LC
TROUBLESHOOTING

Using Dead Time, Retention, and the Capacity Factor as Diagnostic Tools

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Because measuring important parameters can be inconvenient, we often skip such tasks. Here are some shortcuts that minimize the effort.

If you are a regular reader of this column, if you attend an occasional liquid chromatography (LC) short course, or if you read the scientific literature, you're well aware of the importance of keeping an eye on several significant separation parameters, such as dead time (t_0) and the capacity factor (k'). Most of us, however, don't make the necessary measurements, either because of the inconvenience involved or because we aren't sure what to do with the measurements once we've made them. In this month's "LC Troubleshooting," I will show that an estimate of these values is often as useful as an accurate measurement. Furthermore, the increased convenience enables you to estimate parameters routinely, which in turn can result in better methods and faster solutions to separation problems.

COLUMN DEAD TIME

The first important parameter needed is an estimate of column dead time (t_0). Column dead time is the time it takes for an unretained substance to travel through the LC column. We often recognize the dead time by the characteristic baseline disturbance early in the chromatogram, sometimes called the solvent peak (Figure 1). The t_0 disturbance results from a refractive index change as the injection solvent passes through the detector (Figure 1a) or, in reversed-phase LC, from the elution of very polar material that washes directly through the column (Figure 1b). In some cases, identifying the disturbance at t_0 may be difficult, so knowing where to expect it can be useful. In addition, unexpected changes in t_0 can indicate improper pump performance.

You can make a good estimate of t_0 with the help of the column dimensions and the flow rate. First, you need to determine the column dead volume (V_m), which is the volume inside the column, including the pore volume, that is not occupied by the packing particles. You can easily estimate the dead volume for conventional 4.6-mm i.d. columns using equation 1:

$$V_m \approx 0.1L \qquad [1]$$

where V_m is expressed in milliliters, and L is the column length in centimeters. Thus, for a 25 cm × 4.6 mm column, $V_m \approx 2.5$ mL. To determine the dead volume for columns with other internal diameters, use equation 2:

$$V_m \approx 0.5Ld_c^2 \qquad [2]$$

where d_c is the column diameter in centimeters. For a 4.6-mm i.d. column, the estimates obtained using equations 1 and 2 differ by ~5% — close enough for our purposes.

Once you have estimated V_m, you can determine t_0 (in minutes) by dividing by the flow rate (F, in milliliters per minute) using equation 3.

$$t_0 = V_m/F \qquad [3]$$

Of what use is t_0? As I show below, t_0 is necessary for determining the capacity factor, and it also provides information about the performance of the LC pump. If the observed value of t_0 is not within ~10% of the estimated value of t_0 (obtained using equation 3), something is wrong.

As Figure 2 and equation 3 illustrate, t_0 is inversely related to the flow rate: if the flow rate is halved, t_0 doubles. Note that retention times (t_R), measured from the injection point to the peak apex, vary directly with t_0 as the flow rate changes. Thus, if you observe changes in both t_0 and t_R, a flow rate problem is the probable cause. Increases in t_0 and t_R indicate that the flow rate is lower than it should be, possibly because of a leak or an air bubble trapped in the pump. Decreased values of these parameters indicate higher than normal flow rates, which are most likely caused by an improper flow-rate setting. Of course, changing the column length can also change t_0 and t_R, but you are unlikely to overlook this obvious cause of dead-time and retention-time change.

THE CAPACITY FACTOR

Once you know the column dead time and the retention time, you can calculate a value for the capacity factor using equation 4:

$$k' = (t_R - t_0)/t_0 \qquad [4]$$

Determining k' is another way to measure retention because the capacity factor expresses retention in terms of t_0 (or V_m). To find k', first determine the corrected retention time, which is the time between t_0 and the point at which the peak is eluted ($t_R - t_0$). This corrected retention time can be expressed in terms of t_0 by dividing the corrected retention

