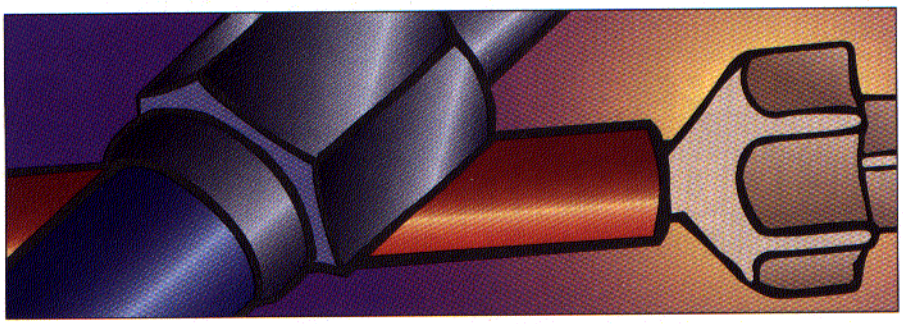
LC Troubleshooting



A Valuable Source of Help

John W. Dolan

Promotional material can be a good source of troubleshooting advice.

Most manufacturers of liquid chromatography (LC) columns and instruments publish newsletters on a periodic basis. Let's face it — the reason these documents are published is to promote a piece of hardware, a service, or some other moneymaking activity. However, just because a newsletter comes on slick paper doesn't mean that it cannot contain useful information. I'm stepping on thin ice with this column because I know I'll get bombarded by vendors that want me to promote their newsletters or products. My intent is not to promote any vendor's newsletter or product. However, I would like to use one example in this month's "LC Troubleshooting" in an effort to encourage readers to consider the valuable technical content of many of the newsletters that come across your desk. From time to time, I will share more useful pointers gleaned from what some consider to be junk mail.

FOUR WAYS TO IMPROVE THE PEAK SHAPE OF ACIDS

With a little editorial license, I've reproduced an article from a manufacturer's newsletter that focused on fixing the peak shape of acidic compound separations (1). Most analysts are familiar with the poor peak shape that can occur with basic compounds. The most common cause of poor peak shape for bases is an undesirable interaction between basic nitrogen atoms in the sample molecule and the acidic silanol groups on the stationary phase in the column. Analysts commonly use triethylamine to reduce peak tailing. Poor peak shape for acidic compounds is less common but still can be problematic. The following suggestions can improve the peak shape of acidic compounds such as ibuprofen.

Increase the salt concentration of the mobile phase: One way to assay for ibuprofen is to use a mobile phase of 60:40 (v/v) acetonitrile–5 mM monobasic sodium phosphate. Figure 1 shows that the peak for ibuprofen appears misshapen with this mobile phase; the tailing factor (T_f) is 3.9. A higher salt concentration (for example, 25–50 mM phosphate) can improve the peak shape of acidic compounds by suppressing solute and silica ionization and the secondary interactions between them.

At this point, analysts should look more critically at this method and the solute. First, ibuprofen has a prominent carboxylic acid functional group (see Figure 1a) with a pK_a of 4.4. Second, 5 mM monobasic sodium phosphate has a pH of 4.4, which is outside the buffering range for phosphate (pH 1.1–3.1). At pH 4.4, ibuprofen's carboxylic acid group is found equally in its ionized and nonionized form. This acid group, especially in the ionized form, can exchange or compete with protons on the silica surface, potentially increasing the tailing and retention. To reduce these interactions, analysts must move the mobile-phase pH away from the sample pK_a.

Reduce the mobile-phase pH: By reducing the mobile-phase pH to 3, the peak shape improves (Figure 1b). At pH 3, the mobile phase is buffered, but the buffer capacity is low. Increasing the salt concentration to more than 20 mM will improve chromatographic reproducibility over time and, as a bonus, improve peak shape even more. With a reduction to pH 3, the ibuprofen is in a single protonated form rather than a mixture of ionized and nonionized forms found at pH 4.4. When protonated, ibuprofen is less likely to interact with the protonated silanols on the silica surface. At both low-pH and -salt conditions, the tailing factor for ibuprofen is reduced to 1.8 (Figure 1b), which suggests that secondary interactions are reduced but not eliminated. Another approach to improving peak shape is the use of an additive.

