Liquid Chromatography Problem Solving and Troubleshooting

Question

My separation is initially fine using a methanol–water mobile phase at pH 7; but over a week or so, the performance rapidly decreases and is not usable. I have called the manufacturer, and they say it's not the column. However, two column failures make me wonder. This is a recently developed method; therefore, I am able to redevelop it if there is hope of increasing the usable life. What do you recommend that I do?

Answer

You may be observing the situation that I commented on indirectly in a previous article (1). In Figure 2 of that article, it was shown that at pH 7, two reversed-phase columns degraded over a 120-day period when stored in a phosphate buffer. One column degraded quickly (approximately 50% of an increase in the retention time of a basic molecule) while the other one changed only slightly (5%). It should be pointed out that a change of 5% in the retention time over a 120-day time period is not a significant change. This figure illustrated the changing retention behavior that reflected the phenomenon that silica dissolves at pH values equal to or greater than 7. This behavior is dependent upon the type of column used. In other words, not all reversed-phase columns are created equally and perform differently when placed in certain mobile phases.

If you are concerned about maximizing lifetime at an intermediate pH, there are several recommendations that can be made to enhance the lifetime. First, make sure you choose an appropriate column. Ask manufacturers which column is recommended at the pH value in which you wish to operate. My experience suggests that you should choose a column made from a silica sol aggregation process (sometimes called a sol-gel process). These columns have thicker column walls than those made by a xerogel process (sometimes called a sil-gel process). In addition to choosing a particle made from a silica sol aggregation process, make sure that the bonded phase is densely bonded so that the coverage is quite complete. Endcapping can accentuate the effectiveness of densely bonded phases, but not all endcapped columns are densely bonded. The key is to use the most densely bonded phase(s) possible. In essence, the densely bonded phase protects the underlying silica from erosion.

For a rugged method using a mobile phase at an intermediate pH value, there are other parameters to control in order to minimize the influence of the chemical environment. First, use citrate instead of phosphate, because citrate has been shown to dissolve the underlying silica significantly less than phosphate (2). Citrate is an underutilized buffer that can be used to adjust pH continually between pH 3 and 6.4. This is contrasted with phosphate, which does not have a continuous buffer capability. As mentioned, with citrate the upper buffer limit of pH is 6.4. However, this usually is not an issue, because most compounds soluble at pH 7 are equally soluble at a pH of 6.4. Another tip is to maintain the buffer concentration at 0.01 to 0.05M. The degree of dissolution of silica is less at lower buffer concentrations (2).

Temperature also influences the solubility of a silica. Keeping the column temperature at less than 40°C will minimize the solubility of the silica. In fact, the column temperature should be constant and as low as possible to maintain a constant retention time and quality of separation. Often, this will be approximately 30°C. Lastly, use acetonitrile as the organic modifier because it has been reported to be less aggressive than methanol in dissolving the underlying silica.

In summary, to achieve maximum life, choose the most appropriate column—one that is densely bonded. Use the column in the most "friendly" mobile phase as possible, and it should be one that eliminates phosphate as the buffer. Generally speaking, using a friendly mobile phase should enable an adequate separation to be achieved. Of course, if you cannot achieve the separation in the friendly mobile phase, then you must make tradeoffs and choices. If you cannot use the ideal less-aggressive mobile phase, you should manipulate the variables in order to obtain the separation and live with the results.

References

1. B.A. Bidlingmeyer. Liquid chromatography problem solving and troubleshooting. *J. Chromatogr. Sci.* **38(6):** 264 (2000).
2. H.A. Claessens, M.A. Straten, and J.J. Kirkland. Effect of buffers on silica-based column stability in reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* **728(1):** 259–70 (1996).

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