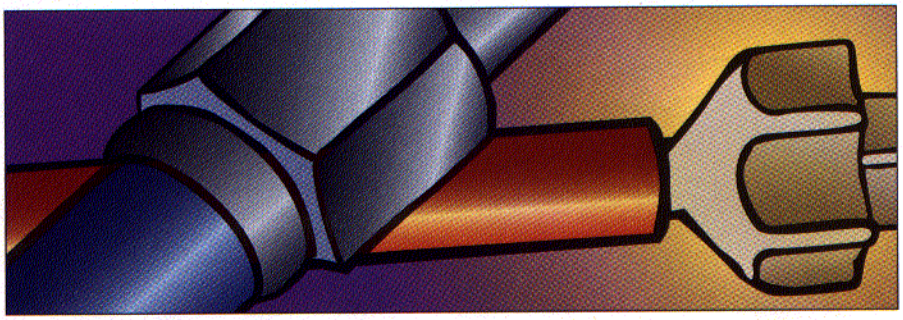
LC Troubleshooting



Anticipating Problems with a New Method

John W. Dolan

Some methods are trouble just waiting to happen.

I recently received a question from a reader who was trying to perform a liquid chromatography (LC) method retrieved as an official method. As we'll see, although a method has been blessed by some authoritative body, it is not necessarily well designed.

THE PROBLEM AND THE SOLUTION

Question: I have been using the following LC method, which originates in the *British Pharmacopoeia,* for validation. The method uses a 25 cm × 4.6 mm, 5-µm d_p C18 column. The mobile phase is 68:19:13 (v/v/v) 1.74% dibasic potassium phosphate–methanol–acetonitrile. The pH is not specified in the method; however, it obviously is greater than pH 8.5. The method calls for a flow rate of 1 mL/min, which creates a back pressure of approximately 2500 psi. The injection volume is 20 µL, and the UV detector is set at 225 nm. The mobile phase and samples are filtered through 0.45-µm filters.

I found that after roughly 100 h of use, the column starts exhibiting extremely high back pressure (>4000 psi), causing the instrument to shut down. This 100-h failure point may be reached after one week or four to five months, depending on how many samples I run. After the column fails, it must be replaced. One of my colleagues suggested that the column should be washed overnight every day (especially if the column is in daily use) to increase the life of the column. I would like to know how to increase the column life and to anticipate any problems validating this method.

Answer: Perhaps the best way to approach a problem like this one is to examine the various aspects of the method that could be causing problems. Several things about this method are not optimum for ensuring extended column life and a rugged method.

The mobile phase: First, let's examine the mobile phase. Three aspects of the mobile phase could be causing problems. First, as you noted, the pH of the mobile phase is high. If you question this assumption, you could measure the pH or just read the label on the buffer bottle (mine says that a 5% aqueous solution has a pH of 9.4). We know that the stability of most LC columns decreases as the pH increases, and the maximum recommended pH for most silica-based columns is pH 8 (see more column comments below). The pH alone could be the source of your trouble.

A second problem is that the mobile phase has low buffering capacity. Although chromatographers frequently achieve the chromatographic objective of ionization or suppression of ionization using an unbuffered mobile phase with the pH above or below a certain value, it is best to use a buffer to control the pH. As do all buffers, phosphate has a buffering region that is most effective within approximately 1 pH unit of the pK_a. So with pK_a values of 2.1, 6.2, and 12.3, phosphate is a good buffer for the pH ranges of 1.1–3.1, 6.2–8.2, and 11.3–13.3. It would be best to add acid (for example, phosphoric acid or monobasic phosphate) to lower the pH to the pH 6.2–8.2 region.

Finally, the salt concentration of the mobile phase is higher than I prefer for routine methods. The recipe calls for 1.74% phosphate, which makes the concentration of the aqueous portion 0.1 M. When diluted with organic solvent, the resulting mobile-phase concentration is 68 mM. I prefer to keep the buffer concentration in the 25–50 mM region, especially when using acetonitrile in the mobile phase. The poor solubility of buffer salts in acetonitrile can result in buffer precipitation in the LC system, especially when using on-line mixing. For example, the buffer may be easily soluble in the presence of organic solvent in a bulk solution, but the interface between the organic and aqueous solvent streams in an on-line mixer can create localized precipitation. This precipitated buffer can cause frit blockage, check-valve malfunction, and other problems.

