

Care & use of Avantor® ACE® Excel® HILIC & Glycan columns

Thank you for purchasing this Avantor® ACE® Excel® column.

Every Avantor® ACE® Excel® column is individually manufactured and validated to exceed stringent specifications. The following measures will enhance its performance and lifetime.

COLUMN INSTALLATION

System compatibility

Avantor ACE Excel columns are compatible with all leading manufacturers' UHPLC and HPLC instrumentation. For improved compatibility onto a Waters Acquity UHPLC system (containing a 1/16" Waters Acquity fitting and ferrule on the inlet tubing), the use of a precolumn filter incorporating the unique Waters Acquity column port profile (P/N EXL- PCF10/ACQ – 10 pack) is additionally recommended.

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 with the internal shoulder of the fitting.

- For HPLC applications (up to 5000 psi / 350 bar), the use of Avantor ACE Fingertight HPLC Column Connectors (P/N ACE-CC10, 10 pack) are recommended for inlet and outlet connections.
- For UHPLC applications (up to 15,000 psi / 1000 bar), the use of Avantor ACE Excel UHPLC Column Connectors (P/N EXL-CC10, 10 pack) are recommended. At the outlet end of the UHPLC column (where pressure demands are lower but a correct connection remains important), ACE Fingertight HPLC Column Connectors (P/N ACE-CC10, 10 pack) may alternatively be used.
- For improved compatibility onto a Waters Acquity UHPLC system (containing a 1/16" Waters Acquity fitting and ferrule on the inlet tubing), the use of a pre-column filter incorporating the unique Waters Acquity column port profile (P/N EXL- PCF10/ACQ – 10 pack) is additionally recommended – for further information please contact our Technical Support Department at chromsupport@avantorsciences.com

Mechanical damage

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

Equilibration

This Avantor ACE Excel 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. For a brand new column, equilibrate for up to 60–80 column volumes. For a previously used column, it may be possible to reduce equilibration time to 20 column volumes. If operating under gradient conditions, equilibration for 10 column volumes between gradient cycles is usually recommended. Further information is contained within the ACE HILIC Method Development Guide.

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

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 $N_{0.5} = 5.54 (t_r / W_{0.5})^2$
– At 10% peak height $N_{0.1} = 18.55 (t_r / W_{0.1})^2$

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 $k = (t_r - t_0) / t_0$

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. Precolumn filters are available for all Avantor ACE HPLC and UHPLC columns – please contact us for assistance identifying the correct precolumn filter for this column.

OPERATIONAL GUIDELINES

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 – 7.0 is recommended. For further information and guidance on maximising lifetime of this column 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.

Vials

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.

Pressure

Avantor ACE Excel columns are suitable for both UHPLC and HPLC applications. Exposure to rapid changes in pressure, pressures > 15000 psi (1000 bar) and/or high linear flows (equivalent to > 0.7 ml/min for 2.1 mm I.D) may reduce column lifetime.

Temperature

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

Storage

Wash out any buffer using 50:50 MeCN/H₂O (ensure that precipitation does not occur) and flush the column onto IPA (propan-2-ol). Replace the end-stops to prevent the packing bed drying out and store in a cool area. Further information is contained within the ACE HILIC Method Development Guide – please contact us to receive your FREE copy.

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 for details of our column disposal program.