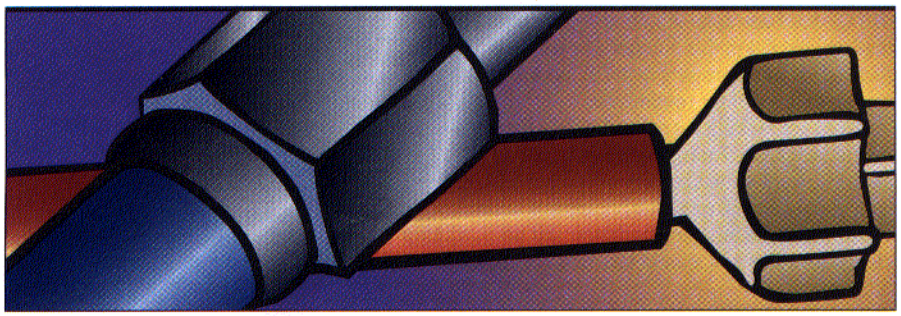
LC Troubleshooting



Basic Compounds — Starting on the Right Foot

John W. Dolan

Maximize your chances for good chromatography of bases.

Historically, liquid chromatography (LC) separations of basic compounds resulted in tailing peaks. Tailing peaks are more difficult to quantify, have poorer limits of detection, require longer runs, and generally are aesthetically unpleasant. Fortunately, current LC technology has reduced the problems associated with bases, so the challenge of analyzing these compounds is minimized. The primary cause of tailing for basic compounds is related to the silanol content of the stationary phase. This problem can be addressed in one of two ways: modification of the stationary phase or modification of the mobile phase. This month's "LC Troubleshooting" column will examine each of these factors to assist analysts in selecting conditions to minimize problems with basic compounds. A description of the history of bonded-phase development and more details on bonded-phase chemistry were covered in an earlier "LC Troubleshooting" installment (1).

THE BONDED PHASE

Retention in reversed-phase LC is thought to occur primarily due to interactions with the stationary phase bonded to the silica surface. Most bonded phases are formed using the reaction generalized in Figure 1. The silanol (Si–OH) groups on the silica surface react with a silane reagent to attach the bonded phase onto the surface. The most common chemistry uses chlorodimethylsilane, as shown in Figure 1. This silane reacts with the silanol group to form a silyl ether bond attaching the bonded phase onto the column. The actual bonded-phase characteristics are determined primarily by the R group, such as $C_{18}H_{37}$ (C18 phase) or C_8H_{17} (C8 phase). It is tempting to assume that the retention characteristics of a column are determined by the bonded-phase chemistry. However, steric crowding prevents the silane reagent from reacting with every silanol group. In fact, approximately half the silanol groups remain unreacted after the bonding reaction. It is possible to react some of these groups with a smaller silane reagent, such as chlorotrimethylsilane, in a process called *endcapping*. However, even endcapped phases still have approximately half of the silanol groups remaining in the unbonded state.

THE SILICA SURFACE

The nature of the column is such an important contributing factor to the shape of basic peaks, it is worth understanding the chemistry of the silica surface. Most LC columns use totally porous, spherical silica particles with 5- or 3-µm diameters. The particles are made of a polymer of silicon and oxygen (–Si–O–Si–O–Si–). Silicon is tetravalent, like carbon, so the polymer is three-dimensional. On the surface, this polymer chain is terminated in silanol groups. These silanol groups are used to attach the bonded phase, as discussed above. In reality, these silanol groups can exist in several different configurations, as shown in Figure 2.

Traditionally, the surface is thought to be covered with free silanols, but that usually is not the actual surface condition. It is possible to have two hydroxyl groups attached as a geminal silanol. If the silanol groups are properly spaced, the associated silanol group exists in which a proton is shared (hydrogen bonding) between adjacent silanols. In addition to the silanol configurations shown in Figure 2, it is possible to have trace metals such as aluminum or iron trapped in the surface. These metal ions can act as ion-exchange sites, or they can interact with adjacent silanols to withdraw electrons from the silanol. Not surprisingly, the chemistry of the various silanol groups shown in Figure 2 differs. In terms of sample interactions, the acid–base characteristics of the silanols are most important. The silanol groups with adjacent metals are the most acidic of those shown in Figure 2 — those groups interact most strongly with basic compounds. The associated silanols are the least acidic, and the remaining configurations fall somewhere in between.

