An increase in t_G or F would be required for the case where the sample elutes later on column 2 (the same as for the case of isocratic elution).

In the case of segmented gradients, there is further uncertainty in the value of b for different bands in the chromatogram. This can lead to further error in values of S.D. from Eq. (B.8).

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