LC Troubleshooting

The Dilemma

John W. Dolan

What do you do when you have to follow the method, but you know it is wrong?

One of the most frustrating aspects of being a chromatographer is working with poorly designed methods. Just because someone has developed a liquid chromatography (LC) separation and claims to have validated the method does not mean that it is reliable. Most methods are fine, but as a troubleshooter, I have found that most problematic methods have several weak spots. These weak spots give the method a kind of metastable state — it may work one day, but not the next. Analysts, especially those performing quality assurance or routine analysis, then find themselves in a predicament because they often have no authority to correct the method's shortcomings. Consequently, my suggestions for fixing methods are often met with "but I'm not allowed to change the method."

Perhaps this limitation is true, but someone in your organization does have that authority. Rather than wasting your time and your company's money collecting useless data, why not spend an hour or two to determine what will fix the problem and present the information to the person with authority to change the method? This month, we'll look at two problems that could be fixed by changing the method.

pH, TEMPERATURE, AND TECHNIQUE

In the first example, an analyst was using an isocratic method for the routine analysis of a pharmaceutical product. The simple separation had four components of interest. The method required that each component be eluted within a certain retention time window for the data to be valid. The analyst observed that after months of successful operation, the peaks drifted to longer retention times, often outside of the acceptable retention window. In addition, one pair of peaks drifted together so that baseline resolution was no longer possible. She mixed up a new batch of mobile phase and changed the column, but neither of these efforts fixed the problem.

The method used a reversed-phase C18 column at ambient temperature with an acetonitrile–buffer mobile phase at a flow rate of 1 mL/min and UV detection. The analyst prepared the mobile phase by mixing a 25 mM monobasic sodium phosphate solution with the proper amount of acetonitrile, then adjusting the mixture's pH to 3.0 with phosphoric acid.

This method has two obvious problems. The first problem is temperature control. A 1 °C change in temperature will shift the retention times by 1–2%. The analyst observed that the laboratory was noticeably colder when the problem began than when the method had been working properly. A decrease in temperature should result in an increase in retention, which is consistent with the experimental observations. Confirming that the change in temperature is the source of a problem should be easy — just adjust the column thermostat to the original temperature and rerun several samples.

I strongly recommend column temperature control to avoid temperature-related drift. The best choice is to use a column heater. Column heaters are available as part of many brands of LC systems, or you can purchase them from third-party parts suppliers. You can make a homemade column heater by placing the column in a water jacket and circulating temperature-controlled water through the jacket.

I like to run the column a few degrees above room temperature so that a constant temperature can be maintained easily — we use 35–40 °C as the operating temperature for columns in our laboratory. If the laboratory has a fairly constant temperature, you may get satisfactory results by insulating the column. A piece of foam pipe-wrap — for example, water-pipe insulation — makes a convenient and inexpensive column insulator. Just snap a piece of foam around the column and make sure there are no direct drafts from heating or air-conditioning vents.

The second and perhaps more serious problem with the present method is the technique of adjusting the pH. When combined with the change in laboratory temperature, I suspect that this is the primary problem source. It is poor practice to adjust mobile-phase pH after addition of the organic solvent. The pH meter will not register accurately under these conditions, and the true pH of the solution may not correspond with the pH meter reading.

The proper technique is to adjust the pH of the buffer in aqueous solution and then add the organic solvent. In the present case, however, the method's mobile-phase preparation procedure specified adjusting the pH after adding the acetonitrile. Temperature affects pH, especially in regions beyond ±1 pH unit from the pK_a of the buffer. I suspect that the temperature change also changed the pH in this case, requiring more or less phosphoric acid to obtain the apparent pH 3.0.

The fresh mobile phase then had a different pH than when the method was validated, which caused the observed change in peak spacing and probably compounded the problem of temperature-related retention drift. The analyst could verify this effect by reformulating the mobile phase at the original laboratory temperature and repeating a run to see if the original retention times resulted.

