

Tips & Tricks GPC/SEC:

How to Install GPC/SEC Columns

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For proper column installation it is important to be familiar with column characteristics such as solvent compatibility, maximum flow rate, and maximum operating temperature. This article focuses on the considerations that should be made when installing a gel permeation/size-exclusion chromatography (GPC/SEC) column.

Installation of a gel permeation/size-exclusion chromatography (GPC/SEC) column is similar to installing a high performance liquid chromatography (HPLC) column. However, a few important differences need to be considered. In contrast to HPLC stationary phase materials, GPC/SEC columns can be grouped into those that are silica-based and those that are polymer-based. Silica-based stationary phases have the advantage of higher pressure stability and solvent compatibility. They often provide an excellent resolution in a narrow molar mass range. Polymer-based materials on the other hand provide wider separation ranges, are easier to combine into column combinations (banks) to increase the separation range, and are often less susceptible to interaction with samples.

For both column types it is important that the maximum column pressure during installation is never exceeded and that air on the columns is avoided.

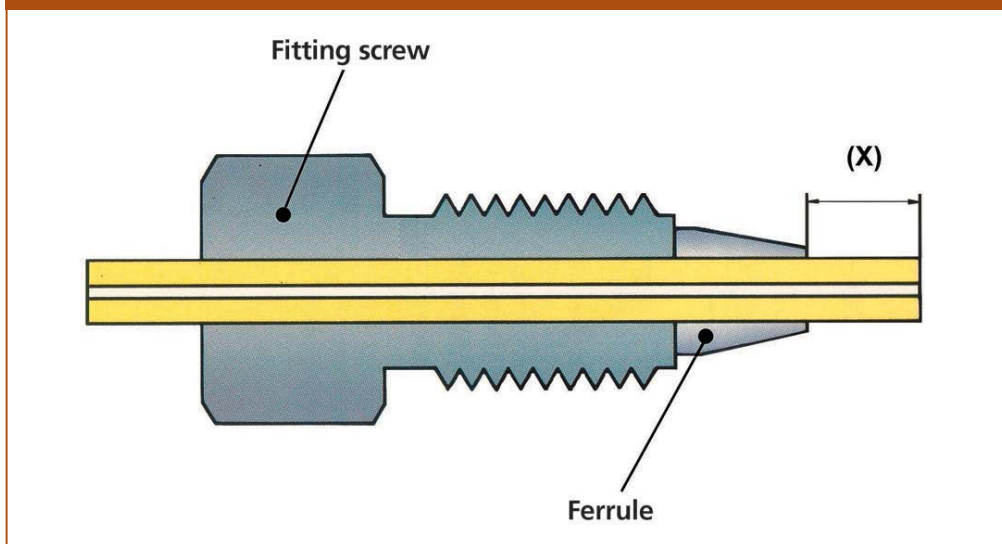
Installation Considerations

Solvent Compatibility: The solvent that is to be used must be compatible with the column(s). If the mobile phase of the GPC/SEC system and the mobile phase in the column are different, the GPC/SEC system solvent should be changed to match that of the column. Use intermediate solvents if the solvents in the system and the column are not miscible. The columns can then be installed and switched to the required solvent following the instructions in the column user documentation.

Tubing, Fittings, and Ferrules:

Tubing length between columns and

Figure 1: The distance between the end of the tubing and the ferrule varies for different manufacturers. Replace existing fittings and ferrules when changing the columns.



detectors should be minimized to avoid dead volumes, which will lead to band broadening. In addition, the inner diameter of the tubing needs to be adapted to the column inner diameter and application. This is especially important when microbore or preparative columns are used. Microbore columns require tubing with a small inner diameter. For preparative applications, which are often operated at higher flow rates, larger inner diameters are often required.

It is good practice to replace (or cut off) existing fittings and ferrules, particularly

when columns from a different manufacturer are installed. Figure 1 shows the tubing complete with fitting and ferrule.

The distance "X" in the figure varies for different manufacturers. When attempting to install columns from a different manufacturer with existing fittings either "X" can be shorter (resulting in a dead volume and poor separations) or "X" can be longer (with the danger of destroying the column head or column frit when fixing the tubing).



Installing the Column(s)

1. Set a low flow rate of, for example, 0.2 mL/min at the pump to keep air out of the system. Verify that solvent is coming out of the tubing. Remove the column end plugs of the (pre)column to be installed and connect the column, all the while taking into account the recommended flow-direction. For most columns the flow-direction is indicated by an arrow.
2. Push the tubing in until it bottoms firmly. Do not over tighten; the fitting only needs to be tight enough to seal.
3. Verify that solvent is coming out of the column. If this is not the case, check if the fitting is tight enough or if air is coming out of the column. An easy test to monitor air bubbles is to put the column end in a beaker with the mobile phase. If there is air trapped at the inlet, install the column in the reverse direction. Fill the column with solvent, using a flow rate of 0.1 mL/min, until no more bubbles appear at the column outlet and change the column to the correct flow direction. Use a flow rate of 0.1 mL/min until no more bubbles appear.
4. Connect the next column only after some eluent has flown freely from the column outlet.

5. Wait at least 3–5 column volumes before re-connecting the detectors.
6. Increase the flow rate slowly to the operating flow rate with an increment of 0.2 mL/min per 5 min. Once the system pressure has stabilized, ensure that there are no leaks at the new connections.

Column Sequence in a Column Bank

In general, the results of a GPC/SEC analysis will not be influenced by the order of the columns in a column bank.

However, if column banks with columns of different porosities are used it is recommended to install the columns with decreasing porosity. This will result in better separations because the viscosity of the injection band is reduced at a faster rate. The high molar mass compounds contribute the most to the viscosity. If the column is able to separate large molecules, the viscosity will decrease fast and the diffusion process into the pores is facilitated.

An exception can be made if columns with a low pressure stability are used. In such cases it is better to install this column at the end to protect it from potential pressure pulses, such as, for example, from a defective pump.

Verifying the Column and System Performance

GPC/SEC columns need a certain amount of time to equilibrate. As a rule of thumb, a minimum of 5 (better 10 when exchanging solvents) column volumes are required until the columns are fully equilibrated.

Before calibrating the new columns it is recommended to verify their performance by measuring the plate count. For this it is always best to follow the manufacturer's test procedure, keeping in mind that the plate count depends on many parameters that are not just column related. A low plate count can also be a result of dead volumes in the system, for example, caused by using the wrong tubing, fitting, or ferrules.

If the plate count test fails when using a column bank, it is recommended to test the single columns to identify which column (or pre-column) is responsible.

After a successful plate count test, the columns should be calibrated and the

resolution determined. Once this has been completed, the system is ready and the samples can be analyzed.

Summary

- When installing GPC/SEC columns it is always recommended to set a small flow to avoid air being trapped in the column.
- Although modern GPC/SEC columns are much more stable than they originally were, it is good practice to install the columns in the recommended flow-direction.
- The sequence of columns in a column bank does not influence the results, but may influence the column lifetime and the resolution. The general recommendation is to install them with decreasing porosity.

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