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Changing reversed-phase high performance liquid chromatography selectivity
Which variables should be tried first?

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Abstract

When carrying out HPLC method development, it is often necessary to vary the relative retention of the sample (values of α) by changing some experimental variable, e.g., solvent type, pH, etc. The choice of which variable will be most suitable for a change in selectivity depends on two conflicting goals: (a) the attainment of maximum changes in α for the better control of resolution and (b) the avoidance of practical problems associated with the use of a given variable to optimize selectivity. This study provides a quantitative evaluation of different variables for their effect on selectivity (α). Various practical problems which must be balanced against this ability of a variable to change values of α are also discussed. The selection of any two variables for their simultaneous use in controlling α is also examined.

Keywords: Selectivity; Relative retention

1. Introduction

The reversed-phase HPLC separation of some samples can be achieved by adjusting solvent strength (%B) for acceptable sample retention; e.g., so that $1<k<10$. More often, a change in band spacing is required, in which case conditions must be changed so as to create large enough values of α (e.g., $\alpha>1.1$) for all band-pairs in the chromatogram. Because of the power of selectivity control in achieving separation, this forms the basis of most published procedures for HPLC method development [1–4]. Changes in selectivity can be based on different variables, as summarized in Table 1 for neutral and ionic samples. In the past, those variables (e.g., solvent type, pH) which were believed to have the largest effect on selectivity, were usually chosen for selectivity optimization. This has led to two widely used method-development approaches: (a) variation of solvent type (acetonitrile, methanol, tetrahydrofuran [THF]) for the separation of neutral samples [5] and (b) variation of pH and ion-pair-reagent concentration (IPR) for ionic or ionizable samples [6]. In each case, various computer programs are able to minimize the number of experiments required to find the best mobile phase composition [1–3,5,6].

Factors other than maximum changes in α need to be considered, however, when creating a HPLC method-development strategy [7–9]: (a) practical problems and/or a lack of method ruggedness associated with the use of certain variables, (b) the ease and applicability of computer simulation for optimizing some variables and (c) the applicability of a given variable for both neutral and ionic samples, especially when the sample composition is unknown at the start of method development. Table 2 summarizes a number of these considerations that either

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Table 1
Variables for changing reversed-phase selectivity

Neutral samples[a]	Ionic or ionizable samples
Solvent type (ACN, MeOH, THF) (*St*)	pH
Column type (C_8 or C_{18}, phenyl, cyano) (*C*)	Ion-pair-reagent concentration
Solvent strength (%B)[b] (*b*)	Temperature
Temperature (*T*)	Solvent type
	Column type
	Solvent strength (%B)

[a] ACN: acetonitrile; MeOH: methanol; THF: tetrahydrofuran.
[b] A change in isocratic %B is equivalent to a change in gradient steepness *b*; see discussion of Ref. [15]
Abbreviations in parentheses (*St*, *C*, *b*, *T*) are used to identify selectivity effects in the discussion of selectivity ($\Delta\log\alpha$) values. Arranged in approximate order of decreasing effectiveness as a means for changing α.

Table 2
Practical limitations in the use of different variables for changing selectivity [7,8]

Variable	Limitation
Solvent strength (%B)	None
Temperature	Equipment: thermostating of the mobile phase and column required
Solvent type	
Acetonitrile	None
Methanol	UV detection (>220 nm)
Tetrahydrofuran	UV detection (>240 nm)
	Slow column equilibration
	Unstable; oxidized by air
	Requires additional runs for computer simulation
Column type (C_8 or C_{18}, phenyl, cyano)	Manual intervention (change of column) required
	Slow equilibration of new column
	Impractical to blend packings of different type
	Selectivity cannot be varied continuously
	Computer simulation less useful
pH	Less rugged methods, due to change of α for random variations in pH (±0.05 units)
	Requires additional runs for computer simulation
	Difficult peak tracking
	UV detection (>215 nm for some buffers)
	Less convenient due to need to adjust buffer pH
	Not effective for neutral compounds
Ion-pair-reagent concentration	Slow column equilibration
	Less rugged methods, baseline problems
	Less convenient, due to more complex mobile phase
	Not effective for neutral compounds

Arranged in approximate order of decreasing convenience and desirability.

make method development more difficult or result in unsatisfactory final methods, with emphasis on "practical" problems (a). Computer simulation (b) is increasingly preferred for its ability to facilitate HPLC method development [3,4,9]. However, its use with some variables requires additional experiments and/or is less reliable. For example, optimizing pH or blending THF with other solvents requires more experimental runs, and peak tracking can be difficult when pH is varied (because the UV spectrum of an ionizable compound is often pH-dependent). Finally (c), pH and ion-pair-reagent concentration have little affect on band spacing for non-ionizable samples. The use of the latter variables to vary selectivity assumes that some of the sample constituents are ionized or ionizable.

