

## Liquid Chromatography Problem Solving and Troubleshooting

### Question:

In past articles, you have pointed out that retention time is one of the most significant HPLC measurements, and you have addressed specific questions on how to troubleshoot the cause of a particular retention time problem. Could you give a general overview of how to troubleshoot reversed-phase HPLC when one encounters retention times that are not constant?

### Answer:

The general overview you request is summarized in Table I. Here are the six most common causes for retention time variations and the suggested corrective actions for each. Leaks are quite common and are the most difficult to uncover. Carefully inspect the HPLC; many leaks will be very slow and difficult for the casual observer to discern. All the fittings should be inspected for leaks, including those that are connected to the check valves. Also inspect the area where liquid would exit the pump if there were a leak in the piston seal.

Next check the flow rate of the pumping system by collecting the eluent in a graduated cylinder for a fixed time. If the flow rate is slower than anticipated and there are no leaks, it is possible that the pump may have sucked in an air bubble and that the bubble is lodged in the pump head(s), interfering with the delivery of constant flow. It is also appropriate to inspect the mobile phase reservoir and tubing attached to the inlet side of the pump for bubbles. It is usually easy to degas the mobile phase and reprime the pump.

Another cause of changed retention time could be that the mobile phase has changed. When using a single pump with a single solvent line, there is usually a one-time jump in retention time, after which the times are constant. In this case, remix the mobile phase. However, when using a single pump with multiple solvent lines and doing "dial-a-mixture" with low-pressure mixing, variations in the accuracy of the mobile phase composition could occur if the proportioning valve is not functioning correctly. Refer to your instrument manual to test the proportioning valve. When using a two-pump system and "dial-a-mix" and mixing on the high-pressure side of the pump, mobile phase composition variations can occur if the individual pumps are not working properly. Again, refer to the instrument manual for the appropriate testing.

Of course, there can be noninstrumental causes to variations in retention time. Injecting an overloaded sample amount will cause a peak to have a different elution time than when a nonoverloaded amount is injected. Another common source of variation in retention time is if the sample is dissolved in a solvent other than the mobile phase. If the solvent is too strong, the retention time of the analyte will generally be short when compared with that from a sample injected in the mobile phase. Lastly, check the temperature of the room; many HPLC separations are done without temperature control. As temperature is increased, retention will decrease. If the room experiences wide temperature fluctuations, the HPLC retention times will probably be affected. The best solution is to run analyses at a temperature that can be controlled by using an oven.

**Table I. What to Do When Retention Times Are Not Reproducible**

| Possible source of problem                              | Corrective action  |
|---|--|
| Leak(s)   | Inspect for loose fittings where liquid is present. Inspect for liquid coming from pump seals and/or around fittings in the check valve. Tighten or replace loose fittings and make new connections.               |
| Air in the pump   | Check flow rate, reprime pump, inspect mobile phase inlet line, and degas if necessary.  |
| Mobile phase composition is incorrect                   | Remake solution if using a single pump. If using "dial-a-mix", test the accuracy of individual pumps and/or the integrity of the low-pressure mixing valve. Replace if necessary.                                  |
| Sample overload   | Dilute the sample by a factor of 10–100 in the mobile phase and reinject.  |
| Sample dissolved in solvent other than the mobile phase | Dissolve sample in mobile phase. If that is not possible, dissolve sample in a solvent that is diluted to approximate the mobile phase strength.   |
| Variation in column temperature                         | Monitor the room or oven temperature, whichever is being used. If column is open in a room, wrap it with insulation to protect it from drafts or place the column in an oven. Repair or replace oven if necessary. |

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC-MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward to JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting*, P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

Brian A. Bidlingmeyer  
Associate Editor