

Liquid Chromatography Problem Solving and Troubleshooting

Question:

After mixing my mobile phase and using it over the course of several weeks, the retention times of my compounds gradually increase, ending up approximately 20–30% longer. Upon preparing a new mobile phase, the retention times almost immediately return to where they originally were. Then, the situation occurs again over the lifetime of the new mobile phase. This cannot be a pump problem, because I rigorously degas my premixed mobile phase (methanol–water, 50:50). Presently, I relate the changes to an internal standard; ideally, I would like to not have to do this. I am stumped as to how to maintain retention times in this real-world chromatographic situation.

Answer:

Your problem is an interesting one and may be a case where an “ounce of prevention” is required, but you applied a “pound of prevention”. Let me explain. Degassing your mobile phase is a correct “ounce of prevention” to protect your pump from swallowing an air bubble and ruining your chromatography. However, I believe that the increasing retention time results from a situation of overdegassing to the point of causing evaporation and loss of the more volatile solvent. Vigorously degassing over a long period of time can change the ratio of volatile to nonvolatile solvent in the mobile phase mixture. In your situation, because you mix 4 L of mobile phase at one time, degassing over a period of approximately 4 weeks can change the mobile phase composition, because the methanol preferentially evaporates. This would result in a weaker mobile phase and a longer retention time. I would suggest that you degas every morning and once or twice during the day rather than using a continuously active helium sparge, or you might consider purchasing a degassing unit that intermittently (rather than continuously) allows gas flow. This periodic degassing minimizes preferential evaporation of one of the solvents.

Coming to this conclusion is a matter of eliminating other possible causes that are improbable because they do not correspond to all of the observations that you made. Because you were experiencing a problem with retention time, only a few other sources could be the cause. The first cause that comes to mind is that your column is changing with time; this could be column fouling with non-eluted sample, a plugged frit, non-equilibration of the column, or overload of the sample injected into the column. However, none of these column-related causes are supported by the observation of preparing a new mobile phase and having the retention times almost immediately decrease to where they originally were.

The second possible source is that the pump is not delivering the correct flow rate. This can be measured by collecting the flow into a small graduated cylinder for a specific time and verifying the flow rate. I believe that a flow rate change is not likely to be responsible for your problem, because the retention times slowly increase (in a nonrandom fashion) and return soon after the new mobile phase is used.

The third consideration is temperature, which in your case is room temperature. Because your changes are only in one direction, a continuous change over several weeks does not relate to problems that temperature variations would probably cause. This leaves the mobile phase as the most probable source of the problem, and the loss of methanol over a long time period is consistent with aggressive degassing activity.

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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