So you can see that your mobile-phase recipe may result in problems. When we consider your column, the problem is compounded.

The column: Most manufacturers of the silica-based columns widely used for reversed-phase LC recommend that the columns be operated in a pH range of approximately pH 2.5–7.5. At low pH values, the bonded phase is cleaved from the silica support. At high pH, the silica itself dissolves. So from this information alone, a red flag should go up regarding the advisability of using the mobile-phase recipe you cited. Chromatographers can use several tactics to minimize problems at extreme pH, but none that I know of can eliminate the problem (except using a polymeric packing material).

Two types of silica are available on the market today. The older one is called *type A,* and a more recent product is called *type B* silica. The main distinguishing feature between these two products is their purity. The older, type A materials tend to contain metal impurities that remain from the manufacturing process. Type A silica tends to have a more acidic surface, which creates more problems with tailing bands, especially with basic sample compounds. The type B material is much purer because it is the product of a manufacturing scheme that greatly reduces the metal content and generates a much more uniform and less acidic surface. These type B materials are good for analyzing basic compounds because of reduced tailing.

The column brand you mentioned in your letter is a type A silica column. The choice of this column has positive and negative aspects in terms of its silica backbone. Perhaps counterintuitively, some type A columns are more stable at high pH than their type B counterparts. This stability results because the metals and other impurities reduce the solubility of the silica; you would expect reduced solubility of any impure substance compared with a pure one. Because of the high-pH mobile phase, I suspect the presence of basic components.

A chromatographer's objective would be to have a mobile-phase pH higher than the pK_a of basic solutes to suppress ionization. But we know that bases are much more susceptible to tailing on the type A silicas than on the type B materials, so your column also has negative aspects.

Another contributing factor to the stability of silica-based columns at high pH is how well the surface is protected from access by the mobile phase. Manufacturers have addressed this problem in three main ways, either alone or in combination. All three depend on making the surface less accessible by protecting it physically or chemically.

One way to protect the silica surface is to make it more hydrophobic ("greasier"), so the conditions for interaction with the polar mobile phase are less favorable. You can adjust the hydrophobicity of the surface by controlling the concentration of bonded phase on the surface. One way to measure this concentration is as the percentage of carbon (% carbon) measured by elemental analysis. A higher percentage of carbon means that the surface is more hydrophobic, which in turn means better protection of the silica from polar materials that do not like the nonpolar environment at the silica surface. It also makes the columns more retentive for most samples.

Your column is a lightly loaded column containing approximately 6% carbon. A higher carbon loading material (12% carbon) is available from the same manufacturer. This higher carbon loading should be more stable at high pH, because it protects the surface better. In addition, the polar components of your sample (likely to be amines) have less access to the surface, so tailing also should be reduced.

Only so much stationary phase can be bonded to the silica surface. Steric considerations dictate that only half of the surface silanols (SiOH groups) can have stationary phase bonded to them. Therefore, half of the surface is unbonded and available for unwanted interactions with polar solutes and attack by high-pH mobile phases. One way to further protect the surface is through a process called *endcapping,* in which a trimethylsilyl functional group is bonded to the surface after the C18 phase has been added. This small molecule can access the surface in places that are not available for further reactions with C18 groups and deactivates the surface a little more. Some manufacturers even endcap their products twice for more protection. Endcapping helps to reduce tailing and increase column stability at intermediate to high pH values (1). Unfortunately, the endcapping is unstable at low pH, and it can be cleaved from the surface rapidly at pH < 3. For this reason, endcapped columns are recommended only for high-pH mobile phases. Your column is not endcapped; columns with higher carbon loads also are endcapped, making them better choices for your mobile phase.

Another approach for protecting the surface from chemical attack and unwanted solute interactions is the use of sterically protected col-

umn packings. This technique uses a bonded phase that has a bulky side chain near the site where bonded phase attaches to the silica surface. This bulky group shields the surface so that it is less accessible. A variation used by some manufacturers is placing a charged functional group near the surface to provide chemical repulsion that counteracts the residual silanols. This sterically protected silica shows good performance even at mobile-phase pH values of 9 (1). Your column uses neither of these techniques for additional surface protection.

