# LC TROUBLESHOOTING

## Mobile Phase Proportioning Problems

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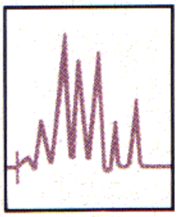

Errors in mobile phase proportioning can create numerous problems with LC analyses, and these problems can be difficult to identify. Some of the causes, such as restricted lines and loose tubing connections, can be corrected by the operator, whereas problems with the valve controller or software must be corrected by a service engineer. The data for this month's case study were contributed by a chromatographer (who wishes to remain anonymous) working in a pharmaceutical R&D lab.

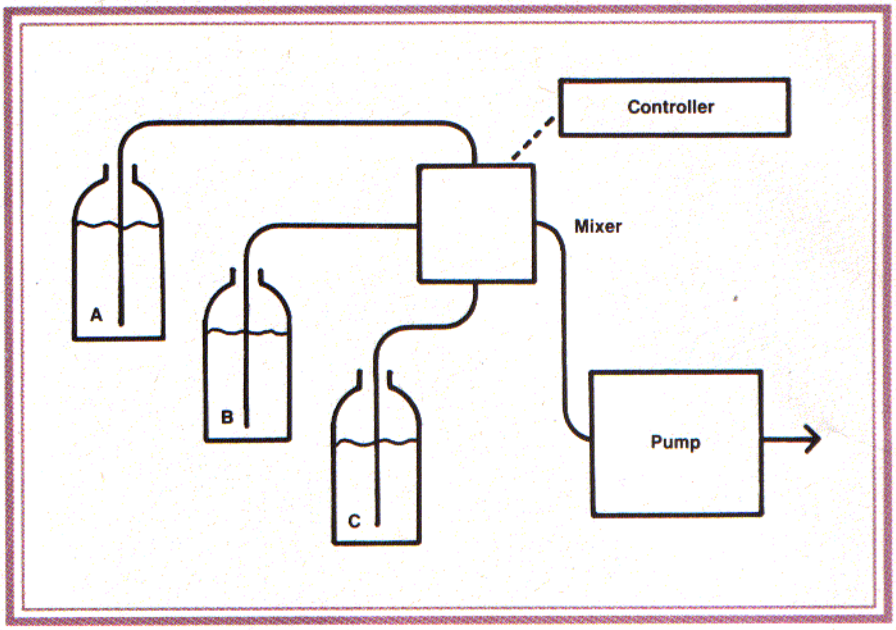

**FIGURE 1:** **Low-pressure mixing. Reservoirs contain solvents A, B, and C. Proportioning valves are mounted adjacent to or directly on the mixer. Proportioning valves typically are under control of the central system processor.**

### LOW-PRESSURE MIXING

Many LC systems use low-pressure mixing to provide the proper blend of solvents to make up the mobile phase. A schematic of a typical low-pressure mixing system is shown in Figure 1. In this design, solenoid-operated proportioning valves open and close under the direction of the controller so that the desired proportions of each solvent are delivered to the mixer. For example, to provide a 60:40 methanol/water mobile phase, the controller opens the methanol proportioning valve for 60% of the time and opens the water valve for 40% of the time. Following mixing, the mobile phase is drawn into the pump and pumped through the rest of the LC system. Low-pressure mixing is increasingly favored over high-pressure (multiple pump) mixing because of the reduced hardware cost (one pump vs. two or more).

