An Overview of Column Selectivity for Reversed-Phase HPLC

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This paper is a progress report on work we've been doing in our lab over the last 4 years to help get a better understanding of column selectivity.

Characterizing RP-LC Column Selectivity

Goals:

- **routine and complete test procedure(s)**
- **ability to compare columns for selectivity**

Challenges:

- **need to identify all selectivity contributions**
- **need to measure all selectivity contributions**
- **different mobile phases and temperature?**
- **different samples?**

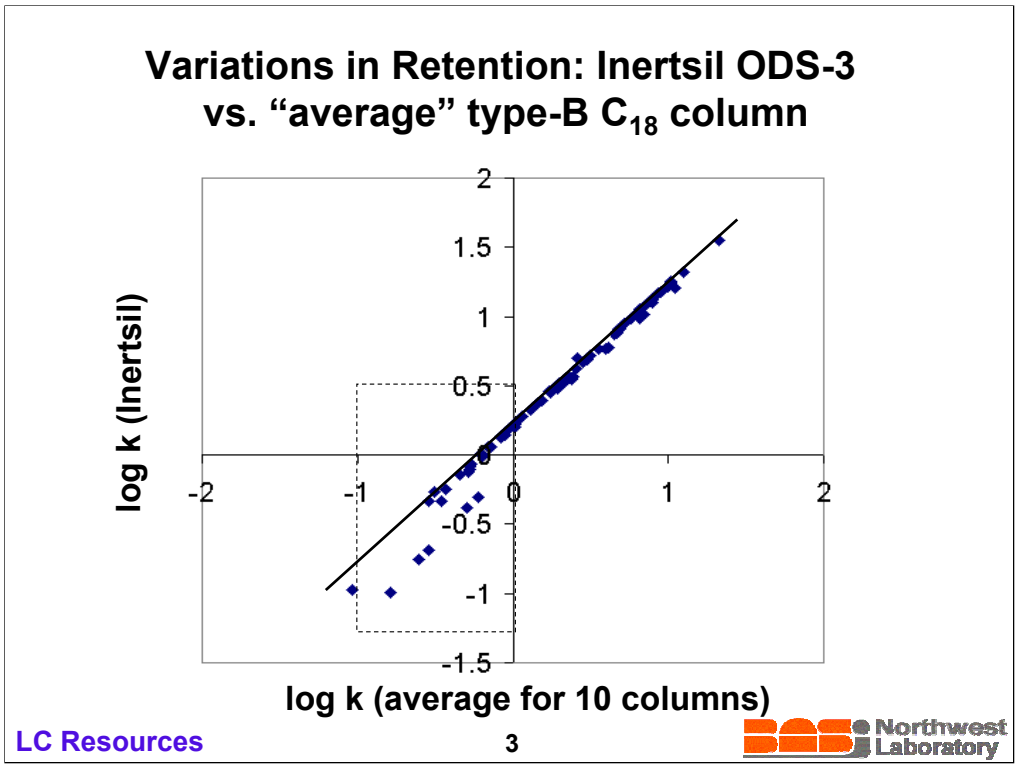
$$\log k = \log k_{EB} + \eta'H + \sigma'S + \beta'A + \alpha'B + \kappa'C$$

hydrophobic **steric** **H-bonding** **cation exchange**

Why do we want to characterize column selectivity? For two reasons: first, in order to select replacement columns that will give the same separation. For example, when a column we have been using is no longer available. Second, to choose a column of very different selectivity. In method development we sometimes need to change selectivity in order to separate overlapping bands. We might also want a very different column for an orthogonal separation.

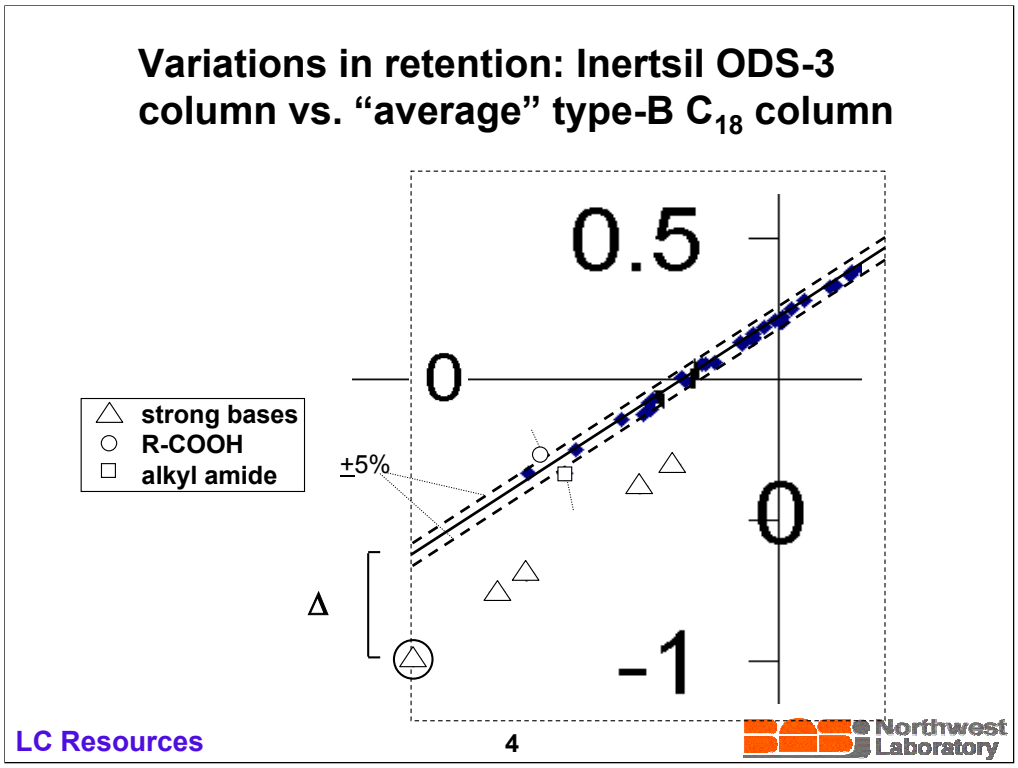
Our goal is to develop a column test that describes all the interactions that contribute to column selectivity: hydrophobicity, hydrogen-bonding, ionic interaction, and so on. Dozens of workers have been working toward this goal for the past 30 years – so far, with what might be termed limited success. The challenges we face include the problem of identifying all the important contributions to column selectivity and then measuring these column properties. We also need to show that column selectivity defined in this way does not change with separation conditions or the nature of the sample. Otherwise, we would need to test column selectivity for every possible condition and all possible samples – not very practical!

