Liquid Chromatography Problem Solving and Troubleshooting

Question:

I am separating sugars using an amino bonded-phase column with an acetonitrile–water mobile phase. Occasionally, the baseline of my refractometer wanders for no apparent reason. When I am in the lab, I often grab the column and check for loose fittings, making sure that the fittings are snug. During and after my checking for the tight fittings, the baseline drifts significantly more and then settles down quickly. When this drift occurs and I am not in the lab, the baseline settles down eventually on its own. I believe the cause is an "on again, off again" leak that I eliminate when I tighten the fittings. My question is, why does this happen, and why does it correct itself if I do nothing?

Answer:

Based on your question, I do not believe that your problem is, as you suggest, a very small, occasional leak. I believe that it is more probably a temperature change in the room that the column experiences which influences the equilibrium of the mobile phase components associated with the stationary phase. When this equilibrium is shifted, the relative concentration of acetonitrile and water is changed in the mobile phase; when this difference in mobile phase composition exits the column, it is detected by the refractive index (RI) detector. When you handle the column to tighten the fittings, you are transferring heat from your hand to the column during the process. This change in temperature is enough to change the equilibrium of the two solvents between the mobile and stationary phases, which is detected by the RI detector.

What many scientists do not appreciate is that the components of the mobile phase are "sorbed" to the stationary phase and are in equilibrium with the mobile phase. Any temperature shift will influence this equilibrium. If the eluent is monitored with a UV detector, the influence of temperature on the change in eluent equilibrium will not be detected. But when an RI detector is used, anything that shifts the RI of the eluent will be recorded. The fact that the shift is a drift in the baseline is consistent with a slow change in the temperature. When your hand heats the column, the shift should be more abrupt, due to a rapid change of temperature influencing the equilibrium.

To test for the cause of your situation, a few straightforward experiments are required. If the cause of your problem is a slow leak, wrap the end fittings and connectors with a piece of laboratory tissue and examine it frequently to see if it becomes wet. Often a slow leak will bleed onto a tissue and cause a small damp spot to be visible. Be careful not to touch the column when you examine it, however, to avoid transferring any heat to the column.

To test for a temperature effect, monitor the temperature of the room and determine if the baseline drifts coincide with shifts in room temperature. Finally, a key experiment would be to set up your system, which you have determined to be leak-free, and when the baseline is stable on the RI detector, grasp the inlet end of the column in your hand for approximately 1 min, making sure to have the skin contact the steel end fitting and outside wall of the column while the baseline is being monitored. Then remove your hand while continuing to monitor the baseline. Observe if there are any upsets or drifts in the baseline. If the equilibrium is shifted when the temperature from your hand is transferred to the column, the baseline should record a shift in approximately the time it takes for one to two column volumes to elute from the column. It is my belief that this temperature change will induce baseline shifts. If it does, the answer to your problem is to isolate your column from thermal variations, perhaps using some thermal wrap or similar product. Alternatively, use an oven to thermostat your column.

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.

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