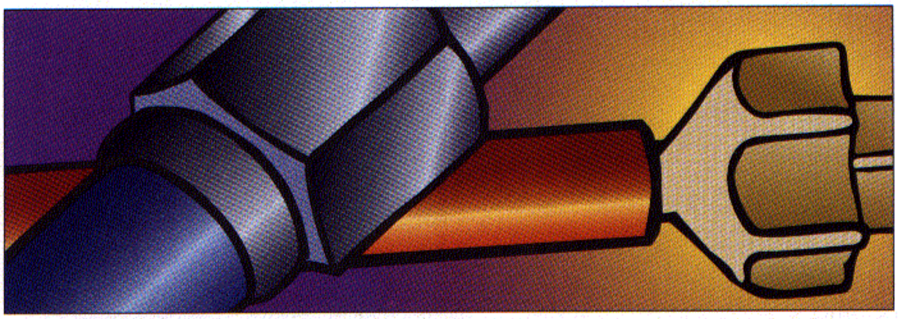
LC Troubleshooting



From the Mail Bag — Dirty Loop, Precolumn Use, In-Line Degassing, and Injector Plumbing

John W. Dolan

Readers share their experiences and troubleshooting tips.

Every week I receive letters and e-mail messages from readers of "LC Troubleshooting" inquiring about specific problems they encounter. Sometimes this correspondence contains examples or operational tips that would interest other readers. I try to respond directly and promptly to each inquiry, but public discussion of this information often is delayed until the column covers an appropriate topic. This month I've pulled some examples from the files to share.

DIRTY LOOP?

Input: My research involves the trace analysis of explosives residues by liquid chromatography (LC) with UV detection at 210 or 240 nm. In an attempt to gain more sensitivity, I recently switched to a larger injection volume. I ordered a 100-µL stainless steel loop from a chromatography supplier, received the shipment, and connected the loop to the manual six-port injector. My research assistant and I immediately began to inject solutions of explosives standards into the 150 mm × 2.1 mm, 5-µm d_p C18 column. To our dismay, very large peaks (on our scale of 0–0.005 absorbance) of unknown origin obscured the expected explosives peaks. We didn't know if these peaks were sample solvent artifacts or the results of the injection loop itself. To check, we injected mobile phase (50% methanol–50% water) and found the same interference (see the upper trace in Figure 1), so we suspected contamination in the injection loop.

We decided to clean the loop by filling it with solvent, waiting 1 h, and then rinsing the loop again. Solvents were used in the following order: toluene, acetonitrile, methanol, and 50% methanol–50% water. After cleaning the injection loop, the chromatogram (Figure 1 lower trace) from an injection of mobile phase improved significantly. After more cleaning in the same manner, the new injection loop yielded acceptable blank chromatograms for our trace analysis.

We were surprised to learn that we cannot use sample injection loops off the shelf. Are dirty injection loops the norm, or were we just unlucky? Should we follow an established cleaning protocol for new injection loops before injecting samples (1)?

Response: Well, I'm just as surprised as you. My inside sources at some of the manufacturers of tubing and fittings for HPLC tell me that they routinely wash all their parts. A typical wash involves degreasing the item with an organic solvent such as methanol and a nitric acid treatment to passivate the part. I'm sure that different vendors use somewhat different cleaning techniques. One of the reasons my laboratory purchases precut tubing and preformed sample loops is that they are clean and ready for use. When analysts in my laboratory cut a piece of tubing from a bulk coil, they take extra time to wash it, but precut pieces are used directly. Supplies vendors aren't perfect, but the quality control in recent years has been very good, so I would hope that your experience is the exception rather than the rule.

ANOTHER PRECOLUMN USE

Input: In a recent "LC Troubleshooting" column (2), you mentioned the use of a precolumn or saturator column as a means to prolong the life of an analytical column that is exposed routinely to aggressive mobile phases. For years we have used a cartridge guard column in this mode for another reason. We found that no matter what the commercial source of water and trifluoroacetic acid, if an analyst connects a new column with pretty, white resin and examines the resin at the head of the column in a week or so, the resin will be yellow. We actually have seen brown resin. I think that the sheer volume of solvent with even minuscule contaminants is responsible for this phenomenon. By placing the guard column upstream of the injector, it does not add to the back pressure, does not affect resolution, and may act as a saturator (although this function is not much of an issue with us), but the guard column absolutely protects the main column from accumulating colored contaminants. Because we use quality reagents, a single guard cartridge lasts for months before we need to replace it. So this application, which is not listed in your article, has greatly prolonged the life of typical columns in our laboratory (3).

