Method Optimization and Maintenance Using a Resolution Map

JOHN W. DOLAN

Determining the best mobile phase strength for an isocratic separation and adjusting the mobile phase to compensate for system drift are two problems that can cause a great deal of trouble in the liquid chromatography (LC) lab. Mobile phase optimization is often accomplished by "successive approximations," in which changes are made based on the results of a previous experiment. This trial-and-error approach generally is successful, but it is time-consuming and may misdirect the user to a false optimum. Once a method has been in use for several days or weeks, column aging or other factors may cause important peaks to drift together, making the method no longer usable. Column replacement or trial-and-error mobile phase adjustment are then used to bring the separation back into specifications. The use of a resolution map can speed the selection of the optimum mobile phase, can give an understanding of method ruggedness, and can guide the chromatographer in making mobile phase adjustments when separation problems occur.

RELATIONSHIP BETWEEN *k′* AND PERCENT ORGANIC

The basis of the resolution map is the relationship shown in Figure 1. You will recall that the capacity factor, k', is a unitless measure of retention based on the equation

$$k' = \frac{t_R - t_0}{t_0} \qquad [1]$$

where t_R is the retention time and t_0 is the column dead time. If we plot log k' vs. ϕ (the volume fraction of organic in the mobile phase), we get a linear plot, such as that shown in Figure 1. (Although this plot is not perfectly linear, it can be considered to be so for all practical purposes.) What information can we get from a plot like this? First, we can use Figure 1 to find out what the retention of the compound will be for any value of ϕ. Second, we can make the plot based on just two experiments, because only two points are required to define a straight line. Now, based on two experimental runs (in a reversed-phase system, for example, 30 vol % and 50 vol % methanol in water), we can determine the capacity factor (and thus the retention time) of the compound at any other value of ϕ (for example, 37 vol % methanol in water). In other words, there no longer needs to be any guessing about how retention time changes when the mobile phase strength is changed.

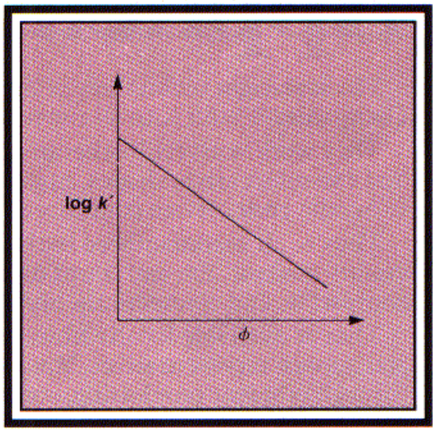

FIGURE 1: Plot of log capacity factor vs. volume fraction of organic in the mobile phase for a hypothetical compound.

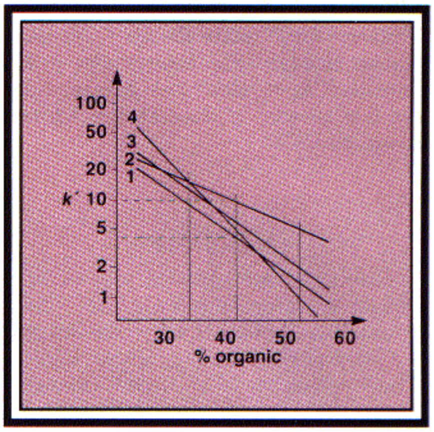

FIGURE 2: Plot of log capacity factor vs. % organic in the mobile phase for a hypothetical sample of four compounds.

All this is well and good, but few of us are interested in just a single compound; rather, we are interested in the movement of one peak relative to other compounds in the sample.

COMPARISON OF RETENTION TIMES

We can compare the retention of the various sample bands by making a plot like that of Figure 1 for each compound in our sample. The resulting composite plot may look something like the one shown in Figure 2. As is typical for most samples, the individual lines are offset slightly from each other, and they vary somewhat in slope. Once again, this plot was made by taking retention data from just two runs (shown by the vertical lines at 34% and 52% organic), converting retention times to k' values, and plotting the data.

Let's see how the data of Figure 2 can help us get a better understanding of our separation. First, we can see that a lot of band reversals take place. For example, band 2 comes out ahead of band 4 when the mobile phase is weaker than 34%; when it is stronger than 34%, band 4 comes out first. At 34%, bands 2 and 4 coelute (only three bands were observed in our initial 34% run). As long as the same pair of bands does not overlap in both initial runs, we can make the plot successfully. We can see quickly that coelution of various band pairs takes place at different mobile phase strengths. Coelution occurs at 30% (bands 2 and 3), 34% (bands 2 and 4), 38% (bands 3 and 4), and 45% (bands 1 and 4). If we are trying to resolve all the bands in the sample, these mobile phase compositions should be avoided.

