# Care & use of Avantor® ACE® HPLC Columns

Thank you for purchasing this Avantor® ACE® HPLC column. Every Avantor® ACE® HPLC column is manufactured and tested to exceed stringent specifications. The following measures will enhance its performance and lifetime.

#### **COLUMN INSTALLATION**

#### System dead volume

Reduce dead volume in the system to a minimum by using connection tubing with an internal diameter of 0.010" (0.25mm) or less for analytical columns. Connections between injector, column and detector should be kept as short as possible.

#### Column connection

The direction of flow is marked on the column. For optimum performance, the tubing connecting the column to injector and detector must abut the internal shoulder of the fitting. For HPLC columns, the use of PEEK fingertight fittings (P/N ACE-CC10, 10 pack) are recommended.

### Mechanical damage

Protect the column from mechanical shock. Dropping a column can impair its performance.

#### **Equilibration**

This Avantor ACE HPLC column is shipped in the storage solvent defined overleaf on the test chromatogram. Flush onto the desired mobile phase (ensure that precipitation does not occur). Ensure that the column is fully equilibrated with the mobile phase prior to starting analysis.

### **Guard cartridges**

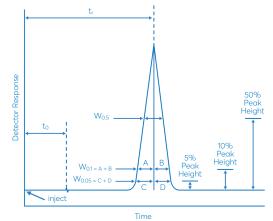
The use of guard cartridges is recommended to prevent both inlet frit blockage and irreversible sample adsorption onto the top of the column. Guard cartridges are available for all Avantor ACE HPLC columns - for assistance identifying the correct guard cartridge for this column, please contact our Technical Support Department at chromsupport@avantorsciences.com.

# **Precolumn filters**

As an alternative to guard cartridges, precolumn filters may be used to protect the column inlet frit from blockage. Due to their ultra low dispersion design, column performance and retention remain unaffected. For 3.0 - 4.6 mm I.D Avantor ACE HPLC columns the use of analytical precolumn filters (P/N ACE-CS210, 10 pack) are recommended. For 2.1 mm I.D Avantor ACE HPLC columns, the use of microbore precolumn filters (P/N ACE-HP210, 10 pack) are recommended.

#### **Performance Testing**

It is recommended that the performance of columns is tested on arrival and periodically during use. Performance parameters are defined below.



Performance - At 50% peak height

- At 10% peak height

 $N_{0.5} = 5.54 (t_r / W_{0.5})^2$  $N_{0.1} = 18.55 (t_r / W_{0.1})^2$ 

**Peak Shape** 

- Overlap to a perfect Gaussian peak

 $% = N_{0.1} / N_{0.5}$ 

Peak asymmetry

(measured at 10% peak height)  $As_{0.1} = B/A$ 

– US Pharmacopeia Tailing Factor (measured at 5% peak height)  $T = W_{0.05}/2C$ 

# **Selectivity**

- Retention factor

 $k = (t_r - t_0) / t_0$ 

#### **OPERATIONAL GUIDELINES**

#### **HPLC** solvents

Use only HPLC grade solvents and freshly prepared aqueous buffer solutions to minimise bacterial growth. A slip-on pump inlet filter will remove extraneous particles.

#### Mobile phase pH

To ensure maximum column lifetime, a pH range of 2.0 - 8.0 is recommended. When operating at high pH, the use of an organic buffer is recommended. Avantor ACE SuperC18 columns provide an increased pH operating range of 1.5 - 11.5 when used with LC-MS compatible buffers. For further information and guidance on maximising column lifetime under your chosen evaluation conditions, please contact our Technical Support Department at chromsupport@avantorsciences.com.

# Sample

For maximum column lifetime, always use freshly prepared sample and filter using a 0.2 µm filter.

For improved reproducibility and greater sample recovery of low abundance analytes, surface deactivated vials which virtually eliminate secondary interactions between sample and vial wall are recommended.

Exposure to rapid changes in pressure or pressures > 4000 psi (276 bar) may reduce column lifetime.

#### **UHPLC** column availability

If a higher pressure rating is required, most Avantor ACE phases are additionally available in a dual compatible UHPLC/HPLC "Excel" hardware format with a 15000 psi (1000 bar) pressure limit.

#### **Temperature**

Exposure to temperatures > 60 °C may reduce column lifetime, dependent upon bonded phase and mobile phase conditions selected.

#### **Storage**

Wash out any buffer (ensure that precipitation does not occur) and flush onto the storage solvent defined overleaf. Replace the endstops to prevent the packing bed drying out and store in a cool area.

# **Column cleaning**

Over a period of time, columns may still become contaminated by strongly adsorbed sample components. This may be indicated by a deterioration in column performance and/or an increase in back pressure. In such instances, specific cleaning protocols may be used in an attempt to regenerate column performance and further extend the lifetime of the column. For further guidance on the recommended cleaning protocol for this column, please contact our Technical Support Department at chromsupport@avantorsciences.com.

# **Column warranty**

All columns are warranted to meet the specifications stated on the Test Chromatogram. Removal of an end fitting to replace a frit or top-up the packing material should be regarded as a last resort to prolonging column lifetime. Removal of the column end fittings will automatically invalidate the column warranty.

### Safety and disposal

This column contains amorphous silica which may be hazardous to health if the column is unpacked and the silica allowed to dry. The silica presents no hazard whilst contained within the column. When the column has reached the end of its useful life, dispose of it in a similar manner to the samples that have been injected onto it. Alternatively, please contact our Technical Support Department at <a href="mailto:chromsupport@avantorsciences.com">chromsupport@avantorsciences.com</a> for details of our column disposal program.