For the past 4 years we have been looking at a new approach to this problem. Some of you will be familiar with the equation we have developed to describe sample retention as a function of column selectivity. We will look at this equation again in a moment, but first let's look at how this equation evolved.



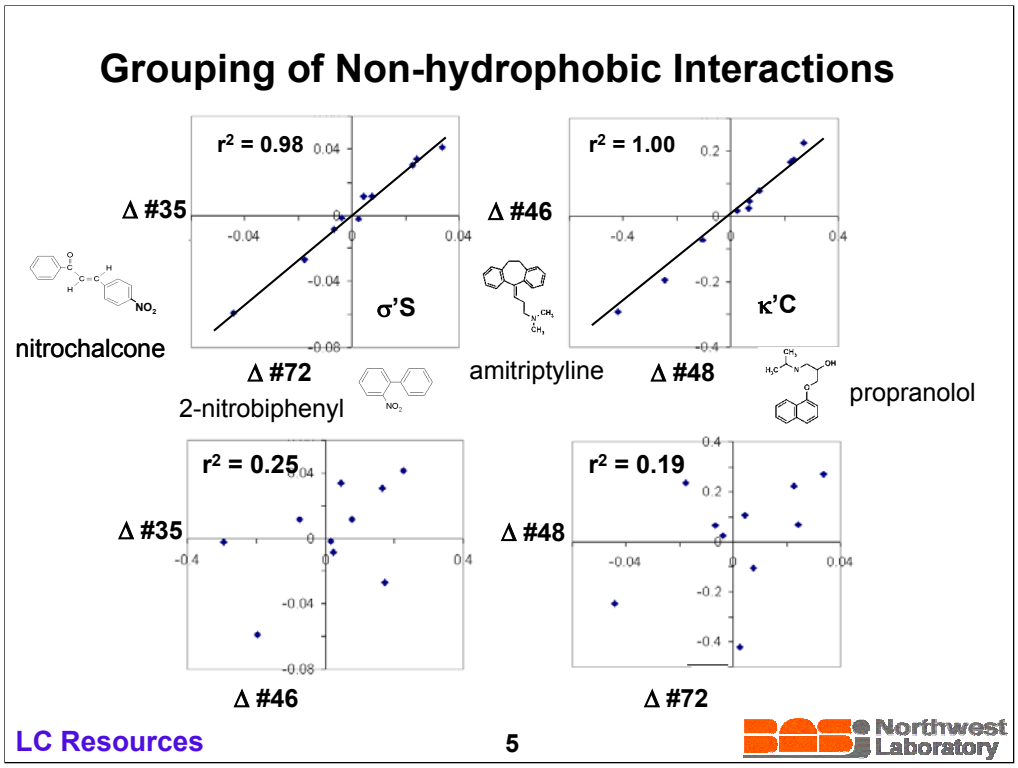
There is one important contribution to reversed-phase retention that everyone agrees on, namely hydrophobic interaction of column and sample. If hydrophobicity were the only column property that affected selectivity, a plot of log k for one column vs another would give a straight line, with all the points on the line. This is illustrated here for data for an Inertsil column plotted vs average values for 10 different C18 columns. And we do see that most of the points, one for each compound, fall on a single line. The slope of this plot depends on the hydrophobicity of the Inertsil column compared to the average of 10 columns. We can determine column hydrophobicity or the value of H from this slope.

There are also deviations from this plot, which all chromatographers should be grateful for. These deviations reflect differences in column selectivity that can be used to our advantage. We can see these deviations better in the next slide.



Here the correlation line is bracketed by dashed lines which represent ±5% deviations in k. Deviations larger than this can result in changes in resolution by a unit or more, and are therefore highly significant. We see for this part of the plot that 7 compounds deviate by more than 5%; for the entire plot of 67 compounds, there were 26 similar deviant points: about one compound out of three. For each of these deviating points we can determine the size of the deviation, which we call delta (circled data).

When we collect values of delta for all the deviating compounds and all of the columns, we find that different compounds can be grouped on the basis of similar delta values as a function of the column.

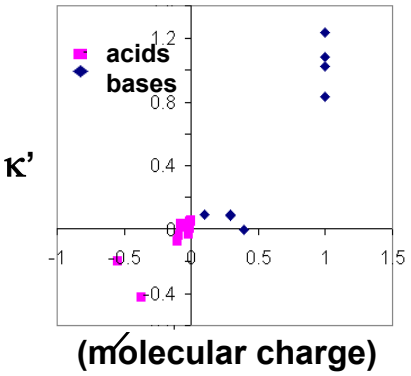


This is illustrated for 4 of these deviating compounds. Compounds #35 and 72 happen to be nitrochalcone and 2-nitrobiphenyl. We see that their delta values are highly correlated, which would be the case if the same interactions are causing these deviations delta. It turns out that delta values for these two compounds determine the sigma'-S term of our equation. Likewise for compounds #46 and 48, a similar correlation of delta values results. These compounds are two strong bases – amitriptyline and propranolol – and their delta values can be assigned to the kappa'-C term of our equation.

When delta values for compounds #35 and 46 or #48 and 72 are compared, there is little or no correlation. The reason is that delta values for these compounds are not caused by the same sample-column interaction.

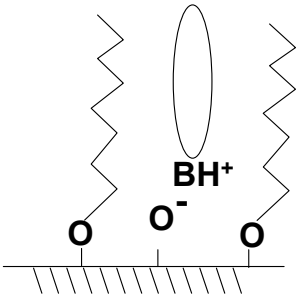
Interpretation of Non-hydrophobic Interactions: κ'C

κ'



C

- decreases with end-capping
- increases with pH
- increases with silica acidity (type-A vs type-B)



Once we have grouped different deviating compounds in this way, and expressed values of delta by different terms in our equation, the next question is: what causes each of these deviations? Can we relate these deviations to specific column-sample interactions? If so, this strengthens our entire analysis, and provides a means of characterizing column selectivity completely and quantitatively.

