

Care and Use of SVEA UHPLC and HPLC Columns

Thank you for choosing a SVEA column. The silica material packed in SVEA columns has been meticulously developed to provide highest quality and superior performance for separation of acidic, basic and neutral analytes. All SVEA columns are individually manufactured and tested to meet strict specification criteria. A Test Chromatogram is provided with each column. Following the recommendations below will keep their performance and prolong column lifetime.

If you observe any signs of damage when you received the column, do NOT use the column. Preserve the shipping/packaging of the column as you received it and CONTACT NANOLOGICA OR YOUR LOCAL DISTRIBUTOR IMMEDIATELY.

Column Installation

Dead Volume

For optimal performance, it is vital to reduce system dead volume to a minimum. Use connection tubing of internal diameter 0.25 mm (0.010") or less. The tubing lengths between injector, column and detector should be as short as possible.

Column Connection

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

Equilibration

The storage solvent used in a new column is specified on the Test Chromatogram. Avoid passing any material through the column that may precipitate in the storage solvent. Ensure that the 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 column.

Operation Guidelines

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. Use of an inline filter before the inlet of the column is highly recommended. This will minimize any particulate matter entering the column and thus extend the column's lifetime. Filter the mobile phase using a 0.2 µm filter to ensure maximum column life.

For recommended mobile phase and pH specific for column types, please refer to the catalogue or the webpage.

Sample preparation

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

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 specific to column types, are available in the catalogue or on the webpage.

Storage

Flush out all buffers from the columns and store the column in an organic solvent mixture (e.g. 70/30 acetonitrile/water). Ensure that the end-fittings of the column are properly sealed to avoid drying of the column

bed. Store at the ambient temperature.

Mechanical Damage

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

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.

Column Cleaning Procedures

The cleaning procedure provided below is for reversed phase columns. Ensure the in-column solvent is miscible with the recommended cleaning solvents. Use the following procedure at maximum 50 % normal flow rate and minimum of 10 CV/step:

- 10/90 methanol/water (heated to 50 °C, washing)
- 95/5 acetonitrile/water (lipophilic rinsing)
- 100 % tetrahydrofuran (lipophilic cleaning)
- 95/5 acetonitrile/water (reintroducing water content)
- 70/30 acetonitrile/water (storage solvent)

Calculated parameters in Test Chromatogram

USP Tailing Factor

$$T_{USP} = \frac{A + B}{2A}$$

Column Efficiency

$$N_{0.5} = 5.54 \left[\frac{t_r}{w_{1/2}} \right]^2$$

