

Liquid Chromatography Problem Solving and Troubleshooting

Question:

I have been having trouble obtaining constant retention time in a normal phase system using hexane as the mobile phase on a silica column. I have the temperature controlled. What else should I do?

Answer:

The trouble you report is a very common occurrence in normal phase chromatography. The most likely cause of this variability is an uncontrolled water content in the mobile phase. In a normal phase system, the separation is dependent on the attraction of the silanol sites to the molecule(s), and anything which blocks the surface OH groups will interfere with a reproducible separation. Therefore, any amount of polar material in the sample or mobile phase will be adsorbed onto the surface silanols and block its retentive ability. Since most solvents used in normal phase chromatography are not miscible with water, people might assume that these solvents contain no water. However, this is not true, and in fact, these solvents can contain small amounts of water. Typically, the water solubility limit can range from a solvent like isooctane, which is 0.006% (v/v) water, to methylene chloride, which is 0.2% water, to ethyl acetate, which can contain up to 3.3% water. For your solvent, hexane, the solubility limit is 0.01%. Remember that the concentration of water in a mobile phase can vary widely depending on its exposure to environmental conditions, and unfortunately, any change in the amount of water in these solvents can deactivate a silica column and result in variations in retention. Thus, if you do not take precautions to control the water content of the mobile phase, wide variability in retention can occur as the water equilibrates with the column and changes its activity.

It is difficult to reproduce water content on a silica column when using a "dry" solvent directly from the supplier's bottle, and it is almost impossible to guarantee a completely dry mobile phase in HPLC, because the solvents can absorb water from the atmosphere. It is often better to control the water content and thereby control retention. Using a mobile phase with controlled water content is appropriate to achieve an equilibrium with the silica surface while also eliminating the very active sites. The best way to achieve a constant water content is to prepare a 50% water-saturated mobile phase. This should make retention times reproducible (1).

For your specific situation, first prepare "dried" hexane. Dried solvent can be made in many ways. One way is by adding activated molecular sieves to the solvent and allowing adsorption of the water onto the molecular sieves to occur during a 2-hr time period with occasional swirling of the container to ensure good contact with the sieves. Then divide the dried solvent into two portions. Filter one half to remove any debris from the molecular sieve, and prepare a water-saturated solvent by adding 1–500 mL water and stirring vigorously for 4 h. Allow the water to settle and decant the hexane through a filter to remove the excess water. This solution is the "water-saturated" hexane. Combine equal amounts of water-saturated solvent with filtered dried solvent to make the final mobile phase. Pump 30–50 column volumes across the silica column to ensure the proper equilibration. It is good practice to inject a sample (standard) three times to verify retention time reproducibility, which implies that the column has reached equilibrium with the mobile phase (1).

Reference

1. B.A. Bidlingmeyer. *Practical HPLC Methods and Applications*, John Wiley & Sons, New York, NY, 1993, pp. 186–204, 332–344.

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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