Agilent

Agilent POLEFIN GPC/SEC Columns

User Manual

Notices

Document Information

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WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Contents

1 Introduction

Thank you for choosing an Agilent POLEFIN column, a high-quality, high-performance product ready for immediate use. To prolong the lifetime and to optimize its performance, please read this user manual thoroughly, paying special attention to the warnings, hints, and tips sections.

Each column has a unique serial number and is quality checked for efficiency in the eluent in which the column is delivered. The test conditions and results are shown on the certificate of analysis (CoA), which is available on our website at:

www.agilent.com/en/ecertificates-of-performance

For additional information about Agilent GPC/SEC products, see

www.agilent.com/en/product/gpc-sec-columns-standards

1.1 Specifications

POLEFIN columns are packed with highly porous particles of polystyrene-divinylbenzene copolymer specially developed for high temperature GPC/SEC analysis and are supplied as standard in Xylene and tested for efficiency with butylated hydroxytoluene (BHT).

Table 1. Operational specifications for Agilent POLEFIN columns.

Column Type	Maximum Operating Pressure per Column (bar/psi)	Maximum Operating Pressure for Three Column Set (bar/psi)	Maximum Operating Temperature	Efficiency Plates/m (BHT in Xylene)
POLEFIN 10 µ	40/580	80/1,160	210 °C	>30,000
POLEFIN 20 µ	40/580	80/1,160	210 °C	>15,000

1.2 Solvent compatibility

POLEFIN is compatible with a wide range of eluents (Table 2) and has optimum performance, when used with eluents that have a polarity index (P.I.) in the range 2.0 to 5.0. Transfer to compatible eluents is easy provided the guidelines in this user manual are followed.

For solvents outside this polarity range, other Agilent column materials are more appropriate to maintain a balanced polarity between, sample, eluent, and packing material for a size only based separation based on the “magic triangle” concept (Figure 1).

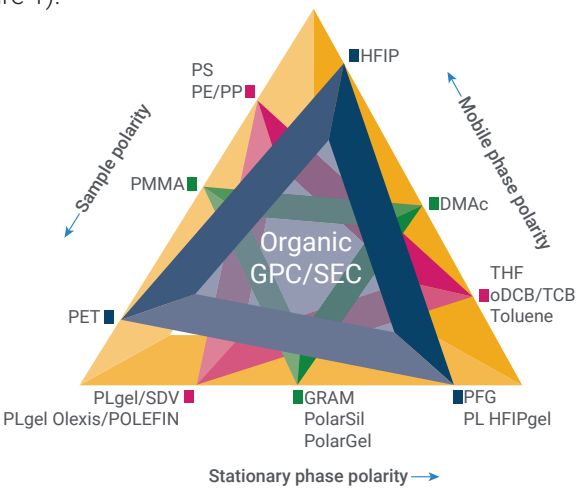

Figure 1. “Magic triangle” illustrating the fact that optimal GPC/SEC performance requires a balance between sample, stationary phase, and mobile phase polarity.

Table 2. The compatibility of solvents with Agilent POLEFIN columns.

Solvent	Polarity Index	Operating Temperature (°C)	Compatibility	Recommended Alternative
Acetone	5.1	25	Unsuitable	GRAM, PolarSil, PolarGel
Acetonitrile	5.8	25	Unsuitable	GRAM, PolarSil, PolarGel
Benzene	2.7	25	Possible	SDV, PLgel
Carbontetrachloride*	1.6	25	Unsuitable	SDV, PLgel
Chloroform*	4.1	25	Possible	SDV, PLgel
Chloronaphthalene*		210	Compatible	
Cyclohexane	0.2	25	Unsuitable	SDV, PLgel
Decalin		150	Compatible	
Dichloroethane*	3.5	25	Possible	SDV, PLgel
Dichloromethane*	3.1	25	Possible	SDV, PLgel
Dimethylacetamide	6.5	70	Unsuitable	GRAM, PolarSil, PolarGel
Dimethylformamide	6.4	70	Unsuitable	GRAM, PolarSil, PolarGel
Dimethylsulfoxide	7.2	70	Unsuitable	GRAM, PolarSil, PolarGel
Dioxane	4.8	25	Possible	SDV, PLgel
Ethanol	5.2	25	Unsuitable	GRAM, PolarSil, PolarGel
Ethyl Acetate	4.4	25	Possible	SDV, PLgel
Hexafluoroisopropanol*		40	Unsuitable	PFG, PL HFIPgel
n-Methylpyrolidone	6.7	70	Unsuitable	GRAM, PolarSil, PolarGel
m-Cresol		100	Compatible	
Methanol	5.1	25	Unsuitable	GRAM, PolarSil, PolarGel
Methyl Ethyl Ketone	4.5	25	Possible	SDV, PLgel
o-Chlorophenol*		100	Compatible	
o-Dichlorobenzene*	2.7	150	Compatible	
Tetrahydrofuran	4.2	25	Possible	SDV, PLgel
Toluene	2.3	25	Possible	SDV, PLgel
Trichlorobenzene*		160	Compatible	
Trichloroethane*		25	Possible	SDV, PLgel
Trifluoroethanol*		40	Unsuitable	PFG, PL HFIPgel
Xylene	2.5	25	Compatible	
Water	10.2	25	Unsuitable	SUPREMA, PROTEEMA, PL aquagel-OH, AdvanceBio

