Column selectivity varies among different reversed-phase columns, and, sometimes, different batches of the same column. For a reversed-phase liquid chromatography (LC) assay that is to be used for several months or years, replacement columns with the same selectivity must be available for the duration of the method. For example, consider the separation in Figure 1a, which uses an Ace C8 column (Hichrom Ltd., Theale, United Kingdom). If for some reason that column became unavailable (or if batch-to-batch selectivity changed), we would require a replacement column of similar selectivity. As Figure 1b shows, a Precision C8 column (Mac-Mod Analytical, Inc., Chadds Ford, Pennsylvania) would serve this purpose. For method development, a column of very different selectivity could be necessary to achieve acceptable sample resolution. For example, an Inertsil C8 column (GL Sciences, Inc., Tokyo, Japan) provides the separation of Figure 1c, in which peak pairs 1–2 and 8–9 overlap. Changing to a column with very different selectivity can lead to an improved separation, as would be the case for this sample by changing from the Inertsil C8 to either the Precision C8 or the Ace C8 columns.

Measurement of Column Selectivity

To select either similar or different columns, we must characterize columns according to their selectivity. The technique we discuss here for evaluating columns has nothing to do with measuring the quality of the packing material — rather it helps us predict how likely it is that one column will replicate the separation obtained on another column. Previous means for measuring column selectivity have relied mainly upon retention data for selected test compounds; column manufacturers commonly use these measurements for the quality control of different production batches of column packing (1). However, as Claessens (2) pointed out in a recent critical review, “There is a need to improve and to develop column test protocols that sufficiently describe the chromatographic properties of columns” [italics added]. This observation has been reinforced by previous “Column Watch” polls, which report that column-to-column reproducibility is the single most important consideration when selecting a column supplier, which implies that manufacturers’ column tests are not always able to detect batch-to-batch differences in column selectivity (3,4).

Figure 1: Separation as a function of the column. (a) Ace C8, (b) Precision C8, (c) Inertsil C8. Conditions: 15 cm × 0.46 cm columns; 2.0 ml/min; 35 °C; 50% A–B, where A is 30 mM, pH 2.8 potassium phosphate buffer and B is acetonitrile. 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. Chromatograms are reconstructed from separate injections of each compound.
One means of comparing two columns in terms of selectivity is by a log–log plot of retention factors (k) for a group of solutes, as illustrated in Figure 2a for the similar Precision and Ace C8 columns of Figures 1a and 1b (5). The figure shows that all data points for this intentionally diverse group of sample compounds fall very close to a straight line, with a standard deviation (SD) of only 0.01 log units (corresponding to a ±2% deviation in values of k and ±3% in corresponding values of the separation factor [α]). For typical separation conditions, a change in α of 3% results in a change in resolution (R) of 0.2–0.4 units, which usually has only a minor effect on separation (6). Thus, when plots for two columns as in Figure 2 show a scatter of ≲2% in k (1 SD) for the sample of interest and same separation conditions, users should obtain essentially equivalent separations for either column; that is, one column can be substituted for the other.

A similar comparison of k values (Figure 2b) for the very different Ace and Inertsil C8 columns of Figure 1 shows a much larger deviation of data points from the best straight line (SD is 0.12 log units or ±25–30% in k). The resulting change in separation on these two columns (Figures 1b and 1c) is much too large to imagine that one column could be substituted successfully for the other. On the other hand, these large changes in k and α make it likely that analysts could achieve useful changes in selectivity during method development by changing from one column to the other.

What should be the goal in column testing when we desire to identify either equivalent or very different columns? One approach is a trial-and-error comparison of a starting column with several different commercially available columns, as in the examples of Figure 1. However, chromatographers can choose from approximately 600 different reversed-phase LC packings, so this approach is inefficient (2). Alternatively, if users can define column selectivity quantitatively and completely by retention data for a small number of test compounds, and if these test results are available for most commercially available columns, then users can select either similar or different columns by using a simple computer program to help identify a column with the desired characteristics.

**Column-to-column reproducibility is the single most important consideration when selecting a column supplier.**

A Different Approach to Column Characterization For the past four years, our laboratory has been investigating means for the quantitative and complete characterization of reversed-phase LC column selectivity (7–9).

Figure 2 shows that values of log k for most solutes fall close to a best-fit line through the data. That is, so-called hydrophobic interactions largely determine values of k in reversed-phase LC; more-polar sample molecules are less retained, and less-polar molecules are more retained. If we first subtract the effect of hydrophobic interaction from values of k, we can examine the remaining and more important contributions to column selectivity effectively.