The diagnosis: Now that we have some background in some of the potential problems that could occur with your method, what really is going on?

I suspect that the major problem is the result of the high-pH mobile phase attacking a column that is not designed for extended use at pH values greater than 8. As mobile phase passes through the column, the silica gradually dissolves. As the silica particles dissolve, they become smaller, and at some point they will move around in the column, increasing the back pressure either by blocking a frit or filling in the interstitial spaces between the particles. When this happens, you have little hope of regenerating the column, so let's look at some ways you can alleviate the problem. Implicit in your question is the restriction that you cannot make major changes to the method. This means that what may be the best approach — reworking the method to use a less-aggressive mobile phase — is not an option.

First, your colleague's suggestion to flush the column daily is a good one. In a case such as yours, the total hours of exposure to the aggressive mobile phase determine the column lifetime. For example, if the column is exposed to mobile phase continuously, the 100-h lifetime would be reached in less than a week. On the other hand, if you use the system only 8 h/day and fill the column with a safe mobile phase for the other 16 h, you might get two weeks or more of use from the column. Usually, I recommend removing the buffer from the mobile phase and flushing it with an equivalent water-based mobile phase. For example, change from 68% buffer to 68% water for the first flush step to remove the buffer and then flush with 100% organic solvent. You could use this procedure, but if it were my method, I'd try flushing with a low-pH (pH 3) mobile phase first, then water–organic solvent, and finally straight organic solvent. You never should store the column in the high-pH mobile phase.

Most compendial methods such as yours allow some leeway for adjustment of the conditions to accommodate interlaboratory and column-to-column variations. Reducing the pH by as little as 0.5 units (for example, to pH 8) may have dramatic effects on the column life. Other tools at your disposal are guard and saturator columns. A guard column packed with the same material as the analytical column also would be attacked by the mobile phase. The trick is to throw out the guard column before it is ineffective at protecting the analytical column. Using a guard column requires some experimentation, but I expect that replacing the guard column every day or every other day could extend the life of the analytical column greatly.

A more extreme measure used by some workers when high-pH mobile phases are necessary is the addition of a saturator column (sometimes called a precolumn). Saturator columns are old analytical columns that are mounted upstream from injectors or autosamplers. They act as sacrificial elements, dissolving in place of the analytical columns. If you use a saturator column, be sure to place a 0.5-μm in-line filter directly after the saturator column, because as the packing dissolves, small particles can pass through the 2-μm frit at the end of the column and cause abrasion problems in the injector or block the frit at the head of the analytical column.

If you want to try another column, a column designed for use with higher pH mobile phases might work for your method. Many methods define a particular brand of column to use but also mention an equivalent column. Be careful if you take this route, because you may see significant changes in retention and selectivity with a different column.

IS IT WORTH IT?

Finally, you need to ask yourself how important it is to extend the life of the column. All the solutions discussed so far involve additional cost, either in parts or in labor. I think if you examine the costs of the current method, then putting much effort into improving it will seem very unattractive. For example, if your method involves a 20-min run time (allowing for standards and controls), you could analyze approximately 200 samples (2/h × 100 h) before the column became unusable. If your sample costs are typical of the pharmaceutical industry, $50/sample is reasonable. Therefore, your laboratory spends approximately $10,000 to analyze 200 samples. A good analytical column costs roughly $400 — approximately 4% of the total analytical cost. If you could double the number of samples that could be run before column failure, you would be saving only 2% of the overall cost. Is this worth the trouble? I think not.

If it were my method, I think I would try lowering the pH a bit and adding the guard column. I'd live with whatever gains these steps provided and invest my time in a more cost-effective manner.

REFERENCE

(1) L.R. Snyder, J.J. Kirkland, and J.L. Glajch, *Practical HPLC Method Development* (John Wiley & Sons, New York, 2nd ed., 1997), pp. 174–232.

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Walnut Creek, California, and a member of the Editorial Advisory Board of LC•GC. *Direct correspondence about this column to "LC Troubleshooting,"* LC•GC, *859 Willamette Street, Eugene, OR 97401, e-mail John.Dolan@LCResources.com.*