Well, it turns out we can do this for each of the 5 terms of our equation. This slide is one example, for the kappa'-C term which measures ionic interaction between ionized acids or bases and the negative charge on the column due to ionized silanols. First, we see that the sample parameter kappa' correlates well with ionic charge. Ionized acids with a negative charge are repelled from the negatively charged column, and have negative value of kappa'. Ionized bases are similarly attracted by the negative column charge and give positive values of kappa'.

If we look at the column parameter C, we find as expected that end-capping results in a decrease in C. When the ionized silanols are blocked by end-capping, they are not as effective. Similarly, an increase in pH leads to an increase in silanol ionization, which also means larger values of C. Finally, acidic silicas (or type-A) are more ionized and also give larger values of C.

Characterizing RP-LC Column Selectivity

$$\log k = \log k_{EB} + \eta'H + \sigma'S + \beta'A + \alpha'B + \kappa'C$$

Challenges:

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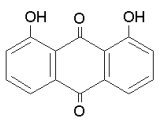
Goals:

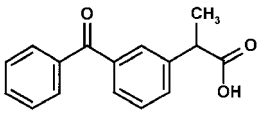
- routine and complete test procedure(s)
- ability to compare columns for selectivity

So, returning to our first slide, we can now see the way in which our equation for column selectivity has been developed and validated. This is only a small part of the story, with a lot of other correlations that back up this simple picture of column selectivity. Because this equation predicts sample retention with an accuracy of 1-2%, we are justified in believing that all important contributions to column selectivity have been identified and measured. We have also studied the effect of changes in the mobile phase or temperature on the column parameters: H, S, and so on. A change in pH changes silanol ionization and changes the value of C, but other changes in conditions do not affect column selectivity. So, measurements of the column parameters for one set of conditions and as a function of pH completely defines column selectivity. Because we have looked at a very wide range in sample structures, we think this equation will work as well for any sample.

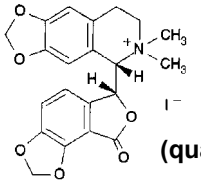
For example, besides the usual substituted alkanes and benzenes, we have looked at a number of very different structures. Next slide illustrates a few of thes.

Illustrative Test Compounds

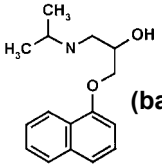




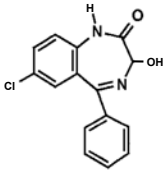
(acid)

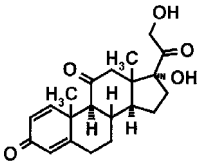


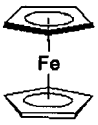
(quat)

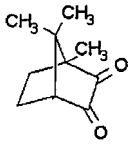


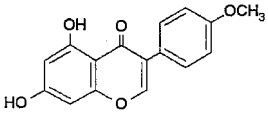
(base)











The 150 or so compounds we have tested so far include a lot of simple molecules such as mono-substituted benzenes and alkanes. These kinds of test compounds have been widely used for column testing by other groups. But we have also included a diverse group of molecular structures, involving in many cases considerable molecular complexity. And these are just a few examples.

Because we have challenged our equation with compounds like this, and found that it is accurate to better than 2%, we are reasonably confident that it will work for most samples.

Characterizing RP-LC Column Selectivity

$$\log k = \log k_{EB} + \eta'H + \sigma'S + \beta'A + \alpha'B + \kappa'C$$

hydrophobic steric H-bonding cation exchange

Challenges:

- need to identify all selectivity contributions
- need to measure all selectivity contributions
- different mobile phases and temperature?
- different samples?

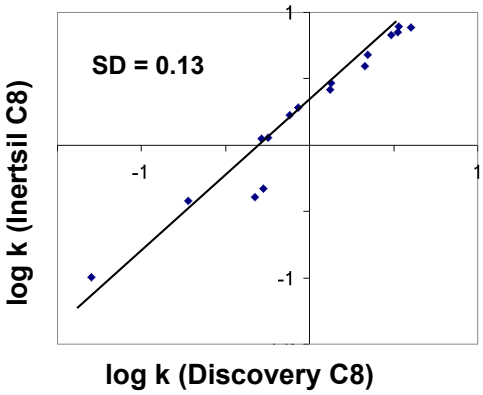
Goals:

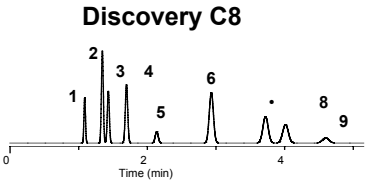
- routine and complete test procedure(s)
- ability to compare columns for selectivity

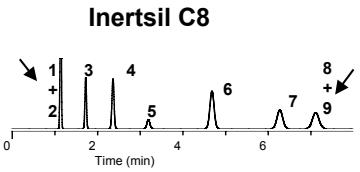
Now that we have developed a way to measure column selectivity, we need a procedure for quantitatively comparing two columns in terms of selectivity.

Comparing Columns

- need similar columns as replacements
- need different columns for method development
- how to compare?







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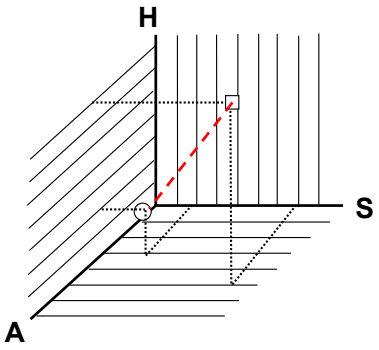
We have measured values of the column parameters H, S, etc. for 150 different reversed-phase columns; how do we select columns that are either similar or different. Similar when we want to replace a column used in a routine procedure or have a reliable backup, or different when we want to change selectivity in method development?

One way of comparing column selectivity is to plot log k for one column against another, using a sample with different kinds of test compounds. In this example, we have plotted data of this kind for an Inertsil C8 column vs a Discovery C8 column. We see some scatter in the data, or a standard deviation of 0.13 log units – that's a deviation of ±35%. Obviously, these two columns are not very similar, as can be seen the the corresponding separations on the two columns.

