LC Troubleshooting

Why Do Peaks Tail?

Truly symmetrical peaks are rare.

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If you look carefully, you will see that almost every chromatographic peak tails to a certain degree. Although many of today's liquid chromatography (LC) columns are less problematic from this standpoint than were their ancestors, the problem has not been eliminated. This month's "LC Troubleshooting" discusses the causes of peak tailing, how to measure it, some preventive steps you can take, and why newer column types are less prone to tailing.

What Is Peak Tailing?

A peak is labeled as tailing or asymmetrical when it deviates from the ideal, symmetrical shape of a Gaussian peak. Figure 1 shows an example of a tailing peak. The later-eluted half of the peak is wider than the front half, and the broadening appears to be emphasized near the baseline. Although fronting peaks can occur in chromatography, they are rare compared with tailing peaks. Because peak tailing can influence the quality of a separation, it is a good idea to quantify the amount of tailing a peak has. Two widely used measurements of peak tailing exist. Workers in the pharmaceutical industry use the U.S. Pharmacopeia (USP) tailing factor (T_f)

$$T_f = \frac{a + b}{2a} \quad [1]$$

where a and b are the front and back half-widths at 5% of the peak height, as shown in Figure 1. Most other workers use the asymmetry factor (A_s)

$$A_s = \frac{b}{a} \quad [2]$$

where a and b are measured at 10% of the peak height. If $a = b$, both measurements yield a value of 1.0 — the value for a nontailing peak.

For values of less than approximately 2, A_s and T_f values are roughly the same, as is shown in Table I. It really doesn't matter which measurement of tailing is used — unless it is dictated by an industry regulation — as long as the same technique is used consistently so that changes in peak tailing can be tracked.

What Is Acceptable?

Some degree of peak tailing is unavoidable, but how much can be tolerated? Figure 2 shows some examples of peaks with various asymmetry factors. When a column is new, most manufacturers will allow it to pass the acceptance criteria if A_s is between 0.9 (slight fronting) and 1.2 (slight tailing). Most practical workers using real samples strive for asymmetry factors of no more than 1.5, but they will settle for less than 2.0 if necessary. Generally, when asymmetry factors are greater than 2.0, something

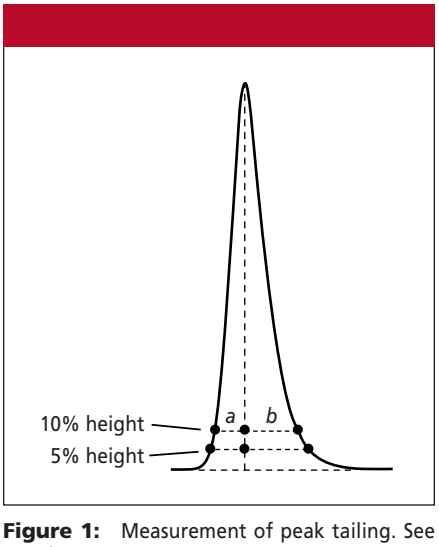

Figure 1: Measurement of peak tailing. See text for details.

Table I: Measurements of peak asymmetry factor and USP tailing factor for the same peak

Peak in Figure 2	Measured	
	A_s	T_f
1.0	1.0	1.0
1.2	1.3	1.2
1.5	1.7	1.4
2.0	2.5	1.9
3.0	3.8	2.9
4.0	5.5	3.5

is wrong with the separation and the problem should be addressed.

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Potential Problems

A look at the peaks in Figure 2 will provide some indication of the potential problems created by tailing peaks. Compare the peak with an asymmetry factor of 1.0 with the peak that has an asymmetry factor of 4.0. The differences are dramatic. For example, although the peak areas are the same, there is a threefold difference in peak height. When using a method for trace analysis, the peak height becomes a critical factor in determining the lower limit of quantification, and the tailing peak clearly is inferior.

Another potential problem is the performance of the data system. Peak-detection algorithms determine the start and end of a peak by detecting a change in the slope of the baseline. For all the peaks shown, the start of the peak is the same, and it is easy for the data system to determine when the peak starts. The more the peak tails, the more gradual the return to baseline. In addition, the end of the peak is difficult to determine, especially if the baseline is not smooth. If the data system uses tick marks to indicate the start and end of the peak, it is likely that the end of the peak would be shown in different places for multiple injections of the same tailing peak. This results in an error in the area measurement.

Tailing peaks also are problematic if minor peaks occur in the same sample as major ones; for example, during a stability-indicating assay or a measurement of metabolites or impurities. With stability-indicating assays, International Committee on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines require reporting of peaks of 0.1% of the main peak or larger. It is easy to see that a minor peak could hide under the peak tail and that ICH requirements for minor peaks might not be met if severe peak tailing occurred.

