



Original-
Manual



YMC Pilot^{PLUS}

Low pressure chromatography column

In order to avoid personal injuries and damage to equipment, please read this manual carefully and act on the recommendations given. Keep this manual accessible for the user at all times.

YMC Europe GmbH



Schöttmannshof 19

46539 Dinslaken, Germany



+49 02064 427-0



Info@ymc.de



www.ymc.de

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1 Information on manual

1.1 General

This manual includes important messages and information for the intended use of this pilot column. The manual must be kept accessible for the user at all times.

In order to avoid injuries and damages, read this manual carefully prior to use and act on the recommendations given.

1.2 Explanation of warnings

Warnings are highlighted by coloured signal word fields. Always read the full text of the warning in order to effectively protect yourself from damage!

The following signal word fields marked by different colours and signal words distinct security levels:



DANGER

Disregarding this warning will result in death or serious injuries.



WARNING

Disregarding this warning may result in death or serious injuries.



CAREFUL

Disregarding this warning may result in minor or moderate injuries.



ATTENTION

Disregarding this warning can result in damage to surrounding property.

Warnings are always in the same structure. They include signal word, type and source of danger, consequences of neglect and security measures / avoidance.

1.3 Structure of instructions

The instructions relate directly to an activity. They are structured in a task oriented manner. Always perform the individual steps in the order in which they are given.

Instructions are structured as follows and are marked by the corresponding levels:

▶ Aim of the instruction

1. Action step

- ✓ Impact of the action step to check whether the step has been performed correctly.

2. Another action step

Figures within instructions:

A figure is only related to the action step which is listed directly below the figure.

1.4 Guidelines

The following guidelines are available in this manual:

- Contents at the beginning
- Index in the end
- Name of the main Chapter in the header
- Links to other Chapters, for example "Information in this manual" (▶Chapter 1, S. 5)
- Additional information and hints indicated with an i-symbol



Example for additional information

1.5 Figures

Figures can differ in the graphical presentation from the product.

2 Safety

This section gives an overview of all the important safety aspects for the protection of personnel and for the safe and trouble-free handling of the YMC Pilot^{PLUS} column. Other task-specific safety requirements are contained in the sections relating to the respective operation. In addition, the national and local legal and other applicable safety regulations must be observed.

2.1 Intended use

The pilot columns are only to be used for the separation of substances via chromatographic techniques.

The intended use of this product must be performed with regards to the specifications ►Chapter 3.2, S. 9. The product is only for use with liquids that have a pressure <0.5 bar above the atmospheric pressure at the intended maximum temperature (40°C). Every use which is outside these specifications is defined as not intended use. The intended use includes the compliance with the advice and terms in the manual.

2.2 General safety instructions

In order to avoid accidents and injuries,

- operate the pilot column only within its intended use.
- follow all general and special safety instructions as well as all warnings in this manual.
- follow danger signs and sign giving instructions.
- follow the rules and regulations for prevention of accidents at the site of operation.
- comply with all inspection and maintenance intervals.

2.3 Personnel requirements

The various tasks described in this manual make different demands on the qualifications and training of the persons carrying them out.





The user must:

- have competent and proficient training.
- be trained in dealing with pressure devices.
- read and fully understand this manual before using this YMC Pilot^{PLUS} column.

2.4 Personnel protective equipment

In order to reduce the risks during working on the column, always use the recommended personnel protective equipment.

The following protective equipment is required:

Symbol	PPE	Phase of life	Action
	Protective gloves	Operation	<ul style="list-style-type: none"> • Column packing • Column unpacking
		Initiation	<ul style="list-style-type: none"> • Cleaning
	Safety goggles	Operation	<ul style="list-style-type: none"> • All actions
		Initiation	<ul style="list-style-type: none"> • Cleaning • Pressure testing
	Dust mask	Operation	<ul style="list-style-type: none"> • Slurry preparation
	Protective clothing	Operation	<ul style="list-style-type: none"> • All actions
		Initiation	<ul style="list-style-type: none"> • Cleaning • Pressure testing

3 Specifications and overview

3.1 Fields of application

The YMC Pilot^{PLUS} pilot column is especially designed for pilot scale use. The column is compatible with common BioLC separation modes (IEX, SEC, Affinity, IMAC, HIC, MMC) and aqueous conditions. Due to the metal-free and the minimal dead volumes, the column is perfectly suitable for the separation of biomolecules (e.g. proteins, oligonucleotides or nucleic acids).

3.2 Specifications

PED* fluid group:	Group 1 referred to EU Guideline 2014/68/EU
PED* safety requirement:	Article 4.3 of EU Guideline 2014/68/EU
Column body:	Borosilicate glass 3.3 Calibrated precision glass (Duran KPG®)
Frits:	PE
Seals:	75 Fluoroprene XP41 blue
Connection:	FEP tubing ¼" OD
Wetted parts:	Borosilicate glass 3.3, FEP, PE, PTFE, PEEK, 75 Fluoroprene XP41 blue
Approved temperature range	4 – 40°C

*Pressure Equipment Directive

Length Glass Body [mm]	500				850			
Inner Diameter [mm]	70	100	140	200	70	100	140	200
Bed Length [mm]	100 - 450 ¹				250 - 800 ¹			
Volume [mL]	0.4 – 1.7	0.8 – 3.5	1.5 – 6.9	3.1 – 14.1	1.0 – 3.0	2.0 – 6.2	3.8 – 12.3	7.8 – 25.1
Pressure Limit [bar]	16	10	7	5	16	10	7	5
Total Weight Empty Column [kg]	19	20	37	38	23	24	45	47
Footprint [mm]	620		750		620		750	
Maximum Height [mm]	1,700		1,750		2,250		2,300	

¹Depending on packing material and packing method

3.3 Chemical Resistance

The chemical resistance depends on the wetted materials used ▶ Chapter 3.2, S. 9.



If the desired solvent is not listed, YMC will be pleased to advise you.

Material Lösungsmittel	Borosilicate glass 3.3	FEP	75 Fluoro- pren XP41	PE	PTFE	PEEK
Acetone	Good	Good	Unstable	Good	Good	Good
Acetonitrile	Good	Good	Limited	Good	Good	Good
Dihydrogen phosphate	Good	Good	Good	Good	Good	Good
Cyclohexane	Good	Good	Good	Limited	Good	Good
Dichloromethan	Good	Good	Limited	Unstable	Good	Unstable
0.1M EDTA (3%)	Good	Good	Good	Good	Good	Good
1M Acetic acid (6%)	Good	Good	Good	Good	Good	Good
Ethanol	Good	Good	Good	Good	Good	Good
Ethyl acetate	Good	Good	Unstable	Good	Good	Good
n-hexane	Good	Good	Good	Limited	Good	Good
Isopropanol	Good	Good	Good	Good	Good	Good
Methanol	Good	Good	Good	Good	Good	Good
2M Sodium hydroxide (8%)	Good	Good	Good	Good	Good	Good
1M Hydrochloric acid (4%)	Good	Good	Good	Good	Good	Good
8M Urea (36%)	Good	Good	Good	Good	Good	Good
1M NaCl	Good	Good	Good	Good	Good	Good
0,5M Na ₂ SO ₄	Good	Good	Good	Good	Good	Good

YMC recommends avoiding the following chemicals:

- Strong oxidizing agents
- Halogenated compounds
- Chlorinated solvents
- Aromatic compounds (e.g. toluene)
- High concentrations of strong acids
- THF

3.4 Overview

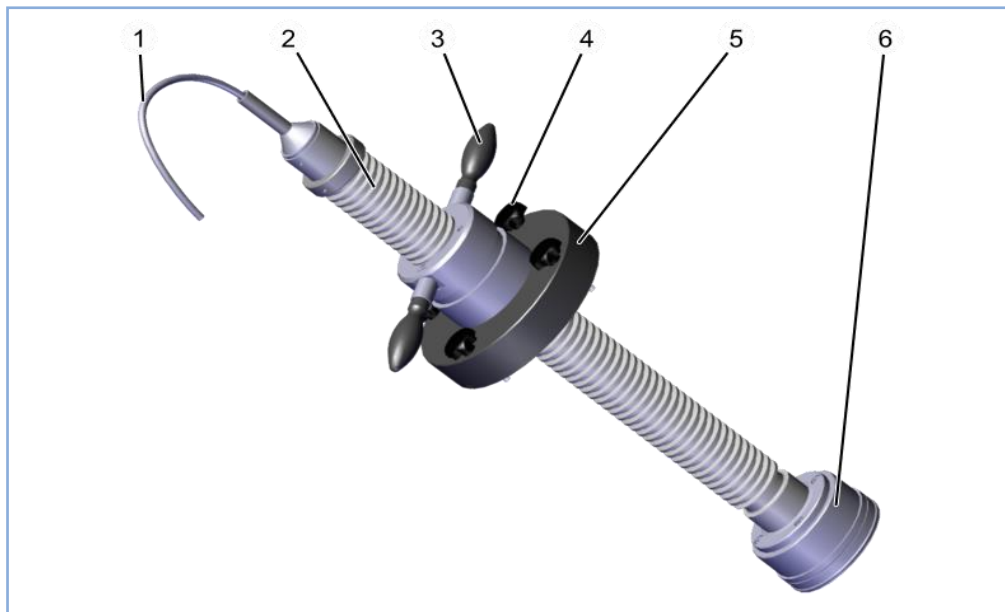


A parts list with all wetted parts and non-wetted parts is in the appendix.