*** When using chlorinated or fluorinated solvents, use silver/titanium frits to avoid corrosion problems. These frits are fitted as standard to POLEFIN columns.**

Mixed solvent systems can be used with POLEFIN columns provided the recommended Polarity Index range is not exceeded.

1.3 Modifiers

It is possible to use small concentrations of modifiers to improve separation performance, reduce tailing, or as a stabilizer, e.g., butylated hydroxytoluene in trichlorobenzene.

2 Column Installation

When using the column for the first time, it is recommended to use the eluent that the column was originally supplied in and to perform a system plate count noting the backpressure due to the column. However, this is not essential.

2.1 Preparing the GPC/SEC system

Remove any existing columns and store according to the manufacturer's instructions. Connect the injector directly to the detector with appropriate tubing and connectors (Section 2.5). Transfer the GPC/SEC system from the current eluent to that required, paying attention to the miscibility and compatibility with the hardware as per the manufacturer instructions.

2.2 Eluent flow rate

The optimum eluent flow rates as well as the volume of the solvent contained in the column (the "column volume") depend on the column dimensions (Table 3).

Table 3. Recommended flow rates.

Column Dimensions	Typical Flow Rate (mL/min)	Optimum Flow Rate (mL/min)	Column Volume (mL)
50 mm x 8.0 mm	0.3 to 2.0	1.0	2.5
300 mm x 8.0 mm	0.3 to 2.0	1.0	15

Avoid subjecting the column to any sudden shocks, such as sudden changes in flow rate. When applying flow to the column, set the flow rate to 0.0 mL/min and turn on the pump. Always increase the flow rate in small increments (e.g., 25% of the optimum flow rate every 15 seconds until the desired operating flow rate is reached).

Even if the flow is in the allowed range, do not exceed the maximum pressure recommended for the column.

2.3 Temperature

When using viscous eluents, increasing the temperature decreases the eluent viscosity and therefore the column pressure, and is also beneficial to the separation. In such cases, use a low flow rate initially and heat the column at a rate no greater than 2 °C/min, ensuring that the final operating temperature is at least 10 °C below the boiling point of the eluent. When at temperature, the flow can be increased to the required rate. To cool the columns down, set a low flow rate and turn off the heating allowing the columns to cool down naturally. The maximum operational temperature is 210 °C.

2.4 Tubing

For best results, use 1/16 in OD stainless steel tubing for connections between columns, injectors, and detectors. The ID of the tubing should be 0.010 in (0.25 mm) for analytical columns (8.0 mm ID). For optimum performance, use short lengths of machine-cut capillary tubing thus ensuring that the tubing fits flush inside the fitting, minimizing dead volume and system dispersion.

Plastic tubing, such as PEEK, is not recommended, as it is incompatible with many of the typical eluents used or must be operated above the temperature and pressure ratings.

2.5 Connectors

Different column types use similar, but subtly different, fittings. The performance of the system can be negatively influenced, or, in the worst case, damage can occur if care is not taken. For best results, use standard stainless steel 1/16 in nuts and one-piece ferrule compression fittings.

WARNING

The distance from the ferrule to the end of the capillary (stop depth or seating depth) must be 1 mm (Figure 2). Different column types have differing lengths of tubing protruding from the ferrule.

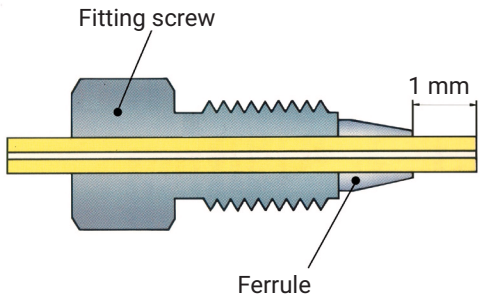

Figure 2. Schematic of the capillary connection.

