Chromatography Solutions

Knowledge note #0004

Column Care, Cleaning and Storage

INTRODUCTION

Correct care and use is essential to maximise the life of any HPLC or UHPLC column. This Knowledge Note summarises simple routine practices that should be followed to help minimise any risk to the integrity of the column, and help maximise the performance of your separations. Column cleaning procedures which may help to regenerate degraded column performance are also discussed.

GENERAL CONSIDERATIONS

Column lifetime is heavily dependent on use and handling. Harsh analytical conditions and injection of dirty samples can considerably reduce column lifetime. Through use, a column may start to show various symptoms of reduced performance including:

- Increase in backpressure, potentially caused by a partially blocked frit or contamination.
- Split/tailing peaks.
- Change in selectivity due to adsorbed sample components.
- Loss of column efficiency, leading to loss of resolution.

Ultimately any column will have a finite lifetime, however the practices and cleaning protocols outlined in this Knowledge Note can help to maximise this. Many of these practices additionally provide benefits to the LC system and therefore help reduce long-term running costs.

The following everyday practices can be employed to enhance the lifetime of a column:

Use only high-purity HPLC solvents and buffers. This will not only help to preserve the lifetime of the column, but also prevent unknown chromatographic peaks due to impurities.

Use freshly prepared mobile phases and buffers to prevent bacterial growth, particularly for low buffer

concentrations and mobile phases around pH 7.

Filter mobile phases to remove particulates or use in-line filters.

Use appropriate sample clean-up procedures. This can prevent particulates reaching the column and also remove sample components that may become strongly bound to the column.

Use a guard column or pre-column filter to protect the column from particulates and other detrimental sample components.

When setting the flow rate, begin at a low flow rate and gradually increase the flow to the desired level. This minimises the physical shock to the column.

Always work within the pressure and flow rate limitations of the column. These are specified on the reverse of the QC chromatogram accompanying the column.

For optimum column lifetime, a mobile phase pH of 2-8 is recommended. To increase lifetime at high pH, consider using organic buffers, high % organic solvent, low buffer concentration and low temperature. The Avantor® ACE® SuperC18 and SuperPhenylHexyl phases have an extended pH range of 1.5-11 when used with LC-MS compatible buffers.

Whilst Avantor® ACE® columns may be operated up to 100 °C, temperatures below 60 °C provide optimal lifetime.

After use, wash buffers from the column and store on the solvent recommended on the test chromatogram.

For methods utilising ion-pairing reagents, it is best to dedicate a column specifically for that method; ion-pairing reagents can alter separation selectivity and be notoriously difficult to remove.

It is advisable to check the performance of the column, before and after any cleaning protocol, using the QC test conditions on the accompanying chromatogram.

CONDITIONING A NEW REVERSED-PHASE COLUMN

Reversed Phase		
C18	SuperC18	Phenyl
C18-AR	SuperPhenylHexyl*	C8
C18-PFP	PhenylHexyl*	C4
C18-Amide	Biphenyl*	AQ
CN-ES	C18-HL	NH_2

New reversed-phase columns are shipped on the relevant storage solvent(s), usually methanol/water, as specified on the accompanying test chromatogram. New Avantor® ACE® reversed-phase columns should be equilibrated with 10-20 column volumes (see Table 1) of the desired mobile phase before the first injection, or until a steady baseline and pressure is achieved.

It is important to check mobile phase compatibility before flushing the column with a new mobile phase. If the shipping solvent is not miscible with the mobile phase, flush the column with 10 column volumes of an intermediate solvent that is miscible with both the storage solvent and the desired mobile phase (Table 2).

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

*Available for Avantor® ACE® UltraCore only

For buffered mobile phases, the column should be flushed with at least 10 column volumes of a water/organic mixture with an organic content the same or lower than that of the buffered mobile phase. This will eliminate the risk of buffer precipitation. The column is then ready for equilibration with the desired mobile phase.

CONDITIONING A NEW NORMAL PHASE COLUMN

Normal Phase	
Silica	NH_2**
CN**	CN-ES**

Avantor® ACE® Silica columns are shipped in heptane:ethyl acetate (as indicated on the test chromatogram) and are ready for equilibration with the required normal phase solvents.

