Liquid Chromatography Problem Solving and Troubleshooting

Question

I believe that I am having column reproducibility problems, and my associate told me that it might be my method that is the problem. Isn't reproducibility the responsibility of the vendor? How can a method contribute to reproducibility?

Answer

When surveys are taken of HPLC users, the number one complaint stated by 70+% of the people is "reproducibility," and your associate raised a good point. It is important to remember that good methodology is important for reproducible methods. Or put another way, reproducibility is the responsibility of both the manufacturer and the user. Let me give an overview of the responsibility of each.

From the manufacturer's point of view, it is very much in their interest to produce a reproducible column. To do this, manufacturers often measure the physical and chemical characteristics of the starting silica and the bonded phase. Such things as pore size, particle size, surface area, and purity are determined for the silica. For the bonded phase, items such as percent carbon, bonding density, and various retention tests of neutral and charged molecules are measured. After all of the chemical and physical tests are performed and passed, the packing is loaded into a column that is often tested for additional performance requirements such as plate count and retention. As each manufacturer measures with a myriad of tests, it is best to contact specific manufacturers to determine if you are satisfied with their testing. When a column arrives, you can verify that the column is what the manufacturer claims by running the manufacturer's test.

Next, let's consider the user's responsibility. This begins with the method development. A well-developed method will minimize "reproducibility" issues later on. Remember that a method should be developed on a new column, not on a used one. Develop a mobile phase that can easily be formulated by everyone. For instance, the mobile phase should be specified as to whether it is weight-to-weight or volume-to-volume. If it is volume-to-volume, use ratios that are easy to measure like 50:50 methanol–water, rather than a ratio of 49.8:51.2. Specify what measurement devices were used (e.g., a 500-mL graduated cylinder).

Develop a mobile phase that minimizes differences in slight column variations. If you will be analyzing ionic compounds, it is imperative that a buffer be used in the mobile phase to adjust the pH to a constant value. I have seen methods with instruction to add five drops of an acid to move the pH of the aqueous portion of the mobile phase to a certain value. While this method may have worked on several batches of columns, it is not appropriate for long-term success, as it does not control the pH. Not controlling the pH leaves the residual silanol form (ionic protonated) as a variable. Additionally, the exact amount of silanol content cannot be measured, and small variations may make a difference in your method when using an unbuffered mobile phase. Make sure pH is adjusted on the aqueous portion of the mobile phase before the solvents are combined. Remember, pH is defined as the hydrogen ion activity in water.

Once the method is developed, use several columns from different batches to test the method. Most manufactures will sell you columns from different batches, and some have what is called a "method development kit" with each column from a different batch. Also, observe whether there is an equilibration time, and specify this in your method. Long equilibration times often are required when using certain additives or ion-pair reagents or both. Long equilibration times are also often required when using ion-exchange packings.

Consider the system contributions. If using a system with a programmed solvent delivery system (either gradient or isocratic) have a test to determine whether the programmed pumping system is delivering accurately and produces the same retention for your standards. Results could be quite different from one system to another depending upon the ratio of A solvent and B solvent, proportioning valves (if there are any), and mixing volume. The automatic system will also probably be very different from a manual mixing of the two solvent components of the mobile phase.

I have attempted to hit the main issues, but I think you can see that the reproducibility is indeed the responsibility of both the user and the manufacturer. If you develop robust methods, it will be easier to work with the manufacturer when you suspect a column issue.

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 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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