The YMC Pilot^{PLUS} column consists of the adjustable end piece, the column body and the column stand. These parts can be assembled whilst operating the column without any tools.

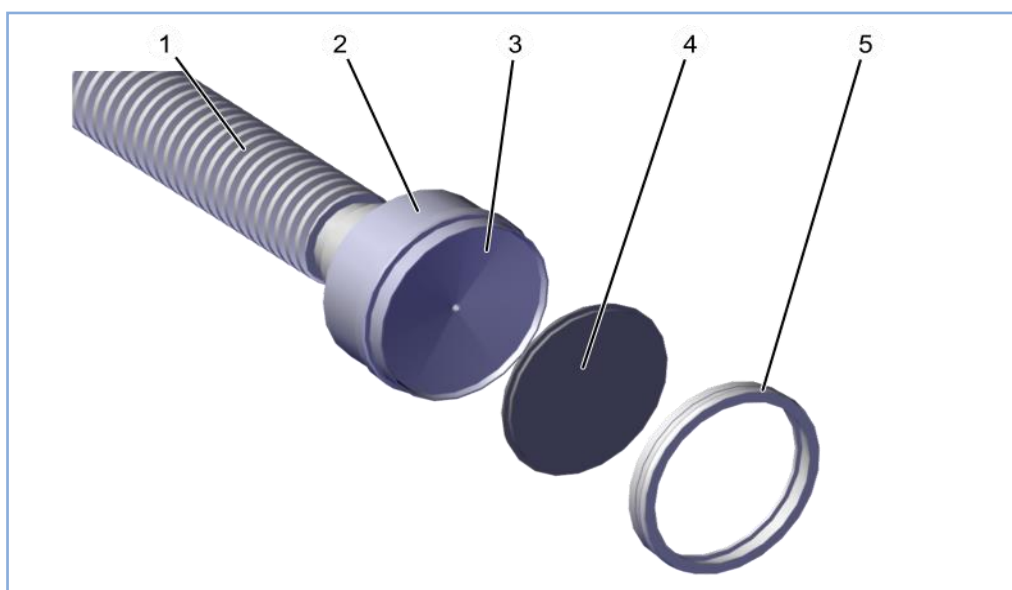
3.4.1 Adjustable end piece



- | | |
|-----------------|----------------------|
| 1 Tubing | 4 Star screw |
| 2 Spindle | 5 Closing disc (AEP) |
| 3 Rotary Handle | 6 Connecting flange |

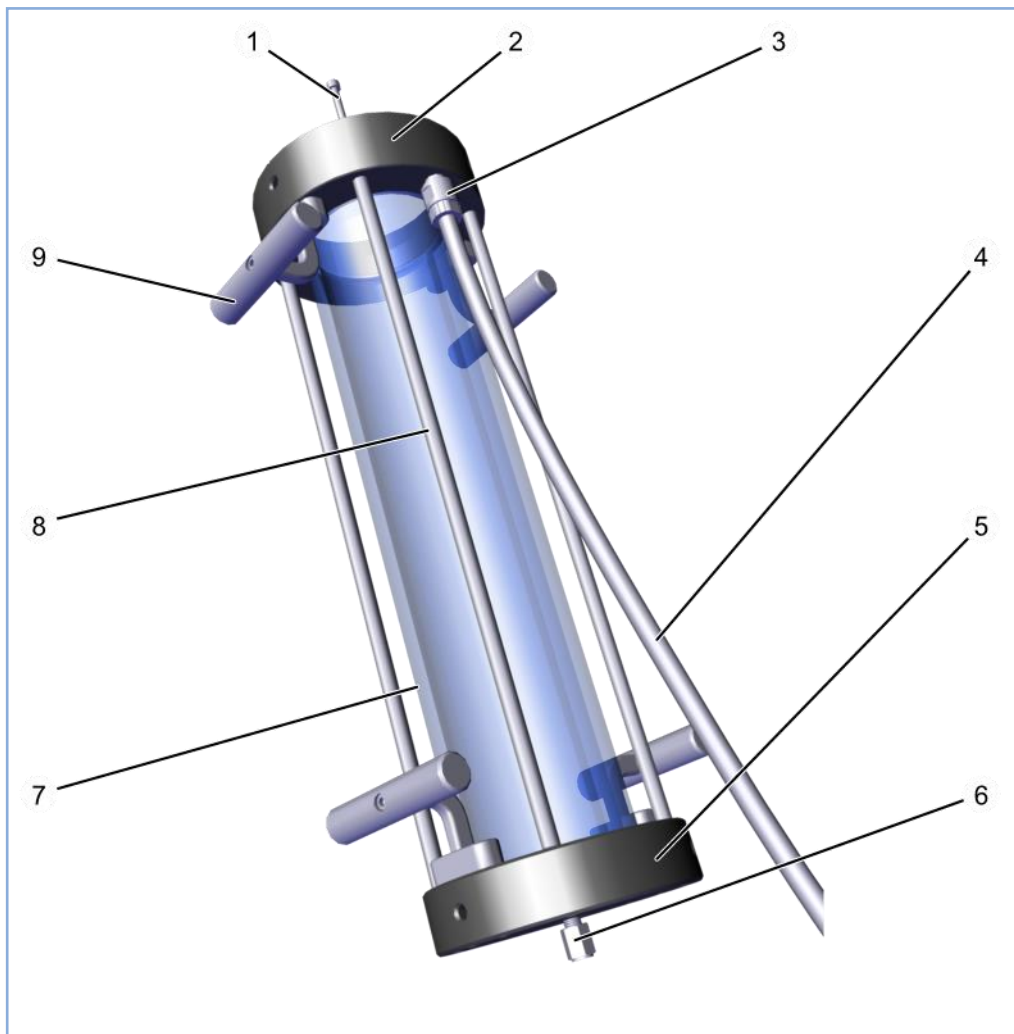
The adjustable end piece consists of a closing disc and the piston. The piston consists of the spindle, the connecting flange, the plunger, the frit and the profile gasket. A tubing goes through the spindle to the connecting flange. With the rotary handles in the closing disc, the piston can be adjusted up and down. The closing disc is attached with star screws to the column body.

Piston front



- | | |
|---------------------|------------------|
| 1 Spindle | 4 Frit |
| 2 Connecting flange | 5 Profile gasket |
| 3 Plunger (AEP) | |

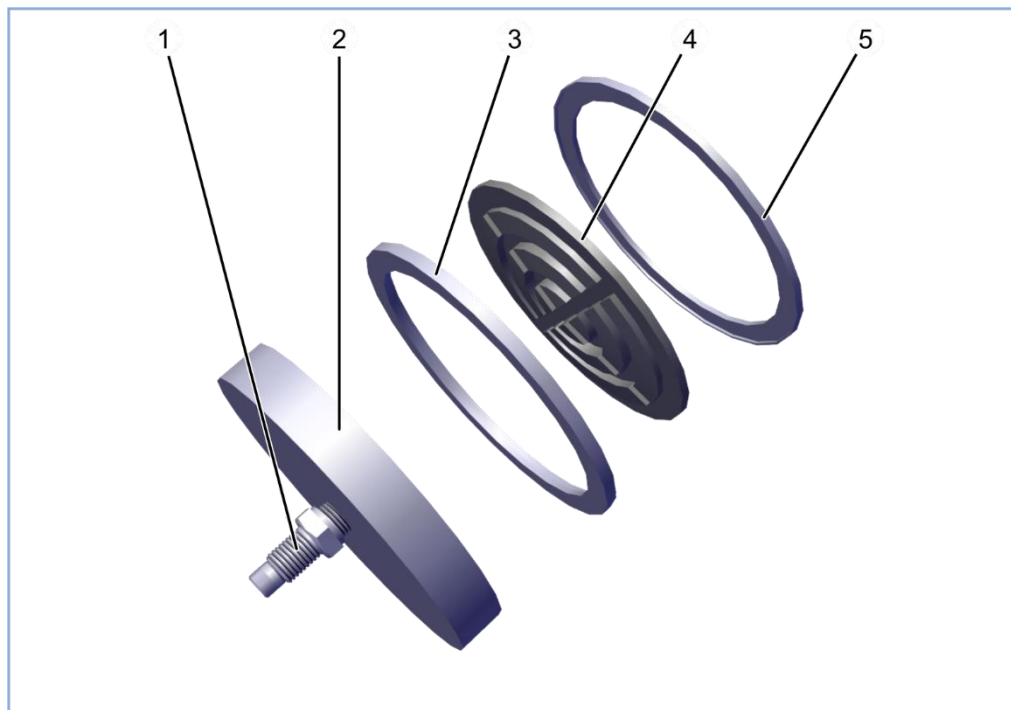
3.4.2 Column body



- | | | | |
|---|---------------------------|---|-------------|
| 1 | Cylinder screw | 6 | Connector |
| 2 | Column disc | 7 | Glass body |
| 3 | Connector drainage tubing | 8 | Rod |
| 4 | Drainage tubing | 9 | Holding bar |
| 5 | Closing disc (CB) | | |