Comparing Columns

- need similar columns as replacements
- need different columns for method development
- how to compare?
- for neutral samples, only H, S, A important



□ (H_1, S_1, A_1)

○ (H_2, S_2, A_2)

difference in selectivity = F_s

= distance between two points

$F_s = \{(H_2-H_1)^2 + (S_2-S_1)^2 + (A_2-A_1)^2\}^{1/2}$

so: small F_s, similar columns

large F_s, different columns

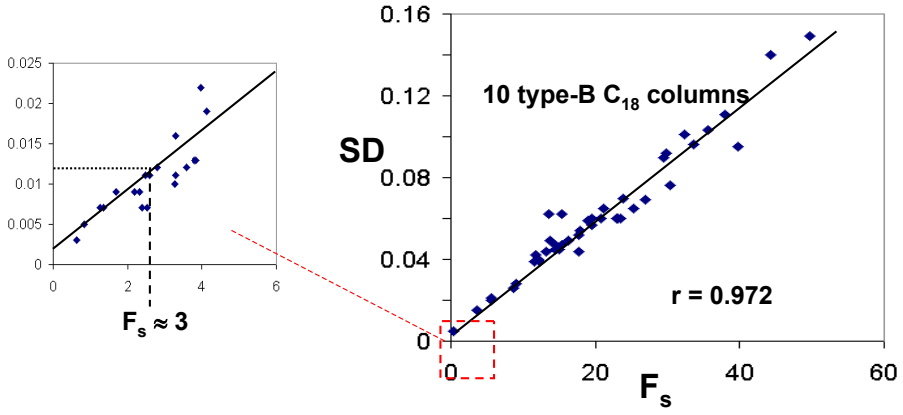
Plotting retention data as we did in the last slide is a tedious way of comparing columns, especially if there are a lot of columns to choose from. Since we now have values of the column parameters for 150 columns, there is an easier way. As a first example, suppose we have a sample that consists of neutral compounds only. In this case, the column parameters B and C will not be important, which simplifies our illustration. That is, column selectivity now depends only on hydrophobicity H, steric interaction S, and column H-bond acidity A.

In the 3-dimensional diagram on the left we show the positions of two columns in a plot of H, S and A. That is, values of H, S and A for each column determine the position of each data point. Now, it is logical to measure column similarity by the distance between two columns in this plot. Let's call that distance, measured in column parameter units, as Fs. By analogy with the Pythagorean theorem, Fs is given by the expression on the right as a function of the values of H, S and A for the two columns.

Comparing Columns

For any sample (both neutral and ionic compounds:

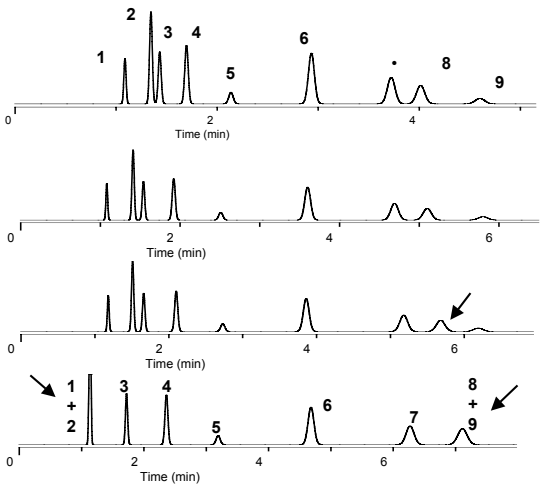
$$F_s = \{(H_2-H_1)^2 + (S_2-S_1)^2 + (A_2-A_1)^2 + (B_2-B_1)^2 + (C_2-C_1)^2\}^{1/2}$$



If we consider all five column parameters, the preceding equation for Fs can be written as in this slide. But how well does Fs correlate with column selectivity as measured by plots of log k for two columns? The plot on the right compares values of the standard deviation from log k plots with values of Fs calculated as in this slide. We see reasonable agreement, so we must be on the right track. The smaller is Fs, the more similar are two columns. But how large can Fs be, and still have equivalent separations for two columns?

This is illustrated in the plot on the left, which is a blowup of a portion of the plot on the right. We have also added some more data points from some other column comparisons. A general rule is that values of the standard deviation should be no greater than about 0.01, or ±3% in values of the separation factors, α. From this plot, we see that Fs should not be greater than 3 for two columns to give the same separation (SD <≈0.01).