Add a competing organic acid: Triethylamine suppresses tailing for bases through its

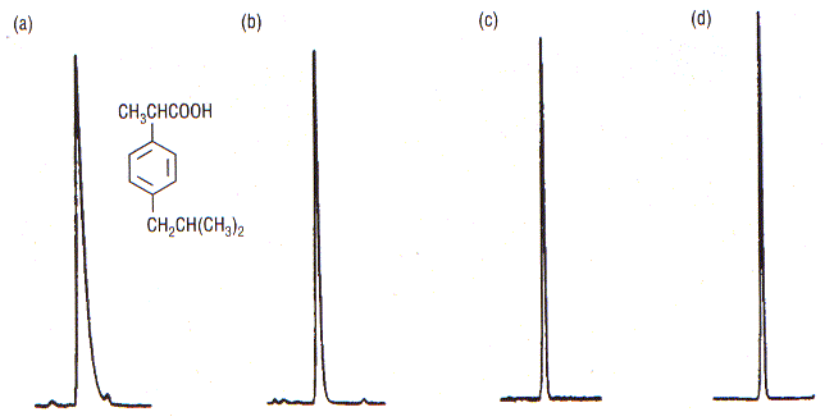


FIGURE 1: LC analysis of ibuprofen using mobile-phase additives (a) 5 mM monobasic sodium phosphate (pH 4.4), (b) 5 mM monobasic sodium phosphate (pH 3.0), (c) 5 mM monobasic sodium phosphate (pH 3.0)–1% acetic acid, and (d) 0.1% trifluoroacetic acid (pH 2.5). Column: 150 mm × 4.6 mm StableBond SB-C18 (Mac-Mod Analytical, Chadds Ford, Pennsylvania); mobile phase: 60:40 (v/v) acetonitrile–additive in water; flow rate: 1.0 mL/min; temperature: ambient. (Reprinted with permission from reference 1.)

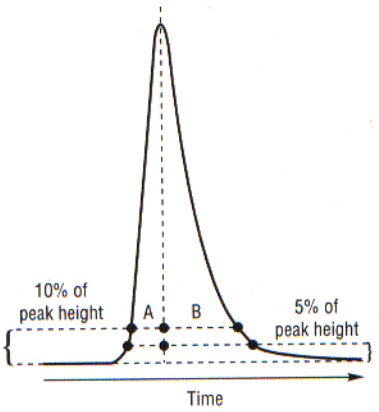


FIGURE 2: Determining peak asymmetry and peak tailing factors (2). Peak asymmetry = B/A, and peak tailing factor = (A + B)/2A. (Reprinted with permission from reference 3.)

superior competition for acidic silanols when compared with many basic sample components. In a similar way, an organic acid can compete with acidic sample components for active sites on the silica surface. The addition of 1% acetic acid to the mobile phase produced the results shown in Figure 1c, in which ibuprofen has a perfectly symmetric band with a tailing factor of 1.0. The mobile phase can be changed again to produce more desirable characteristics.

Substitute 0.1% trifluoroacetic acid: The relatively high concentration of acetic acid resulted in the noisy baseline of Figure 1c. By using 0.1% trifluoroacetic acid (~13 mM) instead of acetic acid and phosphate, the mobile phase is simpler, and ibuprofen still is eluted as a very symmetrical band (Figure 1d). This mobile phase has additional advantages of improved UV transparency and the volatility necessary for LC–mass spectrometry.

TAILING FACTOR OR ASYMMETRY FACTOR?

It is important to keep track of the degree of peak tailing observed in a method. This information helps in monitoring the condition of the column and can serve as a simple way to alert chemists about problems with the method.

Analysts use two common measurements to quantify the degree of tailing that is observed for a chromatographic peak — the U.S. Pharmacopeia (USP) tailing factor and the asymmetry factor (A_s). Both factors are simple measurements based on comparing the peak width for the front and back half of a peak at a specified height, as shown in Figure 2 (2).

Because USP recommends using the tailing factor measured at 5% of the peak height, this technique is the standard used by workers in the pharmaceutical industry. Most other chromatographers use the asymmetry factor measured at 10% of the peak height, because this method is somewhat easier. Although the measurements are simple, many analysts become confused when trying to compare measurements made by one method or the other. When mildly tailing peaks (T_f or A_s <1.5) occur, the tailing measurements are roughly equivalent, as shown in Table I (3). However, when significant tailing is observed, the asymmetry factor tends to produce larger numbers. It really doesn't matter which convention is used, as long as it is used consistently.

TABLE I: Peak Asymmetry and Peak Tailing Factor Relationship

A_s (at 10% of peak height	T_f (at 5% of peak height)
1.0	1.0
1.3	1.2
1.6	1.4
1.9	1.6
2.2	1.8
2.5	2.0

SO . . .

A combination of low pH and the presence of a competing organic acid can improve peak shape. The result was obtained in this case by using either a phosphate–acetic acid additive or trifluoroacetic acid, so analysts are not locked into a single magic formula. One bonus of using these low-pH mobile phases is that, by suppressing the ionization of the silanol groups, the peak shape for basic sample components also is likely to improve.

So don't automatically throw away all those newsletters without at least reading the titles of the articles. You'll find a gold mine of information in these articles that you should be able to put into practical application in your laboratory.

REFERENCES

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