

Column Washing

An altered peak shape and elution time or an elevated column pressure may be resolved by washing the column. This section describes typical warning signs of a deteriorating column and the method for washing the column. For detailed washing procedure, refer to the corresponding operation manual.

■ Typical Findings Observed in a Deteriorated (Contaminated) Column

1. Elevated column pressure
2. Abnormal peak shapes (broad, leading, or tailing) and split peaks
3. Altered retention times
4. Unstable baseline values

■ Points to Consider in the Selection of a Washing Solvent

The solvent should be capable of dissolving the adsorbed substances (contaminants).

The solvent should be able to pass through the relevant column at a high flow rate.

* Use only solvents specified as usable solvents in the operation manual.

■ Standard Washing Methods

To wash the column efficiently, connect the column inversely and reduce the flow rate to one third of the usual value.

Reversed Phase Columns	Flush with a solvent containing a high content of methanol, acetonitrile, THF, or other organic solvent. (Check salt concentration before washing. Flushing too high a concentration of organic solvent may cause the salt to precipitate.)
Ion Exchange Columns (Including IC Columns)	For ionic adsorption Flush with a solution containing a high content of salt or having a pH different from that of the solution used in the analysis. For hydrophobic adsorption Flush with a solvent containing a small percentage of organic solvent. For proteins Inject 1 to 2 mL of 0.1 M NaOH or 30% acetic acid several times.
Sugar Analysis Columns	[SUGAR Series; Ligand Exchange Columns] For counter ion detachment Flush with a salt solution containing the same counter ion or inject such a solution several times. [NH₂P-50; Polymer-based Amino Columns] For amino group modifications Flush 0.1 M perchloric acid, 0.1 M NaOH, and eluent while allowing water to pass through the column ahead of each solution.
Hydrophobic Interaction Columns	For proteins : Inject 1 to 2 mL of 0.1 M NaOH or 30% acetic acid several times.
Aqueous SEC (GFC) Columns	For ionic adsorption Flush with a solution containing a high content of salt or a pH different from that of the solution used in the analysis. For hydrophobic adsorption Flush with a solvent containing a small percentage of organic solvent. (Check salt concentration before washing. Flushing too high a concentration of organic solvent may cause the salt to precipitate.)

* In general, washing with a solvent 5 to 10 times of column volume is recommended. * Avoid pressure elevation during washing.

* A seriously contaminated column will not achieve complete recovery.

For your information:

One cause of elevated column pressure could be clogging of solid substances in the sintered filter mounted at the opening of the column. In this case, connect the column inversely and reduce the flow rate to one third of the usual one or lower. This may decrease the pressure.

* Use only solvents specified as usable solvents in the operation manual.

General Precautions for Handling a Column

For the proper use of a column, follow the instructions described below.

Column Attachment

- Flush your HPLC system (flow lines, sample loop, degasser etc.) with new eluent before connecting the column to it. (When the new eluent is not miscible with the solution that is present in the system or causes salt to precipitate, flush with a solution that is compatible with both before flushing the eluent.)
- Connect the column to the system while flushing with eluent at a low flow rate. Make sure that the eluent flows in the direction indicated by the arrow of the flow mark.
- When warming the column, use a low flow rate until the temperature reaches a desired level and then slowly increase the flow rate to the measurement flow rate.

Column Detachment and Storage

- When the column has been warmed, do not turn off the pump suddenly. Wait until the column has cooled to room temperature while keeping the flow rate low.
- Turn off the pump after the column has cooled to room temperature.

Column Storage

- For long-term storage, substitute the solution remaining in the column with the shipping solvent and remove the column from the system, then cap both ends.
 - Store the column in a temperature-stabilized place.
 - For the long-term storage of SEC columns, it is recommended that you store the column in a fluid.
- * See the operation manual for the method of storing the column in a fluid.

Other

- Avoid impacts to the column. Be careful not to drop the column from a higher position.
- Do not bend the column.
- Avoid opening the end fitting; column performance may deteriorate.