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A Visual Approach to Stationary Phase Selectivity Classification Based on the Snyder-Dolan Hydrophobic-Subtraction Model

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Abstract

A novel type of stationary phase selectivity classification “triangle” has been developed based on the Snyder-Dolan (S-D) Hydrophobic-Subtraction Model, wherein the apices of a set of four triangles represent the relative contributions of steric hindrance (χ_S), hydrogen bonding acidity (χ_A), hydrogen bonding basicity (χ_B), cation exchange capacity (χ_C) to selectivity. We found that “effective selectivity” of a stationary phase is mathematically given by the *ratio* of system dependent interaction coefficients but not their absolute values. Thus by normalizing the S*, A, B and C terms of the S-D model by H, we were able to obtain four parameters which fully define the chromatographic selectivity of the stationary phases. By examining the parameters in groups of three, we can represent all the result in a set of four “selectivity triangles”. The distinctive feature of this approach compared to the S-D phase classification scheme is that it allows the visualization of column selectivity by plotting three-dimensional data in a two-dimensional space. Moreover, it very clearly shows that the RPLC columns thus far characterized cover only a small fraction of separation selectivity space leaving a great deal of room for researchers to develop novel RPC materials. Various applications of these “selectivity triangles” will be discussed in this paper.

Keywords

HPLC; Stationary phase; Reversed phase; Selectivity; Classification Scheme

1. Introduction

The ultimate goal of any separation is to achieve acceptable resolution (R_s) in a reasonable time. As the most essential metric of separation power in chromatography, R_s can be expressed in terms of three parameters: efficiency (N), chromatographic selectivity (α) and retention (k'), where for simple mixtures the most significant impact comes from selectivity [1–3]. Small changes in chromatographic selectivity lead to big changes in resolution. However, there are currently a very large number of phases available for reversed-phase chromatography (RPC) and many show selectivities different from that of a “typical” reversed phase. Extensive work has been done by many research groups, including: Martin, Horvath, Martire, Snyder, Guiochon, Dorsey and Carr [4–22] to understand and rationalize mechanism of retention. Consequently quite a few approaches have been proposed to assess

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differences in phase selectivity of RPC materials [15,16,18,23–54]. One of the most widely used methods [35,36,55,56] is a comprehensive scheme developed by Snyder and his collaborators [15,16,46–52], specifically for the purpose of classifying reversed phases for differences in their selectivities. The work was initiated by examining a few phases using a large set of some 60 probe solutes including non-polar, polar, acidic and basic solutes spanning a reasonable range in shape and size in a fixed eluent [15]. This led to the use of a more manageable set of 16 very judiciously selected but chemically simple probe solutes which were then used to study over 350 different commercial RPLC materials and characterize them with a set of 5 phase parameters by fitting relative retention data of the 16 probe solutes to the equation [15]:

$$\log (k'/k'_{\text{EB}}) \equiv \log \alpha = \eta'H - \sigma'S^* + \beta'A + \alpha'B + \kappa'C \quad (1)$$

Here, ethylbenzene is a “neutral” reference compound. The five phase coefficients represent the five dominant solute-column interactions elucidated by the Snyder group, specifically: hydrophobicity (H), steric resistance (S*), hydrogen-bond acidity (A), hydrogen-bond basicity (B), and cation-exchange activity (C). The first four stationary phase parameters have been shown to be relatively independent of the mobile phase, although the C-term is very pH dependent and C is available at two pHs (2.8 and 7.0) [15,51]. What is important to understand is that a large fraction of all commercial phases have been studied and almost every reversed phase of every major producer is well fitted by this scheme ($\pm 3\%$ S.D).

In this classification scheme two columns are compared by looking at the scatter in a plot of $\log k'$ vs. $\log k'$ for the above 16 solutes. The scatter is measured by the standard error (s.e.) in a linear least square fit of one column vs. the other. The values of the phase coefficients are then deduced based on the scatter in such plots. Note that Snyder's use of the term “selectivity” in this context is different from what is normally meant by “chromatographic selectivity (α)”. The chromatographic selectivity factor is defined as the ratio of retention factors for two solutes. Two phases or columns would have identical *phase selectivity* when a plot of $\log k'$ vs. $\log k'$ has no scatter even if all species were perfectly well separated.

