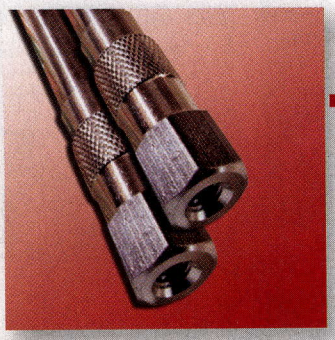


LC TROUBLESHOOTING

Problems from East and West

LC problems know no national boundaries

John W. Dolan
LC Troubleshooting Editor

At the beginning of the new year, it is good to look back in time — are there things that we can learn from the past? As I write this column, I have just finished presenting a series of troubleshooting and method development seminars in Istanbul, Turkey, and Amman, Jordan. Liquid chromatography (LC) is as hot a topic in these cities as it is in London, Bejing, Mumbai, or San Francisco.

As my host, Ibrahim, showed me around Istanbul, he was quick to point out that M.S. Tswett may not have been the first to use chromatography. In fact, as we toured the Basilica Cistern (Figure 1), he noted that the columns were installed more than 1500 years ago and were recycled from even earlier applications — and they still work just as well as they did when new! (You James Bond fans will remember a scene in "From Russia with Love" that was filmed here.) Then, less than a week later, my hosts in Jordan, Khalil and Basela, reminded me that the columns at Petra (Figure 2) are 2000-year-old monoliths that also continue to function in their original manner. (This is where Indiana Jones searched for the Cup of Christ in "The Last Crusade.") And all this time I have felt good if a column lasted a few months!

All joking aside, I had a wonderful time interacting with chromatographers in these two countries. Whenever I make such trips, I am reminded that we are all in the same business — using LC as a tool to separate and measure analytes in pharmaceutical, chemical, environmental, and other types of samples. This month's "LC Troubleshooting" discussion will center on questions from these seminars — you will see quickly that they are no different than the ones you get from your labmates.

How Can I Extend the Life of My Column?

A common question relates to how to maximize the lifetime of a column, and when the column begins to deteriorate, how to restore it to its normal function. First, we have to get the idea out of our heads that the column is a capital purchase and should last like one. As expensive as they can be, LC columns are consumable items. And if you consider the economics of analysis, the cost of a column is a small portion of the total analytical cost. For example, a typical budget number for the LC analysis of samples using UV detection is \$50/sample. If a column costs \$500 and lasts for 500 samples, the cost per sample is \$1, or about 2% of the overall cost. If we can manage to perform some kind of regeneration procedure that doubles the column life — to 1000 samples — we have saved only 1% of the cost. Is this a worthwhile investment? I figure that if I get more than 500 samples through a column, it has paid its way, and in my experience, a column generally lasts for closer to 2000 samples. Contrast this with sample filtration (≈\$1/sample) or solid-phase extraction (≈\$2–3/sample), where we throw away a filter or cartridge without concern.

That being said, how do we get the most out of the column? First, keep it clean to start with. Any effort spent in sample pretreatment usually is paid back in extended column life, but you need to balance the cost of pretreatment with the cost of the column, the quality of the data, and other considerations. Second, operate the column in a manner that keeps it healthy. For silica-based, reversed-phase LC columns, this usually means keeping the pH between 2 and 8. Higher operating temperatures will shorten column life, but in my experience this is a minor factor — methods requiring temperatures as

high as 70 °C don't seem to accelerate column demise greatly. It is a good idea to consult the care-and-use instructions shipped with each column to see if there are other restrictions, especially if the column contains something other than a reversed-phase packing. Third, keep particulate matter out of the column. This means that all samples should be treated in a manner that minimizes particulate matter – either through filtration or centrifugation before injection. I recommend that a 0.5-µm porosity in-line filter be used directly after the autosampler on every LC system to help keep small particles from fouling the column.

Fourth, use a guard column. Those of you who read "LC Troubleshooting" on a regular basis know that I'm not a big fan of guard columns. However, there is no question that they extend the life of the analytical column. My problem is that guard columns are expensive — in many cases I don't think they extend the life of the analytical column sufficiently to justify the cost. And they often degrade the separation, because they are not well packed. If you do use a guard column, you need to replace it on a regular basis — before it no longer offers protection for the analytical column. Usually, it is easiest to replace the guard column after a certain number of samples have been run or on a calendar basis.

Fifth, clean the column regularly. After each batch of samples, the column should be flushed with the strong solvent of the mobile phase, usually acetonitrile or methanol. If a buffer is used, it is best to first flush the column with 10 mL of water or buffer-free mobile phase to avoid buffer precipitation, especially when 100% acetonitrile is used for flushing. A column flush of 20–25 mL of methanol or acetonitrile will help to remove strongly retained materials that tend to foul the column or bleed off as broad bumps in the baseline.

Finally, some people like to wash the column with special cleaning reagents. For example, after flushing with acetonitrile or methanol, washing with another 20 mL of methylene chloride will help to remove additional strongly retained material. But there comes a time when the column cannot be restored to normal use, so heroic measures often are a waste of time and money.