Avantor® ACE® CN, NH_2 and CN-ES columns are compatible with normal phase, however are shipped in typical reversed-phase solvents, as specified on their accompanying test chromatograms. To use these columns in normal phase, they should first be flushed with a solvent that is miscible with both the storage solvent and the required normal phase mobile phase (for example iso-propanol, see Table 2) for 15 minutes at the flow rate indicated on the test chromatogram, or lower. The column is now ready for equilibration with the required normal phase solvents.

Normal phase columns should be equilibrated with the required mobile before the first injection, until a steady baseline and pressure is achieved. Please note that normal phase columns may require longer equilibration times than are typical for reversed phase columns. It is important to check mobile phase compatibility before flushing the column with a new mobile phase. If the storage solvent is not miscible with the mobile phase, flush the column with 10 column volumes of an intermediate solvent that is miscible with both (Table 2).

For buffered mobile phases, the column should be flushed with at least 10 column volumes of a mobile phase containing the desired normal phase composition, minus the buffer. This will eliminate the risk of buffer precipitation. The column is then ready for equilibration with the buffered mobile phase.

CONDITIONING A NEW HILIC COLUMN

HILIC	
HILIC-A	HILIC-B
HILIC-N	NH_2

Avantor® ACE® HILIC columns are shipped on typical HILIC solvents or iso-propanol. HILIC columns should be equilibrated with the required mobile before the first injection, until a steady baseline and pressure is achieved. For a new column, equilibration may require 60-80 column volumes of mobile phase (Table 1). For previously used columns, this can often be reduced to 20 column volumes. It is important to check mobile phase compatibility before flushing the column with a new mobile phase. If the storage solvent is not miscible with the mobile phase, flush the column with 10 column volumes of an intermediate solvent that is miscible with both (Table 2).

For buffered mobile phases, the column should be flushed with at least 10 column volumes of a mobile phase containing the desired mobile phase composition, minus the buffer. This will eliminate the risk of buffer precipitation. The column is then ready for equilibration with the buffered mobile phase. For buffered mobile phases, HILIC columns may require longer equilibration; please refer to AKN0025 for details.

If the Avantor® ACE® NH_2 is to be used for the analysis of reducing sugars, an additional equilibration step may be necessary to ensure the correct ionisation state of the stationary phase surface. Please refer to AKN0022 for further details.