Our study began by collecting k values for 150 compounds of widely different functionality and structure using conditions similar to those of Figure 1 with 10 different C18 columns. Retention plots, similar to Figure 2, enabled us to recognize several compounds — so-called ideal solutes — for which the scatter of log k values from best-fit lines was quite small (≪2% in k). The intercepts of these plots generally were much larger than 2% in k, which can be attributed to differences in column selectivity. By dividing values of k for a given compound by the k value for ethylbenzene, we corrected the resulting values of α for varying column surface area. For these ideal compounds, we can describe values of α accurately by

\[
\log \alpha = \eta' H \tag{1}
\]

where \(\eta'\) represents the hydrophobicity of the solute, and \(H\) is column hydrophobicity. Equation 1 allows values of \(H\) for different columns to be measured. Contributions to retention other than from hydrophobicity (Δ) also can be measured for nonideal compounds, because

\[
\Delta = \log \alpha - \eta' H \tag{2}
\]

When we compared values of Δ for different compounds and columns, we found that Δ values for structurally similar groups of compounds were highly correlated; for example, for various strong bases such as amitriptyline

\[
\Delta = \kappa' C \tag{3}
\]

for the 10 columns, with correlation coefficients \(r\) greater than 0.99, where \(\kappa'\) is a measure of the positive charge on the solute molecule and depends upon the solute and \(C\) is the column cation-exchange activity and varies with the column. Equation 3 suggests that values of Δ for strong bases arise from a single contribution to retention and column selectivity (ionic interaction, in this case). We also observed relationships of the same form as equation 3 for other groups of related compounds.

Continuing our analysis as above (7), we found that retention can be correlated within ±2% in k by

\[
\log \alpha = \eta' H + \sigma' S + \beta' A + \alpha' B + \kappa' C \tag{4}
\]

where \(\sigma'\) is the solute bulkiness or its resistance to penetrating into the stationary phase, \(S\) is the column steric accessibility (values are larger for stationary phases that are less resistant to the penetration of a bulky solute molecule), \(B'\) is the solute

![Figure 2: Comparisons of column selectivity. Conditions, sample, and columns of Figure 1. See text for details.](image-url)
hydrogen-bond basicity, $A$ is the column hydrogen-bond acidity, $\alpha'$ is the solute hydrogen-bond acidity, and $B$ the column hydrogen-bond basicity. The column parameters $H$, $S$, $A$, $B$, and $C$ define column selectivity quantitatively and completely; the corresponding compound parameters $\eta'$, $\sigma'$, $\alpha'$, $\beta'$, and $\kappa'$ represent complementary properties of the solute. Because compound retention can be predicted by these five column properties within $\pm 2\%$, we believe it is unlikely that any other column property contributes significantly to selectivity for the widely used alkyl–silica columns such as C8 and C18.

The Meaning of the Column Parameters of Equation 4
We believe that the five terms of equation 4 correspond to solute–column interactions as summarized in Figure 3. Note that the solute parameters of equation 4 correspond to lowercase Greek letters for hydrophobicity ($\eta'$), steric interaction ($\sigma'$), hydrogen-bond acidity ($\alpha'$), hydrogen-bond basicity ($\beta'$), and cation exchange ($\kappa'$). Similarly, the various column parameters are uppercase letters for the same interactions. Our interpretation of equation 4 as represented by Figure 3 is based upon the dependence of the solute parameters $\eta'$, $\sigma'$, $\beta'$, $\alpha'$, and $\kappa'$ on the compound structure and the relationship of the column parameters $H$, $S$, $A$, $B$, and $C$ to column properties such as ligand length (C8 versus C18), percentage carbon, pore diameter, and the presence or absence of endcapping (9). For example, the ion interaction term ($\kappa'C$), arises from the cation exchange of protonated bases with ionized silanols in the stationary phase (or the ion repulsion of ionized acids). We therefore expect to find a correlation of values of $\kappa'$ with the average charge of an acid or base, and we observed this result ($r = 0.94$ for 22 ionizable compounds). Likewise, we found much larger values of $C$ for columns without endcapping, because ionized silanols can interact more readily with ionized acids or bases in these columns.

The five solute–column interactions of Figure 3 and equation 4 appear to resemble interactions that have been described previously (2). However, in two cases ($\sigma'S$ and $\alpha'B$) the situation appears more complicated than previously reported. Although we define $\sigma'S$ as relating to steric interaction, no correlation actually exists between measurements of column shape selectivity (10) and values of $S$. Shape selectivity appears to occur for the combination of large, rigid molecules such as polycyclic aromatic hydrocarbons and polymeric stationary phases. Steric interaction is important for the interaction of other molecules with monomeric packings. In most separations by reversed-phase LC, it appears that steric interaction affects column selectivity to a greater extent than it does shape selectivity.

In previous studies (11), the hydrogen-bond interaction of acidic compounds with basic column packings ($\alpha'B$) was described as a relatively minor effect, whereas we found that this contribution to column selectivity is of comparable importance to

![Figure 3: Contributions to column selectivity that are represented by equation 1.](image-url)
the other interactions in Figure 3. Because endcapping has no effect on values of B, it is unclear what basic groups within the stationary phase might be responsible for the preferential retention of acidic compounds.