The glass body is fixed between the column disc and the closing disc by the rods. The glass body has a visible scale. Excessive solvent is removed during piston insertion via the connector drainage tubing. The fixed end piece is assembled with the closing disc (CB). The fixed end piece consists of the connector, the sealing washer, the plunger (CB), the ring gasket B, the frit (CB) and the ring gasket A. The column body can be assembled in the stand in two different positions.

Frit column body



1 Connector without cap nut

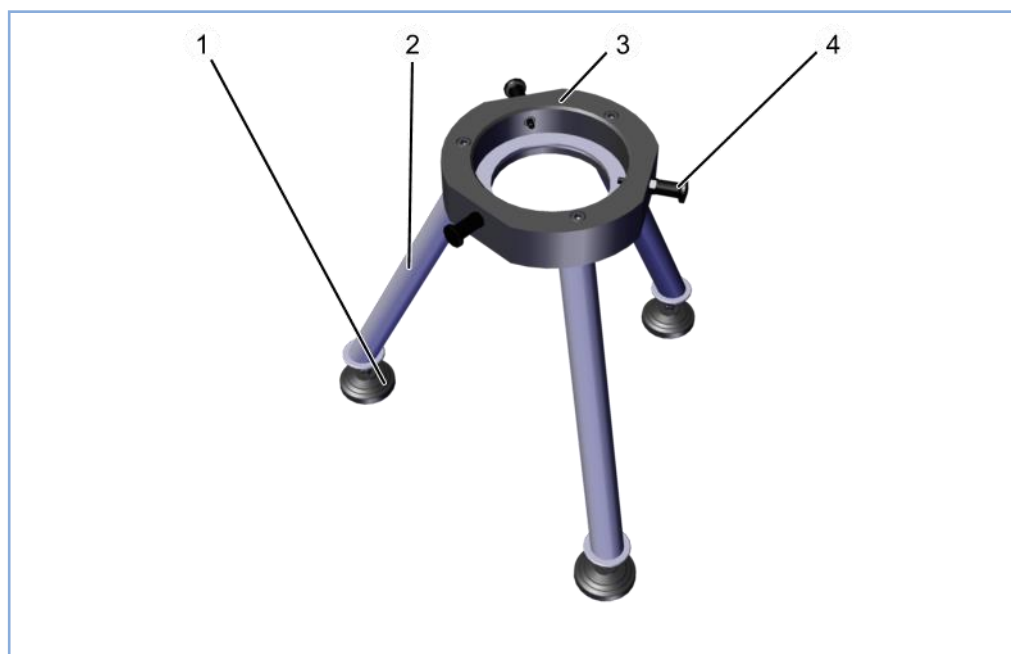
2 Plunger (CB)

3 Ring gasket B

4 Frit (CB)

5 Ring gasket A

3.4.3 Column stand



1 Levelling feet

2 Main pillar

3 Frame disc

4 Locking bolts

With the locking bolts in the frame disc the column body is attached to the column stand. With the adjustable levelling feet, the pilot column can be aligned vertically. Alternatively, the column stand is available with casters.

4 Delivery

4.1 Delivery contents

- Pilot column
- Documentation folder:
 - Brochure YMC Glass Columns
 - Manual
 - Overview drawings
 - YMC Quality Control / Leakage Tests
 - Certificate of conformity FDA/BSE/TSE/USP Class VI

4.2 Delivery inspection



YMC recommends retaining and storing the original packaging and the foam cushion so that the column can always be shipped safely.

Delivery inspection

- ▶ Delivery inspection is performed as follows:
 1. Check the delivery for visible transport damage.
 2. Check the delivery for the completeness of parts.
 3. Compare the column description on the glass body with the data of the delivery note.

Transport damages

- ▶ Procedure, if you observe any visible transport damage:
 1. Refuse or reserve acceptance of the delivery.
 2. Record the transport damage on the delivery note of the transporter or in the transport documents.
 3. Inform YMC Europe GmbH and/or the responsible supplying agent.

5 Requirements for Operation

5.1 Pilot column set up

Required devices:

- Drainage tubing
- Collecting vessel
- ▶ The pilot column is set up as follows:
 1. Make sure that the pilot column is standing on fixed ground.
 2. Make sure that the pilot column is standing vertical in the final operating place.
 3. Connect the drainage tubing to the drainage tubing connector.
 4. Put the drainage tubing in the collecting vessel.

5.2 Pressure test

⚠ WARNING

Operation of pressure tests

Eye or skin injuries possible due to solvent leakage or incorrectly sealed connectors.

- ▶ Perform the pressure test behind a safety screen.
- ▶ During work on the pilot column, wear the prescribed personnel protective equipment.

⚠ WARNING

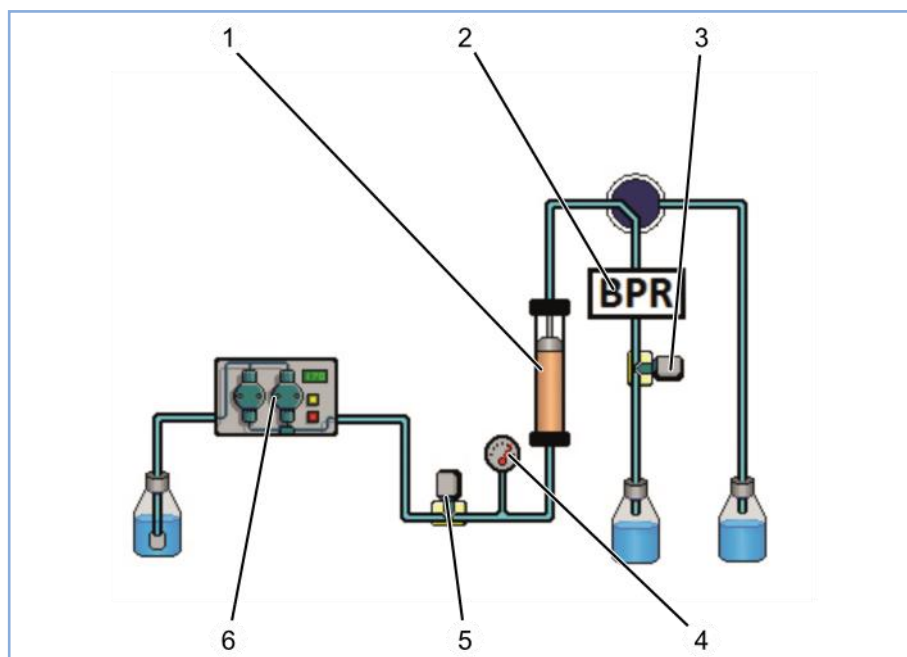
Pilot column under high pressure

Eye or skin injuries possible due to remaining pressure in the tubing or in the pilot column.

- ▶ Stop the pump during work on the pilot column.
- ▶ During work on the pilot column, wear the prescribed personnel protective equipment.

A pressure test is used to check the tightness of both the seals in the pilot column and also the tubing connectors. Before using the column for chromatographic applications, it is recommended that a pressure test is performed to verify the correct assembly. Prior to delivery of the column, the column has been tested according to the standard procedure listed below. The relevant certificate is located in the documentation folder.

