Agilent
Trusted Answers

GPC/SEC Column User Guide

Installation

Tubing and connectors

Stainless steel tubing of 1/16 in outer diameter (od) and 0.12 mm or 0.17 mm inner diameter (id) is recommended for column connections of analytical columns, while 0.5 mm id is recommended for 25 mm preparative columns. Connecting tubing lengths between columns, detectors, and injection volumes should be minimized to avoid excessive dead volume which will diminish system performance. Column connections should be made using compatible 1/16 in nuts and ferrules. The compatibility of column connectors is illustrated in Figure 1.

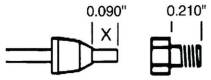


Figure 1. Compatible connectors.

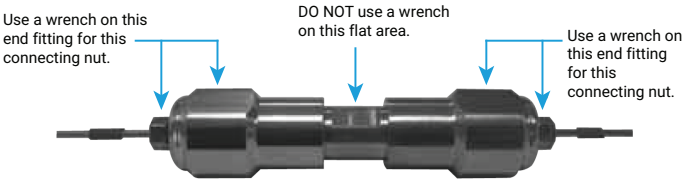
The distance "X" for the standard column end fitting is 0.090 in (2.286 mm) and a minimum male nut length of 0.210 in (5.334 mm) is required. Examples of compatible fittings are Parker and Swagelok. Some fittings from other Agilent GPC columns (not listed in Figure 3) or other manufacturers may not be compatible, for example, Waters and Rheodyne. The most versatile column connectors are the 105 mm long version (0.17 × 105 mm, green color coded, Agilent part number 5500-1193). Agilent recommends connecting them between the columns using Agilent InfinityLab Quick Turn LC fittings (2 × Agilent part number 5067-5966). Agilent also recommends connecting the outlet solvent capillary to the first column with an Agilent InfinityLab Quick Connect LC fitting (Agilent part number 5067-5965). Alternatively, removable Swagelok fittings (Agilent part number 5067-4733) could be used. If unsure, please contact Agilent Technologies.

Column connection

Connect the GPC column in the eluent flow direction indicated and tighten the 1/16 in nut and ferrule using wrenches on the 1/16 in nut and the actual end fitting.

It is recommended that several drops of eluent have been pumped before the column outlet is connected to another column or detector to clean out the end fitting of any particulate matter which may be present.

To avoid loosening the end fittings and causing leaks, wrenches must be used on the end fitting adjacent to the connecting nut and NOT on the column barrel or the opposite end fitting (Figure 2).



WARNING: To avoid loosening end fittings and causing column leakage, use wrenches as indicated above when connecting the guard column to the system.

Figure 2. Do not use wrenches on the flats.

Guard columns or precolumns: These will help to protect the main column set. Solvent compatibilities of guard columns and particle sizes must match with the main column set.

Connecting columns in series: For connections between columns, please use 105 mm long column connectors (Agilent part number 5500-1193) in combination with Agilent InfinityLab Quick Turn LC fittings (order 2 × Agilent part number 5067-5966) as mentioned on page 1. The guard column should be connected to the injector in sequence of increasing pore size so that the largest porosity is closest to the detector. The backpressure will indicate if the guard column has become partially blocked by trapped particulates. Do not exceed the maximum pressure limits of the columns and replace the guard column if required.

Eluent flow rate

For conventional GPC columns using 7.5 mm id columns, 1.0 mL/min is an optimum flow rate for most separations. When column id is increased or decreased, the volumetric flow rate should be adjusted accordingly in order to give an equivalent linear velocity through the column.

The recommended flow rates are given in Table 1, however, in order to avoid excessively high pressures, higher viscosity eluents should be used at reduced flow rates or elevated temperature. Flow rates should be changed progressively and pressure pulses limited. At no time should the maximum operating pressure of the column be exceeded (see Table 4).

Table 1. Recommended flow rate.

