

Liquid Chromatography Problem Solving and Troubleshooting

Question:

My column has an arrow that represents the direction of flow. Are columns unidirectional or can they be used in either direction? Will retention of compounds change when the column is run in reverse?

Answer:

Even though many columns are marked with a "direction of flow" arrow, it is my opinion that a well-packed column of spherical HPLC particles can be used in either flow direction as long as the same porosity frits are used on both ends. For some time now, column-switching has been used in many applications with no reported shortening of column life. In column-switching techniques, special valve arrangements are used to reverse the flow intentionally (backflushing the column) to remove strongly held sample components that would otherwise move through the column too slowly. This technique is used to optimize analysis time.

That having been said, why do some columns come with directional arrows for the recommended flow direction? Some of our "rules of thumb" guidelines were established in the formative years of HPLC when irregular packings were used. In those years, there was concern that irregular packings formed a semi-stable structure when loaded into a column. In other words, the particles did not pack closely but were hypothesized to form "bridges" with other irregularly shaped particles; these bridges could be disrupted when the direction of flow was opposite to that which formed the bridge, causing the column bed structure to collapse. This phenomenon is commonly referred to as "voiding." This flow instability has never been proven, but an ounce of prevention is worth a pound of cure, so the rule of thumb was adopted. In addition to this early influence on directionality, another reason the arrow remains is most probably because modern microparticulate columns are packed in one direction using slurries of packing under very high pressures, and when the loading of the column with packing is completed, conventional wisdom says that this direction of flow will not be harmful to the bed structure. Because it is not clearly known what specific item contributes to the instability of a bed structure, column manufacturers generally practice the safe route of recommendations with regard to usage practices. It is known that flowing in the same direction in which the column is loaded does not contribute to short column life. Therefore, if the direction of flow is restricted to one way, no additional variable is introduced that might contribute to a collapse of the bed structure. It is easier to make this guideline than to conduct a statistically significant study to determine whether the direction of flow is a contributor to bed stability.

Most troubleshooting guides recommend reverse-flowing a column that has developed high backpressure. The reasoning is that because the column is dead or almost dead, no extra risk is induced by reversing the flow, and in fact, this technique often causes any particulate plugging to be washed into a beaker and backpressure to return to normal. Therefore, because columns can be regenerated by flowing in the reverse direction, the stability of the bed structure does not appear to suffer. Remember that flowing a column in the reverse direction can be done as long as the porosity of the end fittings are the same on both ends. However, if a 0.5- μm frit is used on the outlet and a 2- μm frit is used on the inlet, reversing the flow could result in small particles of fines being flushed out of the column.

Sometimes larger porosity frits are used on the inlet of columns to enable a larger amount of particulate matter to build up before the column exhibits high backpressure. The particulates are often introduced from the sample injection and/or from pump seal wear. In this case, reversing a column might not be an appropriate troubleshooting tactic (when in doubt, call the manufacturer). If your column falls into this category in which two different pore-sized frits are used, an alternative would be to replace the end of the frit with an outlet end frit. If column plugging is a common problem in your laboratory and replacing inlet frits is unappealing, use an inexpensive in-line filter or guard column that can be replaced when it periodically becomes fouled.

To answer your second question, for a column in good working condition, the separation resolution will not change from running the column in one direction compared with the other. The resolution is governed by the solute-stationary phase-mobile phase interactions, and this selectivity is not influenced by mechanical factors.

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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