Comparing Columns



Discovery C8

F_s = 0

Ace C8

F_s = 1

Precision C8

F_s = 4

Inertsil C8

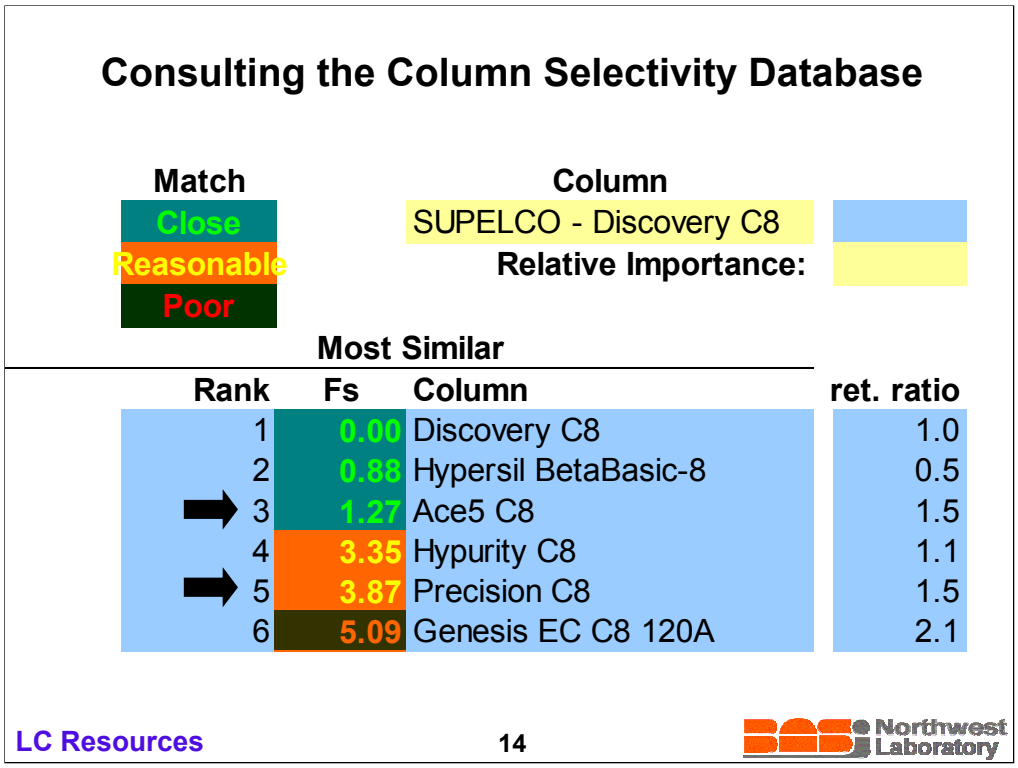
F_s = 38

Here we have an example of the effect of different values of Fs on separation. Our starting column is the Discovery C8 column. We can interrogate our column database for other columns that are similar to this column. One column is the Ace C8, which has Fs = 1 and should give a similar separation. We see that the two separations are in fact very similar. Run time is a little longer with the Ace column, but a small change in flow rate would fix that.

The Precision C8 column has Fs = 4, compared to the Discovery column, and we would expect small differences in separation – differences that might or might not be significant. In fact, the two separations are both acceptable, although we see that peak #8 has moved away from #7. If this peak movement had been in the opposite direction, the resulting separation would have been marginal.

Finally, Fs for the Inertsil C8 column equals 38, which means the two columns are very different. And we see that the two separations are also very different. Had we started with this column in method development, and found overlap of two different peak pairs (arrows), we would have looked for a column with a very different Fs value, in order to change selectivity a lot. This is certainly the case for either the Discovery or Ace columns, each of which has Fs greater than 30 when compared to the Inertsil column.

We're in the process of developing a software package that will do these comparisons of Fs values easily. Presently, this is in an Excel spreadsheet.

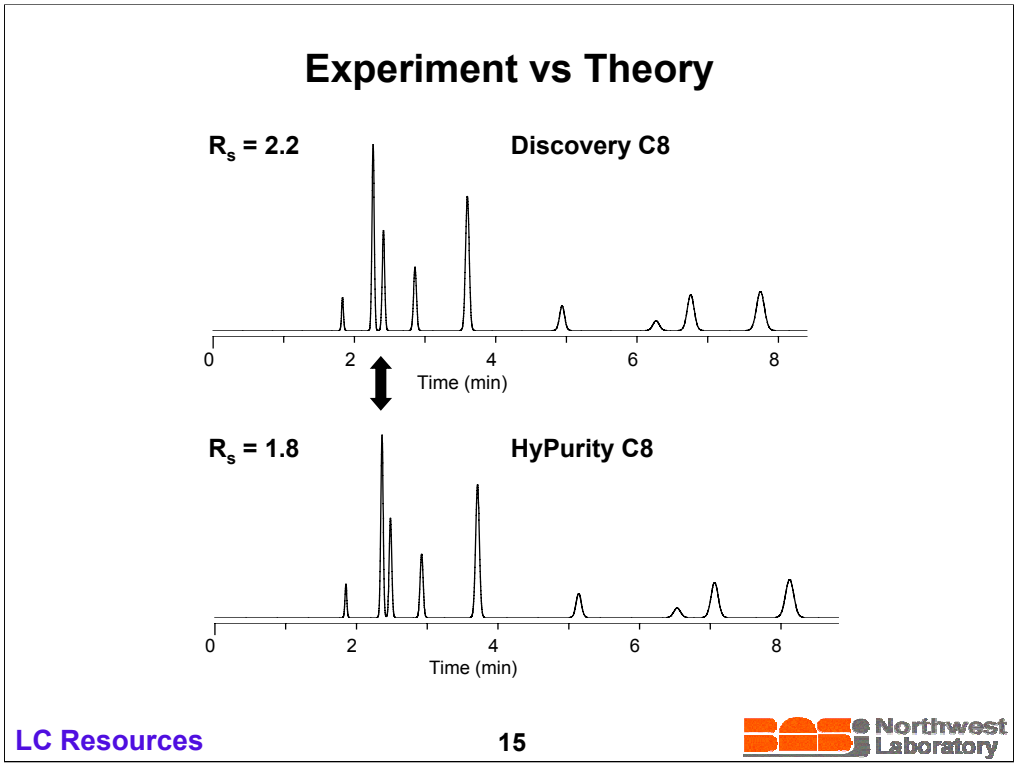


Here's how our preliminary software works.

First we select the column we are trying to match: Supelco – Discovery C8 in this example. This immediately calls up columns that are judged similar on the basis selectivity. Notice the color coding, with green indicating columns that are "close" in selectivity to the column we are trying to match; that is, for which Fs <3. Then we have the "reasonable" columns in red; Finally, we have columns that are poorly matched, for which Fs >5.

The Ace5 C8 and Precision C8 were two of the columns we looked at in the comparison of chromatograms in the previous slide.

We also see a column labeled retention ratio. Even though we may have a good selectivity match, two columns may differ in their relative retention or run time. For example, we see that the Hypersil BetaBasic-8 column is a good match to our Discovery C8 column, but it has half the retention. We could slow down our flow rate to compensate for this, but a better approach is to try for a column with a closer match of the retention ratio. The best match is probably the Hypurity C8 column. It has a good retention match (1.1) and the Fs value is barely greater than 3. The next slide shows the actual separation.



We see a little loss in resolution for peaks #2 and 3, but the HyPurity C8 separation is still acceptable. The run time for the HyPurity run is slightly greater than for the Discovery separation, but an increase in flow rate from 1.5 to 1.55 mL/min would make the two run times the same.

If we know something about the sample, for example, if it contains acids but not bases, we can weight the Fs function to better correspond to reality. That is, we will get more reliable predictions of relative column selectivity. This also means that we can find more columns that are similar to each other. If we do not consider sample type, then our database program may reject columns that would otherwise be equivalent.

What about Other RP-LC Column Types?