It is generally preferable to work with mobile phase compositions that lie midway between compositions at which band overlap occurs; this usually gives maximum band separation. One of these mobile phase selections is shown by the vertical line at 42% organic. In this case, the elution order is band 1, then 4, 3, and 2. We can also see that this mobile phase will give good chromatographic performance. (Remember that the k' value should be between about 2 and 10 for best results.) Contrast this with the separation at 52%, in which band 4 comes out quite early ($k' \approx 1$), and there may be more separation than necessary between bands 3 and 2.

If you use α values to determine the degree of separation between adjacent bands, these can be obtained directly by finding the k' value for each band at the desired organic strength. The α value is just the ratio of k' values for adjacent peaks.

A plot such as that of Figure 2 contains a great deal of information about a separation,

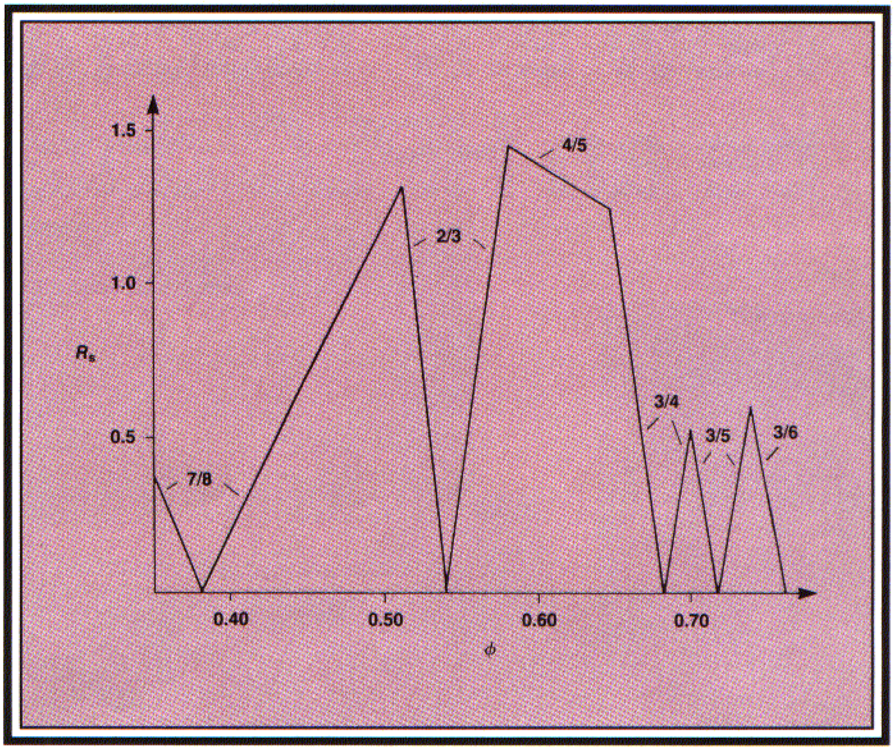

FIGURE 3: Relative resolution map plotting resolution for an N = 10,000 column vs. volume fraction of organic in the mobile phase. Plot shows only the least-resolved band pair; numbers identify that pair. (Data from reference 2.)

but the information is difficult for most of us to grasp quickly because the plot is logarithmic and because no information about bandwidths is given. For these reasons, a resolution map, such as that shown in Figure 3, is much easier to use.

PLOTTING A RELATIVE RESOLUTION MAP

A resolution map is a window diagram that plots the resolution (separation) of the least-resolved band pair vs. the mobile phase strength. It often is easier to use a resolution map for a hypothetical column rather than measuring the exact resolution for each mobile phase composition. Thus, a resolution map based on an arbitrary 10,000-plate column (equivalent to a typical 15-cm, 5-μm particle column) has been referred to as a *relative* resolution map (1,2), because it allows comparison of resolution at one mobile phase strength relative to resolution under other conditions.

To make a plot such as that of Figure 3 from the data of Figure 2, we need to be able to express resolution (R_s) as a function of k'. The standard resolution equation is

$$R_s = \frac{(t_2 - t_1)}{(w_1 + w_2)/2} \qquad [2]$$

where t_1, t_2, w_1, and w_2 are the retention times and bandwidths of the two bands of interest. We can determine the bandwidth for a 10,000-plate column from the plate-number equation

$$N = 16(t_R/w)^2 \qquad [3]$$

if we set N = 10,000 and solve for w. Combining equations 1, 2, and 3 gives equation 4, which expresses resolution for a 10,000-plate column in terms of k' for the two bands of interest.

$$R_s = \frac{50(k_2' - k_1')}{k_1' + k_2' + 2} \qquad [4]$$

Now you can use data such as those shown in Figure 2 along with equation 4 to make a relative resolution map such as that of Figure 4. Plot data for 5% increments in organic over the region of interest; you may want to plot a few more points near the maximum value to improve the accuracy of the plot. Remember to plot data only for the least-resolved band pair. This stage of the process is rather tedious, but you will find that it provides overall time savings; alternatively, you can use commercial software.

