

## Maintaining LC Column Performance

### INTRODUCTION

To maximize the lifetime of an HPLC column and ensure its top performance, it is necessary to properly maintain the column. Many different factors can contribute to loss of resolution or sensitivity, but often times the following factors lead to the vast majority of column performance issues.

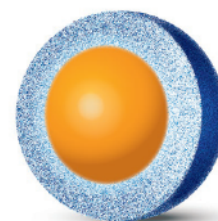
#### Contamination

Often, the sample may contain compounds, originating from the sample matrix, which can be retained by the stationary phase. Salts, lipids, plasticizers, and polymers are some of the possible substances that can come in contact with the stationary phase during the analysis. These substances can then have deleterious effects on the column, the detector, and cause transient peaks during the analysis.

If these substances are not eluted by the mobile phase, they can accumulate on the column. Over time, analytes can interact with these impurities and affect the separation mechanism, resulting in retention time shifts and peak tailing. In addition, these accumulating impurities can create a blockage, causing column back pressure to spike, damaging the pump, and can lead to the formation of a void in the column bed. The use of a guard column is highly recommended to avoid these types of problems. Guard columns are short columns packed with similar packings as the analytical column that are installed between the injector and the analytical column. After a given period of use, they are discarded and a fresh guard column is installed to maximize the lifetime of the analytical column.

#### pH Instability

Always ensure that the analysis is operated within the pH range for the column. Please consult the care and use sheet for the column to confirm the operational pH stability. When not in use, the column should be rinsed from acidic or basic buffer salt modifiers.



## High operating pressure

Superficially porous particles (SPP) based stationary phases provide high resolution separations at lower backpressures than fully porous particles (FPP) of smaller particle size. It is good laboratory practice to design analytical methods to run at the lowest possible operating pressures to maximize instrument and column life. It is also important to avoid pressure shocks on the column. Pressure shocks are sudden increases or decreases of pressure in a short time span. They may lead to the formation of a void in the column bed and contribute to peak splitting in the corresponding chromatogram.

All 2  $\mu\text{m}$  products, and 1000 Å 2.7  $\mu\text{m}$  columns with internal diameters of 2.1 mm and 3.0 mm are rated to 1000 bar. All remaining column dimensions and particle sizes are rated to 600 bar.

### Capillary columns

Particle size	Internal diameter (mm)	Pressure rating (bar)
All	0.75 and 0.1	600
All	0.2 – 0.5	400

### Non-capillary columns

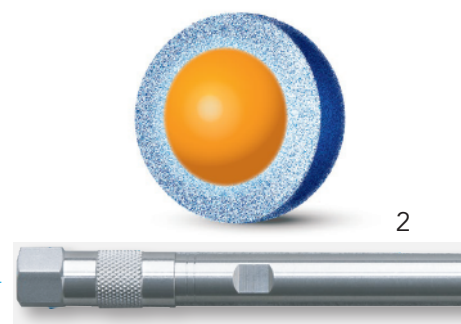
Particle size	Internal diameter (mm)	Pressure rating (bar)
All	1.0	600
2 $\mu\text{m}$	2.1, 3.0	1000
2.7 $\mu\text{m}$ 1000 Å (pore size)	2.1, 3.0	1000
2.7 $\mu\text{m}$	2.1, 3.0, 4.6	600
3.4 $\mu\text{m}$ 400 Å (pore size)	2.1, 3.0, 4.6	600
5.0 $\mu\text{m}$	2.1, 3.0, 4.6	600

NB : a designation of All indicates all particle sizes available

## Poor mobile phase quality

It is essential to use the highest quality solvents available (HPLC or MS grade) for the mobile phase, and to maintain the mobile phase pH at the operational pH range for the column being used. Please consult the care and use sheets for the specified column and phase. Low quality solvents often have impurities that can absorb on the column and interfere with the separation.

It is recommended to filter the mobile phase before use, and the use of a guard column during the analysis will help to ensure the mobile phase is particle free.



## Excessive temperature

It is essential for maximum column life to not exceed the maximum operating temperature rating for the column. The temperature rating can be found on the care and use sheet

## Insufficient equilibration time

The equilibration time of a column depends on the column dimensions. In general, a column is equilibrated after flushing with 20 column volumes. To calculate the column volume, use the following equation.

An example calculation follows.