When choosing among different variables for the purpose of optimizing selectivity, it is useful to know the relative ability of each variable to change α. Practising chromatographers have opinions concerning the effectiveness of different selectivity-changing variables, but little in the way of general quantitative knowledge (except for ionizable samples). A few prior comparisons have been reported in this connection; e.g., changes in solvent-strength (%B) plus solvent type vs. the use of mixtures of ACN, MeOH and THF [10,11]. In the latter examples, these two ways of manipulating selectivity gave comparable results in terms of final sample resolution. A second study compared changes in %B vs. temperature [12,13] and found that %B was somewhat more effective than temperature as a means of changing selectivity.

The trade-off between selectivity control (Table 1) and experimental convenience (Table 2) needs to be assessed for the different variables used to control values of α. For "easy" separations, experimental convenience should be of greater importance. For difficult separations, selectivity will be the major concern. This choice between selectivity and convenience will be facilitated if the qualitative information of Table 1 can be replaced with a more precise measure of the relative ability of each variable to change selectivity. That is one goal of the present study. Because two or more variables can be changed simultaneously for enhanced control over selectivity (as in the procedures of Ref. [5,6]), we also need to know whether or not the selectivity effects for any two variables are independent of each other. When selectivity effects are not independent (i.e. are "correlated"), the second variable will add little to the ability of the first variable to cause changes in α. Thus, a pair of bands that remain unseparated as either variable-1 or -2 is changed, is unlikely to be separated by the simultaneous change of both variables.

The present paper represents a preliminary attempt at addressing these and other issues which relate to the choice of one or more variables for changing selectivity.

2. Background and theory

If a typical column plate number N is assumed ($N=10\,000$), and baseline separation is desired ($R_s>1.5$), a 12% change in α is required for the separation of a totally overlapped band-pair (see related discussion of [14]; assumes $k=1$). Often the initial band-pair is not totally overlapped, k is usually >1, and N can be $>10\,000$. Consequently, changes in α of 5% or more (corresponding to $\Delta\log\alpha>0.02$) are often sufficient to achieve adequate separation of an incompletely resolved band-pair. The question then is: is it possible to anticipate whether a given variable (e.g., %B, temperature, change of solvent) is likely to provide the required change in selectivity for a given sample? When this is the case for different variables, the final choice of variable can be decided with reference to Table 2.

Quantitative assessments of selectivity as a function of sample type have been discussed previously for solvent strength (%B) [11,15] and temperature [15]. For a given sample, an average value of selectivity can be determined: $\Delta\log\alpha$, which represents the average (1 S.D.) change in $\log\alpha$ for each band-pair in the sample, as a result of a maximum change in either temperature (by 60°C) or gradient steepness (by a factor of 10). In this paper, we will designate different kinds of selectivity according to the abbreviations of Table 1. For example, temperature-selectivity will be referred to as T-selectivity and defined as $\Delta\log\alpha(T)$, solvent-strength selectivity is b-selectivity and is defined as $\Delta\log\alpha(b)$, etc. In the present paper, this analysis of selectivity is extended to the additional variables in Table 1 for

neutral samples (which should apply also to ionized samples).

2.1. Solvent-strength (b) selectivity

For a binary-solvent mobile phase A/B, retention is related to the volume-fraction ϕ of B in the mobile phase as [16]

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

where k_w and S are constants for a given solute and $\phi = 0.01\%$B. A value of α for two adjacent bands 1 and 2 will change for a change in ϕ ($\Delta\phi$), provided that the S-values (S_1, S_2) are different:

$$\log \alpha(b) = \log (k_2/k_1)$$
$$= \log (k_{w2}/k_{w1}) - (S_2 - S_1)\Delta\phi$$
$$= (\text{constant}) - \Delta S\, \Delta\phi \quad (2)$$

Solvent-strength selectivity (change in α) is therefore determined by the change in ϕ ($\Delta\phi$) and the difference in solute S-values ($\Delta\phi$).

If a plot of S for the components of a sample vs. retention time (for some value of ϕ and other conditions the same) yields no deviations δS of data points from a best-fit curve through the data, then $\Delta\log\alpha = 0$ and a change in ϕ will be unable to change α for two bands that initially overlap completely ($\alpha = 1.00$ for some value of %B). This is usually the case for a sample composed of compounds formed from identical repeating units (homologs, benzologs, oligomers, etc.), as illustrated in Fig. 1a for eight 1-nitroalkane homologs. Behavior as in Fig. 1a is usually not the case for other samples; e.g., Fig. 1b for a mixture of six steroids. For samples where δS is not zero and for a maximum change in %B (such that values of k change by 10-fold), the average change in α for a given sample is [15]

$$\Delta\log\alpha(b) = \Delta S\, \Delta\phi = 1.4\delta S/S^* \quad (3)$$

S^* is the average value of S for the entire sample. It should be noted that the average change in α due to a change in ϕ can be greater than predicted by Eq. (3), because Eq. (3) is corrected for any overall trend of α vs. ϕ as ϕ increases (see discussion of [15]).

