Liquid Chromatography Problem Solving and Troubleshooting

Question:

I would like to store my HPLC column for a long-term period; what is the correct procedure? What is the best solvent in which to store a reversed-phase column for a short-term and for a long-term period?

Answer:

Because there are many mobile phases used in reversed-phase HPLC, there are multiple answers to your question. The quick answer is that you can use the mobile phase that you are using for short-term, overnight storage. The advantage is that the column equilibration will be rapid when turning on the system the next morning. If the fastest possible equilibration time is required, consider flowing overnight at a very low flow rate (e.g., 0.1 mL/min). In this way, you can simply adjust the flow rate the next morning, wait for one or two column volumes, and begin running analyses.

You know from reading "Liquid Chromatography Problem Solving and Troubleshooting" that columns can change slowly in storage under acidic or neutral conditions (1). Therefore, for intermediate (one or two weeks) and long-term storage (three weeks or more), there are a few things to consider when choosing an appropriate solvent. First, what is the claim of the manufacturer for storage and column lifetime? Because the column is warranted, it is best to follow the recommendation of the manufacturer for the proper storage solvent or to use the solvent in which the column was shipped by the manufacturer. For many reversed-phase columns made with dimethylalkyl silane, acidic solutions (pH < 3) can hydrolyze the bonded phase; do not store the column below pH 3 unless you have a column which is specifically manufactured to have long life at low pH. For many bonded phases, storage at neutral pH (pH 7) or above can dissolve the underlying silica surface, slowly etching the surface and exposing more silanol groups. Therefore, unless you have purchased a column which is documented to have long life at the acid or neutral pH ranges, it is prudent to choose an aqueous portion of the mobile phase having a pH between 3 and 6 for storage of one or two weeks. If you use a buffer to adjust the pH of the aqueous portion, use a small concentration (1–5mM) for use in storage and make sure it is compatible with the organic portion of the mobile phase. For storage of longer than three weeks, it is appropriate that all columns be stored in a solvent which prevents hydrolysis or dissolution of the silica (e.g., acetonitrile).

There are claims that storage of short chain alkyl phases (such as a propylcyano phase) in acetonitrile make the column unstable, such that after storage, the column bed collapses. However, this has not been the experience of this author, and I believe the so-claimed cyano column's instability may be specific to a particular manufacturer and is not a general behavior for all cyano columns. The amino column, on the other hand, can be "reactive" in aqueous solutions and as a general preventative maintenance tactic, this column should be stored for long-term periods in acetonitrile.

When you change from a mobile phase to a storage solvent, it is important to tranistion the solvents in polarity stages so that you do not precipitate any buffers. For instance, if the mobile phase is methanol–water at pH 2 with a 15mM phosphate buffer, the first step is to switch to methanol–water to wash out the buffer, then switch to methanol, and then switch to the final solvent (e.g., acetonitrile).

No matter which storage solvent or solvents you use, it is important to record the date, storage solvent, column type, and the column serial number in your notebook and on a tag placed on the column, so that when you retrieve it from storage, you know what solvent is in the column. In this way, you can develop a history of column lifetime in a specific mobile phase and storage solvent. From this history, specific guidelines can develop for your organization's HPLC operation.

References

1. B.A. Bidlingmeyer. Liquid chromatography problem solving and troubleshooting. *J. Chromatogr. Sci.* **35:** 405–406 (1997).

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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