Liquid Chromatography Problem Solving and Troubleshooting

Question

I am in a university and share an HPLC among many users. Sometimes when I need to use the unit, the system is sitting there with no indication of who used it, with what solvent, and with what column. Many just put their column and mobile phase in the run. I believe that the instrument should be cleaned before I use it. What do you recommend?

Answer

Your problem is a particularly acute problem in a laboratory that uses multiple solvents and methods on a single HPLC system. It is interesting that people normally worry about using solvents to prepare mobile phases that are compatible with the column-packing materials and sample but often give little thought to the compatibility of solvents when changing HPLC solvents using the same pump. Obviously, the old and new mobile phases must be flushed with an intermediate solvent that is miscible with both mobile phases so that the pump and system are "cleared" of the original mobile phase.

If an HPLC pump is delivering a mobile phase that is immiscible with the solvent previously in the pump, the net result is to have tiny "slugs" of different solvents traveling through the HPLC system. Typical indications of this problem are: (*a*) erratic flow and (*b*) noisy baseline, baseline drift, or both. One way to check miscibility is to use a miscible number and measure whether the solvents are miscible (1). Another way is to use an intermediately polar solvent to wash the HPLC system when switching from a nonpolar to polar solvent. Two such solvents that are miscible with both nonpolar and polar solvents are tetrahydrofuran and acetone. Acetone is less desirable to use because it has high volatility, often making it difficult to pump. Also, it is not usually available in HPLC grade, which could result in contamination of the system.

Let me give an example of an embodiment of this situation. An individual was using hexane as a mobile phase in a normal-phase separation mode. When the individual finished the analysis, the column was removed and the solvent inlet line emptied of its mobile phase. Another user arrived and installed a column to do a reversed-phase analysis and filled the solvent inlet line with a mobile phase consisting of methanol–water. Soon the detector baseline was showing "spikes". The operator first thought that the column was malfunctioning, but upon removal of the column, the spikes changed slightly in intensity but continued. This next led to the hypothesis that the lamp was bad, but when the lamp was changed the spikes continued. At this time the operator called the instrument repairperson, and when that service technician arrived it was quickly determined that the spikes were not a result of electronic or system malfunction. The technician then asked the question what solvents had the instrument seen. The answer was that it was not known. Realizing that the system could have been in a normal-phase mobile phase and was now in a reversed-phase mobile phase, the instrument technician flushed the entire system including the column with tetrahydrofuran until a spikeless baseline existed. The instrument was then switched into the reversed-phase mobile phase. The system exhibited a noise-free baseline, and the reversed-phase separation was run successfully.

This was an expensive lesson in miscibility. The expense of a service call could have been avoided if a logbook was kept and some fundamental thinking about solvent miscibility was available.

It is obvious that I agree with your original statement, and the answer to your accompanying question is that the HPLC system should always be left in a known solvent. If the next operator requires a mobile phase that is not miscible with the mobile phase in the pump, the system should be flushed with an intermediate polarity solvent to insure that the old solvent is no longer in the system. When in doubt, remember that miscibility can often be checked in a beaker with visual observation to determine if the two solvents are miscible.

References

1. B.A. Bidlingmeyer. *Practical HPLC Methodology and Applications.* John Wiley & Sons, New York, NY, 1993, pp. 244–46.

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.

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