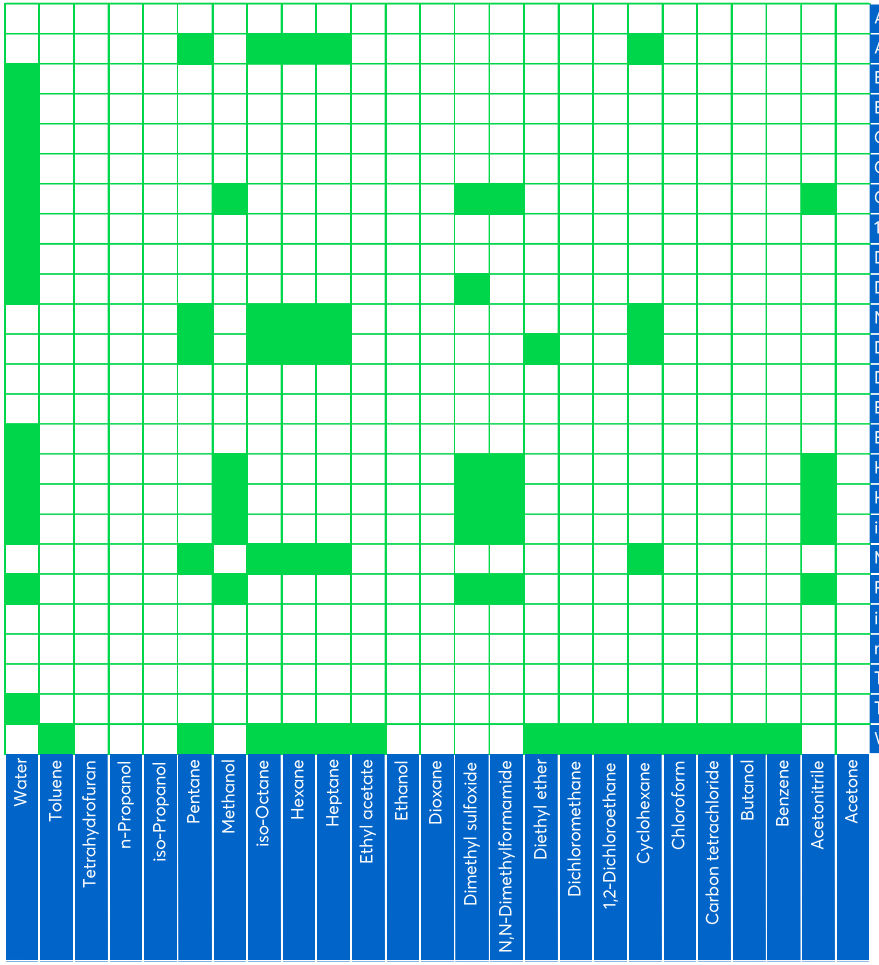
COLUMN CONTAMINATION AND CLEANING

Column contamination is commonly derived from the sample matrix. Some matrix components (e.g. salts) will elute near the void, as they are not retained by the column stationary phase. However, some components could be more strongly retained and adsorbed onto the stationary phase. Any particulate matter in the sample can also accumulate at the head of the column, resulting in increased back-pressure.

To remove strongly retained sample components, the column should be disconnected from the detector and the column outlet directed to waste using suitable

**Shipped on reversed-phase solvents

Table 2: Miscibility and properties of selected solvents



	UV cut-off (nm)	Boiling point (°C)	Viscosity (cP)	Refractive index
Acetone	330	56	0.32	1.359
Acetonitrile	190	82	0.37	1.344
Benzene	280	80	0.65	1.501
Butanol	215	118	2.98	1.399
Carbon tetrachloride	265	77	0.97	1.466
Chloroform	235	61	0.57	1.443
Cyclohexane	210	81	1.00	1.427
1,2-Dichloroethane	225	84	0.79	1.445
Dichloromethane	235	41	0.44	1.424
Diethyl ether	220	35	0.23	1.353
N,N-Dimethylformamide	268	153	0.92	1.430
Dimethyl sulfoxide	280	189	2.00	1.478
Dioxane	220	101	1.54	1.422
Ethanol	210	78	1.20	1.360
Ethyl acetate	260	77	0.45	1.370
Heptane	200	98	0.39	1.387
Hexane	200	69	0.33	1.375
iso-Octane	210	99	0.50	1.404
Methanol	205	65	0.60	1.329
Pentane	210	36	0.23	1.358
iso-Propanol	210	82	2.30	1.377
n-Propanol	210	97	2.27	1.384
Tetrahydrofuran	215	65	0.55	1.407
Toluene	285	111	0.59	1.496
Water	-	100	1.00	1.330

Miscible

Immiscible

tubing. Any buffer components should be removed using a water/organic mixture, the column can then be flushed with 100% of the strong mobile phase solvent.

Below is a list of the solvents to use for cleaning columns of different chromatographic modes. Columns should be flushed with 20 column volumes of each solvent in the order shown. The column can then be flushed back onto the mobile phase.

Reversed-phase columns (e.g. C18, C8)

1) Water/Methanol 95:5 v/v
2) Methanol or Acetonitrile
3) Mobile phase without buffer

HILIC columns (e.g. HILIC-N)

1) Water/Acetonitrile 50:50 v/v
2) Water
3) Mobile phase without buffer

Normal-phase columns (e.g. SIL, NH_2)

1) Isopropanol
2) Methanol or Acetonitrile
3) Ethyl acetate

COLUMN STORAGE

After use, buffers and salts should be removed from the column using unbuffered mobile phase. The column can then be flushed with storage solvent as specified on the reverse of the accompanying test chromatogram. Finally, the end stops should be securely attached to the column to prevent the column drying out.

It is important not to flush or store a column under 100% aqueous conditions (unless specifically stated). Stationary phases bonded with alkyl ligands (e.g. C18) are often incompatible with 100% aqueous conditions. Additionally, storage in 100% aqueous conditions can promote bacterial growth over significant periods of time.

CONCLUSION

This Knowledge Note outlines everyday practices that should be employed to help maximise column lifetime, along with processes that can be carried out to try to restore column performance. This guidance is generally applicable to most columns but refers specifically to Avantor® ACE® columns. For other columns, please refer to guidance from the appropriate manufacturer.