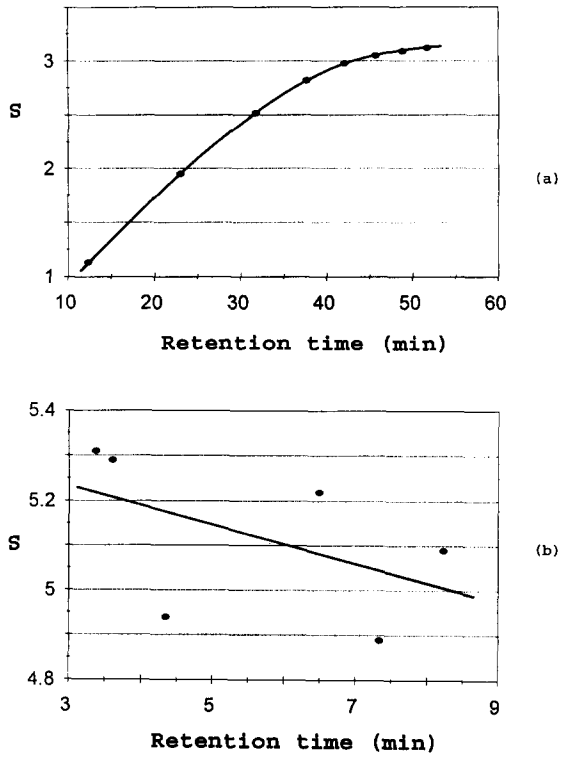


Fig. 1. Determination of solvent-strength (b) selectivity. Plots of S [Eq. (1)] vs. retention time. (a) 1-Nitroalkane solutes, ACN/water gradient, C_{18} column [15]; (b) steroid solutes (Table 4), 56% methanol/water, C_8 column. See text and Refs. [11,15] for details.

Values of S for two sample compounds can differ as a result of differences in their molecular structure [16]. It can be expected that a change in %B (or any variable known to affect selectivity) will have a smaller effect on α when the two compounds are more similar in terms of molecular structure. A corollary conclusion is that the more similar two molecules are, the more difficult will be their separation, and the more important the use of a variable that is capable of larger changes in α. As an example, a homologous series has a value of $\Delta\log\alpha(b) = 0$. Likewise, samples whose compounds differ only in the degree or type of alkyl substitution, or in the presence of other nonpolar groups in the sample molecules, are expected to have small values of $\Delta\log\alpha(b)$.

2.2. Temperature selectivity

Isocratic retention varies with absolute temperature T as

$$\log k = A - B/T \quad (5)$$

where A and B are constants for a given compound, other conditions constant. Temperature-selectivity $\Delta\log\alpha(T)$ can be determined for a given sample in the same way [15] as for $\Delta\log\alpha(b)$:

$$\Delta\log\alpha(T) = 0.0007\,\delta B. \quad (6)$$

Values of δB, analogous to δS, can be determined in similar fashion (from plots of B vs. retention time).

2.3. Solvent-type selectivity

A change of one B-solvent for another leads to changes in selectivity that will be referred to as *St*-selectivity and defined as $\Delta\log(St)$: the average change in $\log\alpha$ for each pair of adjacent bands in the sample (1 S.D.) when one B-solvent is substituted for another (while keeping average sample retention approximately the same by adjusting %B). Values of $\Delta\log\alpha(St)$ can be obtained from a plot of $\log k$ for one B-solvent vs. $\log k$ for a second B-solvent, as illustrated in Fig. 2 from a study reported in 1978 [17]. The deviation of values of $\log k$ ($\delta\log k$) from a best-fit curve through plots such as this (not shown in Fig. 2) are analogous to deviations of S or B from plots vs. retention time. In similar fashion (cf. treatment of [15]), a value of $\Delta\log\alpha(St)$ can be derived:

$$\Delta\log\alpha(St) = 1.4\,\delta\log k \quad (7)$$

The value of $\delta\log k$ from plots as in Fig. 2, is the average of the standard error in values of x and y.