Check the compatibility of the two connectors between the column and the injector and detector. For best practice, use new connectors with the Agilent column. The old connectors, together with the columns being replaced, can be put into storage if these columns will be reused.

Plastic connectors and ferrules, such as PEEK, are not recommended as they are incompatible with the typical eluents used or must be operated above the recommended temperature or pressure ratings.

2.6 Connecting the column to the injector or the preceding column in a set

Please take care when you install and test your new column. Remove the end plugs from the column and set aside for use when storing the column. When making the connections, place the wrenches on the fitting screw and column end fitting. Never use the flats that are machined on the column body.

Noting the direction of flow displayed on the column, connect the inlet fitting with the injector or preceding column using the appropriate nuts and new ferrules at each end of the tubing. Ensure that the capillary is as far as possible into the injector or column head before tightening the nut. Do not overtighten. The nut should be tightened so that the ferrule bites into the capillary tubing and no leak occurs. Excess force should not be required.

To prevent trapped air being pumped into the column, unscrew the nut at the column inlet and slowly pump a few drops of eluent to the waste before reconnecting the column.

2.7 Connecting columns in series

For connections between columns use the column connector delivered with each Agilent column. The precolumn should be connected to the injector and the remaining pore sizes connected in sequence of increasing pore size so that the largest porosity is closest to the detector. Only combine columns of the same particle size. Each time a column is added, the procedure “Connecting the column to the injector or the preceding column in a set” should be followed.

2.8 Connecting the column to the detector

Connect the outlet fitting of the column to a long piece of 1/16 in tubing to take the eluent to the waste. Pump three to four column volumes of eluent through the column to the waste at the recommended operational flow rate. Check for any leaks. Stop the flow, remove the waste tubing from the outlet, and connect to the detector using the appropriate nuts and new ferrules at each end of the tubing. Apply the desired flow rate, again checking for leaks. When the detector has stabilized, the column is ready for use.

2.9 Testing column performance

To verify correct installation, the column can be tested by measuring the plate count/meter and the resolution and comparing with the CoA, noting that the measurements are a reflection of the dispersion in the whole system and not just the column alone. See Sections 2.2 and 3.3 for optimum flow rates, concentrations, and injection volumes.

To measure plate count, inject a suitable low molecular test probe under test conditions as detailed on the CoA.

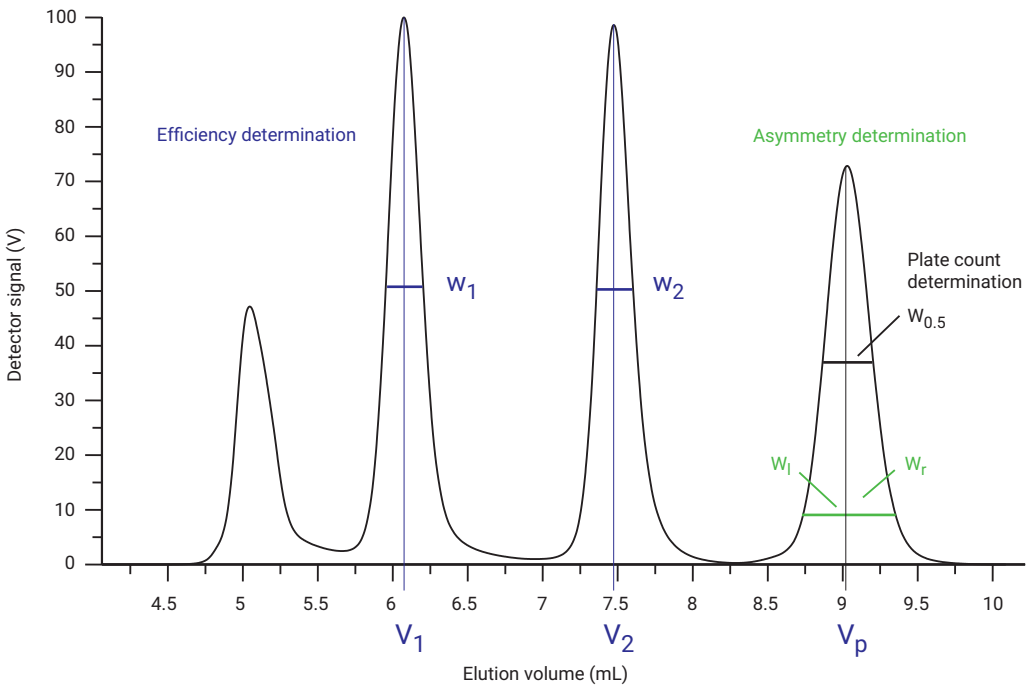

Figure 3. Variables used for determination of plate count, asymmetry, and resolution.

