Peak Tailing and Resolution

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How does peak tailing affect resolution?

Resolution is a measurement used to quantify peak spacing in a liquid chromatography (LC) separation. Although very simple at first examination, resolution can be affected significantly by peak sizes and shapes. This month's "LC Troubleshooting" illustrates how a specific resolution value can sometimes be a misleading description of a separation.

Measuring Resolution

The most common formula for measuring resolution (R_s) is

$$R_s = 2(t_2 - t_1) / (w_1 + w_2) \qquad [1]$$

where t_1 and t_2 are the retention times of the two peaks of interest, and w_1 and w_2 are the peak widths measured at the baseline between tangents drawn to the peak sides. From a practical standpoint, it is much easier to measure the peak width at half the peak height. The resolution equation using the half-height method is

$$R_s = \frac{2\left(t_2 - t_1\right)}{1.7\left(w_{0.5,1} + w_{0.5,2}\right)} \qquad [2]$$

where $w_{0.5,1}$ and $w_{0.5,2}$ are the peak widths measured at half height. The half-height method for measuring resolution is used commonly by data systems because it is much easier to measure the half-height width than the baseline width. This technique is also easier to apply to peaks that are not baseline-resolved. If the peaks are not separated fully, it can be difficult or impossible to measure the baseline width accurately. Equations 1 and 2 will give the same value of R_s if the peaks are symmetric, as is the situation for Figure 1(a).

The valley between two symmetric peaks just touches the baseline when $R_s \approx 1.5$. Because it is a good idea to have a little extra baseline between peaks to tolerate some deterioration in the separation, most workers select a value of 1.75 to 2.0 as the minimum acceptable resolution.

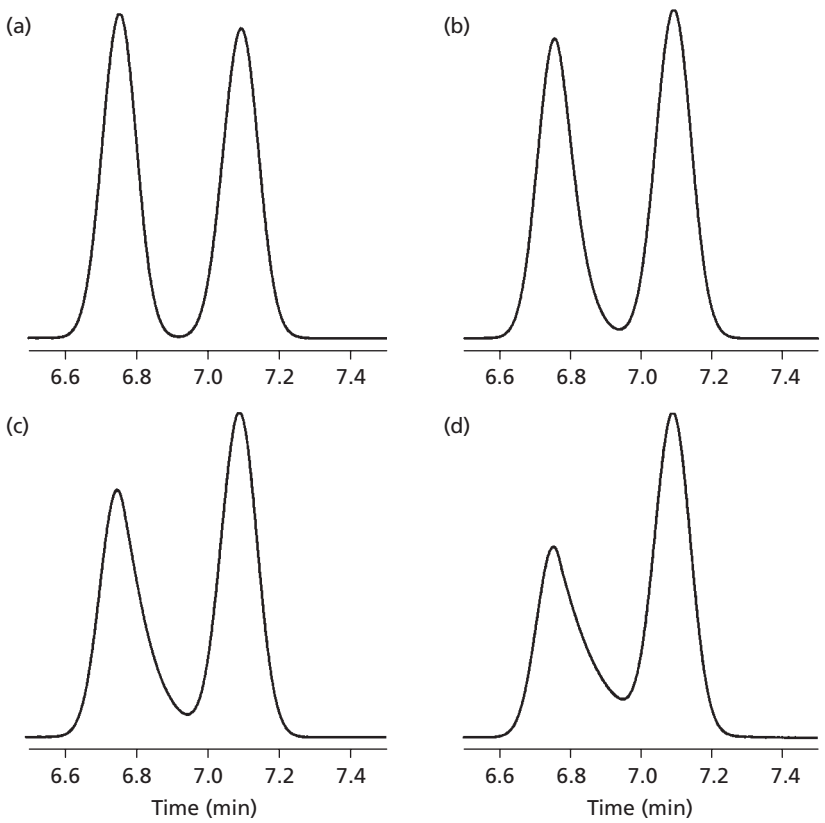
Peak Tailing

In the real world of practical chromatography, perfectly symmetric peaks, as in Figure 1(a), are very rare. More common are peaks that show some degree of tailing. Peak tailing is often measured by the peak asymmetry factor (A_s):

$$A_s = b/a \qquad [3]$$

where a is the width of the front half of the peak, and b is the width of the back half of the peak measured at 10% of the peak height from the leading or trailing edge of the peak to a line dropped perpendicularly from the peak apex. Most analysts outside the pharmaceutical industry use the asymmetry factor.