LC methods used for routine analysis usually must provide baseline resolution of all the peaks and run times that are as short as possible. Figure 2 shows that baseline resolution of tailing peaks will require longer run times than that for peaks that do not tail or that have only minor tailing.

So, tailing peaks not only reduce the quality of the chromatogram, they also reduce the ability to quantify components in the sample. Furthermore, because of questions about peak purity and proper quantification, analysts' confidence in their results is weakened when separations contain severely tailing peaks.

What Causes Peak Tailing?

Peaks tail because more than one retention mechanism is present in a separation, and one of those mechanisms is overloaded. Chemists tend to think of a reversed-phase separation — for example, with a C18 column — as being a pure hydrophobic retention mechanism. It might be true if the stationary phase was only C18 groups. However, approximately one-half of the silica surface is unbonded, which leaves exposed silanol (Si–OH) groups that can interact with the sample.

The silica particles that form the solid support are made of a polymer of silicon and oxygen. Like carbon, silicon is tetravalent, so silica's internal structure comprises four oxygen atoms attached to each silicon atom in a three-dimensional polymer. At the surface, the polymer terminates in silanol groups.

Figure 3 shows the different possible silanol configurations. Chromatographers most commonly draw the surface as free silanols, but silicon atoms with two hydroxyl groups in a geminal configuration also are present. If the silanol groups are positioned next to each other, an associated silanol group can share a proton with an adjacent group. The free silanol groups are more acidic than the geminal and associated groups, and they interact more strongly with basic solutes. The result is peak tailing commonly associated with the separation of bases. To further complicate matters, trace metals (for example, iron and aluminum) can be present in the silica matrix. These trace metals can act as ion-exchange sites or, when adjacent to free silanols, can withdraw electrons, which makes the silanols even more acidic.

In the past, column packings designated as *Type A* silica tended to have all the forms

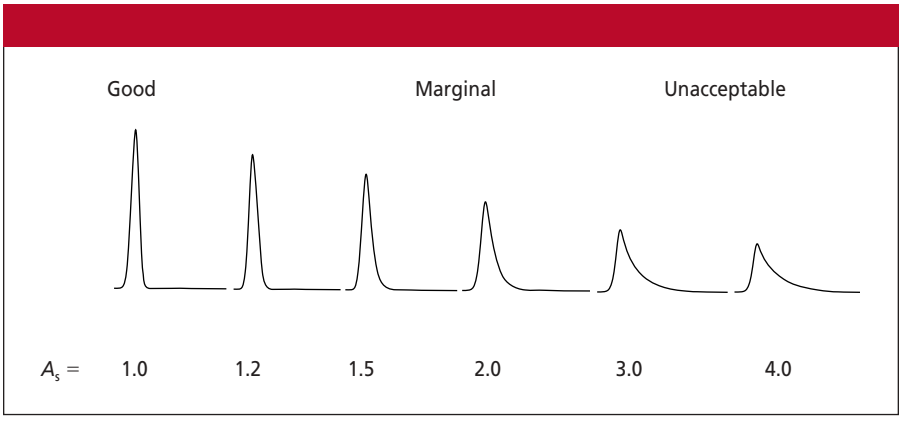

Figure 2: Examples of asymmetric peaks.

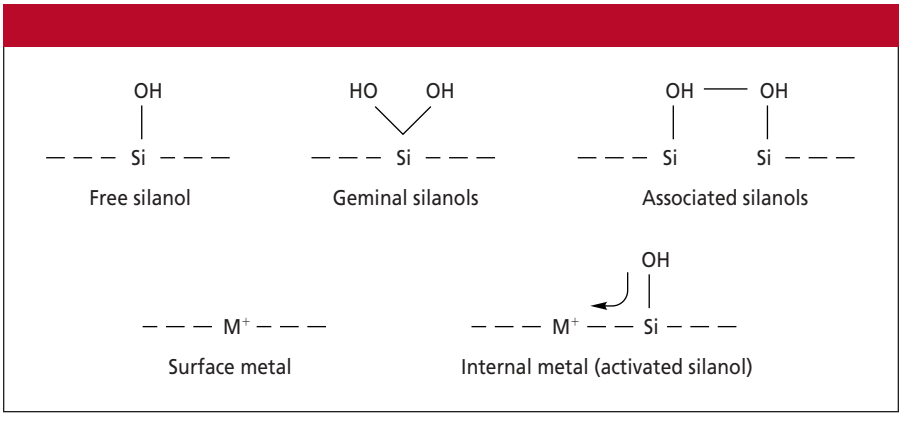

Figure 3: The silica surface. (Reprinted from reference 1 with permission.)

