L C T R O U B L E S H O O T I N G

Shortcuts for LC Measurements

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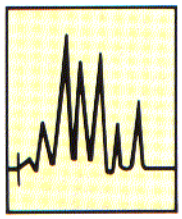

Many workers using liquid chromatography (LC) tend to skip taking basic measurements of such LC separation parameters as k', t_0, and N because it is inconvenient. As discussed many times in "LC Troubleshooting," some of these parameters are important diagnostic tools that can help determine whether problems exist with a separation or with the LC system. The following discussion covers some shortcuts for quickly estimating, rather than calculating, these parameters.

COLUMN DEAD-TIME

The column dead-time t_0 is the time that it takes an unretained sample component to pass through the column. We often recognize t_0 as the first disturbance in the baseline. In gas chromatography (GC), a solvent peak is common, but in LC separations, there is often no baseline disturbance at t_0. A further problem can result when high-molecular-weight components are present in a sample of smaller compounds. If the large molecules are too big to penetrate the pores in the column packing, these molecules elute from the column *before* t_0, just as they would with a size-exclusion column. When this happens, the first "blip" in the baseline is *not* t_0, and, as a result, the behavior of other sample compounds may be misinterpreted.

It is good to double-check that the observed t_0 value is what is expected for the column. This can be done for 4.6-mm i.d. columns with the estimate

$$V_0 \cong (0.1 \text{ mL/cm})\,(L) \qquad [1]$$

where V_0 is the column dead volume, and L is the column length in centimeters. For example, a 15 cm × 4.6 mm column has a t_0 value of ~1.5 mL. Similarly, a 25-cm column has ~2.5 mL of dead volume. The dead volume is converted to t_0 by dividing by the flow rate F:

$$t_0 = V_0/F \qquad [2]$$

So, if you are using a 15-cm column at 3 mL/min, the t_0 disturbance should occur 0.5 min after injection (1.5 mL/[3 mL/min]).

CAPACITY FACTOR

The capacity factor k' may be the most useful, yet the least used, separation parameter in LC. For good chromatographic performance with isocratic separations, k' should be in the range of 1 to 10 (or 0.5 to 20 in extreme cases). If k' is less than ~1, the bands are inadequately separated from unretained material eluting at t_0; if k' is greater than 10, the separation takes too long, and the bands become excessively broadened. If k' is too large or too small, change the mobile phase composition so that acceptable k' values are obtained; otherwise you will be working with a suboptimal separation. If the ratio of k' values for the first and last bands of the chromatogram is greater than ~30, it is unlikely that a satisfactory isocratic separation can be obtained using the present column and mobile phase solvents. In this case, gradient elution should be tried.

Capacity factor can be calculated using the common formula

$$k' = (t_R - t_0)/t_0 \qquad [3]$$

where t_R is the retention time of the band of interest. Calculating k' is fine, but we tend to be too precise in our measurements. That is, we measure t_0 and t_R to the nearest 0.5 mm, if we are doing manual measurements, or to the nearest 0.01 min, if we are using an integrator. We then use a calculator to figure k' to two or three decimal places. For diagnostic purposes, k' values accurate to the nearest integer value are satisfactory. You can make such estimates by using t_0 as a ruler, as illustrated in Figure 1 (this is exactly what is done in equation 3, but less precisely). Usually you can make these estimates visually, although some workers prefer to use a ruler or a pair of dividers.

To estimate k', as in Figure 1, first find the t_0 disturbance (verify this with the t_0 estimate discussed above). Now use the t_0 distance as a ruler and measure the retention of each band in units of t_0, starting at t_0. For example, in Figure 1a, a 25-cm column is operated at 1 mL/min; t_0 is at 2.5 min, the first band elutes at 5 min, and the second band elutes at 10.0 min. Thus, k' is estimated as 1 for the first band (1 t_0 unit past t_0) and 3 for the second band. Remember that although t_0 changes when the flow rate is changed, k' remains constant, as is illustrated for the same separation at 2 mL/min (Figure 1b) and at 0.5 mL/min (Figure 1c).

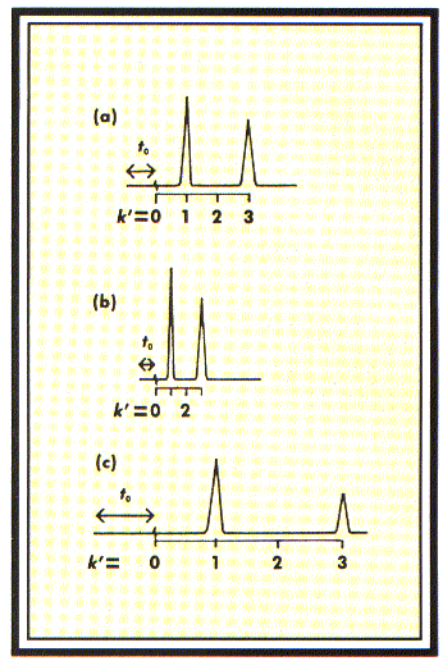

FIGURE 1: Using t_0 to estimate k' values. A 25-cm column at (a) 1 mL/min; (b) 2 mL/min; (c) 0.5 mL/min.

