Liquid Chromatography Problem Solving and Troubleshooting

Question:

Within our company, we have methods that use only organic solvent (methanol or acetonitrile) and water as mobile phases, and the methods do not specify any pH or buffer. We recently had to use an analysis that we had not used in quite a while and were forced to use a new reversed-phase column. We observed bad chromatography, but when we added some acetic acid to the mobile phase, the chromatography improved, and the method was made useful. Why did a previously validated method suddenly not work, and why did our actions improve the result?

Answer:

This is a very good question, and if more people thought about your circumstance when beginning a methods-development activity, there would be fewer problems in the long run. To answer your question, unless a molecule is totally nonpolar, it is always a good idea to use a buffer salt in the mobile phase. There are two main reasons for using this approach. First, water from the "pure water source" can vary in its pH. If the pH varies from one analysis to another, this could be the source of your observation.

Second, you mentioned that you also used a new column. Reversed-phase separations of polar molecules will be impacted by both the hydrocarbon coating on the silica and the underlying silanols. The manufacturers control the production of silica and their bonding reactions, small variations in the silica surface and coating may occur. Therefore, developing a method without any ionic content in the mobile phase will enable these tiny changes in the packing to have a tremendous effect on the chromatography. However, if the mobile phase contains some ionic character, this will often shield the small variations in the silica surface and result in a more robust method.

What should you use as the ionic component? I prefer to use a buffer because it controls both pH and ionic strength. Use a buffer at the highest ionic content that is required to minimize these ionic interactions; usually this results in a concentration in the range of 10–50mM total buffer molarity. As a first choice, try 25mM and adjust from there. I also like using citrate as the buffer salt because I can adjust its pH over a wide range from approximately 3 to 6.4. Using something such as acetic acid may be acceptable, but remember that acetic acid is not a buffer. If you like acetate, use a buffer salt rather than the acid.

What pH should you use? If ruggedness is your concern, you should use a pH of 3 or lower if your column is stable at the pH you choose. At pH 3, surface silanols are protonated. As the pH is raised, the silanols will begin to ionize, and there will be a partial negative charge imparted to the packing, which may interact with your polar compounds and result in different retention. This may give you unique selectivity, but that is another story. If your concern is ruggedness, try to obtain selectivity at pH 3, or, if your column is stable, use a lower pH.

When developing a method, test the ruggedness for both pH and ionic strength by varying both according to the variation you expect might happen in your laboratory. Often this would be ± 10% variation for which you would test. By knowing and documenting the ruggedness of your method, you would have a better method than the one you described, and everyone in your organization would be better equipped to handle any problems that might arise.

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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