Chromatography Solutions

LC Tips #001

Column Cleaning, Regeneration and Storage of Silica-Based Columns

GENERAL CONSIDERATIONS

The routine use of in-line filters and/or guard cartridges is strongly recommended for protection of HPLC columns from both frit blockage and irreversible sample adsorption. However, even with the use of guard cartridges, over a period of time columns may become contaminated by strongly adsorbed sample components. This may be indicated by a sudden or more gradual increase in back pressure generated by the column, loss of column efficiency, an increase in tailing and shouldering or even peak splitting.

The protection of UHPLC columns is also recommended and guard cartridges should be used where available. Guard cartridges capable of withstanding the high pressures required are not available for all manufacturers' UHPLC columns. In these cases, it is advisable to use a precolumn filter which can operate at high pressures.

To maximise column lifetime, particularly with UHPLC columns, the following tips should be considered:

- Use only ultra-pure UHPLC/HPLC grade solvents
- Use freshly prepared aqueous mobile phases to discourage bacterial growth
- Filter all samples, standards and mobile phases (e.g. 0.2 µm filter)
- Use an in-line filter system
- Perform sample clean-up on dirty samples

Please note that in cases of irreversible compound adsorption or column voiding, it may not be possible to regenerate the column.

COLUMN FLUSHING

If a deterioration in column performance or increase in back pressure is observed, then a column cleaning or regeneration procedure can be undertaken, using a series of stronger solvent combinations. It is strongly advised to read the 'Care and Use' instructions provided by the manufacturer before commencing this procedure. While the majority of spherical 3, 5 and 10 µm particle size columns can be reverse flushed or even used in the reverse direction, some 3 µm columns can only be reverse flushed for a short time and should not be used in the reverse direction afterwards. For irregular particle columns, it is advisable to flush in the normal direction of flow.

For UHPLC (≤2 µm) columns, the manufacturer's 'Care and Use' instructions must be consulted before considering reverse flushing the column. In some cases, it may be preferable to flush the UHPLC column in the normal direction of flow.

It is recommended that the column efficiency is measured before and after any clean-up procedure or long-term storage, using either the column test conditions given on the manufacturer's test chromatogram or conditions from the method being followed. This enables the effectiveness of any cleaning procedure to be monitored.

COLUMN CLEANING PROCEDURES

The following general procedures are recommended for regeneration of column performance.

1. Disconnect and if applicable reverse the column.
2. Connect the column to the pump, but not the detector.
3. Follow the appropriate flushing procedure for the type of column (see below), using 10-20 column volumes of each solvent (see table 1). Always make sure that the last solvent used will be compatible with the mobile phase.
4. The flow rate should not exceed that specified on the QC chromatogram for the particular column, but preferably should be maintained at 25-50% of the normal working flow rate.

FLUSHING PROCEDURES FOR VARIOUS TYPES OF COLUMN

Reversed-phase columns

(e.g. C18, C8, C4, Phenyl, CN, 'AQ' type)

a) Mobile phase without buffer

b) Methanol

c) Acetonitrile

d) Acetonitrile/IPA (75:25)

e) IPA

f) Dichloromethane

g) Hexane

In many cases, the sequence a) to e) may be sufficient. If step f) or g) is necessary, flush with IPA before returning to mobile phase.

If metal ions are thought to be causing contamination, flush with aqueous 0.05 M EDTA followed by water.

Columns which have been used with ion-pairing reagents are best dedicated to that method and kept for this purpose.

Reversed-phase columns used for protein/peptide analysis

a) Mobile phase without buffer

b) Gradient of 10 – 90% B where A = 0.1% TFA in water B = 0.1% TFA in acetonitrile

Unbonded silica columns (SIL)

a) IPA

b) Methanol

c) Ethyl acetate

Bonded normal-phase columns
(CN, NH_2, Diol)

a) Chloroform

b) IPA

c) Dichloromethane

d) Hexane

Anion-exchange columns
(SAX, WAX)

a) Water

b) Methanol

c) Chloroform

d) Methanol

e) Water

Cation-exchange columns
(SCX, WCX)

a) Water (inject 4x 200 µl DMSO during flush)

b) Tetrahydrofuran

Size-exclusion columns for proteins

For weakly retained proteins

a) 0.1 M phosphate buffer, pH 3

For strongly retained proteins

a) Gradient of 100% water to 100% acetonitrile over 60 minutes

If you require further information on flushing procedures for specific columns, please contact chromsupport@avantorsciences.com

STORAGE CONDITIONS FOR SILICA-BASED HPLC COLUMNS

The conditions under which a column is stored will affect its lifetime. All buffers, salts and ion-pairing reagents should be flushed from the column before storage. Ideally, the storage solvent should be as shown on the initial column test chromatogram provided by the manufacturer. Column end plugs should be fitted to prevent solvent evaporation and the subsequent drying out of the packing bed.

Table 1: Approximate column volumes in mL for common column dimensions (fully-porous silica).

		Column length (mm)					
		50	75	100	125	150	250
Column ID (mm)	1.0	0.025	0.037	0.049	0.062	0.074	0.124
	2.1	0.109	0.164	0.218	0.273	0.327	0.546
	3.0	0.223	0.334	0.445	0.557	0.668	1.113
	4.6	0.523	0.785	1.047	1.309	1.570	2.617