Liquid Chromatography Problem Solving and Troubleshooting

Question

Recently, I observed split peaks in a chromatogram (shown in Figure 1) and, being a reader of your column, followed your advice and reversed the flow into the column. I observed the elimination of the split peaks (Figure 2). I tried running the column with strong solvent to clean the frit, but it didn't worked. Now what should I do?

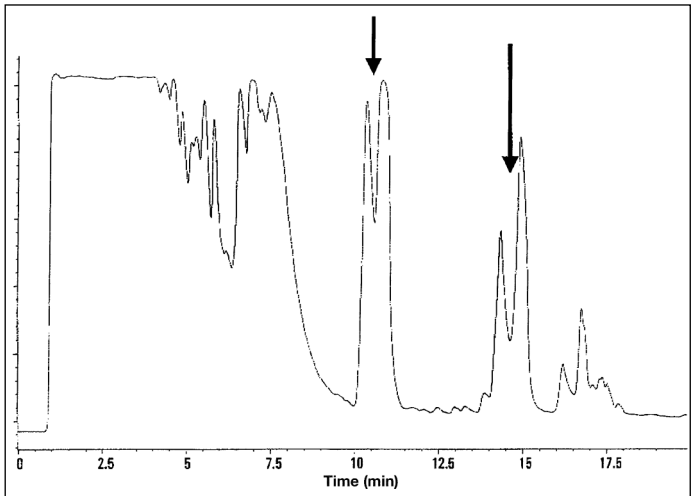

Figure 1. Chromatogram exhibiting peak splitting (indicated by arrows).

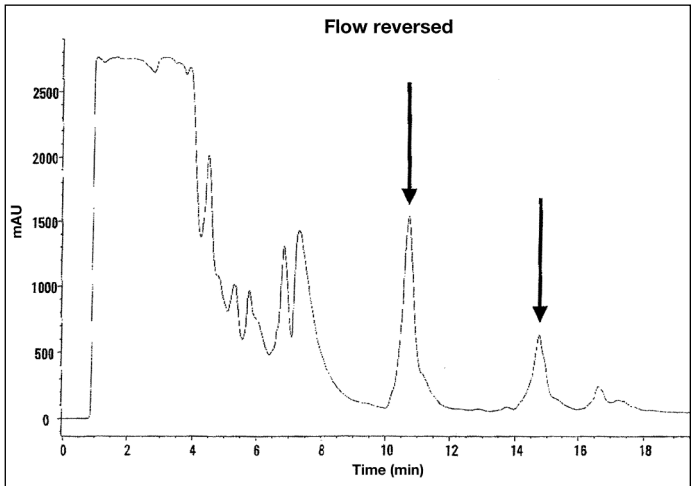

Figure 2. Chromatogram resulting from flowing in the reverse direction (arrows indicate the same two peaks that were previously marked by arrows in Figure 1).

Answer

As has been occasionally mentioned in these troubleshooting articles, split peaks in a chromatogram may be caused by a variety of things. The most common are (*a*) a large injection that overloads the column, (*b*) a mismatch between the strength of the mobile phase and the injection solvent, (*c*) a void or channel in the column, and (*d*) a plugged frit.

Because you are doing large injections on this column, any of the above could be the culprit. By reversing the flow through the column (as shown in Figure 2) and observing the loss of peak splitting, you have eliminated the first two possibilities from being the cause. Therefore, you have taken the correct first step in troubleshooting the problem. By inspecting the chromatogram in Figure 2, it can be observed that the peak shapes are not very symmetrical, especially for the two peaks of interest (indicated by arrows). This may be caused by the material in the sample, but since you mentioned in your notes that the peak was expected to be clean from previous analytical chromatograms, I believe that there is probably material imbedded in the inlet frit that is blocking the smooth introduction of sample evenly onto the column. Also, in the reverse flow direction this debris interferes (although less so) with the elution profile from the column. Because you have flushed the column to clean off debris from the frit and this has not worked successfully, I would suggest that you carefully remove and change the frit (1).

Once you have changed the frit, rerun the chromatogram. If the peak shapes stay the same either you have irreversibly disturbed the column bed when you replaced the frit or the column had a void or channel that was more harmful to destroying the peak shape when flowing in one direction than when flowing in the reverse direction. If the frit replacement does not solve the performance of the column, I would obtain a new column.

References

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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