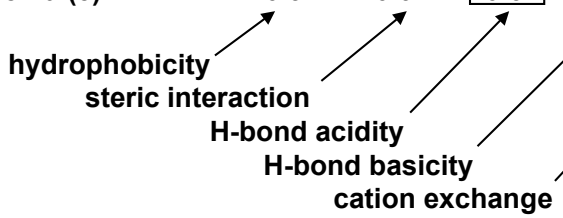
- type-A alkyl-silica columns
- alkyl-silica columns with an embedded polar group
- alkyl-silica columns end-capped with a polar group
- alkyl-zirconia columns
- phenyl, cyano and fluoro-columns

So far, we have looked in detail at four other column types: besides columns made from type-B silica, which is largely free of metal impurities, we have studied columns made from type-A silica which contains metal and is more acidic, columns with an embedded polar group, columns which are polar-end-capped, and alkyl-zirconia columns. Our correlational equation still works for these other column types, and it appears that the same interactions between sample and column are responsible for column selectivity. So, we can still compare columns of one type with another in the same way as the previous examples which used type-B columns.

The next slide summarizes differences in these different column types.

Column type-differences (avg. values)

	H	S	A	B	C
Type-B (95)	0.94	-0.01	-0.11	0.00	0.04
Type-A (38)	0.89	0.06	0.10	0.03	0.76
Embedded polar group (17)	0.68	0.00	-0.53	0.17	-0.28
End-capping polar group (4)	0.94	-0.02	-0.02	0.01	-0.14
Alkyl-zirconia (3)	0.97	-0.02	-0.62	0.00	2.01



Here we have listed average value of each column parameter for each type of column so far studied. The first column type is type-B alkyl silica columns, which now includes 95 columns in the study. Let's compare column parameters for all the other column types and see if they make sense.

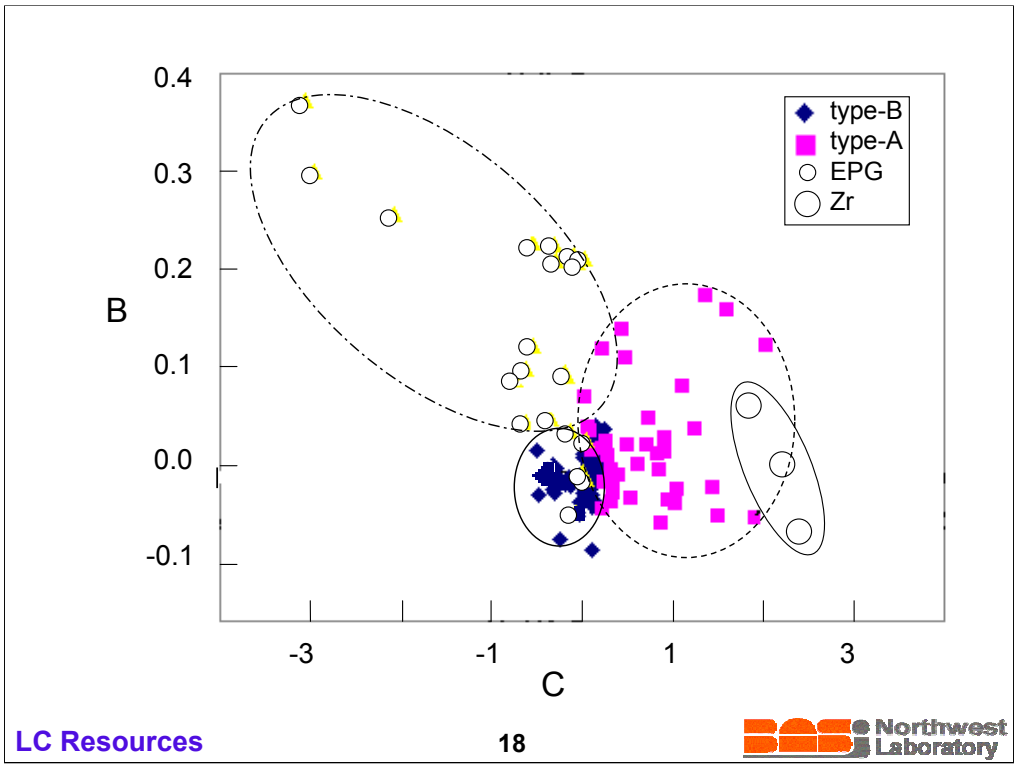
We see for type-A columns that values of A and C are much larger. Because type-A silica is more acidic, this leads to stronger hydrogen bonding by silanols and larger A. It also leads to more ionized silanols and larger C. The results meet our expectations.

Columns with an embedded basic group are much less hydrophobic, or more polar, because of the polar group. Likewise, column basicity B is much larger, while column acidity as measured by A and C is much smaller.

Columns end-capped with a polar group are not much different from type-B columns, presumably because of a smaller effect of the polar group on interactions of sample and column, or a reduced concentration of polar groups.

Finally, alkyl-zirconia columns have no silanol groups, therefore very small values of A. These columns also Adsorb phosphate ions from the mobile phase with increase in column negative charge, therefore very large values of C.

