Agilent

Agilent NOVEMA Max 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 NOVEMA Max 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

NOVEMA Max columns are packed with highly porous particles of a modified acrylate copolymer. Columns are supplied as standard in water with 7.5 mM sodium azide to prevent microbial growth and tested for efficiency with ethylene glycol (EG).

Table 1. Operational specifications for Agilent MCX 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 Ethylene Glycol in Water 7.5 mM NaN_3
NOVEMA Max 5 µ	60/870	150/2,180	80 °C	>40,000
NOVEMA Max 10 µ	40/580	120/1,740	80 °C	>20,000
NOVEMA Max Prep	40/580	80/1,160	80 °C	>15,000

1.2 Solvent compatibility

NOVEMA Max is compatible with a wide range of aqueous eluents and has optimum performance, when used with eluents that have a pH in the range 1.5 to 7.0 for the analysis of cationic macromolecules. Transfer to compatible eluents is easy provided the guidelines in this user manual are followed.

For solvents outside this pH range, other Agilent column materials may be more appropriate to maintain a balance between sample ionic character, eluent pH, and packing material polarity for a size-only based separation based on the "magic triangle" concept (Figure 1).

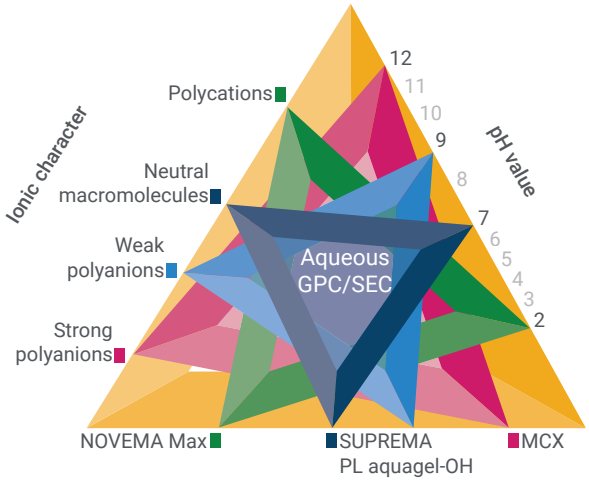

Figure 1. "Magic triangle" illustrating the fact that optimal aqueous GPC/SEC performance requires a balance between sample ionic character, stationary phase, and mobile phase pH.

Table 2. Experimental characteristics of Agilent NOVEMA Max columns.

Parameter	Range	Comment
pH Stability	2 to 12	Chemical stability
Optimum pH Range	1.5 to 7.0	Due to cationic nature of the surface
Temperature	<80 °C	Operate at 10 to 35 °C for optimum column lifetime
Organic Modifiers	<100% v/v	e.g., methanol, acetonitrile, tetrahydrofuran
Salts	< 0.5 M	e.g., NaN_3, NaCl, $NaNO_3$
Buffers		Compatible with most commonly used buffers

1.3 Example conditions

Table 3. Example experimental conditions when using Agilent NOVEMA Max columns.

Eluent	Analyte
Water, 0.1 M NaCl, 0.3 vol % Formic Acid	Polycations, polymeric quaternary ammonium compounds), poly (DADMAC), poly(vinylpyridine), chitosan, poly(ethyleneimine)

NOTE

It is recommended that 2 to 10 mmol/L NaN_3 is added to the eluent to prevent microbial growth.

2 Column Installation

When using columns for the first time, new NOVEMA Max columns must be conditioned. Prepare a 1 vol% solution of formic acid in water (approx. 150 mL per 8 x 300 mm analytical column required). With the column(s) not connected to the detector, pump a minimum of 10 column volumes of this solution at a flow rate of 0.2 mL/min through the column directly to the waste. Following this conditioning step, the column can be used as normal in the eluent of choice. It is also recommended to use the eluent that the column was originally tested 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's instructions.

2.2 Eluent flow rate

The optimum eluent flow rates and the volume of the solvent contained in the column (the "column volume") depend on the column dimensions (Table 4).

Table 4. 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
50 mm x 20 mm	1.0 to 12	6.25	16
300 mm x 20 mm	1.0 to 12	6.25	94

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 hence 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 80 °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) and 0.020 in (0.5 mm) for preparative columns (20 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, should be used with caution, checking 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.

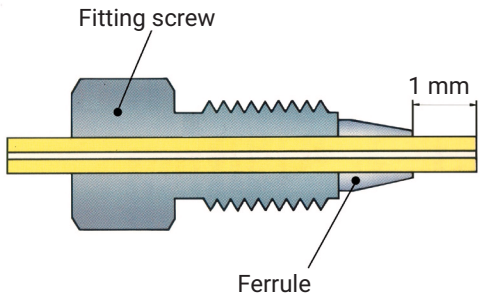

Figure 2. Schematic of the capillary connection.