Standard procedure pressure test



- | | |
|---------------------------|------------------|
| 1 Pilot column | 4 Pressure gauge |
| 2 Back pressure regulator | 5 Gate valve |
| 3 Gate valve | 6 Pump |

► The pressure test should be performed as follows:

1. Assemble the system according to the schematic overview of a pressure test.
 2. Turn the piston of the adjustable end piece in the DOWN direction as far as it will go.
 3. Fill the pilot column with isopropanol.
 4. Increase the testing pressure in the pilot column by slow and stepwise adjustment of the flow rate at the pump.
 - ✓ The testing pressure is the pressure limit of the column plus 10% (70 mm ID = 17.6 bar | 100 mm ID = 11 bar | 140 mm ID = 7.7 bar | 200 mm ID = 5.5 bar)
 5. Keep the testing pressure constant for 10 minutes.
 6. If leakages occur, check the connections. If required, replace tubing and/or connectors.
- If the testing pressure was constant for 10 minutes, the column is ready for use.



If a back pressure regulator is used, the maximum pressure is reached easier.



Leakages at the piston of the adjustable end piece are often related to impurities trapped in the seal.

6 Operation

WARNING

Risk of explosion

Severe injuries possible due to glass splinters.

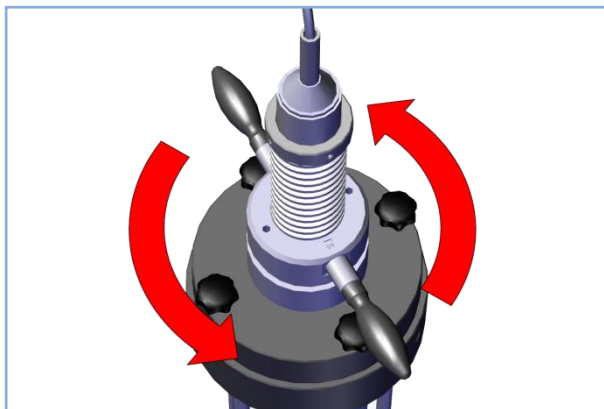
- ▶ Never put the glass column under gas pressure!

In order to guarantee safe operation, the pilot column must be used for the intended use only. (▶Chapter 2.1, S. 7).

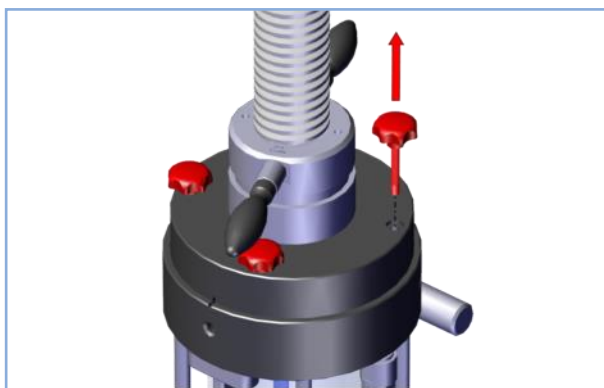
6.1 Removal of adjustable end piece

Required devices:

- Clean and soft surface
- ▶ The adjustable end piece is removed as follows:



1. Turn the piston of the adjustable end piece in the UP direction as far as it will go.



2. Remove the star screws from the closing disc (AEP).
3. Remove the adjustable end piece from the glass body and put it on a clean and soft surface.

6.2 Pilot column packing

CAREFUL

Packing material

Eye and skin injuries possible through contact with packing material.

- ▶ Wear the prescribed personnel protective equipment.

Two different procedures are common for slurry packing the column:

- under flow,
- with sedimentation.

6.2.1 Slurry preparation

CAREFUL

Formation of dust with dry packing material

Lung irritation possible if packing material is inhaled.

- ▶ Read the material safety data sheet from the packing material manufacturer.
- ▶ Wear a dust mask.



The use of a more dilute liquid slurry simplifies the column packing. The total volume of the slurry should not exceed the maximum volume of the column.

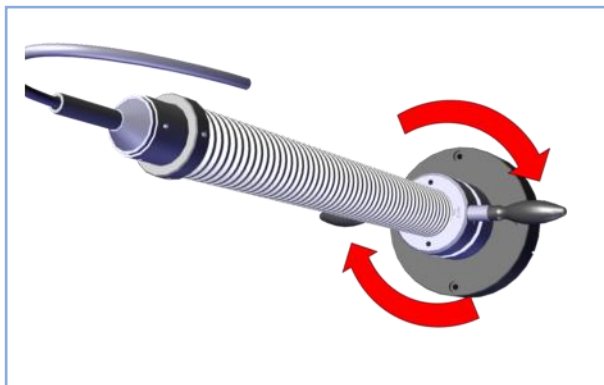


The removal of fines decreases the column back pressure and so improves the performance of the packed column.

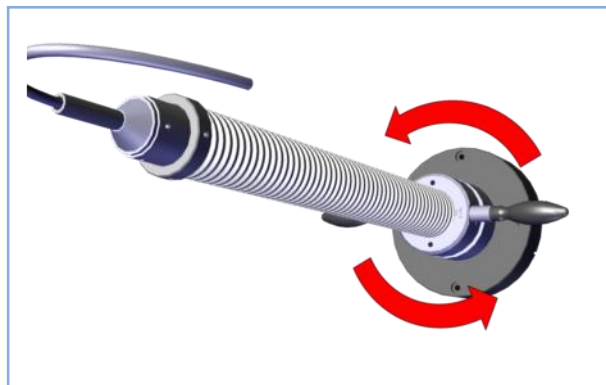
- ▶ The slurry is prepared as follows:
 1. Mix the solvent or buffer with the appropriate amount of packing material in a suitable container (see manufacturer's recommendations).
 - ✓ The total volume of the slurry must be less than the maximum column volume.
 - ✓ The amount of packing material determines the final bed length.
 2. Mix the slurry with a stirring rod until the slurry is homogenous and avoid introduction of air bubbles.
 3. Check if the material is suitable for ultrasonication (see manufacturer's recommendations).
 - ✓ If the material is suitable for ultrasonication, degas the slurry in an ultrasonic bath.
 - ✓ If the material is not suitable for ultrasonication, let the material settle.
 4. Let the degassed slurry settle.
 5. If fines are visible on the surface of the slurry, remove them.

6.2.2 Slurry column packing under flow

- The column is packed with the slurry method under flow as follows:
1. Remove the adjustable end piece ► Chapter 6.1, S. 18.
 2. Check the drainage tubing for correct connection.
 3. Check the drainage tubing outlet for correct position in the collecting vessel.
 4. Connect the pump with the tubing at the column body.
 5. Start the pump.
 6. Fill the glass body with solvent or buffer until 2-3 cm above the frit in the column body (bottom-up).
 - ✓ This ensures that no air is trapped under or is inside the frit.
 7. Close the valve in the tubing on the column body.
 8. Connect the pump with the adjustable end piece.
 9. Flush the piston of the adjustable end piece with slurry solvent until the frit of the adjustable end piece is wetted completely.
 10. Disconnect the pump from the adjustable end piece.
 11. Pour the homogenous and/or degassed slurry into the glass body.
 12. Rinse the inner wall of the column body with slurry solvent for removal of particles.
 - ✓ By removing particles from the inner wall of the column body, a correct seal with the profile gasket is guaranteed.
 13. Fill the pilot column up to the edge of the drain function with slurry solvent.

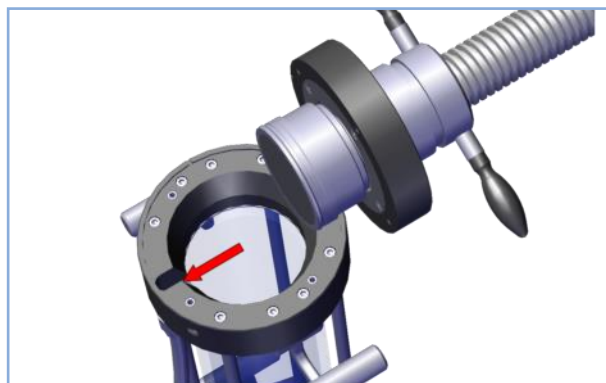


14. Turn the closing disc of the adjustable end piece in the UP direction as far as it will go.



15. Turn the closing disc of the adjustable end piece half turn in the DOWN direction.

✓ The adjustable end piece is ready for insertion into the column body.