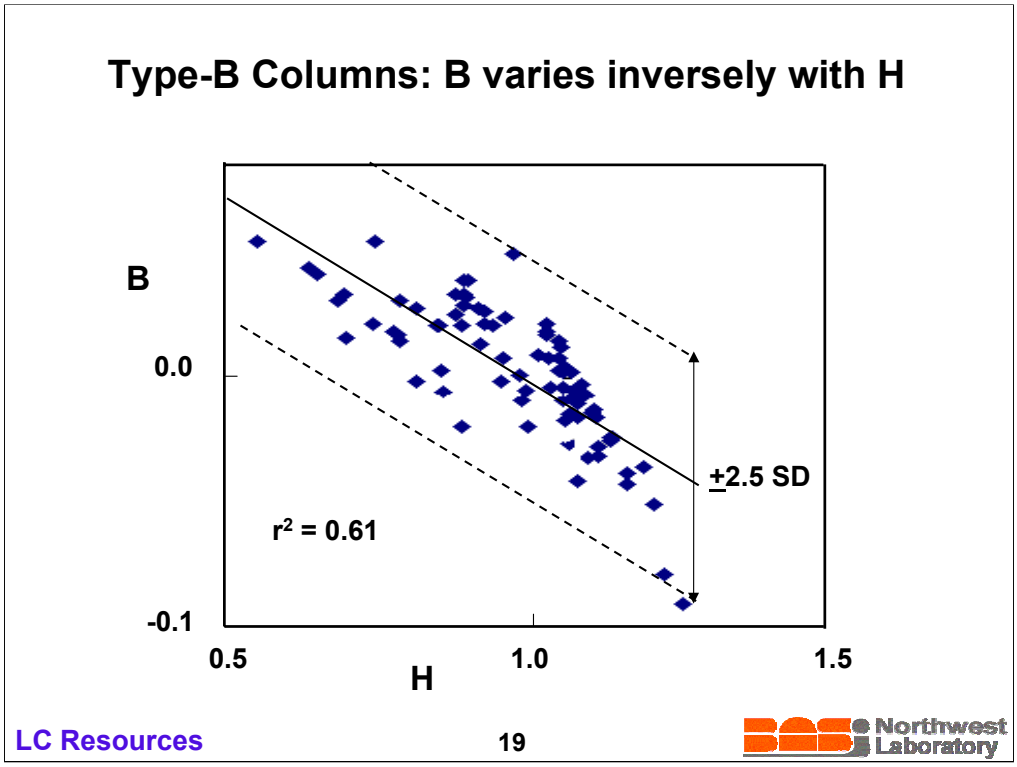
So the differences in the various column parameters fit our expectations. This gives further evidence that the parameters indeed describe the interactions we've identified.



Here's another way to compare the different column types.

The individual column types can be grouped approximately according to column basicity B and acidity C, as seen in this slide. Type-B columns are grouped in a tight cluster, while type-A columns are generally more acidic. Columns with an embedded polar group are more basic and less acidic, while columns that are polar end-capped overlap the type-B group. Alkyl-zirconia columns tend to be even more acidic.

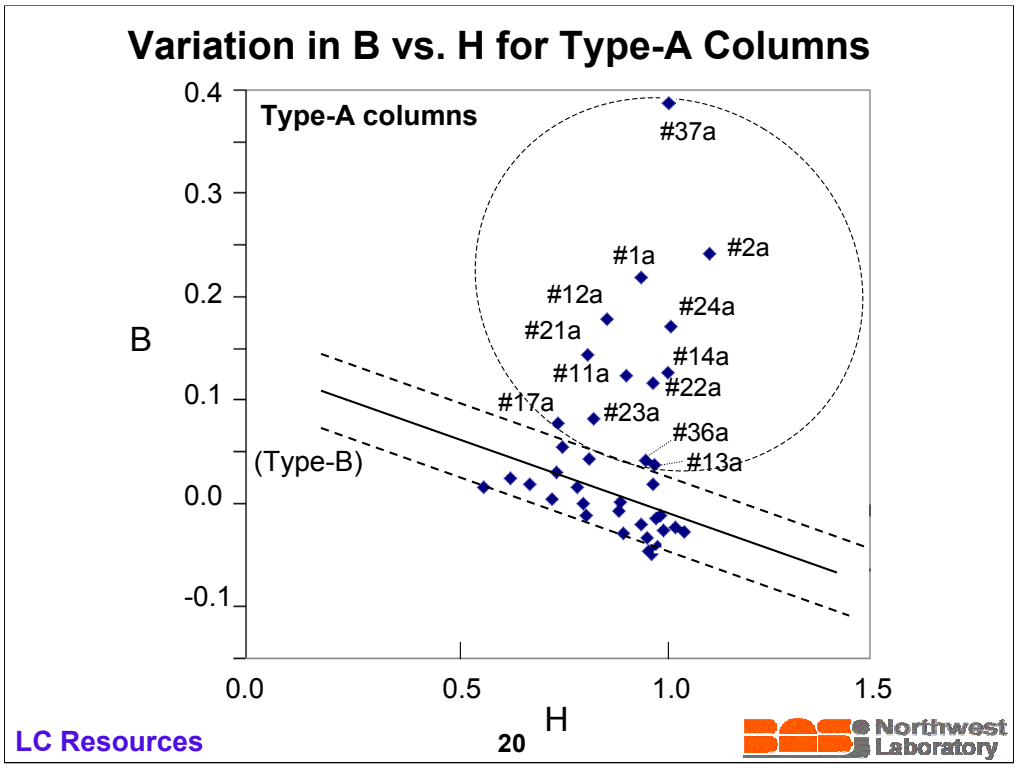
This classification of columns leads to some overlap of columns of one type on another, but this just emphasizes the fact that these column groupings are not completely distinct.



Let's look next at column H-bond basicity or values of B.

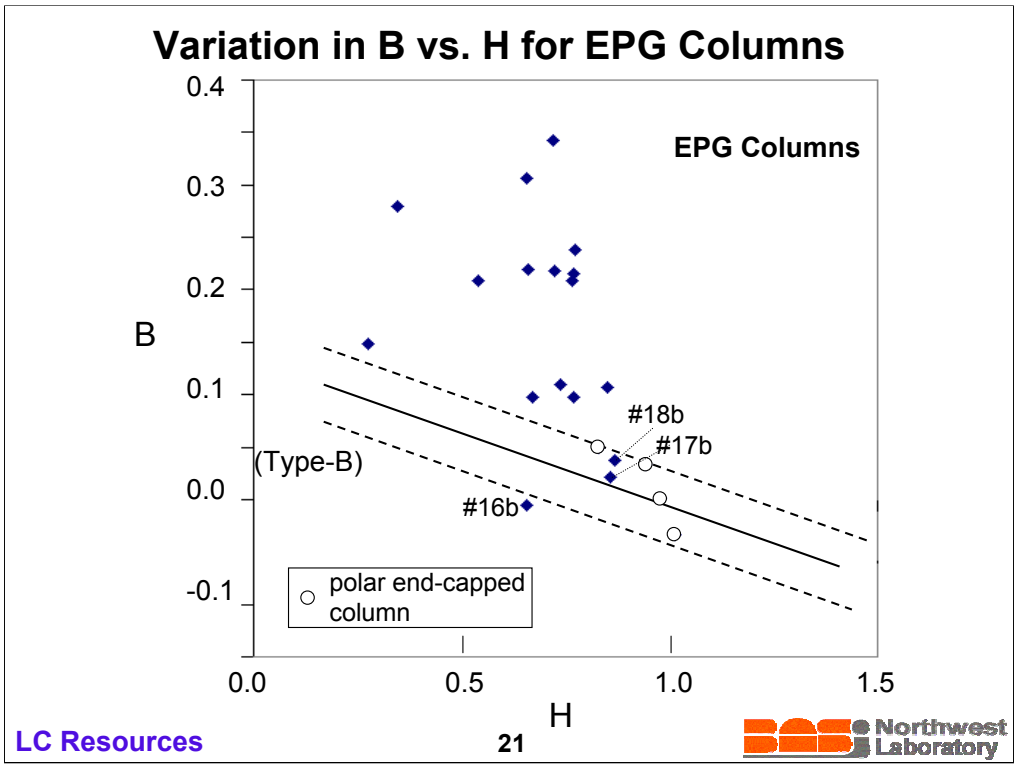
We see in this slide for type-B columns that there is an inverse correlation of values of B and H. This can be explained by column basicity being due to water sorbed by the stationary phase. As H decreases, the column becomes more polar, and more water is sorbed – increasing column basicity and values of B.

