LC TROUBLESHOOTING

Sample Preparation, Guard Columns — Answers to Readers' Questions

John W. Dolan

Sample preparation techniques and the proper use of guard columns can significantly affect liquid chromatographic separations.

This month's "LC Troubleshooting" column addresses readers' problems with precolumn sample behavior. Sample filtration and the use of guard columns often can extend the life of analytical columns, but problems may arise even after taking these precautions.

HIGH COLUMN PRESSURE

Q: I am experiencing a problem with column back pressure when using a preparative separation method. If I make analytical-scale injections on either a preparative column or an analytical column containing the same stationary phase, the system performs as expected, and the pressure remains constant or increases only slightly. However, if I make a preparative injection, the column back pressure rises 500–800 psi (3.4–5.5 MPa) and does not return to the original pressure. The sample is an aqueous, buffered biological extract, and the mobile phase is acetonitrile–buffer. I filter the sample through a 0.22-µm membrane filter to remove any particulates before injection. What could cause this problem, and how can I avoid it?

JWD: I suspect that the problem you have encountered is a result of your sample buffer or another salt precipitating in the column. Many buffers have rather low solubility in acetonitrile, especially compared with their solubility in methanol. When solutions containing sufficiently high buffer concentrations are injected into acetonitrile mobile phases, the buffer salts can precipitate. After precipitates form, they can be very hard to redissolve, and increased system pressure results if the particulate formed is in a quantity large enough to block the inlet frit or the column packing itself. (An analogous situation sometimes occurs when acetonitrile–buffer mobile phases are mixed on-line.)

You should be able to confirm this theory by injecting a sample blank that contains the sample solvent and buffer but no sample components. Try this with your present sample buffer and one diluted 2–10 times with water. Fewer problems should occur with the more dilute buffer.

You can try several possible solutions to the problem without completely reworking the method. One possibility is using a more dilute sample buffer during sample preparation. If a sufficiently dilute buffer is used, precipitation should be precluded. If the use of a dilute buffer is not compatible with your sample preparation procedure, perhaps you can dilute the sample and the buffer after sample preparation is complete. Now, try injecting a proportionally larger sample volume; you should be able to get the same sample mass on the column in a more dilute solution. Usually, the separation will be uncompromised if a large volume of sufficiently dilute solution is injected.

If one of these minor alterations is ineffective, perhaps some changes in the analysis procedure could overcome the pressure problems. One possibility would be to add a column-flushing step after each sample or every few samples. Because buffer precipitation is suspected, the column should be flushed with the aqueous component of the mobile phase. For example, flushing the column with 10 column volumes (~25 mL for a 25 cm × 4.6 mm column) of water or 5% acetonitrile in water might effectively redissolve the precipitate and restore the original column pressure. In general, avoid using 100% water or buffer with reversed-phase columns because they equilibrate very slowly under these conditions. Also be aware that flushing with a highly aqueous mobile phase may require follow-up flushing with a strong solvent to remove late-eluting components from the column.

Another effective procedural change might be to use a 0.5-µm porosity in-line filter between the injection valve or autosampler and the column. If the precipitates form upstream from this filter, you may be able to trap them on the filter instead of on the frit. If this is successful, you could change the filter frit when the pressure becomes unacceptably high.

If the problem persists, consider changing to an alcohol-based mobile phase that is less likely to cause buffer precipitation. As an alternative, you could adjust your sample preparation procedure to use a buffer that is more soluble in acetonitrile.

GUARD-COLUMN PROBLEMS

Q: I perform routine analysis of pharmaceutical-tablet extracts, and my sample preparation consists of dissolving a tablet in the injection solvent, spinning the particulate matter out, and filtering the supernatant before injection. The method works great, but the guard column goes bad so quickly that I have to replace it every 20 samples or so. If I try to use the guard column longer, one of the sample-matrix components interferes with the quantitation of the active ingredient. As soon as I replace the guard column, the separation is back to normal, and typically I can analyze 500–600 samples before the analytical column must be replaced. I don't have time to rework the method because it would require complete method revalidation.

In general, avoid flushing reversed-phase columns with 100% water or buffer because the columns equilibrate very slowly under these conditions.

JWD: I think you may be overconcerned about the performance of the guard column. My guess is that the guard column is doing just what it should, and you may be expecting too much from it. You might be able to extend the life of the guard column by incorporating more thorough sample cleanup or by flushing or backflushing the guard column as it deteriorates. However, if you consider the total analysis cost, a guard column is an inexpensive portion. The guard column contributes perhaps $1–2 per sample, and the analytical column contributes about $0.50. If you add up the costs of solvents and solvent disposal, autosampler vials, sample preparation materials, and sample preparation and data-analysis labor, I'm sure that total analy-

sis costs are >$10 per sample. Even if the guard-column lifetime could be doubled, you would reduce the total analysis cost by only ~5%. However, you would likely increase rather than decrease analysis costs by altering the sample preparation procedure to include liquid–liquid or solid-phase extraction or other such labor-intensive techniques.

Thus, columns and other individually expensive components may account for only a small portion of the total analysis cost. In fact, some workers design their separations with a minimum of labor-intensive sample preparation and cut total costs by using a guard column as the primary cleanup tool. Using these methods, 5–10 injections per guard column may be cost-effective.

GUARD-COLUMN LIFE

Q: How can I check to see if my guard column is still good? If I wait until the separation is obviously degraded, it may be too late and I'll have to replace the analytical column, too.

JWD: Generally speaking, the guard column should not affect the separation. Sometimes the guard column will slightly improve the separation because of the added length of the guard column–analytical column combination, but most workers observe that a system with a guard column provides about the same separation as one without a guard column. Extracolumn effects from tubing connections tend to cancel out any increase in the column plate number caused by the additional column length. In other words, a liquid chromatography system with a good guard column should yield a column plate number within ~10% of the analytical column alone.

You can use three common procedures to monitor guard columns for replacement. The first — which you want to avoid — is waiting for the overall separation to deteriorate before replacing the guard column. In some cases, you can modify this method and replace the guard column before it is too late. For example, you can monitor a pair of peaks in the separation that are separated more poorly than your critical components of interest. By picking a pair of peaks that are not baseline resolved, it is easy to monitor the deterioration in the separation by watching the change in the valley depth between the peaks. You should be able to correlate a change in this separation with the deterioration of the separation of the peaks of interest. This technique may help you anticipate the failure of the guard column.

A second technique is monitoring the number of samples that pass through the guard column before the system fails to give a satisfactory separation. After you've determined the lifetimes of two or three guard columns, you will have a good idea about how long they should last. Alter your method so that the guard column is replaced after ~80% of the expected lifetime. In this manner, you will throw away part of your useful guard-column life but will not compromise the analytical column.

Monitoring guard-column life in terms of solvent volume or the calendar is a third replacement technique. If you choose this route, you might specify replacing the guard column once a week or with every third batch of mobile phase.

Whatever technique you use to estimate guard-column lifetimes, try to replace the guard column well ahead of its expected failure point. As discussed in the answer to the previous reader's question, the guard column usually is a relatively small part of the total analysis cost, so shortening its life in exchange for a more reliable separation probably is a wise compromise.

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Walnut Creek, California, USA, and is a member of the Editorial Advisory Board of LC•GC. *Direct correspondence about this column to "LC Troubleshooting,"* LC•GC, *P.O. Box 10460, Eugene, OR 97440, USA.*