

## ***Shimadzu Column Care and Use Guide***

### **FOR REVERSED PHASE COLUMNS ONLY**

**COLUMN INSTALLATION:** IT IS HIGHLY RECOMMENDED THAT YOU READ THIS GUIDE FOR SPECIFIC COLUMN CONSIDERATIONS BEFORE PROCEEDING WITH THE INSTALLATION.

- Flush HPLC pump and line thoroughly with filtered and degassed mobile phase (without any buffers). Make sure there are no air bubbles in the system.
- Connect the column to the injector corresponding to the direction of the flow label (located on the column). Leave the outlet of the column unattached.
- Set pump to flow at 0.1 ml/min (of lowest setting) and increase to normal flow rate over 5 minutes.
- Stop flow when there is a free flow of solvent from the column outlet, wipe the end and attach to the detector.
- Equilibrate the column by passing approximately 10-30 column volumes of mobile phase at normal flow rate.
- Avoid any sudden pressure changes.

### **STORING THE COLUMN**

- Column storage conditions affect column lifetime.
- Never store columns with buffers.
- Flush with 5 column volumes of mobile phase without buffer to remove any buffers or salts.
- Store column in 65% Acetonitrile 35% water

### **COLUMN CLEANING**

- Rinse with 10 Column Volumes each of:
  - 95% Water
  - 5% Acetonitrile (for buffer removal)
  - THF
  - 95% Acetonitrile/5% Water
  - Mobile Phase
- *If high backpressure is observed reverse flush the column (do not try this on other manufacturer's columns).*
- Use only HPLC grade solvents and water.

### **FOR NORMAL PHASE COLUMNS ONLY**

**COLUMN INSTALLATION:** IT IS HIGHLY RECOMMENDED THAT YOU READ THIS GUIDE FOR SPECIFIC COLUMN CONSIDERATIONS BEFORE PROCEEDING WITH THE INSTALLATION.

- Flush HPLC pump and line thoroughly with filtered and degassed mobile phase (without any buffers). Make sure there are no air bubbles in the system.
- Connect the column to the injector corresponding to the direction of the flow label (located on the column). Leave the outlet of the column unattached.
- Set pump to flow at 0.1 ml/min (of lowest setting) and increase to normal flow rate over 5 minutes.
- Stop flow when there is a free flow of solvent from the column outlet, wipe the end and attach to the detector.
- Equilibrate the column by passing approximately 10-30 column volumes of mobile phase at normal flow rate.
- Avoid any sudden pressure changes.

### **STORING THE COLUMN**

- Column storage conditions affect column lifetime.
- Never store columns with buffers.
- Flush with 5 column volumes of mobile phase without buffer to remove any buffers or salts.
- Store column in Hexane
- Use only HPLC grade solvents.

### **COLUMN CLEANING**

- Please contact your local technical support representative for technical assistance.

**FOR SFC COLUMNS ONLY**

**COLUMN INSTALLATION:** IT IS HIGHLY RECOMMENDED THAT YOU READ THIS GUIDE FOR SPECIFIC COLUMN CONSIDERATIONS BEFORE PROCEEDING WITH THE INSTALLATION.

- Flush pump and line thoroughly with filtered and degassed mobile phase (without any buffers). Make sure there are no air bubbles in the system.
- Connect the column to the injector corresponding to the direction of the flow label (located on the column). Leave the outlet of the column unattached.
- Set pump to flow at 0.1 ml/min (of lowest setting) and increase to normal flow rate over 5 minutes.
- Stop flow when there is a free flow of solvent from the column outlet, wipe the end and attach to the detector.
- Equilibrate the column by passing approximately 10-30 column volumes of mobile phase at normal flow rate.
- Avoid any sudden pressure changes.

**STORING THE COLUMN**

- Column storage conditions affect column lifetime.
- Never store columns with buffers.
- Flush with 5 column volumes of mobile phase without buffer to remove any buffers or salts.
- Store column in Ethanol

**COLUMN CLEANING**

- Please contact your local technical support representative for technical assistance.

**FOR CHIRAL COLUMNS ONLY**

**CAUTION! PLEASE READ BEFORE CONNECTING THE COLUMN TO AN HPLC OR SFC UNIT.**

**Residual solvents that remain in the HPLC and SFC systems such as (Acetone, Chloroform, Ethyl Acetate, DMF, DMSO, Methylene Chloride, THF and Water) will destroy the column. Make sure the system is completely flushed with IPA or ethanol before connecting this column.**

- Flow Direction: The flow direction for this column is indicated by an arrow on the column label.
- Flow Rate and Pressure: To maximize column life, flow rates should be adjusted so that the back pressure does not exceed 6,300 PSI, (450Bar).
- Temperature Range: 0°C-40°C
- Mobile Phase: Typical mobile phases include Hexane/IPA and Hexane/Ethanol. All samples and mobile phases should be filtered through a membrane filter.
- Modifiers: If needed, DEA, TEA, TFA and Acetic Acid can be used to improve peak shape for highly basic or acidic compounds. Modifiers will cause memory effect on the column.
- Storage: Columns should be flushed and stored in Hexane/IPA (90/10) with the end plugs secured to each end of the column.

**SFC**

If the column is to be used in SFC, rinse thoroughly with 100% ethanol before use.

**MOBILE PHASE CONSIDERATION FOR ALL COLUMNS**

- Use only highest purity chemicals and reagents.
- Degas and filter all mobile phases prior to use.
- Trace impurities can dramatically degrade columns.
- When changing to a different mobile phase, make sure the solvents and or buffers are miscible.
- Using solvents that are immiscible with the solvent in the column can permanently damage the column. Salt and buffer precipitation from the mobile phase can permanently damage the column.
- Always check sample solubility and if possible use the mobile phase as the diluent (sample solvent).