LC Troubleshooting

Grandma's Ham

Do your LC habits make sense?

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Both in the classes I teach and the questions that readers send me via e-mail, there is an undercurrent of liquid chromatographic (LC) troubleshooting and maintenance habits that make little sense with today's equipment. Some of these practices were acceptable at one time, and others were flawed concepts from the beginning. Too many are deep-seated in the seven last words of [insert your favorite organization], which are: "We have always done it that way."

This reminds me of the story of Grandma's ham, which goes something like this: A little girl was watching her mother prepare the Christmas ham and asked why her mother cut off a thick slice from each end of the ham before putting it in the pan to bake. Her mother responded that this was the way she learned from her mother and that the little girl should ask her grandmother when she arrived for dinner. The grandmother arrived and the little girl asked why the ends of the ham were removed before baking. In response, the grandmother told the girl that when she was first married, they didn't have a pan large enough for the ham, so she shortened it a bit to fit the pan and never got out of the habit.

Too many of our LC practices are like Grandma's ham. Perhaps they were a good idea originally, but they don't make much sense in today's lab. This month's "LC Troubleshooting" will examine several of these practices to determine their utility today.

Air in the Column

One common question I hear is, "If I run out of mobile phase and fill the column with air, will this ruin the column?" Whoa! Wait a minute. There are two questions here — let's examine them separately. First, what happens if the reservoir runs dry? You will notice that the solvent inlet line connecting the reservoir and the pump goes dry. Then the pump pressure drops to zero. Why is this? LC pumps are designed to pump liquid, not air, so when they fill with air the piston cycles, but no liquid or air is pumped through the outlet check valves. If you don't believe me, block the pump outlet of a dry pump and watch the pressure as the pump cycles. It stays at zero, doesn't it? The pump will continue to pump and piston seal wear will increase because the piston is no longer lubricated by mobile phase. For this reason, it is wise to set the lower pressure limit on the pump to a low value so that the pump will turn off if it fills with air.

So the first part of the answer is that if the pump cannot pump air, how will it fill the column with air? It cannot. The second question has to do with damage to the column if it dries out. This could happen to a certain extent if the column were stored without the end plugs in place, but the amount of air in the column would be minimal. When I worked with a column-development group, we intentionally dried out columns by pumping nitrogen through them and warming them in an oven. Once the column was filled again with mobile phase, the column performed normally. The real difficulty is removal of all of the air once it gets into the column. Pump a low viscosity, thoroughly degassed organic solvent such as methanol through the column to speed this process. For small air pockets, such as when end plugs are left off, the air should clear quickly when the column is brought to operating pressure because the air will be forced into solution.

Another question one reader asked was related to advice he'd been given to always make tubing connections with the pump flow on so that no air was trapped when the fitting was tightened. From the earlier discussion, it is obvious that trapping a small air bubble should not be a concern.

In fact, in one application I developed, an air-segmented sample containing 10–20 µL of air was injected routinely with no adverse effects to the column, so a tiny bubble trapped in a fitting will be of no consequence. As a further suggestion, I strongly recommend *against* making tubing connections when the flow is on. This can be especially problematic when polymeric fittings are used, because it is difficult to determine if the tube end is fully seated in the mating fitting when mobile phase is flowing. This could inadvertently lead to unwanted extracolumn volume in the system and excessive peak broadening.

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Air can be a problem in the pump and can result in low or pulsating flow and pressure. Care should be taken to use degassed mobile phase and purge any bubbles from the pump before beginning to run samples. Similarly, bubbles in the detector will give spurious peaks. Detector bubbles can be prevented in most cases by installation of a postdetector back-pressure regulator. These spring-loaded devices keep 50–100 psi back pressure on the detector so that air stays in solution until it exits the detector. Be sure to check the detector specifications to ensure that the back-pressure restrictor does not exceed the detector cell upper pressure limit.

Step Changes in Flow or Organic

Some workers have been taught to change flow rate in small steps (for example, 0.1-mL/min increases) so that the column is not subjected to sudden pressure changes. This might have made sense at some time in the past when silica-based LC columns were much more fragile. It might still be a good idea for some soft-gel size-exclusion columns (check the care and use instructions), but it is not necessary for the standard reversed-phase columns most of us use. Today's columns are packed at pressures often exceeding 10,000 psi. In this process, the column actually stretches a bit and doesn't return to its normal dimensions for about 30 min after packing. If the endfittings are installed during this time, the column can be thought of as being overpacked, so that the packing is under tension inside the column. This results in a column that has high physical durability. So setting the flow rate to 2–3 mL/min and turning on the pump is very unlikely to cause any damage to the column.