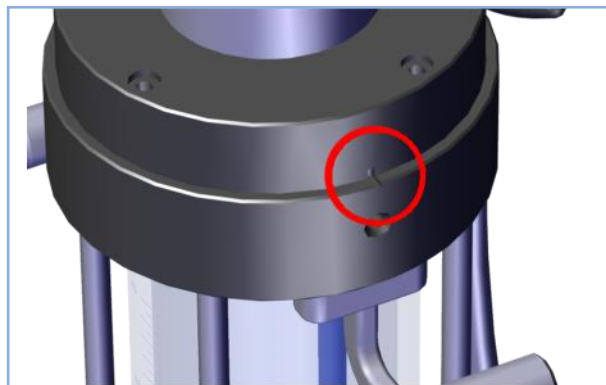


16. Put the piston of the adjustable end piece in a smooth angle opposite the drain function on the column body.

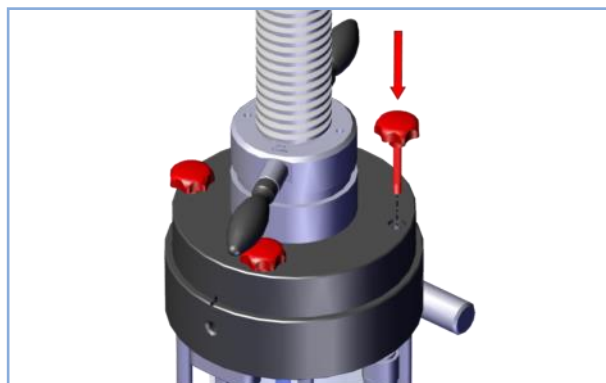


17. Bring the piston of the adjustable end piece slowly to the vertical position.

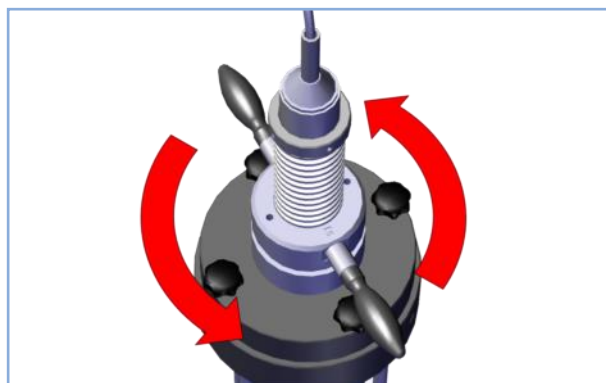
✓ Excessive slurry solvent will flow into a reservoir through the drainage tubing.



18. Align the marks on the closing disc (AEP) and column disc.

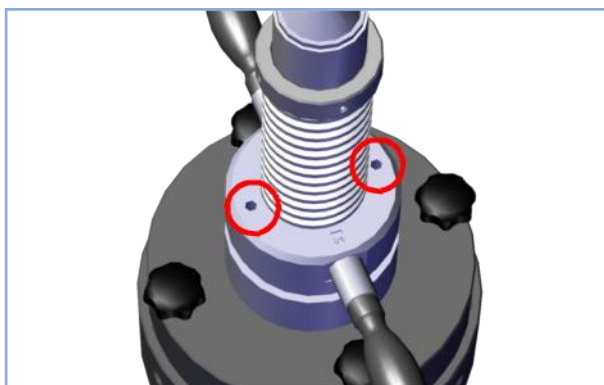


19. Screw the 4 star screws continuously in a diagonal pattern to each other.
20. Open the valve in the tubing on the adjustable end piece.



21. Turn the piston of the adjustable end piece in the DOWN direction until the tubing is completely filled with liquid.
✓ The tubing is now free of air.
22. Connect the pump with the tubing of the adjustable end piece.
23. Open the valve in the tubing on the column body.

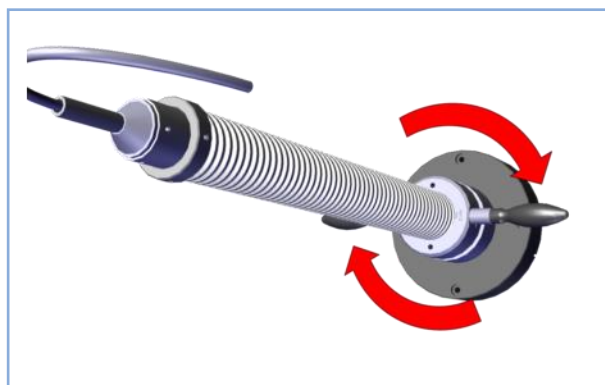
24. Start the flow (top down).
25. Increase the flow rate stepwise until the desired pressure / flow rate is reached. Wait after every step until the pressure is constant. Afterwards, the flow rate can be further increased.
 - ✓ Consider the manufacturer's recommendations for the packing material.
 - ✓ The flow rate should be considerably ($> 20\%$) higher than the operating flow rate.
 - ✓ The column bed is visually compressed.
26. Check and mark the bed length frequently.
27. If the final bed length is reached, stop the pump.
28. Turn the piston of the adjustable end piece in the DOWN direction until the column bed is reached.
29. Start the pump.
30. Check, if the column bed is further compressed. A dead volume would occur.
31. If a dead volume occurs, stop the pump and repeat step 28 to 30.
32. If no dead volume occurs, the pilot column is released ▶ Chapter 6.3, S. 27.



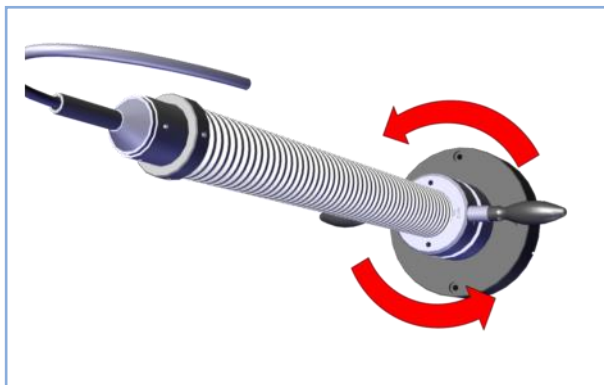
33. Remove liquid above the connecting flange through the two holes in the adjustable end piece.
 - ✓ YMC recommends flushing this area if high salt concentrations are used.
34. Equilibrate the pilot column with the desired eluent and test the column performance.

6.2.3 Slurry column packing with sedimentation

- The column is packed via the sedimentation slurry method as follows:
1. Remove the adjustable end piece ►Chapter 6.1, S. 18.
 2. Check the drainage tubing for correct connection.
 3. Check the drainage tubing outlet for correct position in the collecting vessel.
 4. Connect the pump to the tubing of the column body.
 5. Start the pump.
 6. Fill the glass body with solvent or buffer until 2-3 cm above the frit in the column body (bottom-up).
 - ✓ This ensures that no air is trapped under or is inside the frit.
 7. Close the valve in the tubing of the column body.
 8. Disconnect the pump from the column body.
 9. Connect the pump to the adjustable end piece.
 10. Flush the piston of the adjustable end piece with slurry solvent until the frit in the adjustable end piece is wetted completely.
 11. Disconnect the pump from the adjustable end piece.
 12. Pour the homogenous and/or degassed slurry into the glass body.
 13. Rinse the inner wall of the column body with slurry solvent in order to remove particles.
 - ✓ By removing particles from the inner wall of the glass body, a correct seal with the profile gasket is guaranteed.
 14. Do not move the pilot column anymore.
 - ✓ YMC recommends covering the open column body during sedimentation with some plastic film.
 15. Wait until the packing material has settled (sedimentation).
 - ✓ Depending on the packing material, this can take some hours.
 16. Fill the pilot column up to the edge of the drain function with slurry solvent.

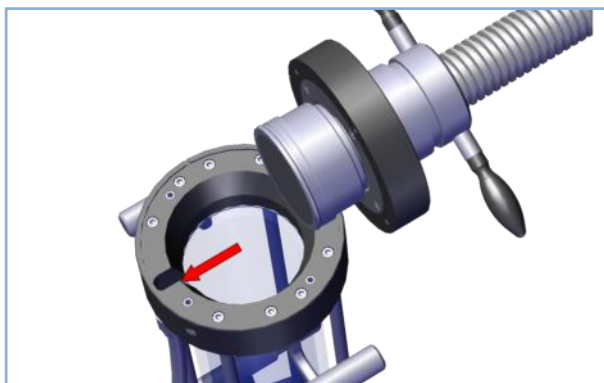


17. Turn the closing disc of the adjustable end piece in the UP direction as far as it will go.



18. Turn the closing disc of the adjustable end piece half turn in the DOWN direction.

✓ The adjustable end piece is ready for insertion into the column body.