Column	Typical Flow Rates (mL/min)	Recommended Flow Rate (mL/min)
2.1 mm id	0.04 to 0.1	0.06
4.6 mm id	0.2 to 0.5	0.3
7.5 mm id	0.5 to 1.5	1.0
10 mm id	2.0 to 5.0	2.0
25 mm id	5.0 to 20.0	10.0

Sample preparation and injection

If maximum resolution and expected column lifetime are to be achieved, care must be taken in sample preparation.

To avoid blockage of the column frits, sample filtration is recommended (0.5 to 2.0 µm porosity depending on MW). A guard column will further protect the columns with little detrimental effect on performance.

Optimum sample volumes and concentrations are best determined for each type of analysis and are dependent on sample MW. Broad distribution polymer can generally be injected at higher concentrations than lower polydispersity samples. Overloading will not damage the column, but distorted peaks and therefore spurious results will be obtained.

Excessive injector loop volume can contribute to band broadening and reduce system performance, particularly with high efficiency or narrow bore columns. Agilent's injection volume recommendations are shown in Table 2.

Table 2. Injection volume recommendations.

Column	Recommended Concentration (%)	Recommended Injection (µL) per Column
2.1 mm id	0.01 to 0.2	0.2 to 2
4.6 mm id	0.01 to 0.2	1 to 20*
7.5 mm id	0.05 to 0.5	20 to 50**
10 mm id	0.05 to 0.5	20
25 mm id	0.5 to 5.0	500 to 2,000

* PL Multisolvent, MesoPore, and ResiPore are 1 to 10 µL.
** PL Multisolvent, MesoPore, and ResiPore are 10 to 20 µL, PL Rapide/PL Rapide Aqua are 20 µL

All eluents should be of high purity and should be filtered and degassed prior to use.

GPC columns can be transferred to other eluents. When transferring to another eluent, miscibility and viscosity of the new eluent are of primary consideration. See Figure 3 for 7.5 mm id columns. For other id columns, apply the flow rate shown in Table 1.

Organic Columns (PLgel, PlusPore, PL Rapide)		
Columns Supplied in Ethylbenzene		
Transfer to Low Viscosity Solvents, e.g. THF, Chloroform, Dichloromethane	**Transfer to Medium Viscosity Solvents, e.g. Toluene, DMF, DMSO, Hexafluoro-2-Propanol (HFIP)**	**Transfer to High-Viscosity Solvents, e.g. TCB, m-Cresol, NMP**
Flush column with acetone at 0.5 mL/min for two column volumes	Flush column with acetone at 0.5 mL/min for two column volumes	Set column oven to 50 °C, flow at 0.1 mL/min
Flush with new eluent at 0.5 mL/min for two column volumes	Flush with new eluent at 0.2 mL/min for two column volumes	Flush column directly with new eluent** at 50 °C at 0.1 mL/min for two column volumes
Increase column temperature to 30 to 40 °C* as required for analysis at 1 °C/min	Increase column temperature to 50 to 80 °C* as required for analysis at 1 °C/min	Increase column temperature to 100 to 220 °C* as required for analysis at 1 °C/min
Operate column in new eluent at required flow rate	Operate column in new eluent at required flow rate	Operate column in new eluent at required flow rate
Aqueous and Polar Columns (PolarGel, PL aquagel-OH, PL Rapide Aqua)		
Columns Supplied in Water Containing 0.02% NaN_3		
Transfer to Aqueous, e.g. Water, Buffer	**Transfer to Polar Organic***, e.g. DMSO, DMF**	**Transfer to Mixed Solvent Systems, e.g. Water/THF*, Water/Methanol***
Flush column with pure water at 1.0 mL/min for two column volumes	Flush column with pure water at 1.0 mL/min for two column volumes	Flush column with pure water at 1.0 mL/min for two column volumes
Flush with new buffer at 1.0 mL/min for two column volumes	Flush with acetone at 0.5 mL/min for two column volumes	Flush with new, premixed eluent at 0.2 mL/min for two column volumes
Increase column temperature* as required for analysis at 1 °C/min	Flush with new eluent at 0.2 mL/min for two column volumes	Increase column temperature* as required for analysis at 1 °C/min
Operate column in new eluent at required flow rate	Increase column temperature to 50 to 80 °C* as required for analysis at 1 °C/min	Operate column in new eluent at required flow rate
	Operate column in new eluent at required flow rate	
Multisolvent Columns (PL Multisolvent)		
Columns supplied in THF containing 0.015% BHT		
Transfer to Aqueous, e.g. Water, Buffer	**Transfer to Organic, e.g. Chloroform*, Dichloromethane****	
Flush column with pure water at 1.0 mL/min for two column volumes	Flush with new eluent at 0.5 mL/min for two column volumes	
Flush with new buffer at 1.0 mL/min for two column volumes	Increase column temperature as required for analysis at 1 °C/min	
Increase column temperature as required for analysis at 1 °C/min	Operate column in new eluent at required flow rate	
Operate column in new eluent at required flow rate		