A related practice is use of a reverse gradient to re-equilibrate the column after a gradient run. For example, a gradient of 5–95% acetonitrile–water might be run over 20 min followed by a reverse gradient of 95–5% in 5 min and a hold at the initial conditions. This practice might have been important for column-packing materials that were subject to swelling or shrinking, such as certain polymeric beads, but not for today's high-quality silica-based packings. It is the total volume of solvent at the initial conditions that is important for re-equilibration after the gradient. The step from final gradient conditions to initial conditions can be made in a single step in the gradient controller, generally 0.1 min.

While we're looking at column durability, what about the column that rolls off the bench onto the concrete floor? Is it ruined? Ten years ago, my laboratory provided technical support for one of the major column distributors. As part of the service we provided, we tested columns that were returned from customers for one reason or another. I will never forget one column we received in its original unopened box. The customer had refused delivery. The box was in poor shape, and when it was turned over, the problem was obvious. There was a tire track across the box. The courier had dropped the box and backed over it with the delivery truck. We opened the box and found the column sufficiently bent that the middle of the column touched the surface when it was set on the lab bench. Of course we all expected that the column was ruined, but we tested it just for fun. It passed the original column specifications, so we put it into service and it lasted as long as any other column. I wouldn't advise dropping columns on the floor, but a single drop is unlikely to ruin the column.

What About That Arrow?

Excessive pressure is one of the most common symptoms of column failure. This results from the build-up of particulate matter on the column inlet frit. With earlier generations of columns, many workers replaced the frit when high pressure was observed, which corrected the problem in perhaps 30% of the cases. Most columns came with spare frits for just this purpose. Spare frits are seldom supplied with today's LC columns. Furthermore, it is inadvisable to remove the endfitting on modern columns because the packing material tends to ooze out of the column due to internal packing tension (as discussed earlier). The remaining technique to reduce the high column pressure is to reverse flush the column. Whenever I recommend this, there is at least one person who objects because of the arrow on the column, which indicates the direction of flow. This arrow is a handy reference, but most silica-based columns can be reversed without damage. When I worked in a column-development group, we discovered that columns were more stable when initially used with the flow in the reverse direction of the original packing flow. As a result, we affixed the flow arrow in the opposite direction of packing flow. (I suspect that this practice is common among column manufacturers.) After a few days of use, the flow direction did not matter. To reverse flush a column, disconnect it from the system, attach the old outlet to the inlet feed tubing, and flush 10–25 mL of mobile phase through the column to waste before reconnecting the detector.

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Note that column-reversal procedures can have some exceptions. If in doubt, check the column care and use instructions included with each column. Columns that contain polymeric particles might have limitations in terms of direction of flow. Some manufacturers of small particle columns (≤ 3-µm particles) can use 0.5-µm poros-

ity frits at the column outlet to keep packing from leaking out, but larger porosity frits (for example, 2 µm) at the inlet to minimize pressure buildup. If this is the case, column damage could occur if the column were reversed. It is best to check the directions.

There is a simple alternative to reverse flushing columns to relieve high pressure. This is to use a 0.5-µm porosity in-line filter between the injector and the column. Such devices are inexpensive and will trap any material that would otherwise get caught on the 2-µm frit at the head of the column (or guard column). Replacement of a blocked in-line frit is quick and inexpensive. I believe that in-line filters are one of the most effective add-ons to reduce pressure problems in the LC system — every LC system should have one.

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A Shocking Experience

As the last topic in this month's discussion, let me share some information reported to me by a reader. A semipreparative normal phase method used ethyl acetate as a mobile phase at 15 mL/min with a silica column. The column was operated at room temperature, and PEEK tubing was used to connect the column to the autosampler and detector. At one point, the user observed a spark jump from the column to a nearby metal component. Her conclusion was that the nonconductive mobile phase and connecting tubing isolated the column from a ground source and allowed static electricity to build as the mobile phase passed through the column. The static charge built, within seconds, to the point of discharge to a grounded component. Fortunately, no further problems were encountered, but one can imagine what might have happened if an open container of flammable solvent or system leak were nearby. The reader asked me to urge readers strongly to make sure the column is always grounded. This might be accomplished by clamping the column in a column oven or by using stainless steel connecting tubing.

I suspect that the combination of conditions for static buildup in the column are not common. Most workers use reversed-phase techniques in which a conductive mobile phase is employed. Also, column ovens are in widespread use, so physical grounding in the oven is likely. However, nearly all of us live in a region in which the humidity is low enough at some time during the year to allow us to build up sufficient static that we get a mild shock after we walk across a carpet and touch a door handle. Although the probability of a system-induced spark is very low, the preventive measure is trivial. I'm going to follow the reader's advice.

Truth or Fiction?

We've looked at several examples of laboratory practices that might have had some basis in truth at some time in the past, but in today's laboratory, many of these no longer make sense. It is important to examine our daily routine to make sure we don't fall victim to the "Grandma's ham" scenario. Fortunately, none of the practices discussed here are likely to cause any harm except wasting time. But we all know that time is money . . .

Do you observe questionable practices in your laboratory? Drop me an e-mail and share your experiences. At some point, we can revisit this topic.

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