Determination of theoretical plates N:

$$N = \left(\frac{V_p}{\sigma}\right)^2 = 5.54\left(\frac{V_p}{w_{0.5}}\right)^2$$

Where:

V_p = Elution volume of test probe in mL

σ = Peak dispersion

$w_{0.5}$ = Width of peak at ½ height in mL = 2.35 σ

L = Column length in meters:

$$\frac{\text{Plates}}{\text{Meter}} = \frac{N}{L}$$

Determination of peak asymmetry A:

$$A = \frac{W_l}{W_r}$$

NOTE

The definition of asymmetry is as per ISO 13885-1. Sometimes, asymmetry is defined as the inverse of this formula.

A better way to determine the separation power for polymers is the calculation of the resolution factor. To do this, a mixture of polymer standards with narrow distribution of molar masses is injected:

$$\text{Resolution} = R_s = \frac{V_2 - V_1}{2(\sigma_1 + \sigma_2)} = \frac{V_2 - V_1}{0.86(w_1 + w_2)}$$

For comparison, it is better and easier to calculate the specific resolution:

$$\text{Specific resolution} = R_{sp} = \frac{0.579}{\sigma \cdot D} = \frac{Rs}{\lg\left(\frac{M_1}{M_2}\right)}$$

Where:

M_1, V_1 and w_1 are the molecular weight, elution volume, and peak width at ½ height of peak 1.

M_2, V_2 and w_2 are the molecular weight, elution volume, and peak width at ½ height of peak 2.

$$\mathbf{D = -\frac{d\lg M}{dV} = \text{slope of the calibration curve}}$$

σ = Peak dispersion obtained from plate count measurements

NOTE

When first installing a column set, it is useful to perform plate count/meter and resolution measurements as well as noting the backpressure in the chosen eluent. These parameters can be used to monitor ongoing column performance and to diagnose problems.

3 Usage Guidelines

3.1 Eluent preparation

Use high-quality HPLC grade eluents, free from large particles, filtered using a 0.45 µm filter if required. Degas the eluent thoroughly, preferably using an online degasser.

NOTE

When using POLEFIN columns with laser light scattering detectors, extra steps must be taken to obtain optimum low noise baselines. Filtering solvents under vacuum through a 0.2 µm filter and avoiding the use of salts and other additives in the eluent can improve performance.

3.2 Transferring to different solvents

Only use POLEFIN columns with compatible eluents. If the column must be used with another eluent, it can be changed by careful solvent exchange without loss of resolution. Differences in plate counts that can occur in these cases are often due to viscosity differences of the eluents.

POLEFIN columns are incompatible with very polar solvents. Residual traces of such eluents, either present in the samples or from prior use of the equipment in such eluents, can cause irreversible damage to the columns.

Before changing eluents, ensure that both the initial eluent and the desired eluent are completely miscible. If they are not miscible, seek an intermediate that is miscible with both, e.g., acetone.

Do not exceed the recommended maximum column pressure while changing solvents.

Frequent transfer of columns between eluents decreases the lifetime.
It is recommended, if possible, to have columns dedicated to certain eluents.

3.2.1 Changing to eluents of similar polarity index (e.g., column in xylene, P.I. 2.0 – 5.0)

1. Prepare the GPC/SEC system with the new eluent. Connect the column/column set to the injector and attach some 1/16 in tubing to the outlet directed to the waste.
2. Pump approximately two column volumes of the eluent at a 10th of the optimum flow rate through the column(s) and then slowly increase the flow rate to half the optimum flow rate and pump a further eight column volumes, e.g., for 300 mm x 8.0 mm columns, start at 0.1 mL/min and increase to 0.5 mL/min.

3. Stop flow and connect the column(s) to the detector.
4. Start flow at 0.1 mL/min. Adjust column temperature to the required value (Section 2.3) and when at temperature, increase the flow rate step by step up to the desired value.
5. Ensure that all detector lines are completely purged and that the system is thoroughly equilibrated before making measurements.

3.3 Sample preparation

Samples and polymer standards used for column calibration should be prepared in the same eluent as the GPC/SEC system. To minimize so-called "solvent peaks" when using RI detection, use eluent taken directly from the solvent reservoir of the GPC/SEC system.