A very useful feature of the hydrophobic-subtraction approach is that a single parameter called the “column selectivity function F_s ” has been defined [15], and can be used to quantitatively compare the selectivity of any two phases:

$$F_s = \{12.5(H_2 - H_1)^2 + 100(S^*_2 - S^*_1)^2 + 30(A_2 - A_1)^2 + 143(B_2 - B_1)^2 + 83(C_2 - C_1)^2\}^{1/2} \quad (2)$$

This equation is based upon the assumption that the differences in phase selectivity for any two columns can be measured by the Euclidean distance between the two phases in the five dimensional space defined by the five phase coefficients. Therefore, the smaller is the distance as measured by F_s , the more similar are the two phases. In the extreme case when two phases are very close ($F_s \leq 3$), the two can be considered to be chromatographically “equivalent” in terms of phase selectivity. On the other hand, two columns with a big F_s are more widely separated; correspondingly they are more different in terms of phase selectivity. This turns out to be the most important application of this model since it allows an easy selection of two phases which are nearly interchangeable and conversely two phases that are dramatically different. Both attributes are very useful in developing new analytical methods as has been demonstrated for a dozen different routine analyses [57].

While the F_s value is undoubtedly useful for identifying columns with *similar phase selectivity*, its application in selecting “orthogonal” phases is still challenging due to some theoretical and practical limitations:

1. Theoretically, it is calculated based upon the *difference* between the five individual column coefficients for two phases. However, in previous work [58] what we at that time called the “effective selectivity”, which is actually the “phase selectivity” as used by Snyder, was mathematically proven to be given by the *ratios* of the system (phase) dependent interaction coefficients and not by their *absolute* values. This is precisely in the same spirit as the studies of Abraham and Poole [25,43,59], and Sandi and Szepeszy [60,61] on the classification of reversed phases selectivity, where ratios of system coefficients were used.

The validity of this concept becomes evident when we compare three hypothetical columns having the coefficients given in Table 1. *These three phases have different absolute values but the same ratios of phase coefficients.* Values of F_s from eq. 2 are shown for phases B and C, each of which is compared with column A. As indicated by Snyder [15], a F_s value of 38 ought to be very different. On this basis the values of $F_s=40$ for column B and $F_s=80$ for column C ought to produce very different selectivities. However, when we plot the retention data for the 16 standard probes, for all three columns, we observe an exactly linear relationship ($r^2=1.000$, $se=0.000$) between the data sets which indicates that the two pairs have exactly the same phase selectivity. In the context of the Snyder-Dolan Hydrophobic-Subtraction model it is the scatter that indicates that the phases have different phase selectivities [8,15]. Thus the example of Figure 1 confirms that *the differences in phase selectivity only exist when the ratios of phase coefficients differ, not when their absolute values differ.* This showed us that differences in phase selectivity ought to be compared based on the *ratios* of the system dependent interaction coefficients but not on their *absolute* values. The significance of this conclusion is that it reduces the number of phase characteristics needed to define phase selectivity. In turn this allows us to represent the properties of all stationary phases in terms of a set of four “triangle plots” similar to the very well known solvent classification triangle of Snyder’s [4,31,62]. The four phase characteristics in sets of three can thus be displayed in two dimensional graphs.

2. Clearly F_s is a composite parameter as it depends on all the column characteristics and therefore it will indicate that two phases will behave differently even when the solute set of interest does not contain solutes which differ in the selectivity characteristic chiefly responsible for the differences in two phases. The importance of this concept were first noted by Gilar et al. [63] and was further illustrated in a recent study by Marchand et al. [35]. As an example, suppose we were to choose a pair of columns having a big F_s value due mainly to differences in the phases hydrogen-bond acidity and examine them with a set of solutes all having similar hydrogen-bonding basicity (B), but differing in hydrophobicities. It is very likely that the separation on these two columns will not be different because the relative retention factors will be dominated by the solute hydrophobicity. The foregoing example challenges the use of F_s alone for the selection of orthogonal columns. To solve this problem, a modified approach was recently proposed by Snyder and coworkers; they now use a solute set dependent correction factor [15]. The corrected F_s can be adjusted for use with a specific solute set; this greatly improved the practical utility of the method. Another limitation associated with any single-parameter measure of phase selectivity is that, as previously concluded by Lesellier [64], it does not tell us the chemical cause of the difference. It simply says the two phases are different.