* Always ensure that the operating temperature is at least 10 °C below the boiling point of the solvent.

** Always ensure miscibility. If unsure, use acetone at room temperature.

*** PolarGel only.

Figure 3. Eluent transfer guide.

When heating or cooling columns in high viscosity eluents (for example, 1,2,4-trichlorobenzene (TCB), N-methyl-2-pyrrolidone (NMP), or dimethylformamide (DMF)), a low solvent flow rate must always be maintained. Typically, 0.2 mL/min for 7.5 mm id and 0.1 mL/min for 4.6 mm id should be used prior to raising the temperature (Figure 3).

Organic GPC columns are compatible with an extensive range of organic solvents ranging in polarity from perfluoroalkanes to DMF. Mixed organic solvent systems can also be used, but water should not be used except at concentrations less than 10% by volume in a miscible organic eluent. Columns are normally supplied in ethylbenzene unless otherwise stated, and can be flushed directly from ethylbenzene to tetrahydrofuran (THF) at 0.5 mL/min. Unstabilized THF (for example, HPLC grade) is not recommended as an eluent due to the attack of peroxide on the gels.

Aqueous/polar GPC columns are normally supplied in water containing 0.02% sodium azide. Buffered eluent systems within the pH range 2 to 10 (of high and low ionic strength) may be used, with no detrimental effect on the column. When transferring from one eluent to another, the compatibility and solubility of any salts or additives must be checked to prevent on-column precipitation which would irreversibly damage the column. PolarGel columns are compatible with an extensive range of organic solvents and with aqueous based eluents. Mixed organic solvent systems can be used, assuming full miscibility of the components. The only organic solvent which is recommended for use with the PL aquagel-OH and PL Rapide Aqua columns is methanol (up to 50% by volume).

PL Multisolvent columns are supplied in stabilized THF, and may be transferred directly to organic solvents, or to pure water and then to buffer. Unstabilized THF (for example, HPLC grade) is not recommended as an eluent due to the attack of peroxide on the gels. Buffered eluents within the pH range 2 to 8.5 (of high and low ionic strength) may be used. When transferring from one eluent to another the compatibility and solubility of any salts or additives must be checked to prevent on-column precipitation which would irreversibly damage the column. Polar organic solvents, such as DMF, dimethyl sulfoxide (DMSO), or dimethylacetamide (DMAc) are not recommended as eluents.

Column Testing and Specifications

Every column is supplied with a test certificate indicating the test conditions and the column performance. Measurements of column performance are described below:

Efficiency (1/2 ht) (Plates/m)

$$N = 5.54\left[\frac{t}{W_{1/2}}\right]^2/L$$

Efficiency (5δ) (Plates/m)

$$N = 25\left[\frac{t}{W5\sigma}\right]^2/L$$

Symmetry a/b

Where "t" is the peak elution time, "$W_{1/2}$" is the peak width at half peak height, "W5σ" is the peak width at 4.4% of peak height, "L" is the column length in meters and "a" and "b" are the peak widths on either side of the perpendicular measured at 10% of peak height.

