Liquid Chromatography Problem Solving and Troubleshooting

Question:

In my assay, the peak area that results from multiple automatic injections of the same sample is not as reproducible as I would like it to be. I obtain only ± 7% relative standard deviation. Do I have a problem with my system, and what should I do to improve this situation?

Answer:

The relative reproducibility of peak areas for isocratic HPLC analyses should be at least 1% or better for a peak that is well-resolved, so it appears that you have an opportunity to improve your situation. But before you begin to troubleshoot the problem, examine the data to determine if there is a trend in the peak area change. Does the area begin small and then increase to a constant value, does the area begin large and have a general decrease to a steady value, or is the variation in peak areas truly random in nature? Knowing if there is a trend will be useful as you search for the cause of the problem.

Because there can be multiple sources of the problem, first check the easiest to isolate and evaluate. Then look at the next easiest. I would first determine if there is a problem with the flow rate. Flow rate variations can cause fluctuation in peak area. Carefully inspect your system and visually determine if there are any leaks. Next verify the flow precision by using an appropriately sized graduated cylinder and collecting the flow for a fixed period of time. Do this several times and determine the average flow and the normal variation for three time periods. Also, as I have stated many times in this column, monitor the real time pressure trace to observe if there is any variation in flow during an analysis. Does the pressure remain constant during the injector loading cycle and during the run? If not, the pump is probably malfunctioning (maybe a leaky check valve or a leaky pump seal).

Second, determine if there is a problem with the integration. Errors in integration are common and can result from improper settings of the integration parameters, especially when peaks are tailed or slightly tailed, which is often the case in HPLC. Look at where the tick marks are occuring on your peak. Are they always at the same point? Does the baseline that is drawn appear to be flat, and does this adequately represent the extention of the baseline of the chromatogram? Consult the user's manual or call the manufacturer's "help line" to determine if you are taking data at the proper data rate. Find out how to change the settings on the integrator in order to "reprocess" the raw data and determine what effect these new parameters have on the area. Also, manually measure the peak heights to see if the variation in the peak area is reflected in the manually measured peak height. In other words, do everything possible to prove to yourself that the integrating device is working properly. If you suspect that the peaks are not being processed correctly, call the manufacturer and talk it over.

Third, focus attention on the autoinjector. Bypass the autoinjector and install a manual injector. Determine if you can obtain constant peak areas with a manual device. This variation in peak area will be your target value when your system is working properly again. A typical problem with autoinjectors is a partially plugged needle. This "partial plug" is often due to bits of the seal or septum covering the vial shedding and entering the needle. Back flush or otherwise clean the injector needle. Next check the draw rate of the syringe that pulls the sample solution into the injector needle. Slow the draw rate to its lowest setting to determine the effect on area reproducibility. If the syringe pulls too fast for the solution to easily enter the needle, air can enter the needle and affect the area. This is particularly true for viscous samples, but it can be a problem in general with some systems. Another cause of air in the needle during the uptake of samples is a vial cover or septum that seals very tightly around the needle, causing a vacuum to be formed in the vial as the sample is withdrawn into the needle. This situation can result in small air bubbles entering the injector.

If you eliminate air in the needle as a source of the problem, consider that there may be carry-over sample from the previous run. This often has the manifestation of an initial high peak area that decreases to a constant value. Determine if your needle wash is working properly and make sure that you are using a strong solvent (for the sample) as the wash solvent. The mobile phase is not always the best solvent to use in the needle wash.

The suggestions I have offered are focused on troubleshooting the items that have the highest probability as potential culprits. Reproducibility of the peak area can be influenced in a minor way by a number of other sources. The above approach should be useful in isolating the source of the problem in 95% or more of the cases.

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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