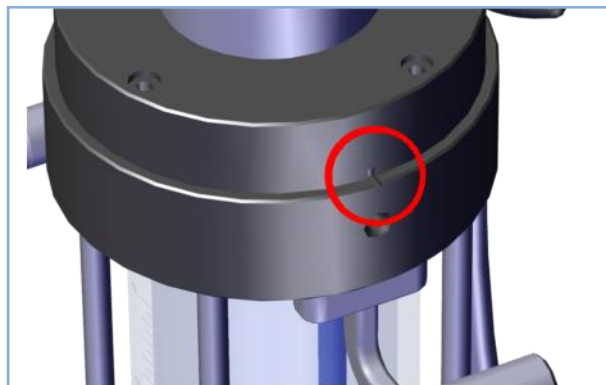


19. Put the piston of the adjustable end piece in a smooth angle opposite the drain function on the column body.

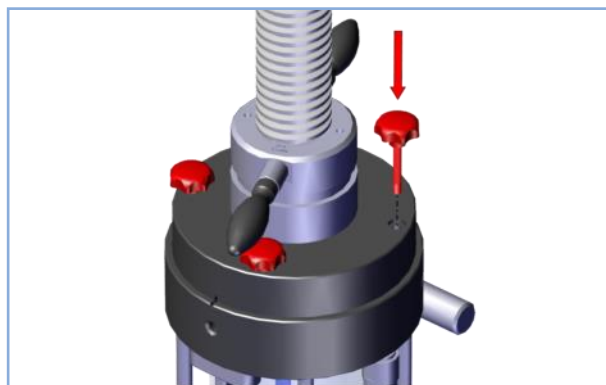


20. Bring the piston of the adjustable end piece slowly to the vertical position.

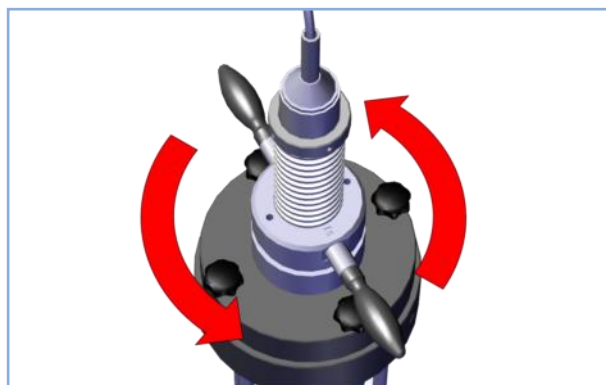
✓ Excessive slurry solvent will flow into a reservoir through the drainage tubing.



21. Align the marks on the closing disc (AEP) and column disc.

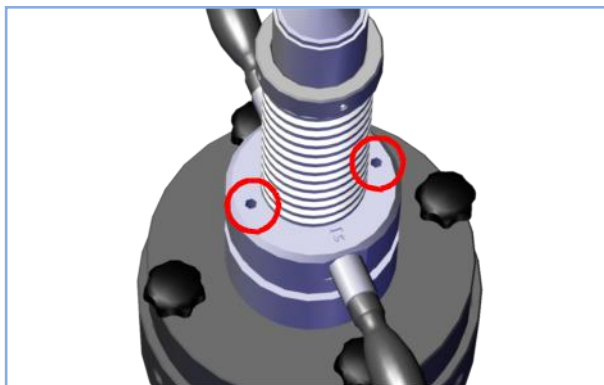


22. Screw the 4 star screws continuously in a diagonal pattern to each other.
23. Open the valve in the tubing on the adjustable end piece.



24. Turn the piston of the adjustable end piece in the DOWN direction until the column bed is reached.
25. Connect the pump to the tubing of the adjustable end piece.
26. Open the valve in the tubing at the column body.
27. Start the flow (top-down).
28. Increase the flow rate stepwise until the desired pressure / flow rate is reached. Wait after every step until the pressure is constant. Afterwards, the flow rate can be further increased.
- ✓ Consider the manufacturer's recommendations for the packing material.
 - ✓ The flow rate should be considerably (> 20%) higher than the operating flow rate.
 - ✓ The column bed is visually compressed.

29. Check and mark the column bed length frequently.
30. If the final bed length is reached, stop the pump.
31. Turn the piston of the adjustable end piece in the DOWN direction until the column bed is reached.
32. Start the pump.
33. Check if the column bed is further compressed. A dead volume would occur.
34. If a dead volume occurs, stop the pump and repeat step 31 to 33.
35. If no dead volume occurs, the pilot column is released. ▶ Chapter 6.3, S. 27.



36. Remove liquid above the connecting flange through the two holes in the adjustable end piece.
 - ✓ YMC recommends flushing this area if high salt concentrations are used.
37. Equilibrate the pilot column with the desired eluent and test the column performance.

6.3 Column test and release criteria

The column test and the release criteria are selected with regards to the later application field. The preparation of the required specifications for the final usage of a method or for the purification of a target molecule is determined individually. YMC will gladly support you.

6.4 Pilot column unpacking

WARNING

Pilot column under high pressure

Eye or skin injuries possible due to remaining pressure in the tubing or in the pilot column.

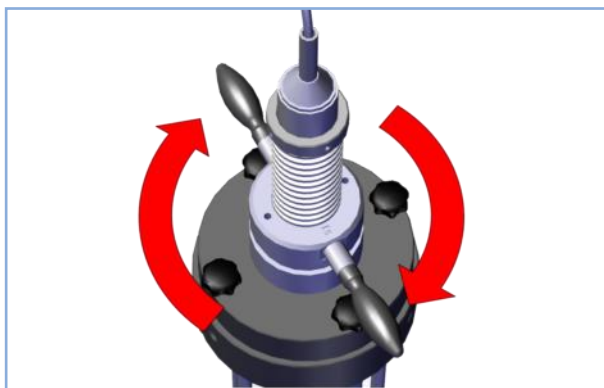
- ▶ Stop the pump during working on the pilot column.
- ▶ While working on the pilot column, wear the prescribed personnel protective equipment.

CAREFUL

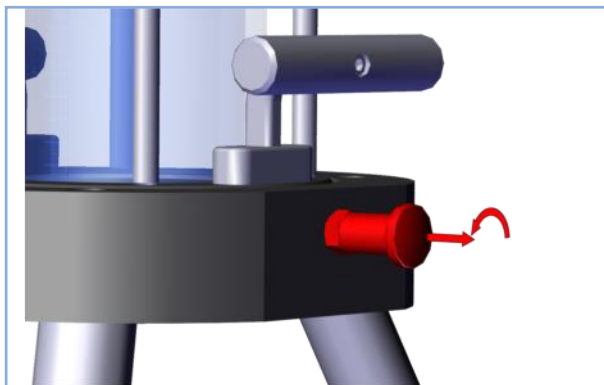
Packing material

Eye and skin injuries possible through contact with packing material.

- ▶ Wear the prescribed personnel protective equipment.
- ▶ The column is unpacked under flow as follows:
 1. Close the valve in the tubing of the column body.
 2. Disconnect the pump from the column body.
 3. Ensure that the valve at the adjustable end piece is open.
 4. Disconnect the pump from the adjustable end piece.



5. Turn the piston of the adjustable end piece in the UP direction until the profile gasket does not touch the glass body anymore.
 - ✓ The loosening of the profile gasket from the glass body is noticeable.
6. Remove the adjustable end piece ▶Chapter 6.1, S. 18.
7. Put a collecting vessel below the column stand.



8. Loosen the locking bolts and lock them by turning slightly.



9. Lift the column body vertical from the column stand using two holding bars which are vertically above each other.
10. Pour any unpacked material and/or solvent into a collecting vessel.
11. If no material now remains in the column body, clean the column body ▶ Chapter 7.3, S. 33.



12. If material remains in the column body, put the column body into the stand with the open end facing down.
13. Turn the column body slowly in the frame disc until the locking bolts lock.
 - ✓ You will hear a noticeable “click” when the locking bolts are locked.
14. Connect the pump with the tubing at the column body.
15. Open the valve in the tubing of the column body.
16. Start the pump.
 - ✓ Because of the flow, the packing material is pressed out of the column body into the collecting vessel.
17. Clean the pilot column ▶ Chapter 7.3, S. 33.

7 Cleaning and maintenance

WARNING

Incorrect maintenance

Severe injuries when used after incorrect maintenance is possible, if the column or column parts do not withstand the operating pressure.

- ▶ Only authorised and qualified personnel should carry out the maintenance.
-

WARNING

Incorrect replacement of spare parts

Severe injuries due to breakage possible if unauthorised spare parts are used.

- ▶ Use only the manufacturer authorised spare parts.
-

WARNING

Faulty maintenance of the pilot column

Severe injuries due to deterioration possible.

- ▶ Meet maintenance intervals.
 - ▶ Perform the maintenance as described.
-



Consumable parts lists are included in the appendix.

7.1 Notes for Cleaning-In-Place (CIP)

CAREFUL

Packing material

Eye and skin injuries possible through contact with packing material.

- ▶ Wear the prescribed personnel protective equipment.
-

ATTENTION

Cleaning with inappropriate solvents

Material damage possible due to corrosion, leaching or deformation.