3. A single table of F_s values relative to a fixed reference column does not allow two or more test columns to be compared to one another. It is evident that two test columns could have exactly the same F_s value relative to a fixed reference yet be quite different from one another [64]. This makes the application of the approach somewhat difficult, especially when columns have to be chosen from over 350 commercial phases. Recently, this problem was greatly reduced when the “Column Match” software developed by Snyder et al.[65], became available. However, there remains a need to develop a method which allows a global view of all stationary phases.

In this work we describe a simple transformation of column parameters based upon the “effective phase selectivity” concept. The data can then be presented as a set of four “selectivity triangles”, which we feel is a convenient and useful way to visualize and compare all phases simultaneously. We caution the reader that the triangle scheme thus derived is mainly for classifying stationary phase selectivities. It does not indicate the absolute retention time, a very important parameter to consider when choosing a column for separations. However, it is our understanding that changes in %B do not substantially effect the S-D method [15,51]. Thus when two phases have the same selectivity, which is based on relative retentivity, but show rather different absolute retentivities, what can be done to make column A have the same absolute retentivity as column B is simply to appropriately adjust the volume fraction of the strong component of the eluent; This will have only a small effect on the phase selectivity but will have a large effect on absolute retention.

2. Development of the Stationary Phase Triangle Concept

The first step in our approach is to calculate the ratios of Snyder’s column parameters. As discussed above, the “effective selectivity” is related to the ratio of the system dependent interaction coefficients. Thus, to compare the selectivities of different stationary phases, we used their S^*/H , A/H , B/H , C/H ratios rather than their absolute H , S^* , A , B and C values. Mathematically we are free to use any of the five parameters as the denominator to calculate the ratios. For convenience and to avoid large ratios, we decided to take the ratio by dividing by H ; its average is close to unity and it has minimal dispersion compared to the other parameters as clearly shown in Figure 2.

After obtaining S^*/H , etc. the second step was the normalization of these ratios. As defined by eq. 3, we arbitrarily adjusted the ratios by first subtracting the minimum value and then rescaling using a set of weighting factors for each term.

$$X_i = (I - I_{\min}) * \phi_i$$

$$i = \frac{S}{H} \text{ or } \frac{A}{H} \text{ or } \frac{B}{H} \text{ or } \frac{C}{H} \quad (3)$$

The last step in developing the new coordinate, to be denoted as three χ values, is normalization to a scale of 0 to 1.000. This is done as follows:

For the S^* -B-C triangle, the three scales (χ_j) are defined as:

$$\chi_j = \frac{j}{X_S + X_B + X_C}$$

$$j = X_S \text{ or } X_B \text{ or } X_C \quad (4)$$

For the S^* -A-C triangle, the three scales (χ_j) are defined as:

$$\chi_j = \frac{j}{X_S + X_A + X_C}$$

$$j = X_S \text{ or } X_A \text{ or } X_C \quad (5)$$

For the S*-A-B triangle, the three scales (χ_j) are defined as:

$$\chi_j = \frac{j}{X_S + X_A + X_B}$$

$$j = X_S \text{ or } X_A \text{ or } X_B \quad (6)$$

Finally for the A-B-C triangle, the three scales (χ_j) are defined as:

$$\chi_j = \frac{j}{X_A + X_B + X_C}$$

$$j = X_A \text{ or } X_B \text{ or } X_C \quad (7)$$

It must be stressed that the selection of the weighting factors is important. Initially, we tested the use of a set of weighting factors chosen so that the same numerical change in two different normalized phase properties had the same size effect on phase selectivity as measured by the s.e. values described above. These weighting factors were obtained as described in Appendix I and are summarized in Table 2. The triangles thus derived can be used directly to measure the phase selectivity differences between stationary phases, wherein the distance between two axis (e.g. $\Delta\chi_C = 0.1$ vs $\Delta\chi_A = 0.1$) in the triangle give the same effect on phase selectivity as indicated by equal standard errors in linear correlations of $\log k'$ vs. $\log k'$. Unfortunately, as clearly shown in Figure 3, this approach to weighting results in a very "tight" clustering of all but the most extreme phases over a rather small fraction of the plot. One major reason for this is certainly the dominant effect of ionized silanols (C), which has been previously noted by Cruz and Euerby through their principle component analysis of over 30 stationary phases [40] and by Marchant and Dolan where the average contribution of individual parameters H, S*, etc to column selectivity were calculated and compared for over 350 phases [35,36]. In our experience, in those triangles which include the coulombic interaction (i.e. all but the S*-A-B triangle), the C term always far outweighs the other parameters and thus dominates the denominator leading to small χ_S , χ_A and χ_B value. This renders the characterization and comparison of phase selectivity very difficult due to the superimposition of so many data points given the large number of stationary phases to be compared.

