Recommended Practices for Proper Column Flushing and Storage

Column lifetime can be thought of as the number of injections that can be carried out or the number of samples that can be analyzed using a given column. Usually, columns can no longer be used for a given method or in general when they no longer perform as they had when they were brand new. Columns often must be retired when they:

- No longer provide results that meet system suitability criteria established for the method (e.g., critical resolution, selectivity (alpha), tailing factor, theoretical plates)
- Produce significantly higher pressure than "normal" for a given set of conditions (e.g., more than 20-40 bar higher than typical values)

There are a variety of ways that column lifetime can be maintained and extended such as:

- Using an in-line filter for particulates from instrument wear and from samples.
- Using the column within the recommended temperature and pH ranges for the stationary phase.
- Using a guard column in front of the column to help minimize column contamination and fouling.
- Practicing regular column flushing, and storing columns properly when not in use.

Occasionally, with some isocratic methods and some sample types, it may be necessary to flush a column periodically while carrying out analyses, because of buildup on the column of strongly retained sample components, which are not eluted under isocratic conditions. Strongly retained components can begin to elute during the normal isocratic run, if the column is not flushed on a periodic basis with a stronger organic modifier concentration, after a given number of samples have been analyzed. This same problem can occur if the final gradient composition is not strong enough to elute all the sample components, and buffer solubility precludes the use of a higher organic modifier concentration as the final composition.

The recommended procedure for proper column flushing for reversed-phase columns, at the end of the day or end of the week, is described below.

First, note that any flushing steps should be carried out with the column oven temperature turned OFF or set to ambient temperature (25–30 °C) to minimize the time that the column is maintained at elevated temperatures, if those had been used for the current method.

If a buffered mobile phase (acetate, formate, phosphate, citrate salts, and their conjugate acids) or a mobile phase with a strong or weak acid or base (trifluoroacetic acid, acetic acid, formic acid, ammonium hydroxide*) has been used, the first step for column flushing before storage is to flush the column with 10–20 column volumes of a mixture of the organic modifier and water in a ratio between 10:90 (v/v) and 50:50 (v/v). This practice helps to remove any acidic or basic additive or any salts from the column.

Following the first flush with the organic modifier/water mixture, the column should then be flushed with an additional 20 column volumes of 100% organic modifier, and the column should be stored in that solvent when not in use. As a rule of thumb, one can estimate the column volume using the following equation:

$$\text{Column volume } (V_M) = \frac{\pi \times \text{ID (mm)}^2 \times \text{length (mm)} \times \text{porosity (0.65 for TPP, 0.5 for SPP)}}{4 \times 1000 \left(\frac{mm^3}{mL}\right)}$$

For example, for a 3 x 150 mm totally porous column, 1 column volume (V_M) ≅ 3.14 x 3^2 mm^2 x 150 mm x 0.65/(4 x 1000) ≅ 0.689 mL (689 mm^3 x 1 cm^3/1000 mm^3). Similarly, for a HALO superficially porous column in 4.6 x 150 mm geometry, V_M ≅ (3.14 x 4.6^2 x 150 x 0.5)/(4 x 1000) ≅ 1.25 mL.

A key point to remember for the next time the column is used is that it was stored in 100% organic modifier, and it should be equilibrated first with isocratic mobile phase or initial gradient mobile phase (minus any buffer), followed by equilibration with the regular starting mobile phase (including buffer). This startup equilibration should help to avoid any possibility of buffer precipitation, caused by too high a ratio of organic modifier to buffer.

Examples

Column Flushing Before Storage

1. Flush column with a mixture of organic modifier with water (10:90 to 50:50 v/v) for 15 column volumes.
2. Flush column with 100% organic modifier for 20 column volumes.
3. Store column with endcaps at room temperature. Make a note that column was stored in 100% ACN or MeOH as needed.

Column Equilibration After Storage Before Use

1. Flush column with isocratic mobile phase or starting gradient mobile phase composition (minus any buffer) for 10–15 column volumes (e.g., 10:90 organic modifier/water).
2. Repeat Step 1 using the respective mobile phase plus buffer (e.g., 10:90 organic modifier/10 mM phosphate).
3. Flush column with the ending gradient point with buffer (e.g., 80:20 organic modifier/buffer).
4. Repeat Step 2 to equilibrate column with isocratic or starting gradient mobile phase.
5. For isocratic separations, inject two or three replicate injections of a standard or sample to assess column performance and retention time repeatability.
6. For gradient separations, carry out two blank gradients with no injection or sample solvent injections, as needed; and perform two replicate standard or sample injections to assess column performance and repeatability.