One additional suggestion is to dedicate a column to each analytical method. If you have two methods that use the same column, you will find that you will have fewer problems and buy fewer columns in the long run if you buy a separate column for each method and use it only for that method, rather than switching one column back and forth.

Bad Peak Shape from Dissolution Samples

One student told me about problems he was having when analyzing samples from a dissolution bath used to test the properties of a pharmaceutical product. The bath is a glass container filled with a simulated stomach-content solution, often containing a surfactant and dilute hydrochloric acid. When injecting 20 µL of sample onto a 150 mm × 4.6 mm C18 column, he observed bad peak shape consistently. If he injected the analytical standard, the peak looked OK. After discussing the problem, I realized that the pH of the injected sample was significantly lower than the mobile phase, so I suspected that the buffer did not adjust the sample pH quickly enough upon injection. Upon suggesting that the pH of the sample be adjusted to something closer to the mobile phase, I was told that any dilution of the sample would not allow a sufficiently strong signal to be generated to reach the detection limits of the method.

Here's a trick that might help to overcome this problem, which comes up in many different methods: Dilute the sample such that the concentration of organic solvent is at least 10% lower — and preferably more — than the mobile phase. For example, with a mobile phase of 40% methanol, dilute the sample so that it contains no more than 30% methanol. Under these conditions, the sample will be slowed or stopped at the head of the column in a process called on-column concentration. This allows you to inject a larger sample without the normal band-shape problems encountered when injecting a large sample with mobile phase as a solvent. So for the present case, it may be possible to dilute the sample by a factor of two with dilute sodium hydroxide so that the pH of the sample matches the mobile phase. Then inject twice as much sample. The same sample mass will reach the column as in the original method, but a less-acidic pH might improve the peak shape.

But I Can't . . .

One of the most frustrating parts of working as an analyst can be using methods that someone else has developed, but that don't work properly. These may be compendial methods or methods developed in another department of your company. Often, when I suggest a change to fix a problem, it is answered with, "But I can't . . . change the sample size, adjust the pH, use a different temperature . . ." and so forth. I understand that changes often are not allowed, but it is necessary to make changes so that we can understand how to fix the method. Let's use the previous dissolution bath problem as an example. If we suspect that the pH of the injection solvent is the problem, as I did in this case, how can we prove this is true or false? The only way that I know of is to try the change to see what happens. If the change is effective, we have ammunition (a.k.a., data) to present to our manager about how to fix the problem. If the change is not effective, we have eliminated a possible problem source and can look elsewhere for a solution. If we refuse to act, based upon "But I can't," we will not be able to find a solution to the problem.

And often, it just isn't true that adjustments cannot be made. The United States Pharmacopoeia (USP) lists guidelines regarding what can be considered an adjustment of a method to meet system suitability requirements (1). Adjustments, such as a change in flow rate by ±50%, can be made to meet system suitability without requiring additional validation, whereas changes to a method will require some amount of validation. There is not universal agreement about what constitutes a change as compared with an adjustment, but the USP guidelines are a good place to start for limits on method adjustment. Of course, one always needs to make sound scientific judgments in such cases. For example, the same USP guidelines list ±10 °C as the limits for adjustment of column temperature. Some methods will tolerate this magnitude of change without problems, whereas other methods can be compromised if the temperature is changed more than ±5 °C.

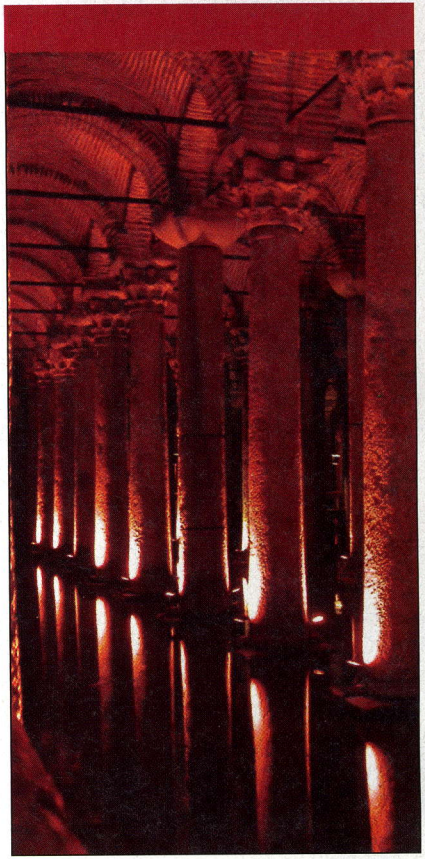


Figure 1: Basilica Cistern (Istanbul, Turkey).

Updating the Method for a New Column

One of the students recently had replaced the recommended column for a method with an equivalent column, because the original column was no longer available. He had successfully found a column that gave equivalent selectivity as the original column by using a database (2) to compare columns, as has been described previously (3,4). His problem centered on the difference in column size and the operating pressure. The original column was 100 mm × 4.0 mm, packed with 8-µm diameter particles and operated at 3 mL/min. The new column was 100 mm × 4.6 mm packed with 3-µm particles, and at 3 mL/min, the pressure was excessive. He wondered how he could adjust the conditions so that it would meet the USP requirements.