Consequently an alternative approach to the choice of weighting factors (see eq. 8) was developed to standardize each individual parameter by rescaling over the range of the phases of interest. As shown in eq. 8 and listed in Table 2, the application of the new set of normalized parameters maximizes the spread of the data points over the triangle. This facilitates visual differentiation of the phases. The resulting triangles are shown in Figure 4. Comparison of the two approaches to the weighting issue discussed above (see Figures 3 and 4) clearly shows that there is very tight clustering of many phases in both cases but there is a significant increase in the number of phases that are not in the cluster when the second approach is used. Consider the S*-B-C triangle as an example, the number of phases that can be clearly differentiated by the first approach is no more than 12; whereas for the second approach, at least 18 extreme phases can be easily identified and the results are summarized in Table 4 and Figure 5. Since the triangles calculated with the second set of scaling factors provide a better view of the overall phase selectivity distribution as more individual phases can be recognized, we chose to use this approach to further classify the selectivity of the reversed phases. However, it should be noted that this approach does not allow quantitative

comparisons of the effect of differences in different phase parameters on phase selectivity as does the Snyder-Dolan F_s metric.

$$\phi_i = \frac{1}{I_{\max} - I_{\min}} \quad (8)$$

$$i = \frac{S}{H} \text{ or } \frac{A}{H} \text{ or } \frac{B}{H} \text{ or } \frac{C}{H}$$

In this study, we included 366 reversed phases *in toto*. Among them, 209 are type-B silica based alkyl silica phases, 58 are type-A silica based alkyl silica phases, 44 are EPG (i.e. polar embedded or polar endcapped) phases, 20 are phenyl phases, 21 are cyano phases, 7 are fluoro phases, 3 are zirconia based stationary phases and 4 are a specialized type of polymer coated silica phases recently developed in our lab [66–69]. All these stationary phases were evaluated under acidic conditions, where the mobile phase pH is 2.8 controlled by a 30 mM phosphate buffer as described by Snyder et al. [50].

3. Results and Discussion

The value of the triangle plots is self-evident. It is all too clear that a very large number of phases are clustered quite tightly in all four triangles. To put it another way *a huge fraction of the available space is under populated and certain regions are extremely over populated*. A large number of similar pairs of phases exist.

3.1 Chemical Interpretation of the Triangles

The new set of selectivity parameters (χ_S , χ_A , χ_B , χ_C) reflect the relative ability of the stationary phases to separate solutes based upon steric hindrance, hydrogen bonding acidity/basicity and coulombic interactions. Thus within the same triangle, phases with large χ_C value will interact strongly with positive charged solutes through coulombic interactions. Similarly, phases with large χ_A , χ_B will interact relatively more strongly with proton acceptors, proton donors via hydrogen bonding. However, as discussed above, the selectivity parameters are normalized (eq. 8) to the range of the respective non-normalized parameters. Thus we must not think that the same numerical change in two different normalized parameters will have the same quantitative effect on selectivity.

Since the intermolecular interactions are primarily determined by the nature of the functional groups present on the stationary phases, we expected to find phases with the same chemical functionality in the same region of the selectivity triangle. As seen in Figure 6(a), this is generally the case for type-B alkyl silica phases. The majority of type-B alkyl silicas, with the exceptions of Platinum EPS C8 and Purospher C18 [70] (see Figure 6a), are very tightly clustered in the center of the triangle. This is consistent with many previous studies [50,71] and the common belief that many alkyl silica phases based on high purity silica are not very different and nearly interchangeable. However, for those types of phases derivatized with different surface chemistry (e.g. cyano, phenyl, fluoro, embedded polar groups) or based upon different substrates (e.g. Type-A silicas), differences in coefficients are generally quite large. Examples of cyano, phenyl and EPG phases are shown in Figure 6(b), where significant variability is seen between the individual data points for all three groups of stationary phases.