2.4. Column-type selectivity

If retention data are obtained for two different columns (e.g., C_8 vs. cyano) and plotted as in Fig. 2,

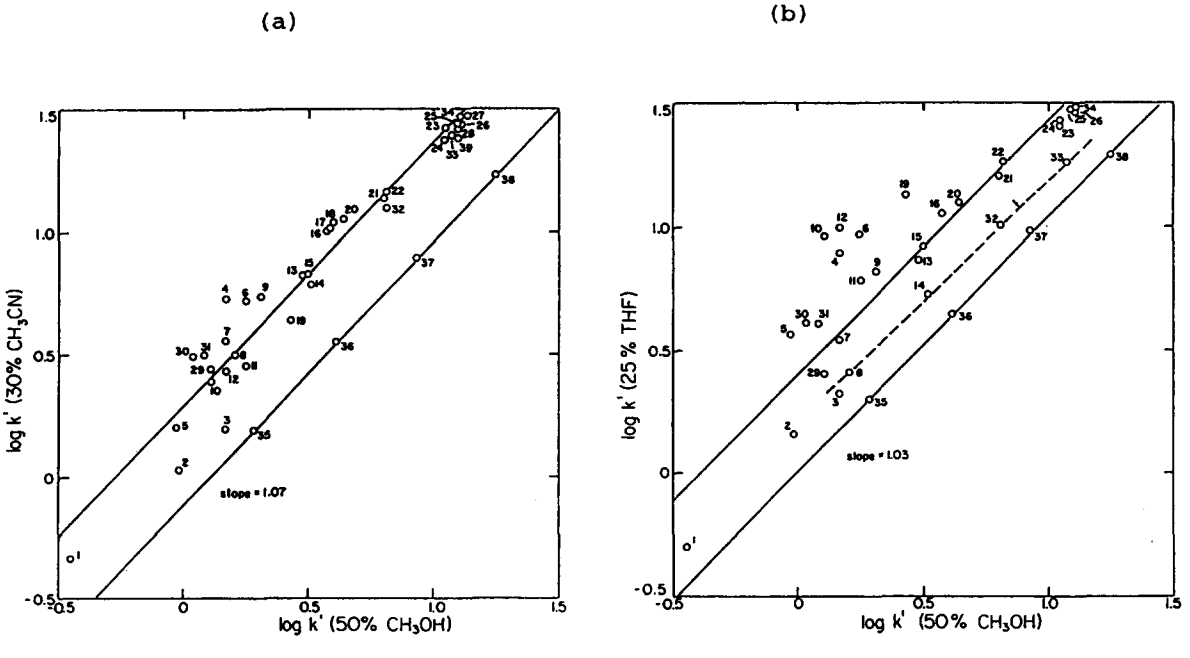


Fig. 2. Determination of solvent type selectivity ($\Delta\log\alpha$) from plots of $\log k$ for two separations with different B-solvents. (a) ACN vs. MeOH; (b) THF vs. MeOH. Substituted benzenes as sample. Reprinted from Ref. [17] with permission.

a value of column-type selectivity can be obtained in similar fashion [cf. Eq. (7)]:

$$\Delta\log\,\alpha(C) = 1.4\,\delta\log\,k \qquad (8)$$

2.5. Selectivity arising from a change in pH or ion-pair-reagent concentration

These selectivity effects are different in two regards. First, they apply only to ionized or ionizable analytes. Second, if the nature of two adjacent bands is known (neutral, acidic or basic), predictable changes in selectivity can be achieved by a change in pH and/or ion-pair-reagent concentration [18]. These changes in selectivity are often quite large, so that a quantitative assessment of values of $\Delta\log\,\alpha$ is less useful.

3. Results and discussion

Data reported elsewhere (see Tables 3–6) allow us to calculate values of $\Delta\log\,\alpha$ for the same samples and two different variables, thereby providing a direct comparison of the effectiveness of different variables in changing values of α. These data also provide some insight into the the role of the sample in determining values of $\Delta\log\,\alpha$ for a given sample.

3.1. Solvent strength vs. temperature selectivity

Data reported in Refs. [12,13] allow this comparison for several different samples. These data are summarized in Table 3 with two sample characteristics that relate to the "difference" between sample compounds: (a) the range in the number of polar substituents for molecules in each sample (x_1) and (b) the number of different polar substituents in the sample (x_2) (cf. discussion of [17]). As an example, consider the "pharmaceuticals" sample of Table 3. These compounds have either one or two polar substituents in the molecule, so $x_1 = (2-1) = 1$. There are four different polar substituent groups represented in the sample, and no one of these groups is found in every compound, so $x_2 = 4$. If one polar group is present in all the sample compounds, as in the fatty acid methyl ester sample of Table 2, this group is not counted, because it does not

Table 3
Relative ability of different variables to change selectivity: solvent strength (b) vs. temperature (T)