1. Void volume( $\mu\text{L}$ )=  $(1/4)[(\text{diameter}^2)(\pi)(\text{Length})(\text{Pore volume})]$
2.  $\pi = 3.14$
3. Pore volume = 0.50 for SPP particles
4. Diameter and length should be kept in mm

For a column with dimensions of (2.1 mm x100 mm):

$$(2.1 \text{ mm})^2 = 4.41 \text{ mm}^2$$

$$(1/4)[(4.41)(3.14)(100)(0.50)] = 173 \mu\text{L} = 0.17 \text{ mL}$$

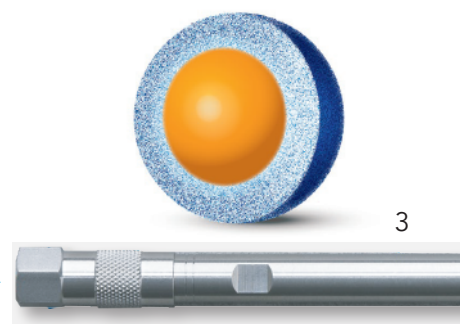
With a flow rate of 0.4mL/min 20 column volumes can be reached in 8.5 minutes

## TIPS ON COLUMN BLOCKAGE, CONTAMINATION AND CLEANING

### Sudden pressure increase - all phases

A large pressure spike, or a noticeable sharp increase in column pressure, is usually indicative of a column being clogged. To remove the clog, removing the column from the instrument and running it in the reverse direction without connecting it to the detector for about 20 column volumes can be attempted to dislodge any clog or particles that may have accumulated on the frit.

—Please note it is not recommended to back flush the capillary or 2  $\mu\text{m}$  particle HALO® columns.



## Column cleaning and regeneration

Absorption of accumulated impurities can be detrimental to the column, but in some cases these impurities can be removed. The following section will outline how this can be accomplished.

### Column cleaning for all non-capillary and particle size $>2\mu\text{m}$

C18, AQ-C18, C8, C4, C30, ES-CN, Phenyl-Hexyl, PFP, RP-Amide, Biphenyl, and Diphenyl stationary phases

To remove strongly retained materials from the column, flush the column in the reverse direction with very strong solvents, such as 100% of the organic component of the mobile phase being used. If a stronger solvent system is needed, a mixture of dichloromethane and methanol (95/5 v/v) is recommended. Extreme cases may require use of very strong solvents such as DMF or DMSO.

—Please note it is not recommended to back flush the capillary or  $2\mu\text{m}$  particle HALO® columns

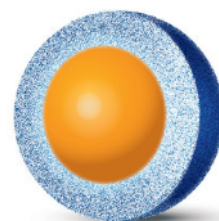
### Column regeneration all particle size

C18, AQ-C18, C8, C4, C30, ES-CN, Phenyl-Hexyl, PFP, RP-Amide, Biphenyl, and Diphenyl stationary phases

If the previous procedure is ineffective, an additional method maybe attempted to regenerate the column. Below is a theoretical procedure that has been effective at regeneration of reversed phase (RP) columns. In general, regeneration procedures are similar, with the common trait that wash solvents used are increased in their solvent strength, and often conclude with a solvent that is non polar. It is critical that all solvents used are miscible in series.

Steps 1-4 are usually sufficient to thoroughly clean most columns, however if this is insufficient, steps 5 and 6 can be added. However, it is critical to wash with IPA after using a strong non polar solvent such as hexane before introducing the re-equilibration solvent system as water will not mix with hexane.

- 1) Methanol
- 2) Acetonitrile
- 3) 50/50 ACN/IPA
- 4) IPA
- 5) Dichloromethane
- 6) Hexane
- 7) IPA
- 8) Re-equilibrate to starting conditions





## HILIC AND PENTA-HILIC STATIONARY PHASE.

### Column cleaning HILIC stationary phase

—Please note it is not recommended to back flush the capillary or 2  $\mu\text{m}$  particle HALO® columns

To remove strongly retained materials from the column, flush the column in the reverse direction with very strong solvents such as 50/50 methanol and deionized water. Extreme cases may require the use of very strong solvents such as 100% of the most polar component of the mobile phase in use. Alternatively, a mixture (95/5 v/v) of dichloromethane and methanol is often effective at this task. To rehydrate a HILIC column after cleaning it, it may be necessary to condition it first by running a mobile phase of 70/30 ACN/H<sub>2</sub>O at a slow flowrate through the column for 2 – 3 hours.

### Column cleaning Penta-HILIC stationary phase

To remove strongly retained materials from the column, flush the column in the reverse direction with very strong solvents such as 10/90 methanol and deionized water. Extreme cases may require the use of very strong solvents such as 100% of the most polar component of the mobile phase in use, which is typically water.

## COLUMN STORAGE (ALL PHASES)

### Long term storage

Long term storage (more than 5 days) of most silica based reverse phased columns is best in 100% acetonitrile. The exception to this is the Biphenyl phase, which should be stored in 100% methanol. After analysis flush out the buffers or salts used during the analysis with the mobile phase minus the buffers or salts, then flush with at least 10 column volumes of acetonitrile before storage.

### Short term storage

Short term storage (less than 5 days) of silica based reverse phased columns can be kept safely stored in most common mobile phases. However, it is recommended, after analysis flush out the buffers or salts used during the analysis with the mobile phase minus the buffers or salts before the column is stored.