For EPG phases, this can be rationalized, at least in part by the fact that a wide variety of chemistries (e.g. amide, urea, carbamate) have been used to make these phases and thus the selectivity of EPG-columns is expected to vary with the nature of the polar group and whether that group is embedded in the chain or used to end-cap the column [15,41]. Nevertheless, upon choosing three EPG phases derivatized with the same group (an amide), we see that they are widely distributed over the triangle plot (see Figure 6(b)). Also there are

significant differences in the example chromatograms shown in Figure 7. It is commonly believed that stationary phases with the same surface chemistry should have similar selectivity. However, the marked diversity observed here strongly suggests that the same surface chemistry does not guarantee the same phase selectivity. This is especially true for stationary phases with novel surface chemistries, as reported by many groups including Snyder and Marchand [46–49], Euerby and Petersson [72], and Neue and et al. [71].

We next compared stationary phases prepared with different chemical functionalities. More particularly type-B alkyl silica phases were compared with non-alkyl silica phases (e.g. cyano, phenyl, EPG, fluoro, Type-A), in this case, EPG phases (see Figure 8). Similarly, we expected to see different chemical types of columns occupying different positions in the triangles, reflecting their different chemical selectivities. However, it is surprisingly to see that some stationary phases from different chemical groups actually lie close to one another and some occupy nearly the same space. Similar results have been shown by Layne wherein the retention of acidic and basic solutes on EPG and ODS columns were compared [41]. This suggests that *different surface chemistries of stationary phases do not necessarily lead to different selectivity*. Clearly we must be cautious in using inferences based not on data but merely on differences in chemical functionality. This is well illustrated in Figure 9, where three columns, from very different chemical classes (i.e. an ACE AQ, an EPG phase; a Betasil Phenyl-Hexyl, a Phenyl phase; Bondclone C18, a Type-A alkylsilica phase,) were selected based on their proximity to one another as indicated by the similarity of their coordinates (χ_S , χ_B , χ_C) in Table 3. As anticipated, the separation of a sample mixture on these three columns are all very similar as suggested by their near unity r^2 values and small s.e. values and also in agreement with their F_s values.

Even though many stationary phases are strikingly similar, there are nonetheless a reasonable number of phases that do spread over a fairly wide area that offer the possibility of very big differences in selectivities. The virtue of the triangle approach is that it allows the easy visualization of these extreme phases. The most “extreme” phases in all four triangles are summarized in Table 4 and examples are labeled in the S-B-C triangle as shown in Figure 5. The results here agree very well with conclusions obtained by Dolan et al. in a recent study [36]. It is clear that most of the extreme phases are non-type B alkyl silica columns. *For the purpose of finding “orthogonal” separations this set of phases are probably the best candidates*. It should be understood that this is a complex issue and is still understudy by us.

3.2 Applications

3.2.1 Selection of “Equivalent” and “Orthogonal” columns—The “triangle approach” developed here can be used in various ways. Basically, it classifies the selectivity of stationary phases based on their positions in these triangle plots. In other words, stationary phases with similar selectivity will be located close to one another; while stationary phases with “orthogonal” selectivity will be very far apart. Thus selection of an “equivalent” or “orthogonal” column is readily achievable by comparing the coordinates of the columns in the triangles. An example of choosing “equivalent” columns is shown in Figure 9 and summarized in Table 3; the result is consistent with the prediction from this approach. An example of the selection of “orthogonal” columns is given in Figure 10 using Zorbax StableBond C18 as the initial column. As noted above, “extreme” phases should be very good candidates for orthogonal separations due to their markedly different selectivity. Here we picked two extreme phases -- Hypersil Prism C18 RP and Purospher C18 to compare to the StableBond C18 phase. As expected there are significant differences in selectivity between the three columns; these differences are supported by the low r^2 value (see Figure 10). The results also agree well with the F_s factors of the S-D approach.

3.2.2 Potential Applications of the Stationary Phase Triangle—We believe that the visual display of information inherent in the set of triangle plots will prove to be useful for method development in HPLC. However this is a broad subject and a detailed presentation is beyond the scope of the present work which focuses on the basic development of the concept. At present we merely wish to suggest that these phase selectivity triangles will be useful for picking phases that should be included in an initial column screening to make sure that columns with maximally different selectivities are included.

A second area of application is in the selection of the proper “orthogonal” pairs of columns to be used in two-dimensional liquid chromatography (2DLC) [73,74]. As Giddings has pointed out not only must the two separation mechanisms used differ but these differences must be complementary to the inherent dimensionality and differences in the properties of the analytes in the mixture [75]. As an example in the separation of complex mixtures of peptides, an ion exchange first dimension is coupled with a reversed phase second dimension separation [71–78]. Similarly if one had a sample whose analytes differed principally in hydrophobicity and in hydrogen bond basicity then one could based on the triangle locate a phase with average properties and pair it with a phase with high phase χ_A .