Sample	ΔLog α		Sample type[a]	
	b	T	x_1	x_2
Aromatic hydrocarbons [13]	0.05	0.02	0	0
Fatty acid methyl esters [13]				
ACN as B-solvent	0.02	0.01	0	0
MeOH as B-solvent	0.04	0.01	0	0
Carotenoids[b] [13]	0.04	0.13	3	3
Pharmaceuticals [13]	0.17	0.03	1	4
Nonbasic drugs [13]	0.24	0.10	3	5
Benzoic acids [12]				
Un-ionized	0.21	0.07	0	5
Ionized	0.13	0.11	0	5
Anilines [12]				
Un-ionized	0.12	0.09	0	5
Ionized	0.25	0.07	0	5
Basic drugs [12]	0.36	0.11	3	5
Herbicides [12]	0.21	0.14	5	3
rhGH peptides [12]	0.31	0.35	30[d]	5
rt-PA peptides [12]	0.30	0.32	30[d]	5
Chlorophylls[b] [12]	0.17	0.21	1	3
Average	0.17	0.12		
Alkylbenzenes[c] [11]	0.00		0	0
Chlorobenzenes [11]	0.01		0	0
Chlorotoluenes [11]	0.00		0	0
Chloronaphthalenes [11]	0.02		0	0
Chlorobiphenyls [11]	0.01		0	0
o-Phthaldehyde amino acids [11]	0.02		1	5
Steroids [11]	0.03		1	1
Substituted aromatics				
Ref. [7]	0.03		0	5
Ref. [19]	0.13		2	5

[a] x_1, range in number of polar groups among different compounds; x_2, number of different polar groups in the sample; maximum value of x_2 arbitrarily set equal to 5.
[b] A more complex mobile phase was used for these samples containing methanol and methyl-*tert*.-butylether.
[c] Data from cited references used to determine $\Delta\log\,\alpha(b)$ from Eq. (3).
[d] Estimated value.
Data of Refs. [7,11–13,19]. Acetonitrile (ACN) or methanol (MeOH) as B-solvent except where noted.

Table 4
Relative ability of different variables to change selectivity: solvent strength (*b*) vs. solvent type (*St*)

Sample	ΔLog α[a]					
	b			*St*		
	ACN	MeOH	THF	A/M	A/T	M/T
Substituted benzenes [7]	0.07	0.08	0.12	0.08	0.14	0.13
Herbicides [11]	0.11	0.14	0.19	0.20	0.24	0.31
Substituted aromatics [19]	0.19	0.10	0.11	0.15	0.20	0.22
Steroids [11]	0.03	0.03	0.05	0.06	0.18	0.14
Average	0.10	0.09	0.12	0.12	0.19	0.20
Nitro compounds [11]	0.10	0.12	—	0.05		
Substituted benzenes [17]	—	—	—	0.15	—	0.20
Substituted benzenes [20]	—	—	—	0.16	0.15	0.19

Data of Refs. [7,11,17,19,20].
[a] ACN, MeOH and THF refer to different solvents for a change in %B; A/M, A/T and M/T refer to comparisons of different solvents for a similar range in values of *k*; A is ACN, M is MeOH and T is THF.

Table 5
Relative ability of different variables to change selectivity: column type (*C*) vs. solvent strength (*b*)

Sample	Δlog α[a]					
	C			*b*		
	C_8/Ph	C_8/CN	Ph/CN	C_8	Phenyl	Cyano
Substituted benzoic acid	0.05	0.08	0.08	0.07	0.06	0.05
Herbicides	0.07	0.05	0.04	—	—	—

Data of Ref. [21].
[a] B-solvent is MeOH for benzoic acid sample and ACN for herbicide sample; C_8/Ph refers to a change from a C_8 to a phenyl column; C_8/CN refers to a change from a C_8 to a cyano column; Ph/CN refers to a change from a phenyl to a cyano column.

Table 6
Relative ability of different variables to change selectivity: column type (*C*) vs. solvent type (*St*)

Δlog α[a]											
C			*St*								
C_8/Ph	C_8/CN	Ph/CN	C_8			Phenyl			Cyano		
			A/M	A/T	M/T	A/M	A/T	M/T	A/M	A/T	M/T
0.05	0.15	0.12	0.05	0.19	0.21	0.05	0.18	0.21	0.05	0.12	0.13

PTH amino acid sample, data of Ref. [22].
[a] ACN as B-solvent for column comparisons; C_8/Ph refers to a change from a C_8 to a phenyl column; C_8/CN refers to a change from a C_8 to a cyano column; Ph/CN refers to a change from a phenyl to a cyano column; A/M, A/T and M/T refer to comparisons of different solvents for a similar range in values of *k*; A is ACN, M is MeOH and T is THF.

comprise a "difference" among various sample solutes. Chloro (and other halogen) substituents, because of their hydrophobicity and retention similar to methylene groups [17], are not counted as "polar" groups in determining values of x_1 or x_2. The compounds of Table 3, for a given sample, may also differ in their carbon skeletons, but these differences are ignored here.