INTERPRETING THE RELATIVE RESOLUTION MAP

The relative resolution map (RRM) displays resolution for a 10,000-plate column on the y-axis and the volume fraction of organic (ϕ) in the mobile phase on the x-axis. The plot shows only the "critical" or least-resolved band pair. The numbers on the plot identify the critical pair; for example, bands 2 and 3 are least resolved in the 50–55% organic region. When the plot drops to zero, there is a band crossover (elution order changes for the critical pair).

There are four significant pieces of data that we can obtain from the relative resolution map. These can help us determine the best mobile phase conditions for our sample, tell us how rugged our method will be, and aid us in adjusting the method if it drifts out of specification. Let's look at each of these areas.

First, the RRM can tell us if it is likely that we will get an acceptable separation by varying the strength of the mobile phase. We can see this by looking at the maximum value of R_s shown on the plot. Let's select a target minimum resolution of about 1.5 for our separation. In Figure 3, the maximum value is $R_s \approx 1.4$ for the 10,000-plate standard column. This indicates that separation is likely to be acceptable on standard columns (for example, 15- or 25-cm, 5-µm columns). If, on the other hand, the maximum R_s value were only 0.5, an acceptable separation would be unlikely. Recall the resolution equation

$$R_s = {}^1\!/_4 \sqrt{N}(\alpha - 1)(k'/[1 + k']) \qquad [5]$$

which tells us that R_s varies with the square root of the column plate number N. In order to increase the resolution to 1.5 in this case, we would need to increase N by a factor of nine. It is not practical to connect nine columns together, or to vary the combination of flow rate, particle diameter, and column length so as to increase N by a factor of nine. So the RRM gives us a quick idea if the separation is possible. If separation is unlikely, it is best to switch to another mobile phase type (for example, from methanol to acetonitrile) or another column type (for example from C18 to cyano).

The second area in which the RRM can help us is in determining the optimum mobile phase strength for the separation. It is easy to see that the best resolution is obtained at ~58% organic. Only a small change in resolution is seen when mobile phases of up to ~65% organic are used. Another optimum is found at ~50% organic. Selection of the best conditions is guided by the consideration of method ruggedness, which is the third important piece of information obtained from the RRM.

When we speak of ruggedness, we mean the ability of the method to withstand changes in operating conditions without compromising the results. Take, for example, the separation obtained at 50% organic in Figure 3. The resolution of ~1.3 may be acceptable for the method, but if a small error in mobile phase composition is made (such as when mobile phases are hand-mixed), R_s decreases drastically. We can readily see that a 1% error in mobile phase composition may reduce R_s by up to 50%! Compare this with the 58–65% organic region. If we were to choose to operate at 61% organic, an error as large as ±2% would make only minor changes in R_s. Thus, the RRM quickly gives us information on how rugged the method is likely to be.

Finally, an understanding of the information presented in the RRM will enable us to adjust the method in the future if a deterioration in the separation is found. Consider the case in which the optimum is on a sharp maximum, such as at 50% organic for Figure 3. After running the method for a few weeks, we notice that bands 2 and 3 are beginning to merge (because of column aging or some other cause). From the RRM we know that we must *decrease* the mobile phase strength to bring the separation back to the optimum. On the other hand, if bands 7 and 8 were observed to come together, we would need to *increase* the organic content of the mobile phase. Thus, the RRM helps us understand how to "tweak" the method from time to time as the separation changes. Without this information, we would have to blindly adjust the mobile phase, or we might choose the more expensive option and change the column first.

SUMMARY

The linear relationship between log k' and percent organic in the mobile phase allows us to predict retention for reversed-phase LC separations quickly and accurately based on just two experimental runs. When this information is converted to a relative resolution map, it is easy to find the optimum isocratic mobile phase for a given sample. Additionally, we can determine if a successful separation is likely and how rugged that separation will be. The relative resolution map also can be used as a diagnostic tool to help guide the adjustment of mobile phase conditions in order to fine-tune the separation or compensate for changes in the separation with time.

REFERENCES

(1) J.W. Dolan and L.R. Snyder, *LC•GC* **5**, 970–976 (1987).

(2) L.R. Snyder, J.W. Dolan, and M.P. Rigney, *LC•GC* **4**, 921–929 (1986).

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Lafayette, California, USA, and is a member of the Editorial Advisory Board of LC•GC.

Bulletin

Equipment grants for small colleges. The Pittsburgh Conference and its cosponsoring societies — the Spectroscopy Society of Pittsburgh and The Society for Analytical Chemists of Pittsburgh — will award at least 10 grants of up to $3000 to small colleges for the purchase of scientific equipment, teaching aids, or library materials. Awards are based on submitted proposals, which are due March 1, 1989. Application and proposal forms for the 1989 Pittsburgh Memorial National College Grants Award Program can be obtained from The Pittsburgh Conference, 12 Federal Drive, Suite 322, Pittsburgh, PA 15235, (412) 795-7110.