### POTENTIAL PROBLEMS

Several potential problems associated with low-pressure mixing systems can result in errors in solvent proportioning (mobile phase composition). One error is related to the fundamental design of the proportioning cycle. Take as an example two different LC systems, one with a 1-s cycle (system 1) and one with a 10-s cycle (system 2). Both use a proportioning valve with a 10 ± 1 ms rise time (the time it takes the valve to open). For a mobile phase of 1% A in B, the A valve in system 1 should be open for 10 ms. To do this, the controller must anticipate the time required to open the valve. In this example, the system's controlling software would add the rise time to the open time and would send a 20-ms pulse. With a ±1-ms error in rise time, this system could be in error by as much as 10% of the target value when a 1% mobile phase is desired. In contrast, the A valve in system 2 would be open for 100 ms. Hence, system 2 would be more likely to accurately proportion a 1% mobile phase than would system 1 because the potential error of ±1 ms would give only a 1% error in the target value. However, longer proportioning cycles require more mixing volume. If the flow rate were 2 mL/min for each of these systems, system 2 would have to have a mixer capable of thoroughly mixing a 3-μL slug of A with a 300-μL slug of B, whereas system 1 would only have to mix a 0.3-μL slug in a 30-μL volume. Thus, system 1 seems to have the advantage in mixer design, but system 2 provides more accurate solvent proportioning.

Manufacturers spend a great deal of engineering effort and use creative software and hardware design to make sure that solvents are well mixed before they reach the column. For the most part, such factors are out of the chromatographer's control. Be aware, however, that properly operating low-pressure mixing systems require a matched set of proportioning valves. When a valve fails, either replace all the valves on the manifold, or be sure that the new valve exactly matches the old one. Mismatched valves can result in proportioning errors because all the valves do not respond in an identical manner to the controlling software.

Another proportioning problem can occur if one of the solvent supply tubes or its frit is restricted. In this case, even though the proportioning valve is open for the proper length of time, the restriction prevents the proper volume of solvent from being drawn into the mixer. For example, if the inlet frit in reservoir A is restricted, a slight vacuum will form in the system when the A valve is open, and the pump will be unable to draw the desired amount of solvent A. When the B valve opens, the vacuum will be relieved as the pump draws an extra volume of solvent B into the mixer. To avoid this problem, be sure to use filtered HPLC-grade solvents and change the inlet frit before it becomes blocked. Bubbles in the supply line can be symptomatic of a restriction or blocked inlet line frit.

Air leaks in the low-pressure fittings or proportioning manifold gaskets also can cause proportioning trouble because air is drawn into the system instead of solvent, resulting in mobile phase composition errors. Air bubbles that form when poorly degassed solvents are mixed can result in proportioning errors as well as pump check-valve problems.

A final proportioning problem can occur if the controller sends an "open" signal of

**TABLE I: MOBILE PHASE PROPORTIONING ACCURACY BEFORE AND AFTER ADJUSTMENT**

| Target % B | Delivered % B | |
|---|---|---|
| | Before | After |
| 10.0 | 3.9 | 9.7 |
| 20.0 | 22.3 | 20.6 |
| 30.0 | 24.3 | 28.5 |
| 40.0 | 42.7 | 40.0 |
| 50.0 | 50.5 | 49.7 |
| 60.0 | 54.4 | 59.4 |
| 70.0 | 72.8 | 70.9 |
| 80.0 | 74.8 | 78.8 |
| 90.0 | 93.7 | 90.0 |
| RSD | 24% | 2.4% |