Figure 1: Simulated chromatograms for peaks with equal peak area. The peak asymmetry factors for the first peak in each pair are (a) 1.0, (b) 1.2, (c) 1.5 and (d) 2.0. The asymmetry factor is 1.0 for the second peak in each pair.



Pharmaceutical workers use the *US Pharmacopeia* tailing factor (T_f) as the most common measure of peak asymmetry:

$$T_f = ac / 2ab \qquad [4]$$

where ac is the peak width at 5% of the peak height, and ab is the front half-width measured from the leading edge to a perpendicular dropped from the peak apex. The two peak measurement techniques yield slightly different numeric values, as illustrated in Table 1. As long as one technique is used consistently, however, it really doesn't matter which is selected. For the rest of this discussion, I'll use the asymmetry factor for the illustrations, but those concepts apply to all tailing peaks, regardless of how the tailing is measured.

Table 1: Peak asymmetry and peak tailing factor relationship*

Peak Asymmetry Factor (at 10%)	Peak Tailing Factor (at 5%)
1.0	1.0
1.3	1.2
1.6	1.4
1.9	1.6
2.2	1.8
2.5	2.0

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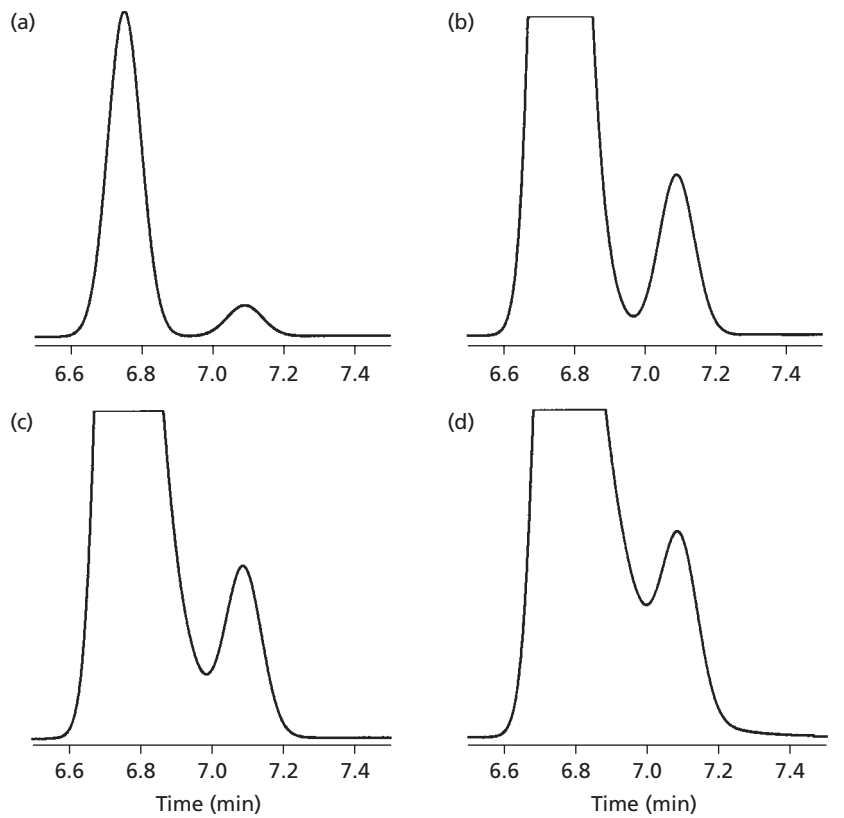
The Effect of Tailing Peaks

I've included several groups of peaks to illustrate the practical effect of tailing peaks on the quality of a separation. In all figures, except Figure 4, the retention times of the two peaks are the same, 6.75 and 7.09 min. Within each figure, the peak area ratios are constant. For illustrative purposes, any peak tailing is applied only to the first peak — the second peak is always a perfect Gaussian peak. These peaks are simulated peaks drawn with the aid of DryLab and Chrom Merge software (LC Resources Inc., Walnut Creek, California, USA), but the discussion applies to real peaks in other chromatograms.

In Figure 1, all peaks have equal areas. Figure 1(a) has an asymmetry factor of 1.0 and a resolution of 1.75. These values would be a satisfactory separation for quantitative analysis — each peak is distinct from the other. A small degree of tailing begins to degrade the separation, as Figure 1(b) shows, in which the first peak has an asymmetry factor of 1.2 and a resolution of 1.5. Most column manufacturers consider asymmetry factors of 0.9–1.2 acceptable for test compounds. However, Figure 1(b) clearly shows the beginning of a degraded separation. Many real methods generate peaks with A_s values of 1.5, as in Figure 1(c), in which the resolution has degraded to a value of approximately 1.3. When peak symmetry has degraded to an asymmetry factor of 2.0, as Figure 1(d) shows, the resolution is significantly compromised ($R_s \approx 1.0$). As peak tailing increases, it becomes more difficult to determine the resolution based upon calculations.