Response: The accumulation of colored material at the head of the column is something I have noticed too, but I always attributed this phenomenon to sample pigments. In many cases sample pigments are the most likely source. For example, if you are analyzing plant extracts, the head of the column

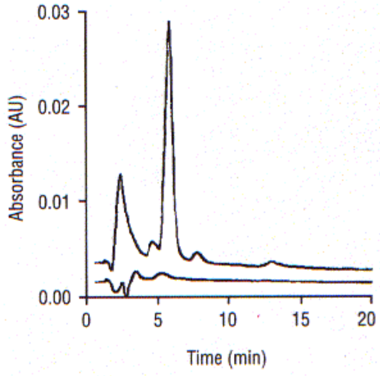


FIGURE 1: Chromatograms obtained from injection of mobile phase using (upper) new and (lower) extensively cleaned sample loop (1).

tends to turn green, whereas if your samples are plasma or serum, the column turns a yellowish color. Your observations suggest that the mobile phase is the source of at least some of this pigmented material. The obvious follow-up question is, "So what?" Is mobile-phase pigment an important problem? How does it degrade column performance? I would welcome reader feedback about this situation.

IN-LINE DEGASSING

Input: Several "LC Troubleshooting" columns in recent years (4–6) have discussed some less traditional problems, such as ghost peaks caused by dissolved gas in the mobile phase. I worked on the development team for one of the popular in-line degassers and would like to share some of our insights. The primary reason for degassing, of course, is to keep a high performance pump pumping. One of the less frequent needs is to remove oxygen from the mobile phase.

We investigated the efficacy of various degassing techniques and dispelled a number of myths. One idea, which we verified experimentally, was that the best on-line method for reducing the oxygen concentration in the mobile phase to a practical minimum was to combine sparging with an efficient in-line degasser. Continuous sparging with an inert gas (either high-grade nitrogen or helium, of low oxygen content) removes the bulk of the oxygen, and an in-line vacuum membrane degasser reduces the total remaining gas load by 80–90%.

Helium sparging alone removes oxygen by displacement but reduces the total gas load only by approximately 45–50%; thus, it fails to degas the mobile phase completely. Newer, more sophisticated pumping systems may still have trouble or suffer in performance when pumping a helium-saturated mobile phase. Nitrogen is less expensive than helium and can displace oxygen, but it is much more soluble than helium, so in-line degassing following nitrogen sparging is necessary to keep a pump operational.

As discussed in instrument manuals (7,8), oxygen forms UV-absorbing complexes with solvents such as methanol and tetrahydrofuran. Removal of oxygen from mobile phases containing these solvents certainly lowers the background absorbance of the mobile phase and can cause the appearance or disappearance of ghost peaks and baseline upsets early in the chromatogram resulting from changes in refractive index or other phenomena. Matching the oxygen levels of both the sample injection solvent and the mobile phase certainly will help (6,9).

Response: As you imply, the mobile-phase degassing phenomenon is very complex. Several potential problem areas exist, and one or more of these areas may be important in one application, but not in another. Most of us think of pumping reliability first, and as is known widely, some pumps are much more tolerant to dissolved gas or air bubbles than others. After the pump problems are under control, analysts still may have problems resulting from retention of dissolved gas (as in references 5 and 6) or from detectors that are responsive to refractive index changes or sensitive to dissolved oxygen, such as electrochemical detectors in reductive mode.

I've stated before that I believe that degassing problems are the number one problem encountered with LC systems today, and routine use of degassing is the simplest preventive maintenance technique for avoiding these problems. Although sparging in combination with in-line degassing may seem to be overkill, every LC system I have seen works more reliably if thorough degassing is practiced.

INJECTOR PLUMBING

Input: Often it is stated that the plumbing configuration on an LC injection valve is critical for optimum performance, especially in the partial-fill mode of injection (for an example, see reference 10). The chromatograms of Figure 2 illustrate this situation clearly. Figure 2a shows the results obtained from a valve that was plumbed properly to backflush the loop onto the column. The distorted chromatogram in Figure 2b was generated after the pump and column connections were reversed so that the loop was flushed onto the column in the same direction as it was loaded (11).

Response: This example is a good illustration of a problem that can occur inadvertently when analysts service an injection valve. Figure 3 shows the standard flow configuration of the injection valve, in which the loop is backflushed onto the column. Reversing the column and pump connections causes no change in back pressure or apparent function of the injector. If analysts use a filled-loop injection technique, the injector will work correctly. However, if the loop is filled only partially, the sample must pass through the remaining loop volume on its way to the column. It is easy to imagine the band spreading that could occur