4. Conclusions

A set of four phase selectivity classification “triangle” plots have been developed based on the Snyder-Dolan Hydrophobic-Subtraction Model, wherein the apices of the triangles represent the relative contributions of steric hindrance (χ_S), hydrogen bonding acidity (χ_A), hydrogen bonding basicity (χ_B), coulombic interactions (χ_C) to phase selectivity. The real benefit of the triangle plots over previous approaches is that it allows the visualization of column selectivity for a large number of phases by plotting three-dimensional selectivity data in a two-dimensional space. It very clearly shows that the 366 RPLC columns studied here cover only a small fraction of the currently available phase selectivity space leaving a great deal of room for researchers to develop novel rather than more redundant phases. Moreover, this approach to selectivity characterization as applied to the non-alkylsilica phases clearly shows that the same surface chemistry (functionality) does not guarantee the same phase selectivity. In contradistinction, the comparison of phases with different chemical functionalities just as clearly shows that different surface chemistry does not necessarily lead to different phase selectivity. Selection of columns of either equivalent or different selectivity is readily achievable with the application of the set of four “selectivity triangles” based on the Snyder-Dolan Hydrophobic-Subtraction approach. It provides an informative and universal approach to classifying the currently characterized set of reversed phases. The most different phases of the 366 phases are listed here.

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(a)

(b)

Figure 1.

Hypothetical plots of $\log k'$ vs. $\log k'$ to illustrate the ratio concept.

(a) column A vs. column B;

(b) column A vs. column C.

The retention data for the 16 standard probes is calculated based upon Eq. 1 assuming $k'(\text{ethylbenzene}) = 1$ on for phases A, B and C.

Figure 2. Statistics of the phase coefficients for 366 reversed phases. Data provided by L. R. Snyder. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Dots above or below whiskers are outlying points.

Figure 3.

Selectivity classification of 366 reversed stationary phases based upon the 1st set of weighting factor defined by standard error of linear regression.

- a) S*-B-C triangle;
- b) S*-A-C triangle;
- c) A-B-C triangle;
- d) S*-A-B triangle.

Figure 4.

Selectivity classification of 366 reversed stationary phases based upon the 2nd set of weighting factor defined by range of stationary phases interested.

- a) S*-B-C triangle;
- b) S*-A-C triangle;
- c) A-B-C triangle;
- d) S*-A-B triangle.

Figure 5.
Placement of the extreme phases based on the S*-B-C triangle.

Figure 6.
Selectivity classification of selected reversed stationary phases in the S*-B-C triangle.
(a) type-B alkylsilica phases;
(b) Embedded Polar Group phases, Cyano phases and Phenyl Phases.

Figure 7.

Comparison of selectivity for a given sample on three EPG phases of the same chemical type. Solutes: 1) amitriptyline; 2) N,N-dimethylacetamide; 3) acetophenone; 4) toluene; 5) trans-chalcone; 6) 5-phenylpentanol; 7) 4-n-butylbenzoic acid; 8) cis-chalcone; 9) mefenamic acid.

Experimental conditions: 50% acetonitrile/pH 2.8 buffer; 35 °C; 1.0 ml/min. r^2 value is compared with Hypurity Advance C18 column.

Figure 8.
Selectivity comparison of type-B alkylsilica phases vs. EPG phases in the S*-B-C triangle.

Figure 9.

Comparison of selectivity for a given sample on three stationary phases of different chemical types. Chromatographic conditions are the same as in Fig. 6. The same set of solutes were used but the elution order is different as follows: 1) N,N-dimethylacetamide; 2) amitriptyline; 3) acetophenone; 4) 5-phenylpentanol; 5) 4-n-butylbenzoic acid; 6) toluene; 7) cis-chalcone; 8) trans-chalcone; 9) mefenamic acid. r^2 value is compared to the ACE AQ column. Note that components 5 and 6 overlapped and thus show as a single peak in all of the three chromatograms.

Figure 10.

Comparison of column selectivity on two extreme phases vs. Zorbax StableBond C18 phase. Chromatographic conditions are the same as in Fig. 6. The same set of solutes were used but the elution order is different as follows: 1) N,N-dimethylacetamide; 2) amitriptyline; 3) acetophenone; 4) 5-phenylpentanol; 5) 4-n-butylbenzoic acid; 6) toluene; 7) cis-chalcone; 8) trans-chalcone; 9) mefenamic acid. r^2 value is compared to the Zorbax StableBond C18 column.