First, the flow rate should be adjusted to give equivalent linear velocity of the mobile phase traveling through the column for both columns. This is accomplished by adjusting the flow rate by the change in the column cross-sectional area (the square of the ratios of the diame-

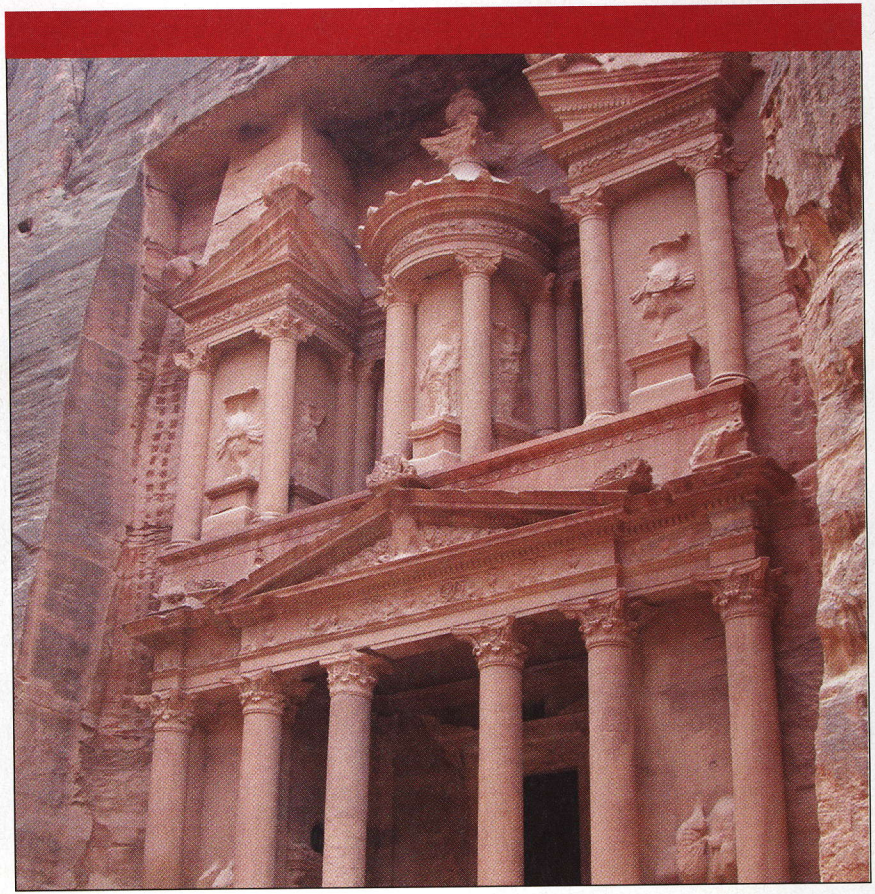


Figure 2: The Treasury (Petra, Jordan).

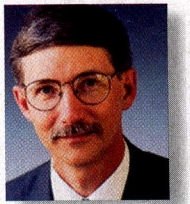
ters). In the present case, the adjustment would be (4.6 mm i.d./4.0 mm i.d.)^2 × 3.0 mL/min = 4.0 mL/min. This means that the linear velocity of the mobile phase for the new column at 4.0 mL/min is the same as the old column at 3.0 mL/min. If the user was concerned about high pressure with the new column operated at 3 mL/min, 4 mL/min will be unacceptable. However, consulting the USP guidelines (1), we see that ±50% change in flow rate is allowed after adjustment for linear velocity. This means that a flow rate of 2.0 mL/min should still meet the guidelines but will lower the pressure, hopefully to acceptable levels. Before we blindly make such changes, we need to stop and think if this change is likely to have unexpected consequences. In the present example, the answer is no — a change in flow rate for isocratic separations will change the column plate number and peak widths but should not affect peak spacing. The run will be longer, but that may be tolerable. Finally, because the 3-µm particle column will have a larger plate number, it may be possible to use a shorter column for the same separation and compensate for some or all of the lost time. For example, in theory, a 50-mm long, 3-µm column should have a larger plate number than a 100-mm long, 8-µm column. A 50 mm × 4.6 mm, 3-µm particle column operated at 2 mL/min should give a faster separation than the original column if the same selectivity (peak spacing) can be obtained.

Summary

Columns will not last forever, but if you treat them well and flush them regularly, they should account for a small portion of the total analytical cost. When problems arise, it might be necessary to explore unauthorized changes just to help you figure out how to fix the problem. After you have data to support a method adjustment or change, it will be easier to justify to whoever can authorize such a change. Generic recommendations for method adjustment have been published, such as in the USP, but it is important to consider if they make sound scientific sense, and if they do, try them to see if you can correct the problem.

References

(1) "Chapter 621, Chromatography," United States Pharmacopeia No. 31-NF 26, (2008).
(2) http://www.usp.org/USPNF/columnsDB.html
(3) J.W. Dolan, *LCGC* **25**(10), 1014-1020 (2007).
(4) L.R. Snyder, J.W. Dolan, and P.W. Carr, *Anal. Chem.* **79,** 3254-3262 (2007).



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