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, should be used with caution, checking the temperature and pressure ratings. However, these connectors offer some practical advantages in that the ferrule does not permanently grip the tubing. The connector position can also be easily adjusted to accommodate the different lengths of protruding tubing used by the different manufacturers while forming a leak-tight connection with little dead volume.

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

Please use 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 etched 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 enough 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. To avoid porosity mismatch, we advise using the recommended column combinations shown in Table 5. Do not combine linear/mixed bed/multipore columns with single porosity columns. Each time a column is added, the procedure “Connecting the column to the injector or the preceding column in a set” should be followed.

Table 5. Recommended analytical column sets.

Separation Range [Da]	Description
100 to 100,000	1 x NOVEMA Max precolumn, 5 µm, 8 x 50 mm (part number NMA080505) 3 x NOVEMA Max analytical column, 5 µm, 100 Å, 8 x 300 mm (part number NMA0830051E2)
100 to 1,000,000	1 x NOVEMA Max precolumn, 5 µm, 8 x 50 mm (part number NMA080505) 1 x NOVEMA Max analytical column, 5 µm, 30 Å, 8 x 300 mm (part number NMA0830053E1) 2 x NOVEMA Max analytical column, 5 µm, 1000 Å, 8 x 300 mm (part number NMA0830051E3)
100 to 3,000,000	1 x NOVEMA Max precolumn, 10 µm, 8 x 50 mm (part number NMA080510) 1 x NOVEMA Max analytical column, 10 µm, 100 Å, 8 x 300 mm (part number NMA0830101E2) 2 x NOVEMA Max analytical column, 10 µm, 3000 Å, 8 x 300 mm (part number NMA0830103E3)
100 to 30,000,000	1 x NOVEMA Max precolumn, 10 µm, 8 x 50 mm (part number NMA080510) 3 x NOVEMA Max analytical column, 10 µm, ultrahigh, 8 x 300 mm (part number NMA083010LUH)

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 3-4 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.

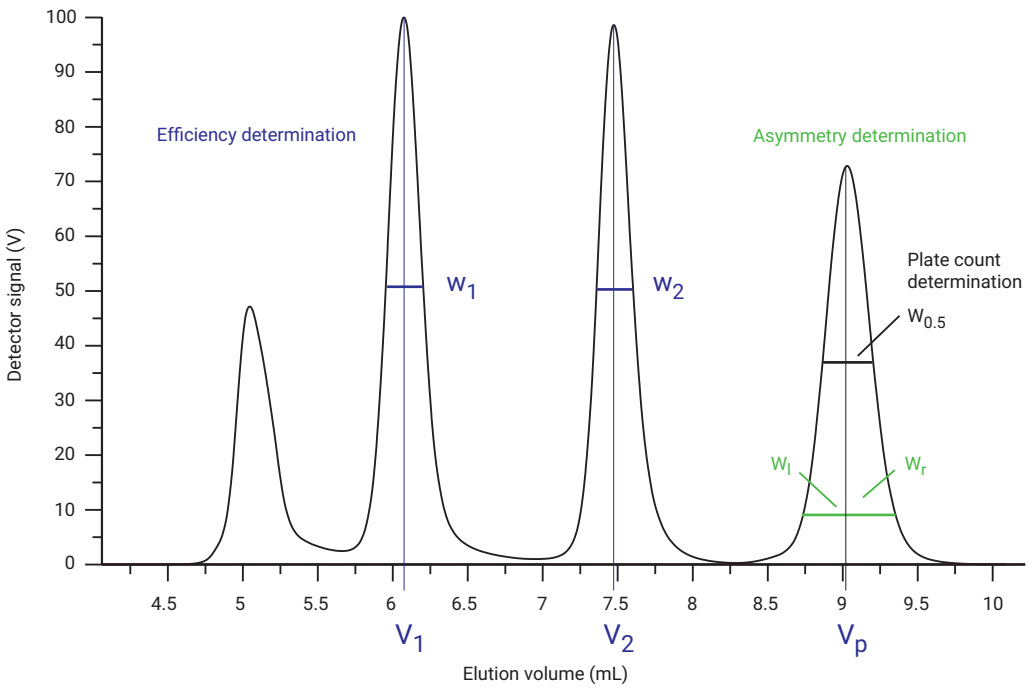

Figure 3. Variables used for determination of plate count, asymmetry, and resolution.