THE 10% RULE

As mentioned above, it is desirable for k' to equal 1–10 for good chromatographic behavior. What happens if the bands don't elute in this k' range for the first run (which is likely to be the case)? If k' is too large, a stronger mobile phase should be used to elute the solutes earlier. Conversely, a weaker mobile phase should be used if the k' values are too small. How much stronger or weaker? For reversed-phase separations, use the 10% rule: **k' changes two- to threefold for a 10% change in mobile phase organic.** For example, if the last band elutes with $k' \cong 20$ in a 70:30 acetonitrile/water mobile phase, k' would be expected to be between about 6 and 10 for a 60:40 mobile phase. Keep the 10% rule in mind and you will be able to find the best mobile phase strength quickly.

TRANSLATING SOLVENT STRENGTH

Even if you have k' in the proper region, the separation still may not be adequate. In that case, you will usually want to try another or-

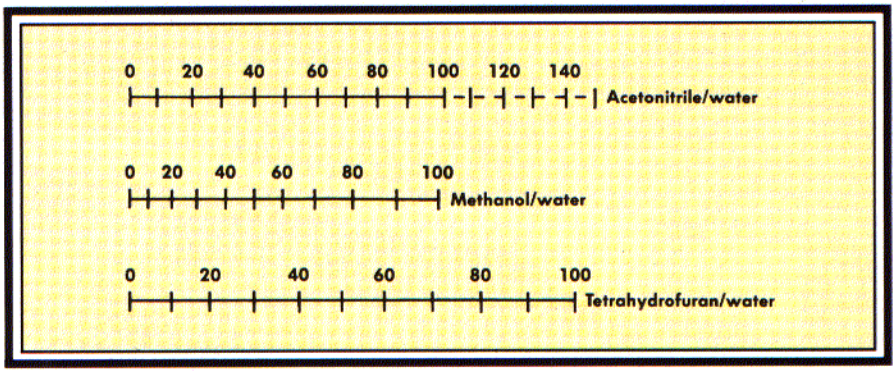

FIGURE 2: Nomogram for estimation of isoeluotropic mobile phases. Based on data from reference 1.

ganic solvent in the mobile phase in order to change the selectivity — for example, change from acetonitrile to methanol or tetrahydrofuran (THF). It would be nice to switch to another solvent without having to start over with the adjustment of solvent strength to get k' in the right range. We can do that with the aid of the nomogram in Figure 2. This figure is based on a large number of compounds (1) and can be used to convert from one solvent to another while maintaining approximately the same solvent strength. Small adjustments of the new mobile phase will be required for most samples, but with Figure 2 and the 10% rule, you should be able to find an isoeluotropic (equal-strength) mobile phase easily.

To use Figure 2 to convert from one solvent to another, locate the present solvent composition on the appropriate line — for example, 50% acetonitrile/water. Now draw a vertical line through the other solvent lines; the intersection of these lines indicates the isoeluotropic mobile phase compositions. Thus, 50% acetonitrile/water is equivalent to 60% methanol/water or about 37% THF/water.

ESTIMATING COLUMN PLATE NUMBER

The column plate number N is a good measure of the quality of an LC column. Columns with large N values will produce narrow peaks and better resolution than columns with lower N values. Low N values can be indicative of extracolumn effects, column voids, unwanted sample interactions, and other chromatographic problems. We can measure N for a test chromatogram using the formula

$$N = 5.54\,(t_R/w_{0.5})^2 \qquad [4]$$

where $w_{0.5}$ is the bandwidth at half its height. We must have an expected N value for comparison if we want to know how good the column is. Many manufacturers include test chromatograms or data indicating the N value for the column under ideal conditions. If you have these data, you can compare your column under the same conditions (and, in fact, you should repeat this test on each new column to verify that the column is good). Often, however, the test sheet is lost, or you would like to know how the column should perform with a real sample. Ideal test conditions result in a reduced plate height h of 2.0–2.5, whereas h values of 3.0–3.5 are more reasonable for real samples. You can calculate the expected value of N with the standard equation

$$N = L/(h\,d_p) \qquad [5]$$

where L is the column length and d_p is the particle diameter (L and d_p are in the same units). As with the example above for calculating k', equation 5 often is more precise than we need, and it requires a pocket calculator for most of us to figure out. A simple estimate of N can be made using the relationship

$$N \cong 3000\,L/d_p \qquad [6]$$

where L is the column length in cm, and d_p is the particle diameter in microns (h is assumed to be 3.3). Thus, a 15-cm, 5-μm column should generate about 9000 plates. Estimating N for a 3-μm particle column is even easier: just multiply the length in cm by 1000. For example, a 10-cm, 3-μm column should give about 10,000 plates.

CONCLUSIONS

I hope that these shortcuts will convince more of you to take LC parameter measurements routinely. By adjusting the mobile phase strength to get reasonable k' values, changing organic solvents to improve selectivity, and verifying that the column is generating a reasonable plate number for a sample, you can develop methods that will give more reliable results with shorter run times. The techniques discussed here should help you reach that goal.

If any of you have other shortcuts, methods for doing estimates, or rules of thumb that you find useful in routine LC work, please share them. Write to The Editor, "LC Troubleshooting," *LC•GC*, P.O. Box 10460, Eugene, OR 97440, USA.

REFERENCES

(1) P.J. Schoenmakers, H.A.H. Billiet, and L. DeGalan, *J. Chromatogr.* **218,** 261 (1981).

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc., of Lafayette, California, and is a consulting editor for LC•GC.