Sample injection volume and concentration

The total amount that can be injected without deterioration in separation performance depends on the total column length and is calculated from the product of injection volume and the sample concentration. A modern approach is to keep the injection volume small to minimize dispersion caused by the loop itself. Only if required, e.g., to improve detection, vary the injected mass. The table below is for guidance only. Large pressure surges related to the injection must be avoided. It may be that for viscous solvents, viscous samples (typically very high molecular weights) and smaller particle size GPC/SEC packings that steps must be taken to reduce the pressure surge. These steps include but are not restricted to, using lower concentrations, larger injection volumes, lower flow rates, and increasing column temperature.

Table 4. Sample quantity recommendations for optimal GPC/SEC separation performance

Sample, Molecular Weight	Concentration (g/L)	Flow Rate	Injection Volume (µL) Column Set
			Column Dimension mm (L × ID)
			300 × 8.0
Plate Count	1	Optimum	20
Narrow Stds. < 1 M Da	1	Optimum	20
Narrow Stds. > 1 M < 3 M Da	<0.5	Optimum	20
Narrow Stds. > 3 M Da	<0.5	50% optimum	20
Broad Stds., Samples < 1 M Da	1 to 3	Optimum	50
Broad Stds., Samples > 1 M Da	< 1	50% optimum	50

Always ensure that samples are completely dissolved before injecting on the GPC/SEC system. High molecular weight materials require several hours for complete dissolution and are best left overnight before injection.

Sample filtration (0.5 µm or, for samples above 1 M Da, 2.0 µm) should be used after complete dissolution of the sample to prevent blockage of the columns. However, it should be noted that filtration may potentially remove some of the sample as well as unwanted particulates.

To avoid damage of the primary column system through adsorption of sample impurities or contaminants within your samples, we strongly recommend the use of a precolumn.

4 Care and Maintenance

4.1 Storage

If the columns are to be used again within one week, then it is not necessary to put the columns into storage provided that the eluent used does not degrade and there is no chance of salt precipitation. If feasible, the GPC/SEC system should either be put into recycle mode or be run at a very low flow rate.

Never leave the columns at elevated temperatures without flow or allow the columns to dry out. If recycle mode is used, replace with fresh eluent when the system is used again.

Replace any eluent that contains salt or acids with pure eluent before storage. All compatible eluents are suitable for storage, but the standard shipping solvent (Specifications 1.1) is recommended to avoid freezing at ambient temperatures. When removing the column(s) from the system, the end plugs must be replaced to prevent the column(s) drying out. If storing in volatile eluents, store the column(s) in a cool place. Never let the eluent freeze.

4.2 Troubleshooting

Table 5. Troubleshooting guidance.

Problem	Potential Cause	Corrective Action	Recommendation
Increase in operating pressure	Blockage of solvent filter between pump and injector	Replace filter	Check pump seals and solvent quality
	Blocked or overtightened connecting tubing	Replace connector	Check sample solubility and filter samples
	Blocked inlet frit of column	Replace frit or replace column	Check solvent quality and sample solubility
	Adsorption of sample on column	Replace precolumn; undertake column regeneration	Alter analysis conditions to prevent adsorption
Decrease in operating pressure	Air in the system; leak	Check degassing of solvent; check connectors	Check if degasser is working; replace worn connectors
Loss of plate count or resolution	Worn or badly made connections	Check and replace connectors if required	
	Problem with injector	Check injector	
	Column frits blocked	Undertake column maintenance	
	Precolumn blocked/saturated	Replace precolumn	
	Analytical column damaged	Replace column	
Changing peak shape	Column damaged	Check plate counts	Replace or repair damaged column
	Sample adsorbing	Clean columns; replace precolumns	Find analysis conditions to prevent adsorption
No peak	Problem with injector	Check injector	
	Problem with detector	Check detector	
	Sample adsorbing	Clean columns; replace precolumns	Alter analysis conditions to prevent adsorption

When diagnosing problems, it is recommended to perform a series of logical experiments by removing components such as columns or connectors from the system one by one to identify which component is the cause of the problem.

4.3 Column repair

For best results, perform repairs on columns individually using pure eluents with no salts or acids. Always flush the columns to the waste.

The performance of a column may deteriorate due to blockage of the frits, sample adsorption, or damage to the packing bed causing an increase in backpressure.

