Column Lifetime and Cleaning Recommendations for Reversed-Phase Columns

Column Lifetime

Column lifetime is usually assessed by the length of usage time or the number of injections of samples and standards that a column can perform, while still providing the desired qualitative and/or quantitative results consistent with the analysis conditions or analytical method for which it was applied or validated. Most chromatographers are satisfied when they can obtain good performance (symmetrical peak shapes, and desired efficiency and resolution) for 500–1,000 injections using an HPLC or UHPLC column.

However, depending on whether the column is used under the manufacturer's recommended conditions (mobile phase pH, column temperature, pressure, etc.) or sample cleanliness, one might obtain 100–500 injections or possibly more than 1,000 injections using a given column. Another factor that can significantly affect column lifetime is whether the column is flushed properly after a set of analyses, and whether the column is stored either overnight or for extended time periods in the recommended or appropriate solvents or solvent mixtures. For some stationary phases that are designed to be used under mid- or high-pH conditions, the manufacturer may state that a column will perform under those conditions for a minimum finite number of column volumes (for example, 20,000, 30,000 or 40,000 column volumes).

Reversed-phase columns can become fouled from samples due to insoluble components, strongly retained components, as well as particulates from sample solutions, mobile phases, and instrument wear and tear. Analyses of formulated pharmaceuticals or agrichemicals (tablets, granules, powders, creams, ointments, etc.) have been known to affect column performance adversely due to a buildup of strongly retained excipients, which directly affects column lifetime. Certainly, a precolumn filter and/or an appropriate, phase-matched guard column can help to extend column lifetime.

Column Cleaning

What is most important, when you believe that column cleaning is necessary, is to know what the likely contaminants are and in which solvents they are most soluble. For RPLC columns, it's wise to flush the column with a mixture of organic modifier and water first, 10:90 (v/v) to 50:50 (v/v) organic modifier/water for 15–20 column volumes, unless you believe that a formulation ingredient such as sucrose, lactose, etc., may have fouled the column. In the latter case, it may be appropriate to flush the column first with heated HPLC-grade water (50–60 °C using a hot plate) for 15–20 column volumes to solubilize and elute any sugar or similar water-soluble component(s).

Subsequently, a column flush should be carried out with 20–30 column volumes of 100% (v/v) of the organic modifier from the mobile phase. If desired, one can flush with another organic modifier such as methanol or acetonitrile. For the most difficult-to-remove contaminants, the column may be flushed for 20–30 column volumes with 2-propanol (isopropanol), at half the typical flow rate for the column ID, because of IPA's much higher viscosity.

If the 100% organic modifier and/or isopropanol flush does not restore the column to normal performance, it is not likely that additional stronger solvents flushes using (in order) dichloromethane or even hexane will be useful and, therefore, we do NOT recommend them for column cleaning purposes.

Special Considerations for Protein Buildup from Serum or Plasma Sample Analyses

If column backpressure has risen or the column appears to be contaminated due to proteinaceous buildup on the head of the column, it is likely because protein precipitation or removal during sample preparation has not been adequate prior to the HPLC or UHPLC analysis.

Based on published articles, the most effective way to remove proteins from plasma samples is to use solid-phase extraction, usually with 96-well plates. The next most effective way is to use protein precipitation followed by high-speed centrifugation (13,000 rpm). Research has shown that 1:1 (v/v) mixtures of methanol and ethanol, or 1:1:1 (v/v/v) mixtures of methanol/acetonitrile/acetone with a 2.5:1 (v/v) to 4:1 (v/v) ratio of solvent to plasma, were best for protein precipitation, whereas methanol was found to be the best solvent for sample reconstitution following any evaporation step.

To clean a column that has become fouled due to protein buildup, it has been reported that large bolus injections of trifluoroethanol (TFE) are effective in solubilizing bound or adsorbed proteins. Several (≥ 3) large injections of TFE using 10- to 15-minute, programmed gradient runs from 5–10% isopropanol/water to 100% isopropanol with 40–50 °C column temperatures at 50% of the normal flow rate for the column ID (e.g., 2.1 mm ID, 0.2 mL/min; 3.0 mm ID, 0.3 mL/min; 4.6 mm ID, 0.5 mL/min) should be quite effective. Some examples of large injection volumes for various column diameters are: 2.1 mm ID, 20–30 µL; 3.0 mm ID, 30–50 µL; 4.6 mm ID, 50–100 µL.

References

1. LC-GC Magazine, 17(4), April 1999, 354ff. (Reference available on request to technical@mac-mod.com)
2. Investigation of Human Blood Plasma Sample Preparation for Performing Metabolomics Using Ultrahigh Performance Liquid Chromatography/Mass Spectrometry; Anal. Chem. **2009**, 81, 3285–3296.