The presence of different types of silanol groups and the existence of a significant proportion of silanol groups on the bonded phase surface means that the chemical interaction between the sample and the residual silanols

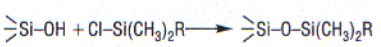


FIGURE 1: Chemistry of bonded phase packings: reaction of surface silanol with chlorodimethylsilane. (Reprinted with permission from reference 2.)

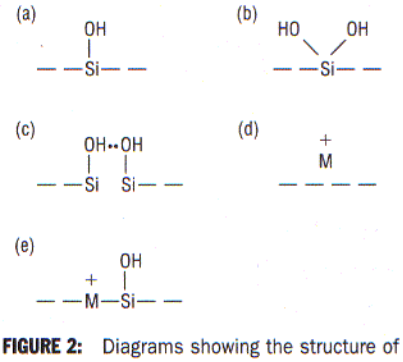


FIGURE 2: Diagrams showing the structure of silica particle surfaces. Shown are (a) a free silanol, (b) geminal silanols, (c) associated silanols, (d) a metal atom exposed on the surface, and (e) activation of a silanol group by an adjacent metal atom. (Reprinted with permission from reference 2.)

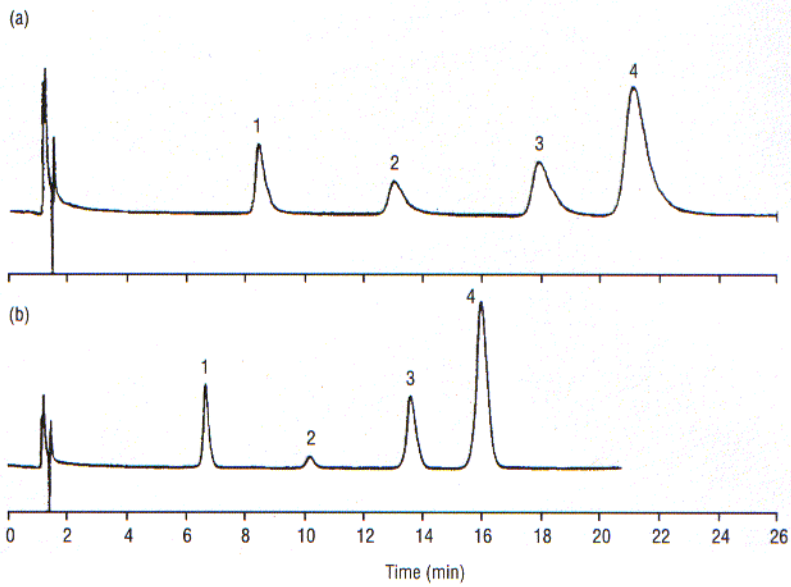


FIGURE 3: Separation of basic drugs on bonded phases attached to (a) type A and (b) type B silica supports. Mobile phase: 30:70:0.2:0.2 acetonitrile–sodium phosphate (pH 2.5)–triethylamine–trifluoroacetic acid. (Reprinted with permission from reference 2.)

can be very important in an LC separation. If it is impossible to eliminate the residual silanols, is it possible to control the nature of the silanols? Fortunately, yes.

Most manufacturers have figured out how to prepare the silica so that they control the nature of the silanol groups. For example, silica can be prepared in a metal-free environment so that problems with metals are eliminated. It also is possible to create a silica surface that comprises primarily associated silanols with a minimal content of the more acidic free silanols. These silanol surfaces are much less acidic and tend to provide much better peak shape for bases, as shown in Figure 3. In the column based on the older silica chemistry, the bases interact strongly, increasing retention and peak tailing. The newer column chemistry reduces the retention and peak tailing for the basic compounds.