Column efficiency is dependent on many experimental factors (system dead volume, eluent, flow rate, test probe, temperature, and so forth) and test results may differ from those quoted on the column certificate due to variability in these parameters. Band broadening effects are more severe when using high efficiency and/or narrow bore GPC/SEC columns. It is vital to ensure that the system dispersion is minimized in order to obtain the full potential of Agilent columns.

Agilent recommends measuring performance when a column set is first used and at regular intervals after that. Consider replacing columns when efficiency falls below 80% of the starting value.

Storage

On removing the column from the system, the end plugs must be replaced to prevent the column from drying out by evaporation, since subsequent shrinkage of the gel and disruption of the packing will occur. The end plugs need only be applied finger-tight. Columns are best stored in the shipping solvent. However, all eluents previously mentioned are suitable for storage provided they contain no salts. Unstabilized THF and halogenated solvents (unless shipping solvent) should not be used.

For long term storage of aqueous columns, flush with water and store in water containing 0.02% sodium azide.

Table 3. Column specifications.

Column	Typical Operating Pressure[1] psi (bar)	Maximum Operating Pressure psi (bar)	Maximum Operating Temperature (°C)[2]
PLgel 3 µm	750 (50)	2,700 (180)	110
PLgel 5 µm	450 (30)	2,200 (150)	150
PLgel 10 µm	150 (10)	2,200 (150)	220
PLgel 20 µm	50 (3)	1,500 psi (100)	220
PLgel Olexis	90 (6)	2,200 (150)	220
PLgel Prep 10 µm	350 (25)	2,200 (150)	150
PolyPore	750 (50)	2,700 (180)	160
ResiPore	750 (50)	2,700 (180)	120
MesoPore	750 (50)	2,700 (180)	120
OligoPore	450 (30)	1,500 (100)	120
PL Rapide-F	350 (25)	1,500 (100)	150
PL Rapide-L	225 (15)	2,700 (180)	150
PL Rapide-M	225 (15)	2,700 (180)	220
PL Rapide-H	150 (10)	2,200 (150)	220
PolarGel	450 (30)	1,600 (112)	80
PL aquagel-OH 5 µm	900 (60)	2,200 (150)	90
PL aquagel-OH 8 µm	450 (30)	1,600 (112)	90
PL aquagel-OH 15 µm	150 (10)	1,600 (112)	90
PL Rapide Aqua	225 (15)	1,600 (112)	90
PL Multisolvent (Water)	1,450 (100)	5,800 (400)	80
PL Multisolvent (Organic)	750 (50)	5,800 (400)	80
PL HFIPgel	150 (10) (in methanol)	1,600 (112)	50

[1] Based on THF (organic columns) or H_20 (aqueous columns) at 20 °C, 4.6 mm id at 0.3 mL/min, 7.5 mm id at 1.0 mL/min, 10 mm id at 1.8 mL/min (2 mL/min for PL Rapide 10 × 100 mm) and 25 × 300 mm at 10 mL/min.

[2] At very high temperatures, column lifetimes are likely to be reduced. OligoPore and PL Rapide-F may show deterioration to lifetimes at > 80°C, while PL Multisolvent may degrade at > 50 °C. Amend flow rates and temperatures accordingly.

Warranty

The columns are covered by warranty for 90 days following delivery. For columns used at or above 170 °C, the warranty period is reduced to 30 days. Agilent Technologies cannot accept liability from improper handling and use of columns above their maximum operation temperature and pressure (see Table 3) and void the warranty. For a full warranty statement, please request Agilent's General Conditions of Sale.

Maintenance

Deterioration in column performance may occur as a result of damage to the packed bed or as a result of blockage in the column frits. In the case of frit blockage, the column can be reverse-flushed for at least 20 minutes or two column volumes, starting at lower flow rates than used for standard operational conditions, and it should be ensured that p_{max} is not exceeded. To avoid capillary blockages, the waste solvent should not go through the detector. If a column blockage exists despite back flushing, it is recommended to replace the column.

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