

## Liquid Chromatography Problem Solving and Troubleshooting

### Question:

Recently, I have experienced high backpressure for several new or almost new HPLC columns. I assume that the high pressure is due to a plugged frit. Isn't this unusual and what can I do about it? I regularly filter my samples.

### Answer:

Yes, from what you described, your early column plugging is unusual and it could be an "out of specification" column. Your best indication of this is good documentation of the history of the column. When you receive a new HPLC column, it should be installed and the pressure should be monitored by using the mobile phase flowing at the flow rate that you will use for your analysis. Then the test sample should be run and the plate count should be measured. If either of these parameters appear to be out of specification, the manufacturer should be called and the column returned for replacement. These readings, when "in spec," become the reference to which the parameters are compared during use. Doing the tests once each day is usually sufficient.

Assuming that your column tested "good" initially and that the high backpressure developed early in use, it is probably due to a plugged frit. Often, a plugged frit has other indications including peak doublets or peak splitting that occurs where there used to be only single peaks or noticeable peak tailing or fronting. These changes in peak shape are probably due to debris blocking one side of the frit and allowing preferential flow to distort peak shape during entry onto the column. The most common reasons frits plug include (a) debris from the HPLC system; (b) particulates from the sample; and (c) insoluble sample. On the basis of your question, sample particulates are probably not the source of the problem, but check that you are using a 0.45- $\mu\text{m}$  pore size filter. Sometimes larger filters are inadvertently used.

If it is not the filter, the next step is to consider the HPLC system. The HPLC system can become a "particle generator" when the system is wearing quickly. Pump seals and injector seals experience wear during operation, and the debris from these seals can clog the inlet frit of a column. Usually inlet frits can be replaced, but there is a slight risk that you will disturb the bed structure when doing this. However, since the column is plugged, you have nothing to lose. To aid in the elimination of plugged frits in columns, use an inline filter or an inline guard column. Then, when the pressure increases, simply change the frit in the filter or change the guard column rather than changing or repairing the column. If the plugging is due to sample insolubility, elevating the temperature should aid in enhancement of the solubility.

An alternative to frit replacement is to remove the column and to reverse it such that flow now enters what was the exit. In this way, the column can be backflushed with mobile phase (20–30 column volumes) into a waste container. (Do not allow the mobile phase to flow through the detector when doing this operation or the debris being washed off of the inlet frit will enter the detector cell and possibly clog the cell.) Column backflushing is noninvasive and it is the only way to rejuvenate a column that has a pressed-in inlet frit. Pressed-in frits are common in cartridge columns. If you are uncertain of whether the frit is pressed-in or changeable, ask the manufacturer.

If the inlet frit does not appear to be the problem, the outlet frit may be clogged due to column packing entering and plugging the pores of the frit. This has been reported for some small particle columns (less than 3  $\mu\text{m}$ ).

Finally, remember that there is no "standard frit," and different manufacturers use different types of frits. Some frits can be "prone to plug" if the area of porosity is small compared with the available surface of the frit or if the pores in the frit are larger than the packing size. Because there are differences, you must become a more enlightened consumer if you suspect early frit clogging. If you have problems with a particular manufacturer, ask questions about how the frit is made, its pore size, et cetera. Some "frits" are, in fact, screens, and screens will behave differently than porous, traditional frit material.

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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