Table 1

Phase parameters for the three hypothetical phases shown in Figure 1.

Phase	Column selectivity parameters					Relative column selectivity parameters <i>a</i>				
	H	S	A	B	C	H/H	S/H	A/H	B/H	C/H
A	0.5	0.2	-0.2	0.2	0.2					
B	1	0.4	-0.4	0.4	0.4	1	0.4	-0.4	0.4	0.4
C	1.5	0.6	-0.6	0.6	0.6					

^aCalculated by taking the ratio of column selectivity parameters by dividing by H.

Table 2**a Statistics of the “effective selectivity” parameters based on 366 RPLC stationary phases.**

	S/H	A/H	B/H	C/H
Min	−0.391	−1.525	−0.373	−3.213
Max	0.235	0.611	0.384	2.593
Range	0.626	2.136	0.757	5.806

b Weighting factors to normalize the selectivity parameters in the selectivity triangles

	ϕ_S	ϕ_A	ϕ_B	ϕ_C
Snyder-Dolan's ^a	100	30	143	83
1 st ^b	100	62	141	96
2 nd ^c	100	29	83	11

^a Coefficients reported by Snyder et al. [9];^b Weighting factors are calculated based upon the method of Appendix 1.^c Weighting factors are calculated based upon eq. 8 where the range of selectivity ratios was further normalized to $\phi_S = 100$.

Table 3

Candidate phases for orthogonal and equivalent separations.

	Column	H	S	A	B	C(2.8)	k' _{ref}	F _s	γ _S	γ _B	γ _C	R ^{2a}	Type
Equivalent ^b	ACE AQ	0.804	-0.051	-0.129	0.034	0.009	5.0	0	0.335	0.279	0.386	1.000	EPG
	Betasil Phenyl-Hexyl	0.707	-0.053	-0.294	0.028	0.054	4.3	6.4	0.329	0.280	0.392	0.999	Phenyl
	Bondclone C18	0.824	-0.056	-0.125	0.044	0.078	4.5	6.0	0.329	0.283	0.387	0.998	C18-A
Orthogonal ^c	Zorbax StableBond 80A C18	0.996	-0.032	0.264	-0.001	0.136	7.6	0	0.355	0.255	0.390	1.000	C18-B
	Purospher RP-18	0.841	0.235	0.155	0.300	-0.964	4.9	104	0.429	0.331	0.239	0.798	C18-B
	Hypersil Prism C18 RP	0.645	0.089	-0.459	0.301	-2.817	4.8	250	0.423	0.447	0.130	0.752	EPG

^a Chromatographic conditions: 50% acetonitrile/pH 2.8 buffer, 35 °C; 1.0 ml/min. Solutes: 1) 5-phenylpentanol; 2) amitriptyline; 3) acetophenone; 4) N, N-dimethylacetamide; 5) anisole; 6) 4-n-butylbenzoic acid; 7) cis-chalcone; 8) trans-chalcone; 9) mefenamic acid;

^b F_S and R² are obtained by comparing with ACE AQ as reference.

^c F_S and R² are obtained by comparing with Zorbax StableBond C18 as reference.

Table 4

Summary of Extreme phases based on the four triangles ^a.

Labels	Stationary phases	Types	Extreme phases on triangle			
			S-B-C	S-A-C	S-A-B	A-B-C
a	Inertsil CN-3	Cyano	✓	✓	✓	✓
b	EC Nucleosil 100-5 Protect	EPG	✓	✓	✓	✓
c	Hypersil Prism C18 RP	EPG	✓	✓	✓	✓
d	Bonus RP	EPG	✓	✓	✓	✓
e	BetaMax Acid	EPG	✓	✓	✓	✓
f	Purospher RP-18	B-C18	✓	✓		✓
g	Discovery HS PEG	EPG	✓	✓	✓	✓
h	Hypurity Advance	EPG	✓	✓	✓	✓
i	Vydac 218MS	Type-A	✓		✓	✓
j	HC-OH	Special	✓		✓	
k	HC-C8	Special	✓		✓	
l	ZirChrom-PS	Special	✓	✓	✓	✓
m	Nova-Pak CN HP 60A	Cyano	✓	✓	✓	
n	BioBasic Phenyl	Phenyl	✓	✓	✓	✓
o	-SO ₃ -HC-C8	Special	✓	✓	✓	
	Inertsil Ph-3	Phenyl			✓	
	Prodigy Phenyl-3	Phenyl			✓	
	Platinum EPS C8	B-C8			✓	
	HC-T	Special			✓	
	ProntoSil CN	Cyano		✓		✓
	Kromasil KR60-5CN	Cyano			✓	✓
	Discovery CN	Cyano		✓	✓	✓
	Genesis CN 300A	Cyano		✓	✓	✓
	Genesis CN 120A	Cyano		✓	✓	✓
	Thermo CN	Cyano			✓	✓
	ACE 5CN	Cyano		✓	✓	✓
	Inertsil ODS-EP	B-C18		✓	✓	✓