Our experiments showed that there was no effect of end capping on column basicity, which indicates that the basic column contributions are not due to silanols. Further support for adsorbed water is the observation that for type-B columns, phenols and alcohols do not interact with the column basic groups; this can be explained by two-fold interaction of water with acids (forming a 5-membered ring).



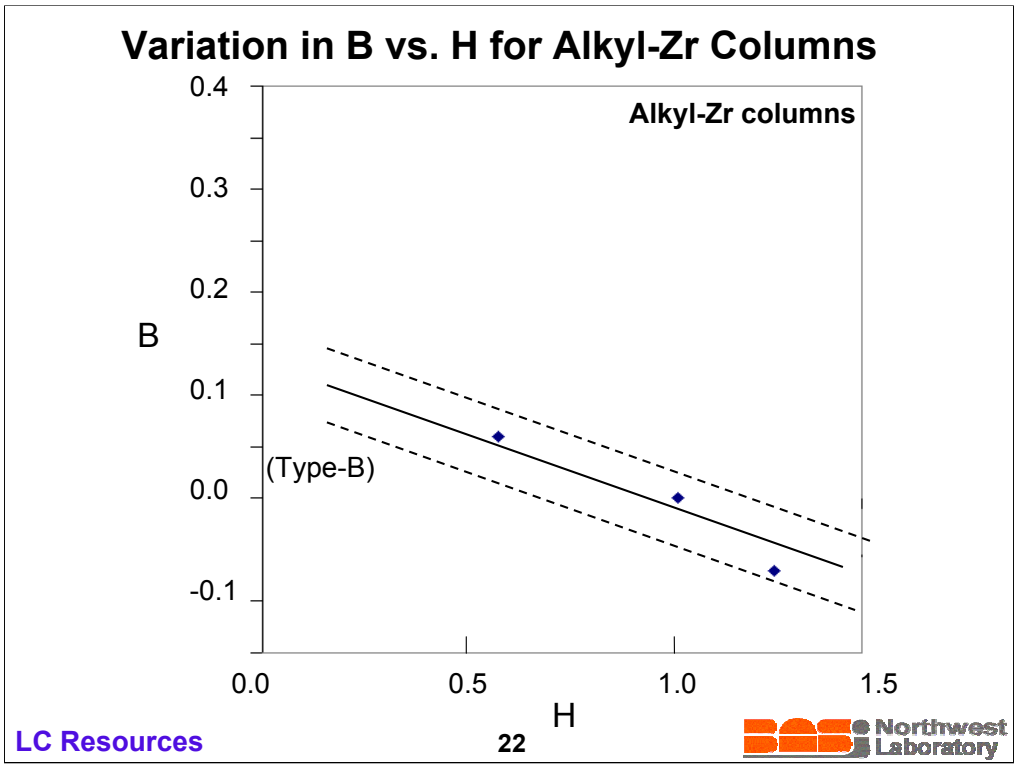
In the next few slides we compare values of B and H for other kinds of columns, with the data spread for type-B columns superimposed.

Looking at type-A columns first, we see that about 2/3 of the data points fall within the dashed lines from the previous slide. However, a remaining 1/3 of the points fall above the dashed lines, corresponding to some additional contribution to column basicity. We believe that metals in the silica lattice can interact with carboxylic acids by chelation, hence accounting for the extra column basicity.



Here we see results for the embedded polar group columns compared to type-B results. The polar-end-capped columns all fall within the error limits for the type-B columns, suggesting that the basicity of these columns is due to sorbed water, not the polar end-capping group.

All but three of the embedded-polar-group columns, all but three fall above the error limits for type-B columns. This suggests that the basicity of these columns is due to something other than sorbed water, in agreement with our belief that the embedded basic polar group is responsible for this greater basicity.



In the case of alkyl-zirconia columns, the three columns fall within the lines which include all type-B columns. That is, sorbed water appears responsible for the basicity of these columns. This is a pretty small data set to support a strong conclusion, so it would be a good idea to gather more data on the zirconia columns.

Conclusions

- column selectivity can be measured
- a database exists for ≈ 180 RP-LC columns of all kinds (>200 columns planned by end of year)
- columns in the database can be compared

  -- either similar or different columns can be selected
- minor differences in selectivity can be corrected by method adjustment
- all of these capabilities will be supported by commercial software

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Let's summarize what we've just looked at.

First, we have reported a means of measuring column selectivity that seems to describe the physical processes that take place when a solute interacts with the stationary phase. We've been able to identify a small set of solutes (8) that allows us to predict the behavior of a given column toward other solutes.

We have developed a database of about 180 columns including those with Type-A and Type-B silica, zirconia, and several different POLAR-bonded phases. This allows us to find columns closely related to the initial column so that there is an alternate column. We can also find a column that is drastically different from the current column so that different selectivity can be obtained during method development.

We haven't talked about method adjustment, but recently we've published techniques that allow small adjustments in conditions to fine-tune selectivity matches between similar columns.

Finally, we're in the process of converting our database into a commercial software package that will make this column comparison capability available to all workers.