4.3.1 Partially dried out columns

During storage, some of the solvent may evaporate through the threads (this typically happens during long storage times and with high storage temperatures and volatile eluents). It is a good practice to keep columns with volatile eluents in a refrigerator when not in use, to prevent solvent evaporation. It is an indication of a partially evaporated column solvent if the expected pressure does not build up or the pump constantly readjusts the flow. In this case, reduce the flow down to 0.1 mL/min at once (to prevent damaging the packing) and fill the column until no more bubbles appear at the column outlet and column backpressure is stable. Now add the next column using the previously mentioned procedure for installation until each column is installed in your system.

NOTE

If you want to install a complete set of columns where the solvent has been partially evaporated, you can connect all columns when solvent appears from the previous column. The flow rate can be set at 0.1 mL/min unattended (for example overnight) with no negative effects.

4.3.2 Partially blocked columns

Columns become partially blocked when particulates get trapped in the frits or in the packing bed and/or sample components adsorb on the frits or packing material. Due to the varying chemical nature of possible samples and column history, there is no single treatment that is successful in all cases. It is often not possible to recover a column. Certain steps can be taken, either individually or together, to try to at least partially recover performance. These steps should be undertaken on individual columns to avoid potential contamination problems.

Reverse flush the column to the waste at a low flow rate (25% of the optimum flow rate) for at least four column volumes. Afterwards, reconnect the column in the correct direction. Slowly increase the flow rate to see if the backpressure generated on the column has reduced and if it can be operated under normal conditions. If the pressure is now reasonable, perform a plate count test and also, if desired, a resolution test. If the pressure remains high, either try the following additional steps below or directly change the frits.

1. Increase the temperature of the column in combination with the procedure in Section 4.3.2 following the guidelines in Section 2.3. Flush for 10 column volumes.
2. Change the eluent and/or use modifiers to try to dissolve any adsorbed components in combination with the procedure in Section 4.3.2 and, if appropriate, step 1 in this section. Flush for 10 column volumes.

4.4 Changing frits

This procedure can be used for changing both the inlet and outlet column frit. The inlet frit is particularly susceptible to blocking. The column should only be opened to change frits if a blockage could not be removed by the above measures.

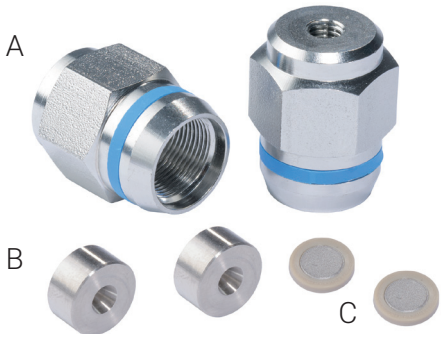

Figure 4. Components of the column head
A) End fittings
B) Fitting adapters
C) Frits

1. Remove the column from the system and replace the end plug in the end that is not being changed.
2. Using two wrenches, one placed on the flat of the column body nearest the end fitting (Figure 4) to be changed and the end fitting itself, slowly unscrew and remove the end fitting, the end fitting adapter, and finally slide off the frit and seal from the top of the column.
3. Inspect the top of the gel packing to ensure that there are no holes in the gel packing.
4. Inspect the end fitting adapter to ensure it is not damaged.
5. Rinse end fitting and fitting adapter to ensure that they are entirely clean and free of particles. Re-assemble the fitting adapter (flat end towards the frit) and a new frit in the end fitting.
6. Holding them at an angle of 45° to each other, screw the end fitting, the adapter, and new frit back onto the column until finger tight. Then use the wrenches to tighten by a further 60° turn.
7. Connect the column in reverse direction and flush for three column volumes to the waste, ensuring that gel particles do not elute due to a poorly seated frit and seal.
8. Connect the column in the correct direction and check the pressure and plate count.

5. Ordering Information

Table 6. Ordering information for spare parts.

Part Number	Description
299-2003	Replacement Ag/Ti frits for 8.0 mm ID column (pk of 2)
PL1310-0008	Tubing ferrules, 1/16 in, 5/pk
PL1310-0007	Column connecting nuts 1/16 in, 5/pk
PL1310-0048	Connecting tubing for inter-column connection, 100 mm length, ID 0.25 mm, 10/pk, for 8.0 mm and 20 mm ID columns, combine with ferrules (PL1310-0008) and nuts (PL1310-0007)
5022-6508	Connector from column to injector/detector, 280 mm length, ID 0.25 mm, for 8.0 mm ID columns, combine with ferrules (PL1310-0008) and nuts (PL1310-0007)

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