Figure 1 also illustrates a second cost of tailing peaks — loss in peak height. Because detection limits are directly related to peak height, the detection limits are also compromised by tailing peaks. If I wanted to regain the resolution of Figure 1(a) from the situation of Figure 1(d), I would need to either move the peak centres apart, generally at the cost of run time, or make chemical changes to reduce the peak tailing. Peak tailing can often be minimized by using the newer Type B silica columns or mobile-phase additives, but it is rarely possible to eliminate peak tailing completely.

Figure 2: Same as Figure 1, except the peak area ratio for the two peaks is 10:1. The peak heights are expanded to aid visualization of the smaller peaks.



When Peak Size Changes

Figure 2 shows the same chromatograms from Figure 1, except the area of the second peak is one-tenth that of the first peak. When the peaks are symmetric, as in Figure 2(a), an improvement is apparent in the peak separation when compared with Figure 1(a), even though the calculated resolution is the same in both instances, because the peak width is independent of the peak height as long as the column is not overloaded. However, as the peak tail increases, the quality of the separation decreases dramatically. The R_s value doesn't tell the whole story because the calculated resolution for each peak pair in Figure 2 is the same as its counterpart in Figure 1. This problem occurs in part because the trailing edge of the first peak contains all the added width of the tail, which distorts the calculation.

I can obtain a more practical measure of the separation's quality by observing the depth of the valley between the peaks when compared with the height of the shorter peak. For example, rather than specifying a value of R_s, I could require that the valley between the two peaks must be

no larger than 20% of the shorter peak, as in Figures 1(d) and 2(c).

The chromatograms of Figure 3 are based upon a peak area ratio of 100:1. In this separation, the problems encountered for the peak area reduction are exacerbated. When peak asymmetry is much greater than 1.2, the second peak is lost in the tail of the first.

must often be separated by several minutes to escape the broad tailing skirt at the base of the parent. Resolution calculations fail to tell the proper story, especially if analysts use the half-height resolution measurement technique. For example, I could inject standards of two compounds separately. The half-height widths of the two peaks might suggest that the

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A loss of separation can be of practical significance when peaks that differ greatly in size occur in the same run, such as in trace analysis. When the major peak is expanded sufficiently, the tail of even a well-shaped peak can extend for some distance beyond what is normally considered the peak width. I commonly observe this situation in my laboratory during the development of stability-indicating assays. In these methods, chromatographers must quantify all peaks larger than 0.1% of the parent peak. If a minor peak is on the tail of a parent, it

combination should appear as in Figure 3(a), yet when the large peak is examined carefully, it tails moderately near the base and causes a nearly complete overlap, as in Figure 3(c).

With real sample compounds, even peaks with the most symmetrical appearance when viewed at full scale will show significant tailing when they are enlarged 100-fold. This outcome is the result, at least in part, of the fact that most peaks are retained by a combination of reversed-phase and silanol retention. Any molecule that contains a basic nitrogen will tend to interact more strongly with silanol groups at the silica surface of the column packing. Because the population of free silanols is limited compared with the bulk bonded phase, the silanols become overloaded more easily. This overload causes a tail for nearly every nitrogen-containing peak in a run. When this peak is enlarged greatly, the tail is exaggerated and can hide a closely eluted minor peak. Figure 4(a) (same as Figure 3(d)) illustrates this situation in which the second peak is nearly hidden. It is difficult, if not impossible, to eliminate peak tailing completely in these instances, so the problem must be solved by increasing the separation between peak centres, as in Figure 4(b). Often this adjustment is no trivial task.

Conclusions

Chromatographers can learn several practical lessons from these fairly simple examples. First, be careful about relying too heavily upon the numeric value of resolution, especially when any peak tailing is present. Second, although the baseline and half-height methods of measuring

Figure 3: Same as Figure 2, except the peak area ratio for the two peaks is 100:1.