- ▶ Check the chemical resistance of the wetted parts for the cleaning method before the CIP procedure.
-

With the cleaning-in-place procedure, a packed pilot glass column can be cleaned without unpacking or dismantling. Depending on the contamination, three different procedures can be used:

1. Regeneration,
2. Sterilisation,
3. Depyrogenation.

Regeneration

Regeneration removes inorganic and organic contamination, which binds to the chromatography matrix and substantially affects the capacity and resolution of the column. Commonly these are: lipids, pyrogens, protein aggregates, pigments, polyphenols or metal complexes.

Sterilisation

Sterilisation is the removal and/or destruction of microorganisms and spores that will contaminate the purified product. The most common method is the treatment with sodium hydroxide, acetic acid or ethanol solution.

Depyrogenation

Depyrogenation includes the removal of endotoxins, which can be bound on the chromatographic material or the column hardware (frits, tubing, etc.) and which can contaminate the purified product.

Cleaning methods for CIP

Treatment	Type of contamination	Sterilisation	Depyrogenation
Pepsin, pH 1.5 - 2	Proteolysis of adsorbed proteins	No	No
Non-ionic detergents (Triton X-100; Tween 80)	Removal of hydrophobic proteins and lipids	No	No
Cationic detergents, pH 9 - 11	Removal of hydrophobic proteins and lipids	No	Partly
Urea, 6 - 8 M	Removal of protein aggregates	No	Unknown
1 - 100 mM EDTA, pH depending on the stationary phase	Removal of metal complexes	No	No
0.1 - 1 M NaOH	Removal of bounded hydrophobic proteins, lipopolysaccharides	Good	Good
0.5 - 1 M acetic acid in 60% ethanol	Removal of lipids, pigments, lipopolysaccharides, and other lipophilic substances	Very good	Good

7.2 Cleaning and maintenance plan

The specified maintenance plan here is to be regarded as a general recommendation. Depending on the frequency of use and specific application, the appropriate maintenance plan and the intervals must be defined locally.

Interval	Maintenance
After every operation	<ul style="list-style-type: none"> Clean the pilot column.
Daily	<ul style="list-style-type: none"> Check the column bed, adjust the piston if necessary. Check all tubing and connections regarding leakage ▶ Chapter 5.2, S. 16. Fix loosened fixing screws again or replace tubing and connectors.
Every 3 months	<ul style="list-style-type: none"> Check the profile gasket in the adjustable end piece. If damaged, replace it. Check the ring gasket of the column body. If damaged, replace it.
Every 6 months	<ul style="list-style-type: none"> Check the frit in the adjustable end piece. If damaged, replace it ▶ Chapter 7.4.1, S. 36. Check the frit in the column body. If damaged, replace it ▶ Chapter 7.4.3, S. 40.

7.3 Cleaning

WARNING

Pilot column under high pressure

Eye or skin injuries possible due to remaining pressure in the tubing or in the pilot column.

- ▶ Stop the pump while working on the pilot column.
 - ▶ When working on the pilot column, wear the prescribed personnel protective equipment.
-

CAREFUL

Packing material

Eye and skin injuries possible through contact with packing material.

- ▶ Wear the prescribed personnel protective equipment.
-

Required devices:

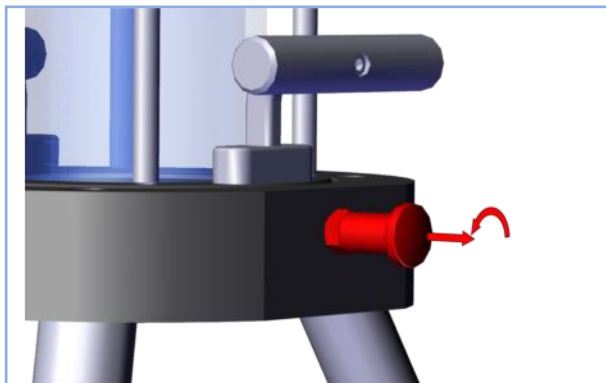
- Wetted and lint-free rags
- Collecting vessel
- Solvent for column body and adjustable end piece cleaning ▶ Chapter 3.3, S. 9
- Cleaning agent for cleaning the outer wall of the column body

Authorised cleaning agents for cleaning the outer wall of the column body:

- Commercially available dishwashing cleaners
- Aliphatic hydrocarbons
- Aliphatic alcohols
- Aromatic hydrocarbons
- Aldehydes
- Diluted and weak acids
- Bases

► The pilot column is cleaned as follows:

1. Remove the adjustable end piece ►Chapter 6.1, S. 18.
2. Clean the outer wall of the column body.
3. Clean the column body with an authorised cleaning agent and a wetted, lint-free rag.
4. Put a collecting vessel below the column stand.



5. Loosen the locking bolts and lock them by turning slightly.

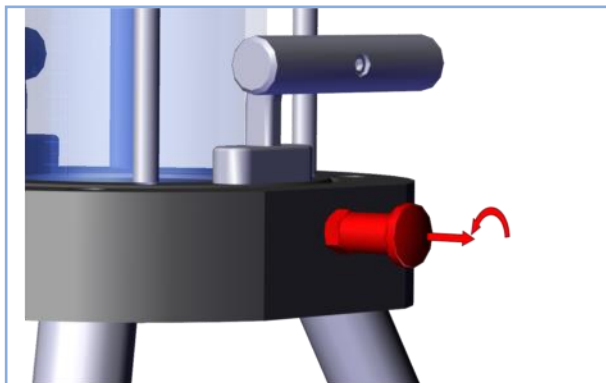


6. Lift the column body vertical from the column stand using two holding bars which are vertically above each other.



7. Put the column body with the open end facing downwards in the column stand.
8. Turn the column body slowly in the frame disc until the locking bolts lock.
✓ You will hear a noticeable “click” when the locking bolts are locked.

9. Connect the pump to the tubing in the column body.
10. Start the pump.
11. Flush the column body with an appropriate solvent and/or 1 M sodium hydroxide.
12. Stop the pump.
13. Disconnect the pump from the tubing in the column body.



14. Loosen the locking bolts and lock them by turning slightly.



15. Lift the column body vertical from the column stand using two holding bars which are vertically above each other.
16. Insert the column body in the starting position again.
17. Turn the column body slowly in the frame disc until the locking bolts lock.
 - ✓ You will hear a noticeable “click” when the locking bolts are locked.
18. Clean the adjustable end piece with an appropriate solvent and/or 1 M sodium hydroxide.



For a rigorous cleaning, dismantle the pilot column.

7.4 Replacement of spare parts

ATTENTION

Faulty assembly

Damage to the glass body possible due to faulty assembly.

- ▶ Take care during the assembly of the glass body that the maximum torque of 5 Nm for columns with 70 and 100 mm ID respectively 10 Nm for column with 140 mm and 200 mm ID are not exceeded.
- ▶ Assemble the cylinder screws in a diagonal pattern and always tighten on the opposite sites.
- ▶ Take care during the assembly of the glass body that all parts are correctly aligned.

7.4.1 Frit replacement in the adjustable end piece

- ▶ The frit in the adjustable end piece is replaced as follows.
 1. Remove the adjustable end piece ▶ Chapter 6.1, S. 18.



2. Remove the profile gasket.
3. Remove the frit.
4. Insert the new frit. The distribution profile faces the plunger.



5. Reattach the profile gasket and press firmly in position.

7.4.2 Replacing the glass body

ATTENTION

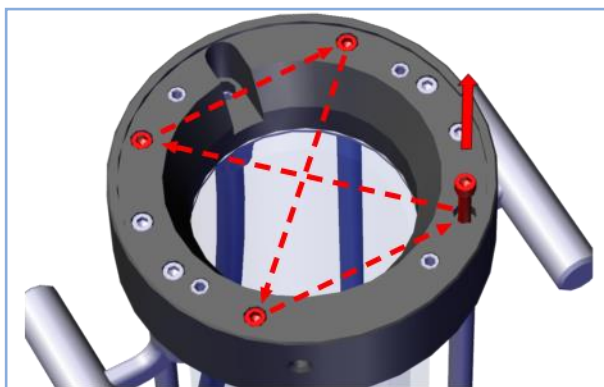
Faulty assembly

Damage to the glass body possible due to faulty assembly.

