Liquid Chromatography Problem Solving and Troubleshooting

Question:

My reversed-phase column changes irreversibly during use. Is this a normal occurrence? Should I always expect my column to change during use?

Answer:

Yes, columns change during use; this is why column-to-column reproducibility is of concern to most chromatographers. The instant you place the column in the instrument and start mobile phase flowing, the column begins the "aging process". Sometimes this aging is slow, and other times it is fast. The degree to which the column changes during use depends on the type of column and the mobile phase that is used. The best way to ensure long life is to follow the manufacturer's operating instructions during use, but there are times when columns must be used in mobile phases that will cause "wear and tear" on the bonded phase and/or silica. In these situations, you will have to accept the change in column performance and should search for a column that will minimize the change.

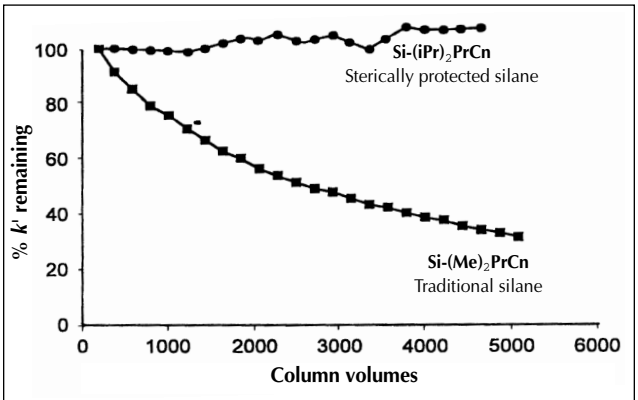

Figure 1. Stability of alkylcyano bonded phases at low pH; relative retention of toluene as a function of mobile phase (column) volume pumped across the column. Two different silanes were used to make the bonded phase: an isopropyl–alkylsilane (•) and a dimethyl–alkylsilane (▫). The conditions were pH 2.0 at 50°C.

For instance, many reversed-phase column manufacturers recommend that columns be used at pH values from 3 to 8. The recommended pH range is intended to protect the column from encountering mobile phases that can degrade the performance. At acidic pH, the silane linkage of the bonded phase can be hydrolyzed. This is particularly troublesome for short-chain alkyl bonded phases such as C_4 groups and below. This behavior will vary from manufacturer to manufacturer and will depend on how the alkyl silane is attached to the silica. If the silane is attached using a dimethyl alkyl group, the bond is more prone to hydrolysis than if the silane has protecting side groups. The demonstrated stability in Figure 1 is for a cyano column that is often reported to be unstable in comparison with a C_8 or a C_{18}. As seen in Figure 1, the problem is in the lability of the alkyl linkage to the silica and not the cyano phase itself. The change in capacity factor *k'* represents a loss of stationary phase; one of the bonded phase hydrolyzes, and the other phase is stable.

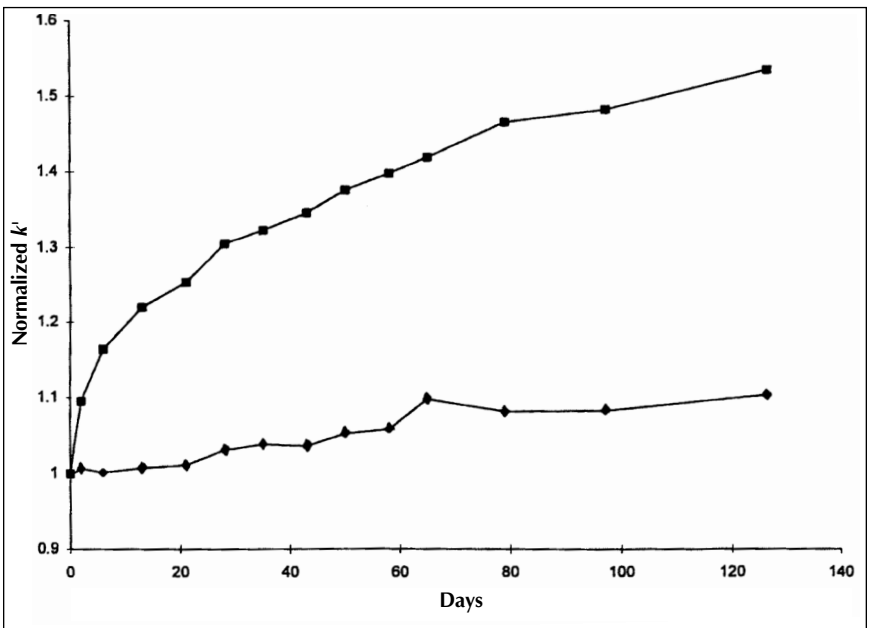

Figure 2. Comparison of relative retention of amitriptyline on two different columns: a xerogel silica bonded with a C_8 and singly endcapped (▫) and a solgel silica bonded with C_8 and doubly endcapped (▫). Conditions: methanol–water (65:35) containing 7mM phosphate buffer at pH 7 at 23°C.

An extrapolation of the findings that short chain groups are labile at low pH is that endcapping groups will be cleaved from the silica at pH values less than 3. This is a commonly believed phenomenon, and although a completely bonded phase of C_3 will lose phase at low pH, a study of the loss of only the endcapping group in the presence of a longer alkyl chain group has not been done. The stability of phases at low pH is particularly important to those researchers using trifluoroacetic acid (TFA) in their mobile phases. It may be worth investigating several columns to see if the stability might be improved using a different column bonding type.

In the basic pH range, the column can

change during use not due to silane hydrolysis but due to the dissolution of the silica. Even at pH values of 7, the column can change. The change is generally believed to be the result of the creation of additional hydroxyl groups as the silica is dissolved and exposes more silanols. Compounds sensitive to the silanol population will exhibit retention time changes and often peak shape changes. An example of this is shown in Figure 2. In this example, two C_8 columns are compared. One has a retention time change of 55% over a 125-day exposure to a pH 7 phosphate buffer mobile phase, whereas the other column changes only 10%. In this example, there were two main differences between the two columns. One difference was the type of silica. The more quickly degraded material was a xerogel type of material, and the other was a solgel type of silica. The second difference was that the more quickly changing column was singly endcapped, whereas the other column was doubly endcapped. Clearly, both columns "aged" during use as exemplified by the change in the relative *k'* of the analyte. To use an analogy of steel rusting, steel is painted to keep the "corrosive agent" away from the steel. In HPLC, the endcupping may be thought of as a coating (like the paint), and a double endcapping my be a more effective coating to protect the silica from the "corrosive" mobile phase.

If there were agreement on how to make a stable, long-lasting column, there would be only one type of column manufactured. But there are perhaps well over 50 manufactured reversed-phase column types available, so there is no agreement on the "best way" to make a column. The user must become involved in trying different columns and observing their performance during use. In this way, the most appropriate column for the application will be chosen, and the column will give the most satisfying performance during its operational lifetime.

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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Associate Editor