Column Care, Cleaning and Storage

AKN0004

ACE Knowledge Note #0004

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 ACE SuperC18 and SuperPhenylHexyl phases are compatible with an extended pH range of 1.5-11.

Whilst 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 Column

New columns are shipped on the relevant storage solvent(s), usually methanol/water for reversed-phase columns and isopropanol for HILIC columns. A new column should be equilibrated with 10-20 column volumes (see Table 1) of mobile phase before the first injection. HILIC columns may require longer equilibration; please refer to AKN0025 for more details.

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 compatible with both the storage solvent and the desired mobile phase.

For buffered mobile phases, the column should be flushed with at least 5 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.

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 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 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.

Column i.d. (mm) \ Column Length (mm)	50	75	100	125	150	250
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

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

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 ACE columns. For other columns, please refer to guidance from the appropriate manufacturer.