**Practical Aspects of Equation 4**

The measurement of the column parameters H, S, and so forth, as described elsewhere, is relatively tedious and time consuming (7). We have simplified this procedure so that column testing requires no more than 1–2 h per column (12). This simplification has allowed us to collect data for more than 100 alkyl–silica columns, with an eventual goal of several hundred columns, including other column types such as embedded-polar–group, phenyl, and cyano columns.

Apart from mobile-phase pH, we found that the values of H, S, A, B, and C do not vary much with separation conditions (8). If the mobile-phase pH is changed, the ionization of column silanols will vary, and this variation can cause changes in C; as the pH increases, silanols become increasingly ionized, and the values of C tend to increase. For many columns, however, this effect is fairly small, and in any case measurements of C at pH 2.8 and 7.0 are sufficient to define C as a function of pH. Values of the column parameters for a given pH define column selectivity, regardless of other separation conditions.

Given values of the column parameters for a large number of columns, we need a simple procedure to compare the relative selectivity of any two columns. An obvious approach is to plot values of log k as in Figure 2, in which case, relative selectivity is defined by SD for the best fit. Thus, larger SD values reflect greater differences in selectivity. Practical Aspects of Equation 4 suggests a very different column selectivity compared with the Ace C8 column, as confirmed by the chromatogram.

Table I lists some representative values of H, S, A, B, and C for different reversed-phase LC columns, grouped according to alkyl chain length (C18, C8, or C4) or the presence of an embedded polar group (Symmetry Shield C18, Waters Corp., Milford, Massachusetts). Column hydrophobicity (H) generally increases for an increase in chain length and is smaller for embedded-polar–group columns of the same chain length. Because S measures the ease of inserting a sample molecule into the stationary phase, S tends to decrease with stationary-phase chain length. Values of A, which measure the accessibility of nonionized silanols in the stationary phase, tend to increase with chain length, because alkyl-group concentration (measured as micromoles per square meter) tends to decrease from C4 to C8 to C18 (and, therefore, silanol concentration increases). Embedded-polar–group columns generally have smaller values of A and C because of silanol suppression by the polar group, whereas the basic polar group — amide, urea, and carbamate — leads to larger values of B.

The last column of Table I lists the values of the column selectivity function (F₁) relative to the Symmetry C18 reference column (similar tables can be constructed to compare F₁ values relative to other reference columns). The generally large values of F₁ for these columns suggest that a close match in column selectivity (F₁ < 3) will occur only occasionally. This conclusion is overly pessimistic, however, because it assumes that the sample contains all kinds of compounds, including acids and ionized bases. If either of these compound types are absent, the effects of B (acids) and C (ionized bases) on column selectivity become less important. The latter terms in equation 5 for F₁ become less important and can be deleted, which reduces all F₁ values and makes finding a column replacement with F₁ of less than 3 more likely.

Small changes in separation conditions — method adjustment — also can be used to minimize differences in column selectivity and to provide some compensation for

| Table I: Comparison of selectivity between Symmetry C18 and other columns |
|-----------------------------|---|---|---|---|---|---|
| Column Type | H | S | A | B | C | F₁* |
| Symmetry C18 | 1.06 | -0.06 | 0.06 | -0.02 | -0.29 | 0 |
| Symmetry Shield C18 | 0.90 | 0.04 | -0.42 | 0.21 | -0.66 | 49 |
| Genesis C4 | 0.64 | 0.07 | -0.43 | 0.03 | 0.05 | 35 |

* Compared with the Symmetry C18 column.
† Eka Chemicals Inc. (Marietta, Georgia).
‡ Phenomenex Inc. (Torrance, California).
§ Agilent Technologies Inc. (Wilmington, Delaware).
¶ Argonaut Technologies, Inc. (Foster City, California).
cases when $F_s$ is greater than 3. Dolan and co-workers (13) described a simple spreadsheet procedure that can determine optimum method adjustment conditions; adjusted conditions typically lead to a reduction in values of $F_s$ by threefold. Thus, the combination of a reasonably complete column database — values of $H, S, A, B,$ and $C$ — with method adjustment software should allow the replacement of most columns by a different column with adjusted conditions.

**Conclusion**

We have described a procedure that allows analysts to use a simple six-component sample to adequately define column selectivity for all other samples. This procedure can be used to help identify columns that are similar or dissimilar. Columns of similar selectivity are desired to allow for an equivalent or backup column in case the first-choice column is no longer available. When separation difficulties are encountered during method development, chromatographers might want to select another column that displays selectivity characteristics distinctly different from the first. From a database of well-characterized columns, users can select alternative columns for the task at hand.

During this study, we have collaborated with several companies that manufacture and sell reversed-phase columns. Other companies who want their columns to be represented in our planned column database should contact the authors (john.dolan@lcresources.com and lloyd.snyder@lcresources.com).

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