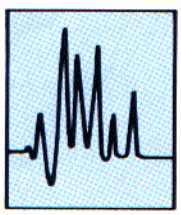
TROUBLESHOOTING

Readers' Questions

JOHN W. DOLAN



This month's Troubleshooting discussion centers on questions submitted by readers. Among the items covered are a persistent contamination problem, isolation of bubbles in a low-pressure-mixing system, and an elusive check-valve problem. Two readers share tips on preventing LC system problems. Some possible criteria for determining if a column is no longer usable also will be explored.

SAMPLE DEGRADATION

When a large batch of samples is prepared for unattended analysis, the samples often remain on the autosampler carrousel for several hours before injection. Under these conditions, degradation can occur in many types of samples. One reader shared a technique that he uses to determine sample stability (1). Samples are prepared in the normal manner and placed in the autosampler tray, and the LC system is programmed to inject a sample regularly over an extended period (for example, 1 injection/h for an overnight run). A strip-chart recorder is used at a slow speed (to conserve paper), or an integrator can be used if the chart can be run continuously. If sample degradation occurs, you will probably see a regular change in peak height and/or shape over time. In some cases, a band may disappear or a new band appear. It is important to use a continuous recording because degradation products often have retention times that are longer than those of the sample components. If an unanticipated peak elutes after the recorder is shut off — that is, between runs — it could be missed entirely. Later, however, if an assay is run on actual samples (with shorter run times), the new band might elute, interfering with a peak of interest and otherwise confusing interpretation of the assay. Thus, long run times for the degradation tests will, one hopes, allow all peaks to elute before the next sample is injected, and problem bands can be identified before actual samples are run.

STICKY CHECK VALVES

Q: I am using a 50 m*M* buffered mobile phase at pH 10.5 for my assay. The mobile phase is mixed on the low-pressure side of the pump. I flush the entire LC system at the end of each day to remove any residual buffer, so I don't have problems with pump-seal life. I do, however, have problems with check valves sticking on one brand of pump but not on another. The problem pump will work for 30 min to several hours before the inlet check valve(s) closes and stays closed. I have to remove the check valve and force water or mobile phase through it with a syringe to reopen the valve, which then works well for awhile but eventually fails again. The manufacturer has told me that there shouldn't be problems as long as the pH is kept below 11. Replacing the check valves does not solve the problem. Do you have any ideas about what is causing the problem or how to correct it?

JWD: To my knowledge, all check valves are made of materials such as stainless steel, ruby, sapphire, and Teflon (or another fluoropolymer) that are stable at the pH of your mobile phase. The buffer concentration is low enough that you shouldn't have problems with salting out when the organic component is mixed with the aqueous component of your mobile phase. I cannot think of an obvious reason why you have problems with one brand of pump and not another. You could determine whether the problem is with the check valves or mixing system by installing check valves from another manufacturer in your problem pump (often, check valves from one pump fit into another). Unless you are sure that the new check valves do not add dead volume or create other problems, however, exercise caution if you choose to use them routinely. You also could vary the pH and the buffer concentration independently to determine if either is responsible for the problem. Of course, the easiest solution is to run the assay only on the pump that does not fail under your test conditions. If readers have encountered this or similar problems that they would like to share, they should write to me c/o *LC·GC*, P.O. Box 50, Springfield, OR 97477.

CONTAMINATED HELIUM

Q: I inadvertently used a piece of copper tubing that was contaminated with cutting oil to connect the helium tank to the mobile phase sparging manifold. When I saw peaks in a blank chromatogram, I realized what I had done. I replaced the Teflon inlet lines from the reservoirs to the pump, but a small contaminant peak still appeared in every chromatogram. The peak is becoming smaller with time, but I would like to eliminate it. Do you have any suggestions?

JWD: I suspect that you still have some oil in the portion of the manifold between the supply line and the mobile phase reservoirs. A small drop of oil that slowly evaporates could continuously contaminate the mobile phase long after the supply line is replaced. Disassemble that part of the system, and replace all tubing, sparging frits, and other parts that are readily replaceable. Wash the remaining parts in a strong organic solvent such as methylene chloride or tetrahydrofuran to remove any traces of the oil; allow the solvent to evaporate, and reassemble the system.

Two other possible causes of your problem are that either the regulator or the helium tank is contaminated. You can test these by substituting for the questionable components ones that are known to be good. You should use 99+%-pure helium for sparging, and many workers use a molecular-sieve trap after the regulator, as is common practice in gas chromatography.

BUBBLE TROUBLE

Q: I am using methanol–water as mobile phase with low-pressure mixing. The reservoirs are continuously sparged with helium. I cannot get rid of the bubbles in the line between the outlet of the mixer and the pump, so of course I have problems with the pump as well. How can I isolate the source of the problem?

JWD: First, determine if the bubbles arise from outgassing of solvents during mixing, if they result from cavitation, or if they are caused by air leaks. Place both solvent supply lines in the methanol reservoir, and run the LC system with the settings adjusted to mix the same proportions of solvents A and B (both methanol, in this case) as normally would be used. If the problem disappears, the bubbles are a result of improperly degassed mobile phase.

Once you have eliminated degassing problems, check for restrictions in the lines. Restrictions can cause a partial vacuum to form in the supply lines, and even well-degassed solvents will release bubbles under those conditions. The problem is sometimes referred to

as "cavitation." Check for restrictions by removing the pump's inlet line at the inlet check valve while the proportioning valves are operating. In systems in which the reservoirs are above the pump, mobile phase often will siphon freely from the reservoir under these conditions; if not, a syringe should be used to draw solvent through the line. If a restriction is detected, remove the inlet filters from the reservoir end of the lines; if the lines are now clear, replace the filters with new ones; otherwise, locate and clear the blockage.