Values of $\Delta\log\alpha$ appear to correlate with sample characteristics x_1 and x_2, as expected. For the first set of samples of Table 3 (from Refs. [12] and [13]),

$$\Delta\log\alpha(b) = 0.045 + 0.0035(\pm 0.0029)x_1 + 0.034(\pm 0.016)x_2 \quad (9)$$

with $r^2 = 0.54$, and a standard deviation of $Y = 0.09$ ($0.02 < Y < 0.36$). Similarly,

$$\Delta\log\alpha(T) = 0.025 + 0.0073(\pm 0.0015)x_1 + 0.0188(\pm 0.0081)x_2 \quad (10)$$

with $r^2 = 0.85$ and a standard deviation of $Y = 0.05$ ($0.01 < Y < 0.36$). It appears that differences in the kinds of polar substituents (x_2) are more important than differences in the number of polar substituents (x_1) in affecting selectivity [Eq. (9)].

Table 3 lists several additional samples (from Refs. [7,11,19]) for which only values of $\Delta\log\alpha(b)$ are available. Application of Eq. (9) to the prediction of these values gives a standard deviation of ± 0.03 units, which is comparable to the error in Y for the original data set [± 0.07 for a wider range in $\Delta\log\alpha(b)$].

For the same set of samples in Table 3 (from Refs. [12,13]), the average values of $\Delta\log\alpha$ are $0.17(b)$ and $0.12(T)$. Thus, on average, a change in %B will produce a 1.4-fold greater change in selectivity ($\Delta\log\alpha$) than will a change in temperature. However, individual samples can differ markedly from this average.

It has been shown previously [12,13] that there is little correlation between b- and T-selectivities, so these two variables can be used together for a more effective optimization of selectivity.

3.2. Solvent-strength vs. solvent-type selectivity

Data from the literature can be used to compare values of $\Delta\log\alpha(b)$ and $\Delta\log\alpha(St)$ for the same samples. In each case, retention values (k or retention time) are reported as a function of %B for the three common B-solvents: ACN, MeOH and THF. The application of Eq. (3) and Eq. (7) to these data is summarized in Table 4. Not enough different samples were used to justify correlations with sample characteristics x_1 and x_2, as for the samples of Table 3 and Eqs. (9,10). However, it is reasonable to assume that samples with larger values of x_1 and/or x_2 will yield larger values of $\Delta\log\alpha$ for any variable used to change selectivity (as noted in [17]). Average values of $\Delta\log\alpha$(b) in Table 4 are similar for ACN and MeOH as B-solvent (0.10, 0.09), but are slightly greater for THF (0.12). Therefore, the use of THF instead of these other solvents may yield somewhat larger changes in α for a change in %B. St-selectivity is greater for a change from either ACN or MeOH to THF (avg. values: 0.19, 0.20) than for a change from ACN to MeOH (0.12), by a factor of 1.6. The special advantage of THF for a change in selectivity was noted as early as 1978 [17], and is today widely appreciated by practical workers.

The correlation of S vs. $\Delta\log k$ for a change in solvent type was tested for several samples. Values of r^2 ranged from 0.0 to 0.2, suggesting little correlation of the selectivity effects for these two variables.

3.3. Column-type vs. solvent-strength and solvent-type selectivity

Tables 5 and 6 summarize values of $\Delta\log\alpha$ for C-, b- and/or St-selectivities. These data show an average larger value of $\Delta\log\alpha$ for a change from a C_8 (or presumably C_{18} [23]) to a cyano column (0.09), vs. a change from a C_8 (or C_{18}) to a phenyl column (0.06). Values of $\Delta\log\alpha$ for a change in column type are similar to values for a change in solvent-strength and smaller than a change in solvent-type. Solvent-strength or solvent-type selectivity is reduced by about 1/3 when a cyano column is used, vs. the use of either a C_8 or a phenyl column. The columns compared in these studies are all from the same manufacturer, and it is possible that comparisons among columns from different manufacturers (involving differences in bonded-phase concentration and other characteristics) would give different results.

These conclusions agree with those of Ref. [23], which also found that column-type selectivity tends to correlate with solvent-strength selectivity. In view of this correlation, simultaneous changes of column type and %B are predicted to be less useful. However, in one case [21] the variation of %B for different column types did result in a substantial improvement in separation.