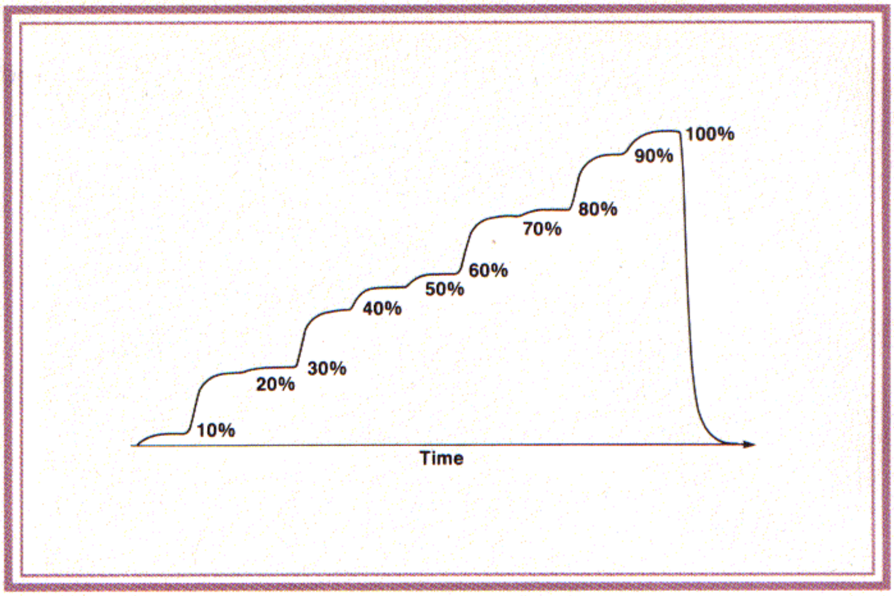

**FIGURE 2:** Step gradient trace from a poorly operating LC system. Steps are nominally 10%, changed every 5 min. Steps are labeled with the target values of %B in the mobile phase. See text for details.

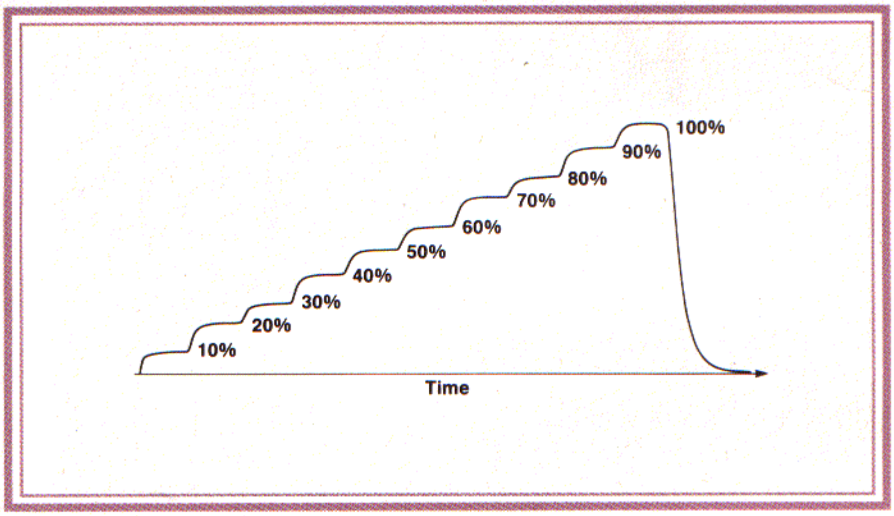

**FIGURE 3:** Step gradient trace from same LC system as in Figure 2, after it was adjusted to improve proportioning accuracy.

the wrong duration to each valve. This is the basis of the problem in the case study discussed below.

## ERRORS MAY NOT BE OBVIOUS

Air leaks or flow restrictions usually result in proportioning irreproducibility, showing up as precision or accuracy problems with the method. It should be noted that errors in proportioning that are due to valve limitations or improper control signals often will go unnoticed if the instrument is used for isocratic analysis, as was the case for the example below. While the pump may be inaccurate, it may also be precise (for example, the pump *always* delivers 43% A in B when 50% is requested). Hence, as long as a method is developed and used on the same system, an inaccuracy in proportioning may not be obvious. To a certain extent, differences in proportioning accuracy among LC systems are overlooked as well. Such errors often are attributed to column-to-column variation or unexplained variables; the method is "tweaked" to produce the desired results, and the problem is forgotten. Even when gradient runs are used, the instrument often will be very reproducible, but the gradient shape may not be what is expected. In the case study below, the chromatographer used commercial LC method optimization software to convert initial gradient runs into isocratic predictions, and it was found that the predictions did not match the experimental runs. At first the software was suspected, but a proportioning check was performed and the instrument was found to be at fault.