Many manufacturers now offer the less reactive column media, called *type B* silica (as opposed to the older *type A* silica) or *base-deactivated* silica. Because of the dramatic improvement in peak shape for bases on the newer columns, analysts should use these columns when developing new methods. As I conclude below, the alternative techniques to reducing peak tailing of bases can be cumbersome — why not start out with the cards stacked in your favor by using a column that minimizes tailing? Many newer columns are named to indicate that they are designed for better peak shape by using words or word-variants such as *inert, base deactivated,* or *symmetry.* If in doubt, you should consult the manufacturer's literature when selecting a column to ensure that it uses this less acidic silica as a base material.

MOBILE-PHASE ADDITIVES

Analysts know that columns using acidic silica tend to produce more peak tailing than corresponding columns using less acidic silica, as illustrated in Figure 3. However, in this case even the base-deactivated column produces tailing peaks for basic compounds. For example, compare the chromatograms in Figures 4a and 4b. The column packing used for the chromatogram in Figure 4a is a traditional acidic silica, whereas the column packing used in Figure 4b is deactivated for improved performance with basic compounds. The two columns are very similar in retention and peak shape for neutrals (caffeine) and acids (vanillylmandelic acid, homovanillylmandelic acid, and salicylic acid), but the performance with basic solutes is dramatically different. Two basic drugs, procainamide and *N*-acetyl procainamide, were used. With the acidic column, the basic peaks were so strongly retained and tail so badly that they are barely discernible. The base-deactivated column, on the other hand, shows much weaker base–column interactions, which are exhibited as lower retention and better peak shape.

Triethylamine: Even with a base-deactivated column, however, peak tailing for the bases is apparent, which suggests that unwanted silanol interactions are occurring. One very simple way to further reduce the peak tailing for bases is to add a basic compound to the mobile phase that competes with the sample for the residual silanols. The chromatograms in Figures 4c and 4d show this result after the addition of triethylamine to the mobile phase. In this case, the columns were overloaded with triethylamine, which interacts with the acidic silanols, thus reducing their availability for interaction with bases in the sample. This addition of triethylamine or other small bases

to the mobile phase is a technique used commonly to reduce peak tailing for bases in the sample.

Low pH: Another way to reduce the activity of the residual silanol groups is to use an acidic mobile phase, thus suppressing ionization of the silanols. Analysts can use a mobile-phase pH of less than 3 to accomplish this task. Low-pH mobile phases often are advised as preferred starting conditions for separation of any sample type (2). Another advantage of using a low-pH mobile phase is that the ionization of acids tends to be suppressed, so retention is increased and unwanted ionic interactions are minimized. It would be nice to adjust the mobile-phase pH to suppress the ionization of basic solute, but under the required high-pH conditions, the silica columns dissolve. As a general rule, silica-based columns need to be operated in the pH 2–8 range. At pH values less than 2, the bonded phase is unstable, whereas the silica dissolves at pH values greater than 8. So from a practical standpoint, analysts can choose a pH to suppress acid ionization, but not base ionization.

ELIMINATE THE SILICA

If the primary cause of band tailing with bases is related to unwanted interactions with silanol groups on the packing surface, why not totally eliminate the silanol groups? Analysts can use a nonsilica solid phase such as a polymer- or zirconium-based material. You can buy reversed-phase columns with a polymeric solid phase. The peak-shape improvement for basic solutes with these columns, when com-

