Care and Use of Kanak UPLC and HPLC Columns

Thank you for choosing a Kanak column. The silica material within the Kanak column is rigorously examined with acidic, basic and neutral analytes to ensure the highest quality and superior performance. All Kanak columns are individually manufactured and tested to meet strict specification criteria. A Test Chromatogram and Column Characteristics Summary are provided with each column. The following measures will maintain their performance and lifetime.

If you have any questions regarding your test results or the column quality, or if there are signs of damage, CONTACT NANGAHVI OR YOUR LOCAL DISTRIBUTOR IMMEDIATELY.

1 Column Installation

1.1 Dead Volume

For optimal chromatographical performance, it is vital to reduce system dead volume to a minimum. Use small internal diameter connection tubing, for analytical columns 0,25 mm (0.010") or less and keep the tubing lengths between injector, column and detector as short as possible.

1.2 Column Connection

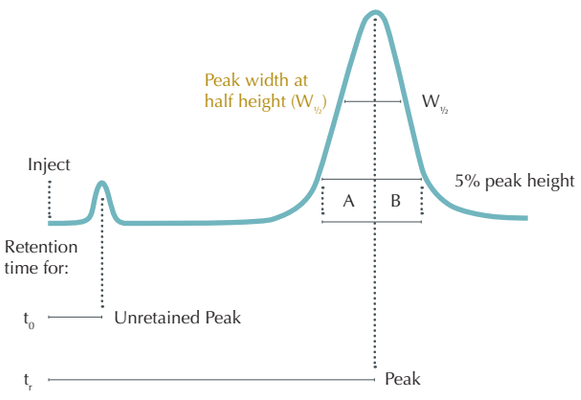
For optimum performance, it is important that the tubing used to connect the column to the injector or detector is swaged into position such that it touches the internal shoulder of the fitting.

1.3 Equilibration

The storage solvent in a new column is specified on the Test Chromatogram. Avoid passing any material through the column that may precipitate in the storage solvent. Furthermore, make sure your in-column solvent or mobile phase is miscible with the equilibration solvent(s). Ensure that the column is fully equilibrated to the mobile phase prior to starting an analysis. Flow direction is shown on the columns.

Figure. Calculated paramaters in the Test Chromotogram.

USP Tailing factor	Column Efficiency
$T_{usp} = \frac{A+B}{2A}$	$N_{0.5} = 5.54 \left(\frac{t_r}{W_{½}} \right)^2$



2 Operation Guidelines

2.1 Solvents

To minimize bacterial growth, only HPLC grade solvents and freshly prepared buffer solutions should be used. A pump inlet filter will remove particulate matter from the solvents. It is suggested that an inline filter is used just before the inlet of the column to minimize entry of particles to the column and extend the column´s lifetime.
If large volumes of mobile phase are prepared at one time, the unused solution should be kept in the refrigerator to avoid bacterial growth and unnecessary evaporation. Always filter the mobile phase using a 0.2 µm filter to ensure maximum column life.
The recommended mobile phase pH for Kanak columns packed with bonded silica phases, is specified in the Column Characteristics Summary.

2.2 Sample

Always use fresh sample and filter it through a 0.2µm filter.

2.3 Pressure and Temperature

Exposure of a column to rapid changes in back pressure may reduce column life. It is suggested that the column is operated below 75% of the maximum system pressure. Avoid any sudden pressure changes. The recommended pressure and temperature limits are stated in the Column Characteristics Summary.

2.4 Storage

Make sure to wash out all buffers from the columns and then store the columns in an organic solvent mixture (e.g. 80/20 methanol/water). Ensure that the end-fittings of the column is completely sealed to avoid drying of the column bed. Store in a cool area.

2.5 Mechanical damage

Protect the column from mechanical shock, as this could impair its performance.

2.6 Fittings

Do not use excessive tightening of the column end fittings, as this would result in damage to the column fittings. Use ferrules with seating depth of the nib of 2 mm (Phenomenex or Valco type), or preferably polymeric finger tight fittings, to install column on the LC-system. Replacing a frit or top-up the packing material should be considered as a last resort to prolong the life of the column.

2.7 Column Cleaning Procedures

The described cleaning procedure is meant for reversed phase columns. Before starting any kind of cleaning procedure, make sure your in-column solvent is miscible with the recommended cleaning solvents. Run column at half the normal flow rate for at least 10 CV/step of the following:

1. 10/90 methanol/water (heated to 50 ºC, removing buffer)
2. 95/5 acetonitrile/water (lipophilic rinsing/equilibrating)
3. 100 % THF (lipophilic cleaning)
4. 95/5 acetonitrile/water (reintroducing water content)
5. 80/20 methanol/water (equilibrating column bed)
6. Run with mobile phase