Once restrictions are eliminated as a cause of the bubble problem, you can assume that it is a result of air leaks. First check to be sure that all the fittings are snug. Be very careful not to overtighten the plastic fittings on the proportioning manifold because the threads can strip, the manifold block can be warped, or the seal can be broken. If your system uses flared fittings for low-pressure connections, visually inspect the fittings to be sure they are properly made. Any questionable fittings should be replaced. It is often convenient at this time to convert from flared fittings to low-pressure fittings that use a ferrule (for example, General Valve, East Hanover, New Jersey; Omnifit, Metuchen, New Jersey; or Upchurch, Oak Harbor, Washington).

After the fittings have been checked for leaks, the remaining problem source is the proportioning valve manifold itself. A leaky valve or valve diaphragm or a warped manifold block can result in air leaking into the system. Replace the entire manifold to confirm it as the problem source. You can exchange individual parts to pinpoint the exact cause if the manifold is indeed the problem, but it is generally more cost effective to replace the entire unit than to spend more time on the problem. When you have finished, don't forget to record the symptoms, the problem, and the solution in the system record book.

PURGING THE WISP

One of the most repeated recommendations in the Troubleshooting column is that buffered mobile phases be flushed from the LC system at the end of each day's operation to prevent buffer precipitation and subsequent accelerated system wear. Replacing the buffer reservoir with a container of water and pumping does not guarantee that all parts of the system that should be flushed will be reached, however. One reader (2) experienced poor precision that was traced to incomplete flushing of the Wisp autosampler (Waters Chromatography Division, Millipore Corp., Milford, Massachusetts). He was using a mobile phase that contained 0.005 *M* phosphate buffer and was finding poor precision if a sample larger than 200 μL was injected. He discovered that the low-pressure waste valve was blocked with precipitated buffer. When the system was flushed with water, the high-pressure syringe valve was positioned so that water never reached the low-pressure waste valve. The problem was remedied by purging the autosampler several times during system flushing.

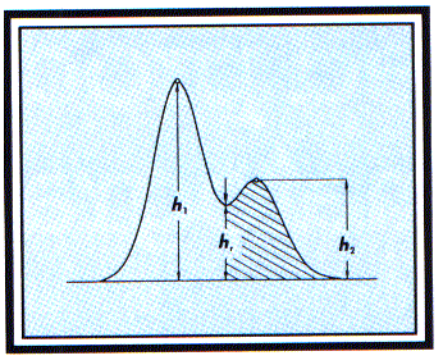


FIGURE 1: Measurement of peak valley/height ratio. (Reprinted with permission from reference 3.)

TABLE I: USE OF PEAK VALLEY/HEIGHT RATIO TO DETERMINE PEAK PURITY*

h_r/h_2	Relative Error in Apparent Area (minor band) (%)
0.25	– 1
0.4	– 2
0.6	– 5
0.75	– 10

*reprinted with permission from reference 3

WHEN IS A COLUMN BAD?

Q: We in the pharmaceutical industry are faced with increased pressure from government regulatory agencies to keep records on the efficiency of our columns and to establish limits for discontinuing the use of a column for a particular assay. Do you have suggestions on how best to accomplish this task within the routine of running assays? All of our assay methods are multicomponent and use the internal-standard method of calibration.

JWD: The best way to build record keeping into your daily routine is to use a reference run taken at the beginning of each day as a yardstick for determining if the system is running properly. In most labs, a standard or calibrator is run as the first sample each day; it serves as an ideal reference. You can use the column plate number as a criterion for whether a column is good or bad. The plate number can be measured manually, or you can use the height/area ratio for a peak (for example, your internal standard) as a measure of plate number. As the plate number drops, the height/area ratio will also drop because the peaks get wider while the area remains constant. The ratio is convenient because many data systems automatically calculate it; thus you can continuously monitor the plate number.

A more useful criterion for column performance, however, is resolution, because the separation of peaks of interest is the purpose of the assay. If possible, focus on a pair of bands for which the separation is most likely to degrade first. You can use a variety of methods to measure resolution (3), although the valley/height ratio shown in Figure 1 is one of the most convenient methods. Measure the height of the minor component of a peak pair (h_2 in Figure 1) and the height of the valley between the two peaks (h_r in Figure 1). As shown in Table I, the valley/height ratio can be used to determine error in measuring the area. Once you determine the level of error that is acceptable in your assay, you can determine the maximum valley/height ratio that allows you to stay within the desired limits. If the ratio exceeds the set limit, the resolution has degraded to a point at which you should regenerate or replace the column.

EQUIPMENT REVIEW

I regularly receive letters from manufacturers sharing information about products or product features that are designed to address common HPLC problems. For example, a detector manufacturer shared test data indicating that their variable-wavelength UV detector has lamp lifetimes that are about 10 times the nominal 500 h to 1000 h of useful life most of us expect from a deuterium lamp. Another manufacturer called to my attention a product that can be retrofitted to most autosamplers and allows smaller-than-normal injection volumes.

I would like to devote some attention in Troubleshooting to special product features that are designed to reduce or eliminate commonly encountered LC system problems. If you manufacture such equipment or are a reader who has practical experience with these products, please write to me c/o *LC•GC*, before December 1, 1986, so that I can present a review of this material early in 1987.

REFERENCES

(1) M. Miller, personal communication.
(2) N. Chamkasem, personal communication.
(3) L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd Ed. (John Wiley & Sons, New York 1979), Chapter 2.

John W. Dolan is president of LC Resources Inc., of San Jose, California, and is a consulting editor for LC•GC.

Readers are invited to contribute their troubleshooting tips to this column or to submit topics or questions for discussion in future columns. Write to: The Editor, *LC•GC*, P.O. Box 50, Springfield, OR 97477.