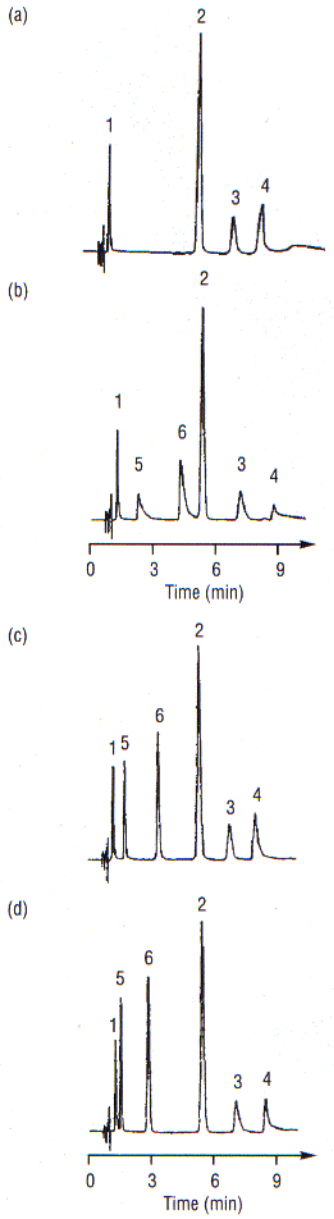


FIGURE 4: Chromatograms showing the effect of silica deactivation and mobile-phase additives on peak shape for bases. Columns: (a, c) Supelcosil LC-18 and (b, d) Supelcosil LC-18DB; mobile phase: (a, b) 7% acetonitrile–water (pH 3.5); (c, d) 7% acetonitrile–water (pH 3.5) with 10 mM triethylamine. Peaks: 1 = vanillylmandelic acid, 2 = caffeine, 3 = homovanillymandelic acid, 4 = salicylic acid, 5 = procainamide, 6 = *N*-acetyl procainamide. (Courtesy of Supelco [Bellefonte, Pennsylvania].)

pared with the silica column, can be dramatic because no unwanted silanol interactions take place. The absence of silica also removes the upper pH limit. Thus, analysts can operate at pH values high enough to suppress base ionization and reduce tailing for bases.

Zirconium also is stable at high pH, and bonded-phase columns using zirconium as the solid support are available. Figure 5 shows the peak shape advantage that can be obtained when zirconium-based columns are used for basic samples.

If analysts can use columns such as polymer- or zirconium-based columns to eliminate unwanted silanol interactions, why bother with silica? Although the nonsilica columns are quite useful for specific applications, these columns are not without problems. One problem is that plate numbers for nonsilica columns generally are much lower than those of silica columns. Another problem is that eliminating silanols seems to be desirable, but the silanol groups are very important in obtaining the desired selectivity to produce a useful separation. In other words, nonsilica columns generally produce worse separations than silica-based columns.

CONCLUSIONS

Several factors contribute to poor peak shape when analysts use LC to analyze basic compounds. These factors have led to some wise practices for developing a separation of samples containing bases.

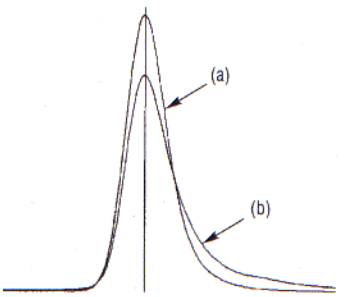


FIGURE 5: Comparison of peak tailing for pyridine between (a) zirconium-based (USP tailing factor 1.4, retention factor =0.6) and (b) type B silica C18 (tailing factor =2.0, retention factor = 0.3) columns. (Courtesy of ZirChrom Separations, Inc., Anoka, Minnesota.)

Use a base-deactivated column: Base-deactivated silica yields better peak shape for bases and more-reproducible columns. Analysts should not begin developing a new method using older, type A silica columns.

Use a low-pH mobile phase: By using a low-pH mobile phase, ionization of the remaining silanols is reduced, thus reducing their reactivity with bases. Low pH has the added advantage of suppressing ionization of acidic sample components. Because silica-based columns generally are unstable at pH values high enough to suppress base ionization, ionization of bases is ignored and the best alternative conditions are selected — low pH.

Add triethylamine: If peak tailing persists with a base-deactivated column is used at low pH, adding triethylamine can further reduce tailing for basic compounds. This step may or may not be necessary, and in interest of simplicity, it is recommended that the sample should be separated without added triethylamine, if possible.

Use special techniques: If the above methods are unsuccessful, it may be useful to examine alternative separation techniques, such as the use of polymer- or zirconium-based columns. Another alternative is to use ion pairing for the separation of basic compounds.

REFERENCES

(1) J.W. Dolan, *LC•GC* **16**(4), 350–354 (1998).
(2) L.R. Snyder, J.J. Kirkland, and J.L. Glajch, *Practical HPLC Method Development* (John Wiley & Sons, 2nd ed., 1997).

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