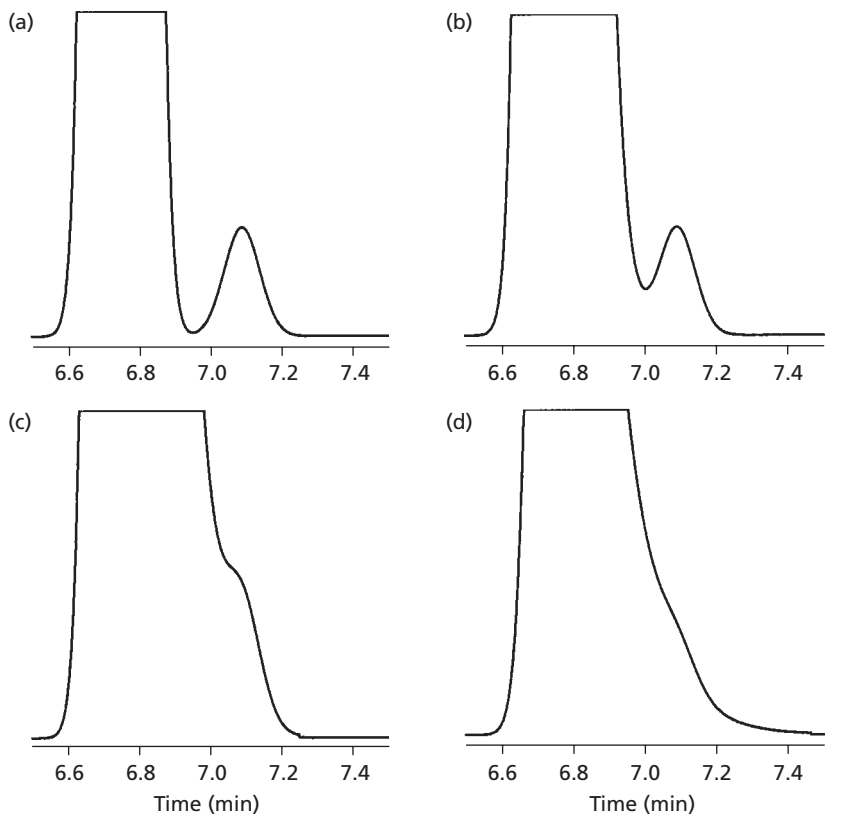
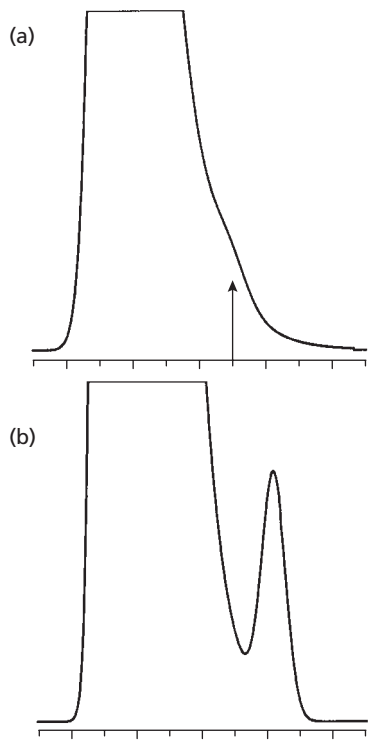


Figure 4: To increase resolution when a minor peak (arrow in (a)) is buried in the tail of a large peak, the peak centres must be moved apart (as in (b)) at the cost of run time.



resolution provide the same values for symmetric peaks, they can differ greatly when applied to tailing peaks. This difference means that a data system set to use the half-height method can give you a false sense of security if you apply its measurement to a system-suitability sample with tailing peaks. The number in the report might say that the resolution is satisfactory, but without visually examining the peaks, analysts could have an incomplete qualitative understanding of a separation. It is a good idea to require baseline resolution between peaks, not just a numeric value of resolution. Overlooking this basic principle can have disastrous consequences as a column ages with natural increases in peak tailing.

Finally, it is important to realize that the problems discussed in this month's "LC Troubleshooting" apply almost exclusively to small peaks that follow large peaks that have some peak tailing. If the small peak leads the big one, the problem is reduced or nonexistent because the occurrence of fronting peaks is much less common than tailing ones. If chromatographers have a choice about where to place peaks in the chromatogram during method development, they should always put the minor peaks in front of the larger ones.

Reference

1. L.R. Snyder, J.J. Kirkland and J.L. Glajch, *Practical HPLC Method Development*, (John Wiley & Sons, New York, USA, 2nd ed., 1997), 211.

"LC Troubleshooting" editor **John W. Dolan** is president of LC Resources Inc., of Walnut Creek, California, USA, and a member of the Editorial Advisory Board of *LC•GC Europe*. Direct correspondence about this column to "LC Troubleshooting," *LC•GC Europe*, Advanstar House, Park West, Sealand Road, Chester CH1 4RN, UK.

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