3.4. Relative selectivity for different variables

Table 7 is an approximate attempt to combine the data of Tables 3–6 in order to arrive at the relative effectiveness of different ways of changing selectivity. It is assumed that a value of $\Delta\log\alpha$ is the product of some intrinsic characteristic of the variable and some property of the sample (e.g., values of x_1, x_2). This implies that the ratio R of $\Delta\log\alpha$ values for two variables (e.g., T and b) will be roughly constant for the same sample. Values of R for different pairs of variables can then be compared. While very different values of R are found in Table 3 for individual samples, the large number of samples should tend to average out these variations for comparisons of b- vs. T-selectivity. A smaller number of samples comprise the comparisons of Tables 4–6, and average values of R from these samples must be considered less reliable.

Table 7
Summary of relative ability of different variables to change selectivity

Variable[a]	Relative value of $\Delta\log\alpha$
Temperature	
ACN, C_8 or C_{18}	0.5
Solvent strength (%B)	
ACN, C_8 or C_{18}	0.7
MeOH, C_8 or C_{18}	0.8
THF, C_8 or C_{18}	1.0
Column type (A, M, or T)	
C_8/phenyl	0.6
C_8/cyano	1.0
Phenyl/cyano	1.0
Solvent type	
A/M, C_8, C_{18}, phenyl	(1.0)[b]
A/T, C_8, C_{18}, phenyl	1.7
M/T, C_8, C_{18}, phenyl	1.8
A/M, cyano	1.0
A/T, cyano	1.0
M/T, cyano	1.1

Approximate ranking based on data of Tables 3–6.
[a] For each variable, the B-solvent (A=ACN, M=MeOH, T=THF) or column type (C_8, C_{18}, phenyl, cyano) is specified.
[b] Reference value; all values calculated relative to this.

With the latter caveat, what does Table 7 tell us? If an initial chromatogram contains two overlapping bands and our goal is to increase the resolution of these two bands as much as possible, a single variable used to change selectivity should have as large a value of $R=\Delta\log\alpha$ (relative) as possible. The best choice from Table 7 is a change from either ACN or MeOH to THF as B-solvent (relative $\Delta\log\alpha=1.7$–1.8). However, in many cases a smaller change in α will suffice, and a more favorable variable according to Table 2 might then be considered. When beginning method development, it is preferable to choose variables that are free from practical or other problems, as summarized in Table 2. The preferred choices in this case are solvent strength, temperature or a B-solvent composed of varying proportions of ACN and MeOH [7,8]. Selectivity based on changes in these variables can be further increased by changing two or three of these variables simultaneously. There is also the possibility that, for a given separation, some or all of these latter variables will prove more effective, despite their usual lesser effect on selectivity.

3.5. Simultaneous use of two variables

Changes in two variables at the same time can be used to increase control over selectivity, providing that selectivity effects for the two variables are uncorrelated. Table 8 summarizes what is known about the correlation of selectivity effects for different pairs of variables. The possible correlation of temperature and %B (or b) has been examined in detail [12,13] and found to be unimportant, as has the correlation of %B and solvent type (this paper). Data for other pairs of variables are less compelling. Column type selectivity is most pronounced for columns of different "strength" [23], e.g., C_{18} (strong) vs. cyano (weak). As a result, the use of columns of different strength necessarily involves a change in %B to maintain $1<k<10$. It has been

Table 8
Correlation of selectivity effects for different variables

Variables	Comment
%B–temperature	Effects appear to be uncorrelated [12,13]
%B–solvent type	Effects appear to be uncorrelated (this paper)
%B–column type	Effects appear to be correlated [23]
%B–pH	Effects appear to be correlated for basic samples [24]
%B–ion-pair-reagent concentration	Effects are correlated (see text)
Temperature–solvent type	Correlation not expected
Temperature–column type	Correlation not expected
Temperature–pH	Correlation not expected
Temperature–ion-pair-reagent concentration	Correlation expected
Solvent type–column type	Possible correlation
Solvent type–pH	Correlation not expected
Solvent type–ion-pair-reagent concentration	Correlation not expected
Column type–pH	Correlation not expected
Column type–ion-pair-reagent concentration	Correlation not expected
pH–ion-pair-reagent concentration	Correlation expected

estimated [23] that about 75% of column-type selectivity can be attributed to this change in %B, i.e. %B and column-type selectivity are definitely correlated. For ionic samples, a change in ion-pair-reagent concentration should lead to changes in selectivity similar to those produced by a change in %B or temperature, because the uptake of the reagent by the column will depend on %B [25]. Because selectivity effects for two variables are correlated to some degree, this does not mean that their combined use in method development will never be worthwhile. Selectivity effects arising from changes in pH and ion-pair-reagent concentration are generally similar for ionic samples, yet these two variables are often varied together for improved control over selectivity. In this case, selectivity effects are large and additive for each variable, and the use of pH and reagent concentration together provides more control over band spacing. For the case of other pairs of variables, values of $\Delta \log \alpha$ do not correlate exactly, so there will always be some incremental advantage in the use of the second variable.