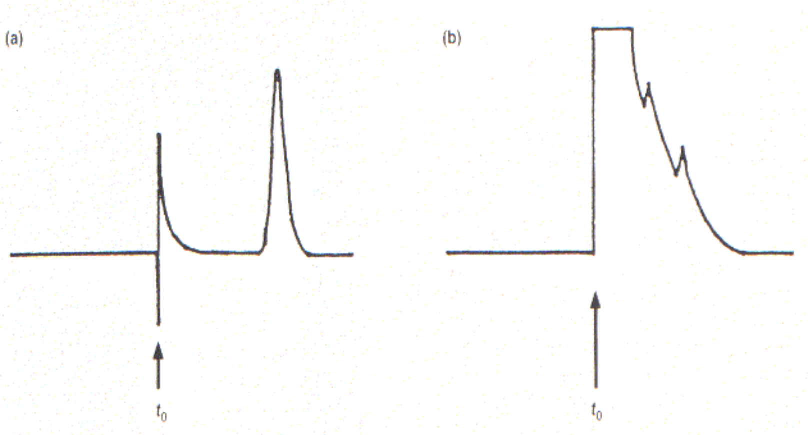

FIGURE 1: Determination of column dead time using (a) refractive index change and (b) elution of unretained sample components. (Reprinted with permission from reference 1.)

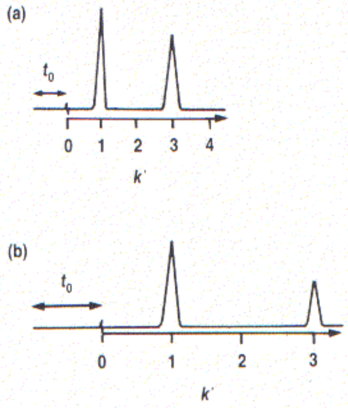

FIGURE 2: Estimating capacity factor and column dead time from chromatograms generated at flow rates of (a) 1.0 mL/min and (b) 0.5 mL/min. (Reprinted with permission from reference 2.)

time by t_0. You can measure t_R and t_0 either in units of time or in distance (using a ruler on the chart paper) because the units cancel out, leaving k' as a unitless quantity. You can accurately measure t_0 and t_R and then use equation 4 to calculate k' to several decimal places, but for most purposes, estimating k' to within ± 0.5 is satisfactory.

Figure 2 shows how to make a quick estimate of k'. First, measure t_0 by holding a piece of paper next to the baseline and marking the distance from the injection point to t_0. Next, use this distance to divide the rest of the chromatogram into t_0-size units, starting at t_0. The first peak occurs one t_0 unit past t_0, so $k' \approx 1$ for this peak. Similarly, because the second peak is eluted three t_0 units from t_0, its k' value is ~3.

In contrast to t_0 and t_R, k' does not change with the flow rate (compare Figures 2a and 2b). The factor that most commonly affects the value of k' is mobile-phase composition. Using stronger mobile phases (or more organic solvent in reversed-phase systems) reduces k'; using weaker mobile phases increases k'. Thus, when k' changes, the most likely cause is a change in the mobile phase. A change in the column packing type and gradual aging of the packing can also affect k'. Column temperature changes can affect k' to a certain extent, but unless the temperature change is dramatic, the capacity factor changes only minimally.

THE BEST CAPACITY-FACTOR RANGE

Estimating k' can help you identify mobile-phase conditions that are likely to provide the best chromatographic performance for your separation. Figure 3 is a plot of resolution (R_s) vs. k'. For very low k' values, resolution is also low. Small resolution values indicate a poor separation; in addition, the "garbage" being eluted at t_0 probably interferes with the peaks. At the other end of the plot, R_s rises only slightly as k' increases. In fact, under these conditions, sensitivity is lost because as retention increases, the peaks broaden and are thus shorter and harder to detect.

The plot of Figure 3 indicates the range of k' values that can provide the best chromatographic performance. Ideal separations have capacity factor values between 2 and 10, but, in practice, eluting a sample within the 1–20 k' range is a more realistic goal. Within this range, relatively small changes in mobile-phase composition result in significant resolution changes. In addition, you can obtain reasonable run times and fairly narrow peaks. Adjusting mobile-phase conditions for problem separations so that sample peaks are eluted in the recommended k' range can solve many practical separation problems.

For reversed-phase separations, k' increases as the percentage of organic solvent decreases. As a guide to adjusting mobile-phase strength and thus the k' range, you can use the "rule of three," which states that a 10% change in the amount of organic solvent in the mobile phase causes k' to change by a factor of ~3. Thus, if $k' = 15$ for a substance eluted by 60:40 (v/v) methanol–water, k' should be ~5 for the same compound eluted by 70:30 (v/v) methanol–water. The rule of three is meant to be used as a general