of silanols shown in Figure 3. These Type A materials usually yielded tailing peaks for basic compounds. In the past 10 years, column manufacturers have learned how to make high-purity silica or *Type B* silica. These products are made carefully in metal-free environments so that trace metal levels are extremely low. Proprietary technologies have enabled manufacturers to simultaneously reduce the free-silanol content and increase the number and spatial distribution of geminal and associated silanols. The result is a much less acidic silica surface. In addition, Type B silica columns have less of a tendency to generate severe peak tailing with basic compounds.

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What to Do about Tailing?

With Type A columns, reducing peak tailing usually meant modifying the mobile phase to include a tail-suppressing compound. Triethylamine was a popular mobile-phase additive for reducing peak tailing. Used at concentrations of 20 mM or more in the mobile phase, triethylamine could reduce peak tailing significantly with Type A columns. In my laboratory, my coworkers and I perform contract separation services for the pharmaceutical industry. When we depended on Type A columns, triethylamine was present in most mobile phases.

Today, with the availability of Type B silicas, peak tailing for basic compounds is much less of a problem. In my laboratory we now use Type B columns almost exclusively; very seldom do we add triethylamine to the mobile phase. As a general rule, low-pH mobile phases (pH < 3) also act to suppress silanol ionization, which further reduces tailing problems. Today, the added reliability of the columns and the simplicity of the mobile phases make for more-dependable and robust LC methods. I see no reason to develop a new separation on a Type A column — you are just asking for problems that easily could be avoided by using a Type B packing material.

Peak tailing primarily is a problem with silica-based packing materials. Reversed-phase stationary phases attached to organic polymers, zirconia, or other nonsilica supports are available as alternatives to silica-based columns. These specialty columns could solve your separation problem and produce peaks with little or no peak tailing.

What about Gradients?

Generally, analysts are most concerned about peak tailing with peaks generated by isocratic separations because gradients have two characteristics that minimize tailing. The first characteristic is more of an illusion than a real difference. All the peaks in a gradient separation are approximately the same width, whereas an isocratic separation generates peaks that increase in width with retention. Tailing often is more visually obvious with broader peaks such as the later-eluted peaks in an isocratic separation. Because gradient peaks tend to be narrower, tailing is less obvious.

The second characteristic that minimizes the problem of peak tailing with gradients is *peak compression,* a process that acts on all gradient peaks. Think of a gradient on a microscopic scale — the concentration of organic solvent gradually increases over time in a reversed-phase gradient. At any point in a column, the solute molecules passing through it experience a weaker mobile phase than those that pass through it later. For a given peak, this phenomenon means that the molecules at the front of the peak are traveling in a weaker solvent than those at the back edge of the peak. A stronger solvent (more organic) elutes molecules through a column faster than a weaker solvent does. Thus, under gradient conditions, the molecules at the trailing edge of a peak always are traveling faster than those at the front edge of the peak. In other words, the tail is constantly being pushed back into the main peak, and peak tailing is reduced. For this reason, peak tailing in gradient separations is less of a problem than the tailing for the same compound under isocratic conditions and with the same column and mobile-phase components.

Conclusions

Peak tailing is a real and unavoidable problem with most LC separations. Manufacturers have made great strides in the development of Type B silica support materials, and although these materials significantly reduce peak tailing, they seldom eliminate it. Peak tailing can cause qualitative and quantitative problems with LC methods, so it is important to monitor peak tailing so that it does not compromise your analytical results. Measure peak tailing using either the USP tailing factor or the asymmetry factor. With today's Type B packing materials, adding reagents to suppress peak tailing generally is unnecessary. However, strongly basic solutes still might require the use of triethylamine or another reagent to reduce peak tailing. Another alternative is to explore the use of a nonsilica-based packing material.

The use of silica-based packing materials can be a love–hate relationship. I remember when organic polymer–based supports were introduced in the mid-1970s. Many workers thought they would be the death knell for silica because they did not have the tailing problems associated with silica. But then they realized that the silanol effects of the stationary phase played a very important role in selectivity of the chromatographic separation. Although polymer-based columns could generate symmetrical peaks, they were not as useful as silica-based columns for separating complex mixtures. The perfect LC column has yet to be invented.

Reference

(1) L.R. Snyder, J.J. Kirkland, and J.L. Glajch, *Practical HPLC Method Development* (John Wiley & Sons, New York, 2nd ed., 1997), p. 180.

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