Liquid Chromatography Problem Solving and Troubleshooting

Question

I am performing separations of small molecules present in a matrix that is soluble in the injection solvent. I suspect that the loss of column performance I observe is because of the contamination of my reverse-phase alkyl column with material in my original sample that is not eluting from my column. How do I clean off this contamination?

Answer

Often when contamination exists, system backpressure will increase, and when it is removed, the backpressure should drop. However, with chemical contamination as you describe, the backpressure may not have increased significantly. Nevertheless, it is a good idea to note the backpressure before you begin the cleaning process. As you perform various cleaning steps, also enter the backpressure in your log book. Therefore, once you have successfully returned the column to its original performance, you will have the system backpressure values to benchmark against in the future should the situation arise again.

Your problem may be quickly and easily solved by simply flushing the column with a large injection of the strong solvent after a finite number of injections. For example, after every 10 injections, inject 100 to 500 µL of the strong mobile phase solvent. Also, perhaps the approach might be for you to perform a full day of analyses and note the retention time, peak shape, and efficiency changes until the performance degrades 10 to 15% of the original values. Then, wash the column with 100% strong solvent for 10 column volumes, equilibrate with the mobile phase, and continue the analyses.

If your contamination is not removed with this simple approach, a more structured approach may be needed. For bad chemical contamination, you can regenerate or rejuvenate a reverse-phase column by first disconnecting and reversing the column. Connect the column outlet to the line from the solvent delivery system, but do not connect the former inlet to the detector. Allow the outlet line to go into a suitable reservoir. Again, note the backpressure and then flush this column with strong solvents that will dissolve the suspect chemical contamination. If the suspect contaminate is believed to be a water-soluble (even slightly soluble) compound, I have found that flushing with the following solvents can usually remove the contamination and bring the column back to its original performance: 10 column volumes of methanol, followed by 10 column volumes of acetonitrile, and then followed by 10 column volumes of tetrahydrofuran. This flushing should take place at the normally used flow rate (i.e., 1 mL/min for a 4.6-mm-i.d. column). After flushing with this wash protocol, reverse the column and again pump 10 column volumes of each solvent mentioned, only in the reverse order returning to the original mobile phase.

If this does not return the performance of the column to its original behavior, repeat the previous washing steps to tetrahydrofuran and add the additional steps of washing with 10 column volumes each of methylene chloride and hexane. Then as before, reverse the wash procedure back to the mobile phase and evaluate the column performance. Efficiency, peak symmetry, and retention should be within 5% of the original value. If the performance has not returned to the original, I am afraid that you may have to live with it or replace the column.

If you believe the column contaminates are because of compounds that are not soluble in water, then the second flushing procedure mentioned previously should be followed. This involves using progressively stronger solvents—methanol to acetonitrile, followed by tetrahydrofuran to methylene chloride, and finally hexane. Return to the original mobile phase by reversing the solvents used in the wash procedure. In all cases, flush for 10 column volumes.

In the future, if chemical contamination is anticipated to be a possibility, consider using a guard column and discard the guard column after the performance has declined 10 to 15%. Replacing with a new guard column (although somewhat costly) may be more economical, because it saves considerable time versus the time it takes to clean the column. Another approach is to perform an off-line solid-phase extraction (SPE); however in this case, the time of the SPE step and the cost of the cartridges may be similar to using a guard column.

Once you know how to regenerate the column, you can implement the best strategy for improving the analysis in the future. All of the trade-offs mentioned for the various approaches (washing, guard columns, and SPE) can be quantitated in order to determine the best approach for your specific situation.

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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