## CHECKING PROPORTIONING ACCURACY

The present example used a popular liquid chromatograph that incorporates low-pressure mixing. The proportioning test was a simple one. The A and B solvent reservoirs were filled with 50% methanol/water and the B solvent was spiked with 2–3 mL of acetone. A column was installed that produced 1000–2000 psi back pressure at 2 mL/min. A mobile phase of 100% B was pumped and the detector attenuation and wavelength were adjusted so that the signal was ~90% full scale. The system was then programmed to pump isocratic steps from 0 to 100% in 10% increments with a change programmed every 5 min. The results for the initial run were suspect, and the test was repeated. The same results were found and are presented in Figure 2.

The plot in Figure 2 should resemble even stairsteps, yet it obviously does not. Ideally, the edges of the steps should be square, but some rounding — caused by slow mixer washout — is common and generally is not a great problem unless step gradients are used in analysis. A quantitative presentation of these results is shown in Table I. Steps 1 and 3 were the worst, delivering 3.9 and 24.3% instead of 10 and 30%, respectively. Yet at a setting of 50%, the delivery was just 0.5% too high. This table illustrates the potential danger of checking proportioning accuracy by taking data at only one mobile phase composition. Overall, the relative standard deviation from the target value was 24%, which is unacceptable.

A service call was made, and after the system was adjusted, the trace shown in Figure 3 was observed. Although the results were still imperfect, Table I shows that the RSD was reduced from 24 to 2.4%. The LC system used for this case study was more than five years old, so this may be the best it can do. Specifications for new LC systems often quote proportioning accuracy of about ±0.1% RSD. Generally, if you repeat this test on an LC system that is performing well, RSDs of <1% should be satisfactory. To achieve the manufacturer's specifications, you generally have to be extremely careful about how the test is run.

*Exposing problems by changing applications:* It should be emphasized that the system discussed above had been performing very reliably for a long time. Typical quantitative results were better than 0.5% RSD for replicate determinations, and retention-time reproducibility was satisfactory. Only when the application changed did the problem become apparent. Changing from one application to another often exposes previously unknown problems. Most often these problems are blamed on the application, but as the present example has demonstrated, an existing equipment problem may be the underlying cause.

## PREVENTING PROPORTIONING PROBLEMS

Some proportioning problems can be prevented, and often you can keep those problems that cannot be prevented from surprising you. Change the inlet frits (sinker frits) in the solvent reservoirs before they become blocked. Most labs can use inlet frits for a month or more if filtered HPLC solvents are used. Use fresh buffers daily to help extend frit lifetime by avoiding microbial contamination. Solvent should siphon freely through the frits and inlet tubing when no restrictions are present. Be sure that the low-pressure fittings are sealed properly so that air cannot leak in and solvent cannot leak out. Thoroughly degas the solvents with helium sparging or another method to prevent most bubble problems. Elevation of the solvent reservoirs above the proportioning manifold will also improve the reliability of solvent delivery, as will other means that place a slight head pressure on the solvent reservoirs.

Mechanical problems with the proportioning valves and problems with the controlling software cannot be prevented, but you should be able to isolate them quickly if you have a good knowledge of how the system works when all is well. Two reference tests should be performed when the LC system is new, when you suspect proportioning problems, or when a major change is made to the system (for example, replacing the mixer). The first test is the step test described in the case study above. This is just a series of 10% isocratic steps from 0 to 100% of the B solvent (which is spiked with a UV absorber). The steps should be the same height and fairly square. The second test is to run a 20-min blank gradient at 4 mL/min using the same B solvent. Ideally, this trace should be straight with angular intersections at the 0% baseline and 100% plateau. In practice, there will be some rounding at the ends, but the region between 5 and 95% B should be very close to a straight line. If you are dissatisfied with either of these traces, correct the cause of the problem before proceeding. Keep good examples of both of these traces for later reference. If in the future you suspect a proportioning problem, rerun the tests and compare the results with your reference charts.

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*"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Lafayette, California, USA, and is a member of the Editorial Advisory Board of* LC•GC.