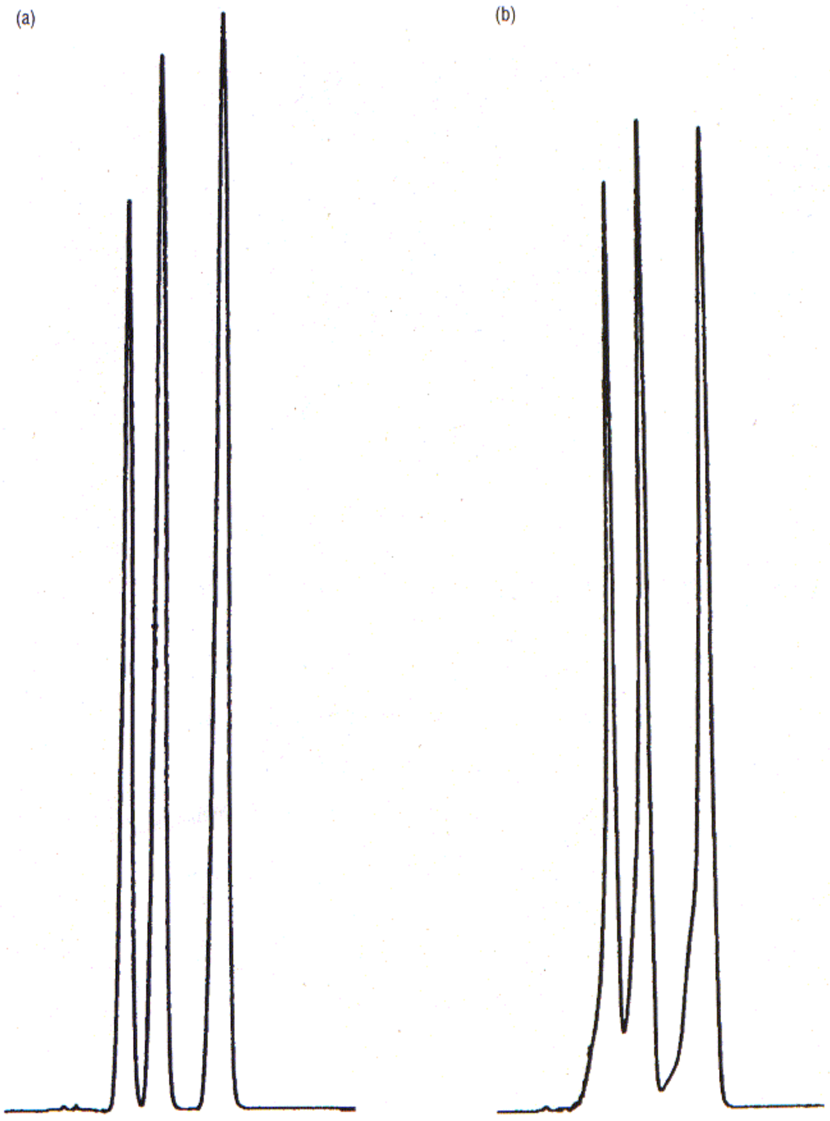


FIGURE 2: Chromatograms generated by partial-loop injections using (a) a correctly plumbed valve (see Figure 3) and (b) a valve for which the pump and column connections have been reversed (11).

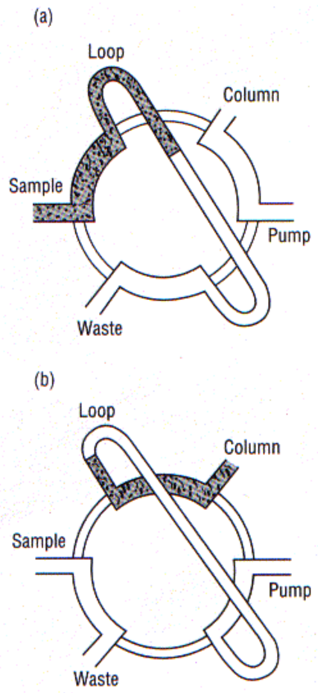


FIGURE 3: Valve configuration for a partial-loop injection. Shown are (a) load and (b) inject positions. (Reprinted with permission from reference 10.)

if a 1-mL loop were mounted on the injector and a 10-µL sample were injected. Dilution would broaden the small sample as it traveled through the loop, yielding an excessively large injection volume or a distorted injection profile, as shown in Figure 2b. Whenever analysts service an injection valve, it is wise to label all the tube connections before disassembly. Manufacturers generally stamp numbers into the valve body next to each fitting for reference.

CONCLUSIONS

After chromatographers have a few years of experience under their belts, they often discover special techniques that enable more reliable instrument operation. Often these techniques and observations never are shared beyond their immediate colleagues. This month's "LC Troubleshooting" column shares some of these experiences with a broader audience in hopes that others will benefit from their practical use.

Please feel free to contribute similar observations for future columns. As I've stated before, I give credit to the ideas that are shared but tend to keep the source of problem illustrations anonymous. Brand names, compound names, and equipment specifics are included only if they are necessary to illustrate a point and do not compromise proprietary information.

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