How do you correct a method like this? The method was developed by adjusting the pH of the organic-containing mobile phase to obtain the desired retention characteristics. To obtain a more repeatable method, we need to convert the pH-adjustment technique while maintaining the same true pH. I would mix the unadjusted buffer–organic solution at the original laboratory temperature. Next, I carefully would measure the amount of phosphoric acid required to obtain a reading of pH 3.0. For exam-

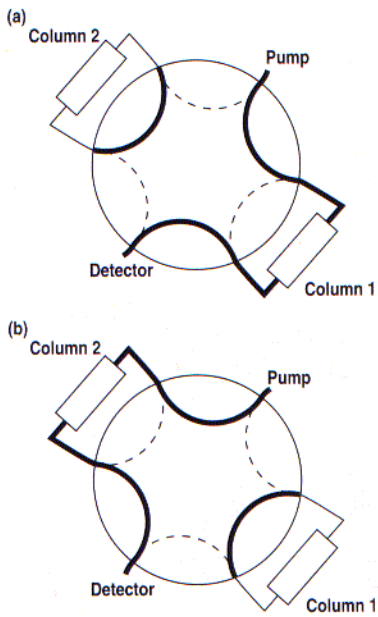

FIGURE 1: Column-switching setup, in which either (a) column 1 or (b) column 2 are in use.

ple, the starting solution might be ~1 L, with 500 mL of 25 mM monobasic sodium phosphate and 500 mL of acetonitrile. Let's say it took 3.7 mL of the acid solution to obtain a pH of 3.0. Next I add 3.7 mL of the acid to 500 mL of the 25 mM buffer (no organic solvent) and obtain a pH measurement of ~2.5. The new mobile-phase preparation method should specify adding acid to the aqueous buffer until you obtain a pH of 2.5, then adding the organic solvent. This should yield a consistent mobile phase that will be less sensitive to changes in pH when the temperature (or some other factor) is changed.

BLOCKED COLUMNS

In another case I encountered recently, an analyst was performing a single-component analysis on a final product. The separation was very stable over the life of the column, but the column pressure built up and exceeded the acceptable pressure limit every two or three days. Reversing the column and flushing for a few minutes with mobile phase washed the offending material from the frit and restored normal back pressure. This interruption usually caused ~1 h of downtime every couple of days, and the sample load was too great to accommodate the downtime. The laboratory workers were looking for a way to resolve the problem without revalidating the method.

The most obvious way to reduce the particulate load in the system is to filter each sample before injection. But the added cost of ~$1/sample for this 4-min assay was undesirable and adding filtration would also tack on the expense of at least a limited revalidation of the method. Furthermore, the laboratory staff was hesitant to use filtration because of a bad experience with it in another method. Also, they were unwilling to use guard columns.

Two other options could minimize the downtime for the method. Perhaps the easiest option would be to add an in-line filter between the injector and the column. This filter, fitted with a 0.5-µm porosity frit, should trap any particulate matter that would normally collect on the 2.0-µm frit at the head of the column. When the pressure increased to an unacceptable level, users could shut off the system, replace the filter frit, and return to operation in just a minute or two. Alternatively, workers could change the frit daily as part of system maintenance, just like replacing the mobile phase. This maintenance would alleviate the need to shut the system down during a series of runs. The added cost (a catalog lists 0.5-µm frits at ~$2.50 each) is negligible and certainly lower than shutting down the system for an hour to reverse-flush the column.

The most obvious way to reduce the particulate loading in the system is to filter each sample before injection.

Another option to minimize downtime would be to use a second column. When the pressure in the first column becomes too high, analysts could quickly substitute the second column and restart the system. Then, the users could backflush the first column off-line using another LC pump and prepare it to relieve the column in use.

Using a standard six-port injector valve, as shown in Figure 1, would refine the column switching process. In Figure 1a, column 1 would be in use, and column 2 could be removed for cleaning. Column 2 could then be reinstalled, and the valve would switch to the position shown in Figure 1b when the pressure rose again. Then column 2 would be in use, and column 1 could be removed for backflushing. This simple column-switching system would alleviate shutting off the pump each time the column was changed, so downtime should be insignificant. Some commercial LC instruments have automated column-switching options, so if you're buying a new instrument for an application similar to this, the option might be worthwhile.

SUMMARY

In both cases presented here, the analysts were severely restricted in the type of change they could make to the method. However, changes could improve the method or enable the analysts to identify the problem source as justification for method modification. Remember that you're doing no favors by continuing to use a method that gives erroneous or questionable results. Your frustration level will be high and the resulting data will be of questionable value — sooner or later the method will cause an expensive revision or replacement.

"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. *Direct correspondence about this column to "LC Troubleshooting,"* LC•GC, *859 Willamette Street, Eugene, OR 97401, USA.*

Erratum: As several readers have pointed out, two mistakes appeared in the January 1994 "LC Troubleshooting" column. Both errors related to the calculations associated with Figure 2. First, the width of the 10-min peak should have been 8.0 mm, not 0.8 mm. Second, I inverted the millimeter-to-minute conversion factor in my calculation, so the true retention time for the 10-min peak should be ~38.5 min, not 84 min.

JWD