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 NOVEMA Max columns with laser light scattering detectors, it is recommended that they be purchased pre-equilibrated for light scattering (NOVEMA Max Lux, see part numbers in Table 6). Extra steps must be undertaken 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.

Table 6. NOVEMA Max Lux columns pre-equilibrated for use with light scattering detectors.

Part Number	Description
NMA080505LS	NOVEMA Max Lux precolumn, 8 x 50 mm, particle size 5 µm
NMA0805010LS	NOVEMA Max Lux precolumn, 8 x 50 mm, particle size 10 µm
NMA0830051E2LS	NOVEMA Max Lux analytical column, 8 x 300 mm, particle size 5 µm, 100Å, molecular weight separation range 100 to 100,000 Da
NMA0830051E3LS	NOVEMA Max Lux analytical column, 8 x 300 mm, particle size 5 µm, 1000Å, molecular weight separation range 1,000 to 1,000,000 Da
NMA0830053E1LS	NOVEMA Max Lux analytical column, 8 x 300 mm, particle size 5 µm, 30Å, molecular weight separation range 100 to 30,000 Da
NMA0830101E2LS	NOVEMA Max Lux analytical column, 8 x 300 mm, particle size 10 µm, 100Å, molecular weight separation range 100 to 100,000 Da
NMA0830103E3LS	NOVEMA Max Lux analytical column, 8 x 300 mm, particle size 10 µm, 3000Å molecular weight separation range 1,000 to 3,000,000 Da
NMA083010LUHLS	NOVEMA Max Lux analytical column, 8 x 300 mm, particle size 10 µm, ultrahigh, molecular weight separation range 100 to 30,000,000 Da

3.2 Transferring to different solvents

Only use NOVEMA Max columns with compatible eluents. If the column has to 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.

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

3.2.1 Changing eluents

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

Frequent transfer of columns between eluents decreases the column lifetime.

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. Then 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 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.

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. If required to improve detection, vary the injected mass. The information provided in Table 7 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 7. Sample quantity recommendations for optimal GPC/SEC separation performance.

Sample, Molecular Weight	Concentration (g/L)	Flow Rate	Injection Volume (µL) Column Set	
			Column Dimensions mm (L × ID)	
			300 x 8.0	300 x 20
Plate Count	1	Optimum	20	50
Narrow Stds. < 1 M Da	1	Optimum	20	<1,000
Narrow Stds. > 1 M < 3 M Da	< 0.5	Optimum	20	<1,000
Narrow Stds. > 3 M Da	< 0.5	50% optimum	20	<1,000
Broad Stds., Samples < 1 M Da	1 to 3	Optimum	50	<1,000
Broad Stds., Samples > 1 M Da	< 1	50% optimum	50	<1,000

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 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 dry out. If recycle mode is used, replace it with fresh eluent when the system is used again.

Replace any eluent that contains salt or acids before storage. Store the columns in water/NaN_3 to prevent microbial growth. When removing the column(s) from the system, the end plugs must be replaced to prevent the column(s) drying out. Store in a cool place but never let the eluent freeze.

4.2 Troubleshooting

Table 7. 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 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 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, immediately reduce the flow down to 0.1 mL/min (to prevent damage to the packing) and fill the column until no more bubbles appear at the column outlet and column backpressure is stable. Then add the next column using the previously mentioned installation procedure 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 then be set to 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. There are some steps either individually or performed together to try to at least partially recover performance. These 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 extra steps, or directly change the frits.

1. Increase the temperature of the column in combination with the procedure from 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 from 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 previously mentioned measures.

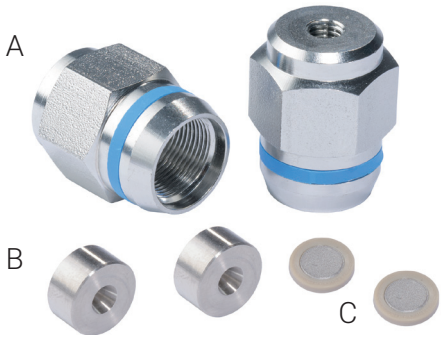

Figure 4. Components of the column head
A) End fittings
B) Fitting adapters
C) Frits

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 8. Ordering information for spare parts.

Part Number	Description
299-2045	PEEK/titanium replacement frits for 8.0 mm ID column (pk of 2)
299-2047	PEEK/titanium replacement frits 20 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)
5022-6510	Connector from column to injector/detector, 300 mm length, ID 0.5 mm, for 20 mm ID columns, combine with ferrules (PL1310-0008) and nuts (PL1310-0007)

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