Labels	Stationary phases	Types	Extreme phases on triangle			
			S-B-C	S-A-C	S-A-B	A-B-C
Prontosil 120-5-C8 ace-EPS	BioBasic CN	EPG		✓	✓	✓
		Cyano		✓	✓	✓
	ZirChrom-EZ	Special		✓	✓	✓
		Cyano		✓	✓	
	ZirChrom-PBD	Special			✓	
Nucleosil 100-5-C18 Nautilus	Spherisorb S5 ODSB	Type-A				✓
		EPG				✓
	Hypersil Prism C18 RPN	EPG				✓
		EPG				✓
	Alltima HP C18 Amide	EPG				✓

^a All phases which we consider to lie well outside the main cluster in any of the four triangles are listed here. Many of the phases appear in several triangles. Some show up in all four triangles, very few are present in only a single triangle. Note these are not necessarily “unique” phases as they may well have close neighbors.

Appendix I

Calculations of weighting factors to normalize phase selectivity coefficients based on the standard error in plots of $\log k'$ vs. $\log k'$.

Case	Column #	Column coefficients					Linear regression			Normalized weighing factor ^c
		H	S	A	B	C	i/H	Standard error ^b	R ²	
Case a i=S	reference	0.864	-0.015	-0.094	0.009	0.104	-0.017	0.000		
	1	0.864	0.246	-0.094	0.009	0.104	0.284	0.064		
	2	0.864	0.740	-0.094	0.009	0.104	0.856	0.185		
	3	0.864	0.391	-0.094	0.009	0.104	0.453	0.099	1.0	0.2544
	4	0.864	0.124	-0.094	0.009	0.104	0.143	0.034		100
Case b i=A	5	0.864	0.440	-0.094	0.009	0.104	0.509	0.111		
	1	0.864	-0.015	0.082	0.009	0.104	0.095	0.050		
	2	0.864	-0.015	0.930	0.009	0.104	1.076	0.292		
	3	0.864	-0.015	0.304	0.009	0.104	0.352	0.114	1.0	0.1572
	4	0.864	-0.015	0.257	0.009	0.104	0.298	0.100		62
Case c i=B	5	0.864	-0.015	0.448	0.009	0.104	0.519	0.155		
	1	0.864	-0.015	-0.094	0.061	0.104	0.071	0.010		
	2	0.864	-0.015	-0.094	0.808	0.104	0.936	0.151		
	3	0.864	-0.015	-0.094	0.534	0.104	0.618	0.099	1.0	0.3593
	4	0.864	-0.015	-0.094	0.117	0.104	0.135	0.020		141
Case b i=C	5	0.864	-0.015	-0.094	0.935	0.104	1.083	0.175		
	1	0.864	-0.015	-0.094	0.009	0.192	0.222	0.028	1.0	0.2451
	2	0.864	-0.015	-0.094	0.009	0.513	0.593	0.129		
	3	0.864	-0.015	-0.094	0.009	0.644	0.745	0.170		
	4	0.864	-0.015	-0.094	0.009	0.239	0.276	0.042		
	5	0.864	-0.015	-0.094	0.009	0.257	0.298	0.048		

^a Hypothetical column sets with one of stationary phase coefficients randomly generated by Excel.

^b Standard error were obtained by linear correlation of the retention of 16 standard solutes in ref [9] on each phase vs. the reference column. All the retention data are calculated based upon eq. 1, assuming chromatographic conditions: 50% acetonitrile/pH 2.8 buffer; 35 °C; 1.0 ml/min.

^c Weighting factors are calculated by normalizing slope to $\phi_S = 100$.