

## ***Liquid Chromatography Problem Solving and Troubleshooting***

### **Question:**

I am running a mobile phase of 98% aqueous acetonitrile, and over a period of a day or so, I see retention times decrease. If I run a gradient up to 100% acetonitrile and then return to the initial conditions, the retention returns to the original values. I think I have clean samples, so what can I do to clean up the mobile phase?

### **Answer:**

I do not believe that the cause of your problem is impurities in the sample or solvent. I believe that you are observing a phenomenon thought to be caused by wettability. The situation you observed, using a column which has been solvated (wetted) in a highly aqueous environment (i.e., 98% water) and having the retention slowly decrease with time, may be due to what some have called "bonded phase collapse". I suggest that you perform an experiment to confirm that it is phase collapse. Start your analyses, and after a few injections, measure the consistency of retention. Then, stop the HPLC overnight and start up again in the morning. The retention of the sample components will probably shift to a lower retention. If it does, the most probable explanation of both behaviors is that the bonded alkyl chains have lost their solvation, because water will not wet them. The alkyl chains have changed from being fully extended into the mobile phase to a collapsed state, either wrapping themselves together (self-association) or collapsing onto the surface (think of the analogy of putting a drop of oil into a beaker of water compared with putting a drop of oil into 100% organic solvent). Once the column is rinsed with 100% organic solvent and returned to the original highly aqueous mobile phase, retention is regained. In methods where a highly aqueous mobile phase causes retention to decrease, placing a rinse with 100% organic solvent into the protocol at a specific time or frequency will often return ruggedness to the method.

The term "wettability" is generally used to describe the act of solvating the alkyl chains of a bonded phase packing material. Another manifestation of the phenomenon occurs when using cartridge columns that are shipped dry (i.e., the cartridge does not contain a significant amount of solvent). If these cartridges are used immediately in a mobile phase containing highly aqueous contents, the retention of the analytes will be short, because the alkyl chains of the bonded phase will not be solvated. The current theory is that the chains are not extended into the mobile phase because they are not fully solvated; therefore, with the functional surface not being totally accessible, there is little retention of the analyte. By pumping a mobile phase of 100% organic solvent through the cartridge column before use in an aqueous mobile phase, the bonded phase is wetted, the chains become fully solvated, and the bonded surface is available for retention. It appears that once a "dry" phase is solvated, it stays solvated; therefore, most bonded phases can be used in a wide range of aqueous mobile phase concentrations.

Some bonded phases have better wettability than others and should be selected if a highly aqueous mobile phase is required. Unfortunately, in some cases, the desired retention and selectivity are not always found in a phase with good wettability characteristics. In these cases, as mentioned previously, the operational protocol must contain a periodic wetting process to insure ruggedness.

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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