4. Conclusions

A HPLC method development strategy for all but "easy" samples will require changes in selectivity. Different variables can be used for this purpose, either alone or in combination. Those variables that are convenient to use and which do not cause problems during method development or subsequent use of a routine procedure (%B, ACN/MeOH mixtures and temperature), tend to be less effective for effecting changes in band spacing. Thus, a compromise is necessary when choosing variables for HPLC method development. The relative ability of different variables to achieve changes in α was determined in this study.

For most samples, achieving an adequate HPLC separation is not difficult, and it is therefore recommended to first vary such conditions as %B, temperature and solvent type (MeOH or ACN) either alone or in combination. If this strategy is unsuccessful, variables such as solvent type (MeOH, ACN and THF), column type, pH and/or ion-pair-reagent

concentration can be considered next. When using two or more variables for the simultaneous control of selectivity, it is preferable to select variables whose selectivity effects are uncorrelated. The relative correlation of different pairs of variables has been reviewed.

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References

[1] J.C. Berridge, Techniques for the Automated Optimization of HPLC Separation, Wiley, New York, 1985.
[2] P.J. Schoenmakers, Optimization of Chromatographic Selectivity, Elsevier, Amsterdam, 1986.
[3] J.L. Glajch and L.R. Snyder (Editors), Computer-assisted Development for High-performance Liquid Chromatography, Elsevier, Amsterdam, 1990 [J. Chromatogr., 485 (1989)].
[4] L.R. Snyder, J.L. Glajch and J.J. Kirkland, Practical HPLC Method Development, Wiley-Interscience, New York, 2nd ed., 1996.
[5] J.L. Glajch, J.J. Kirkland, K.M. Squire and J.M. Minor Jr., J. Chromatogr., 199 (1980) 57.
[6] J.S. Kiel, S.L. Morgan and R.K. Abramson, J. Chromatogr., 485 (1989) 585.
[7] J.A. Lewis, L.R. Snyder and J.W. Dolan, J. Chromatogr. A, 721 (1996) 15.
[8] L.R. Snyder, Today's Chemist at Work, 5 (1996) 29.
[9] A. Drouen, J.W. Dolan, L.R. Snyder, A. Poile and P.J. Schoenmakers, LC·GC Int. Mag. Liq. Gas Chromatogr., 9 (1991) 714.
[10] L.R. Snyder, J.W. Dolan and D.C. Lommen, J. Chromatogr., 485 (1996) 65
[11] L.R. Snyder, M.A. Quarry and J.L. Glajch, Chromatographia, 24 (1987) 33.
[12] P.L. Zhu, J.W. Dolan and L.R. Snyder, D.W. Hill, L. Van Heukelem and T.J. Waeghe, J. Chromatogr. A, (Part III) submitted for publication.
[13] P.L. Zhu, J.W. Dolan and L.R. Snyder, N.M. Djordjevic, D.W. Hill, J.-T. Lin, L.C. Sander, L. Van Heukelem and T.J. Waeghe, J. Chromatogr. A, (Part IV) submitted for publication.
[14] J.A. Lewis, D.C. Lommen, W.D. Raddatz, J.W. Dolan and L.R. Snyder, J. Chromatogr., 592 (1992) 183.
[15] P.L. Zhu, L.R. Snyder and J.W. Dolan, J. Chromatogr. A, (Part II) submitted for publication.
[16] K. Valko, L.R. Snyder and J.L. Glajch, J. Chromatogr. A, 656 (1993) 501.
[17] N. Tanaka, H. Goodell and B.L.Karger, J. Chromatogr., 158 (1978) 233.
[18] A. Bartha and G. Vigh, J. Chromatogr., 485 (1989) 383.
[19] P.J. Schoenmakers, H.A.H. Billiet and L. De Galan, J. Chromatogr., 185 (1979) 179.
[20] S.R. Bakalyar, R. McIlwrick and E. Roggendorf, J. Chromatogr., 142 (1977) 353.
[21] J.J. DeStefano, J.A. Lewis and L.R. Snyder, LC–GC Int. Mag. Liq. Gas Chromatogr., 10 (1993) 130.
[22] J.L. Glajch and J.J. Kirkland, J. Chromatogr. Sci., 25 (1987) 4.
[23] P.E. Antle, A.P. Goldberg and L.R. Snyder, J. Chromatogr., 321 (1985) 1.
[24] J.A. Lewis, J.W. Dolan and L.R. Snyder, J. Chromatogr., 592 (1992) 197.
[25] A. Bartha, G. Vigh and Z. Varga-Puchony, J. Chromatogr., 499 (1990) 423.