Liquid Chromatography Problem Solving and Troubleshooting

Question:

I purchased a column with greater than 14,000 plates; however, when I ran my analysis, I only measured 8,000 plates. What's wrong?

Answer:

You have identified a common truth in HPLC; namely, a column does not have a single plate count. Stating that columns do not have a fixed plate count may sound confusing at first, but this is why operators need to interact with and understand their HPLC methods and not simply "plug and chug" analyses. Before you conclude the situation is hopeless and that it is not important to measure plate count, let me explain exactly what I mean. A column has an innate plate count which is often the best case performance, and the column has a method plate count which is its performance in the analysis. The innate plate count is determined by the mechanics and fluid dynamics of the packed bed structure, hardware design, packing material properties, etc. The method plate count is determined by the method and HPLC instrument used for the separation and is affected significantly by the nature and quantity of the sample and the physicochemical characteristics of the real-world analysis. In other words, the innate plate count is determined under ideal circumstances and the method plate count is determined under actual circumstances.

The innate plate count is measured by the manufacturer and uses a "neutral" molecule that is retained only by its interaction with the bonded phase with no secondary chemical interactions. It is determined under very lightly loaded conditions and in the linear region of the isotherm. The key point is that the testing of a column should be performed under conditions that verify that the column is packed as inherently efficient as possible. This is the only way to determine whether or not the column is packed as well as it can be. The innate plate count measures the quality of the packed column. Variation from the innate efficiency is caused by the following factors: mobile phase factors, such as viscosity and linear velocity; solute factors, such as chemical nature of the molecule, sample load, solvent used for injection, and rate of diffusion in each phase; mechanical factors, such as the dead volume of the HPLC and homogeneity of the packed bed; and numerical factors, such as the method of calculating the plate count (1).

As an example, consider a typical reversed-phase column. Its innate plate count should be measured on a low-dispersion HPLC using a low concentration of a small-molecule hydrocarbon sample such as toluene with a mobile phase containing a high amount of organic such as acetonitrile, which has a low viscosity. In this way, the innate plate count will reflect how well the column has been packed. However, in practice, most users will often be using polar molecules which can chemically interact with both the bonded phase and the underlying silanols on the surface of the silica. Separations of polar molecules may require higher water content in the mobile phase which means higher viscosity. Also, analyses are often run at a higher flow rate than has been used for the testing. All of these differences contribute to a lowering of the plate count in the method. The conclusion is that you, as an operator, need to measure the innate plate count as the manufacturer does to ensure that you are using a well-packed column. Each time you receive a new column, you also need to measure the plate count in your method to ensure that the new column has the same chemistry for your separation. Using both the innate and method plate count will also be helpful in troubleshooting problems with the HPLC.

References

1. B.A. Bidlingmeyer. Column efficiency measurement. *Anal. Chem.* **56:** 1583A (1984).

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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