Liquid Chromatography Problem Solving and Troubleshooting

Question

My reverse-phase HPLC column develops high pressure after running a specific analysis over approximately two weeks. The performance is good, but it is just that the pressure is too high for routine operation. I am using two pumps to mix an isocratic mobile phase. After experiencing the high back pressure, I have tried washing my column with a number of solvents, and the only clean-up method that works is to wash the column with 100% water. Is it possible that my sample contains a minor impurity that precipitates on the column and that this is the cause of my problem? If so, how can I improve the situation?

Answer

Not every high-pressure problem is caused by the contamination of insoluble material from the sample. In fact, I believe that it would be very unusual that your sample contained water-soluble trace components, which having dissolved in the aqueous mobile phase would also precipitate on the column head. However, to rule this out as a possible cause, you should test the possibility by injecting approximately 50–100 µL of sample into a vial containing approximately 1 mL of mobile phase. Observe whether the sample dissolves readily or if there is evidence that part of the sample is insoluble. Evidence of insolubility would most often be in the form of small particles (flecks) forming in the vial.

I think that there is another more probable reason for your situation. Because your mobile phase contains 3% aqueous buffer, 15% methanol, and 82% acetonitrile, it is my suspicion that the buffer only has limited solubility. Therefore, with limited solubility the buffer would slowly precipitate on the inlet frit that acts like a seed crystal. Over time this precipitate would build up, and, of course, the pressure on the column would also increase until it reached the limit of your system. The reason I believe that this is a more reasonable hypothesis is that the resolution is constant and only the pressure increases. Usually, if it is chemical contaminates fouling the column, the resolution will change. The other reason I believe that this hypothesis is correct is that the pressure dissipates after you wash the system with water. To test this possibility of insolubility of the buffer in the mobile phase, mix your mobile phase in a flask and let it sit for a time and observe whether any precipitate is visible.

The observation of a high back-pressure blockage resulting from buffer precipitation is also commonly observed when running gradients from an aqueous buffer to a high concentration of acetonitrile. After a flush with 100% water, the system returns to the normal back-pressure. In this situation, the suspicion of precipitation resulting from insolubility can be readily tested by pouring a small volume of the buffer solution into a large volume of acetonitrile.

How can you improve the situation? If you are not able to modify your method, you should consider doing a column wash with water every week (or so many number of injections). In this way, the operation becomes predictable by using a "maintenance activity" rather than an abrupt system clean-up. On the other hand, if you can modify your method, consider using a more soluble buffer, investigate a lower concentration of buffer, or both. In fact, in this system you may even wish to test whether a buffer is needed. If the buffer type or buffer concentration negatively impacts the separation quality, nothing should be changed; and the first approach would then be the fall-back position.

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 them 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 it 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 Bidlingmeyer
Associate Editor