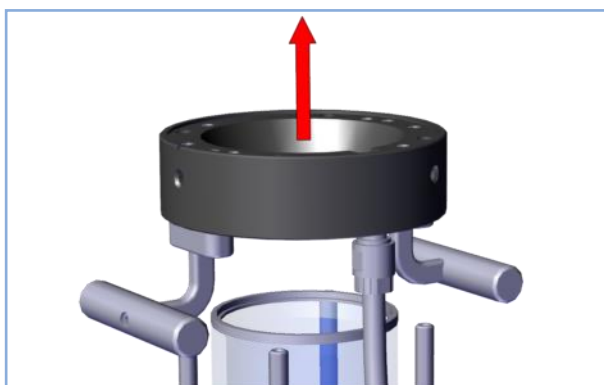
- ▶ Take care during assembly of the glass body that the maximum torque of 5 Nm for columns with 70 and 100 mm ID respectively 10 Nm for column with 140 mm and 200 mm ID are not exceeded.
- ▶ Assemble the cylinder screws in a diagonal pattern and always tighten on the opposite sites.
- ▶ Take care during assembly of the glass body that affected parts are correctly aligned

Required devices:

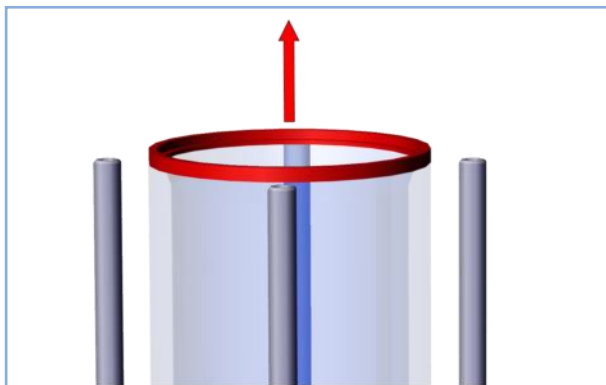
- Cylinder screw key / torque wrench
- ▶ The glass body is dismantled as follows:
 1. Remove the adjustable end piece ▶ Chapter 6.1, S. 18.



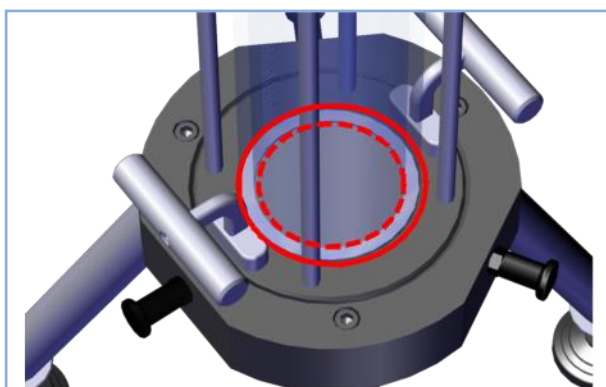
2. Loosen the cylinder screws stepwise half turn. Do it diagonally: Loosen cylinder screws always on opposite sites.
3. Remove cylinder screws.



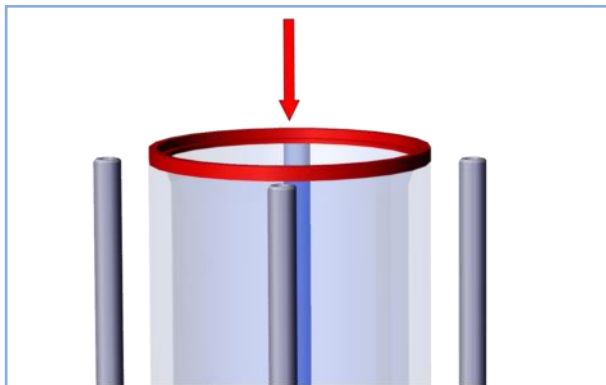
4. Remove column disc from the column body.



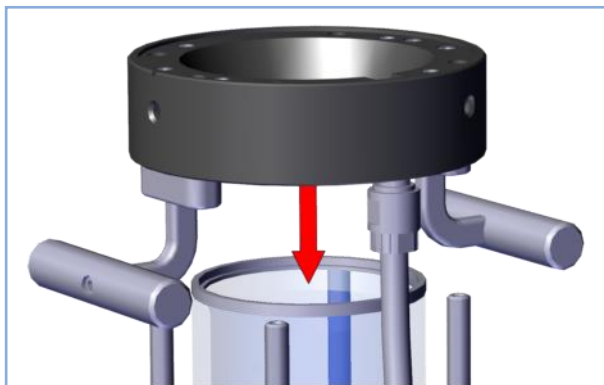
5. Remove flat gasket.
6. Remove glass body carefully.
7. Position the new glass body on the ring gasket A.



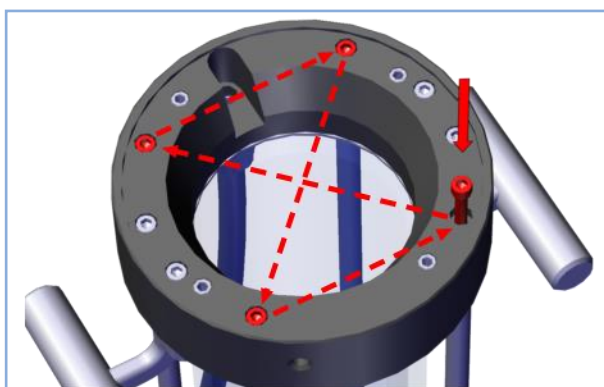
8. Check that the glass body is concentric with the gaskets.



9. Put the flat gasket corresponding to the structure on the top edge of the glass body.



10. Adjust the column disc over the rods and lower it onto the glass body.
11. Check that the flat gasket is in the groove in the column disc.
12. Check that the glass body is concentric with the column disc.



13. **ATTENTION** Damage to the glass body is possible due to faulty assembly. Note the maximum torque during the assembly of the glass body.
Insert the cylinder screws in a diagonal pattern and tighten them by gradually increasing the torque to screws on the opposite sides. Adjust to a **maximum torque of 5 Nm for columns with 70 and 100 mm ID respectively 10 Nm for columns with 140 and 200 mm ID.**

7.4.3 Frit replacement column body

ATTENTION

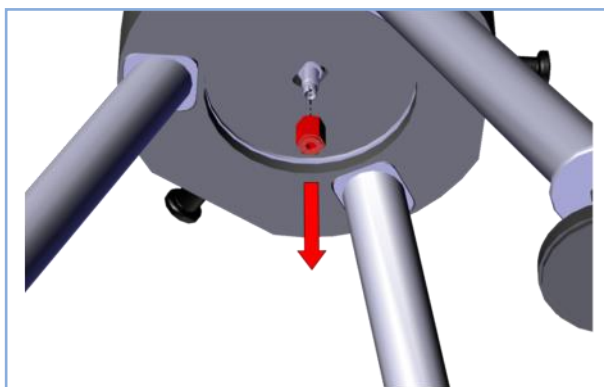
Faulty assembly

Damage to the glass body is possible due to faulty assembly.

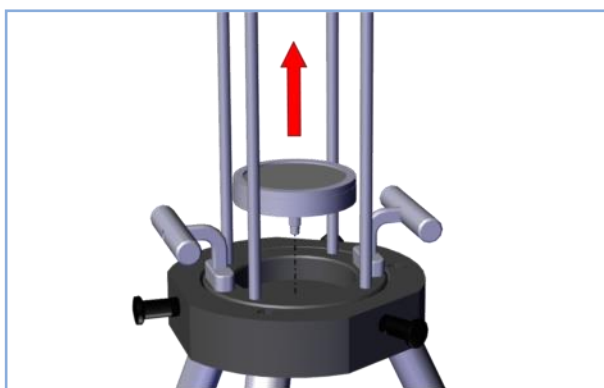
- ▶ Take care during assembly of the glass body that the maximum torque of 3 Nm is not exceeded.
- ▶ Assemble the cylinder screws in a diagonal pattern and always tighten on the opposite sites.
- ▶ Take care during assembly of the glass body that affected parts are correctly aligned.

Required devices:

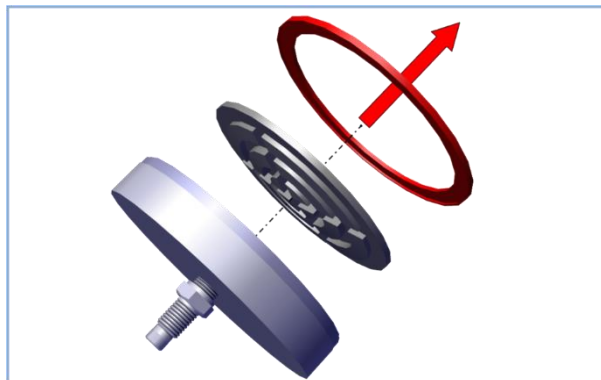
- Cylinder key/torque wrench
- ▶ The frit in the column body is replaced as follows:
 1. Remove the adjustable end piece ▶Chapter 6.1, S. 18.
 2. Dismantle the glass body ▶Chapter 7.4.2, S. 37.



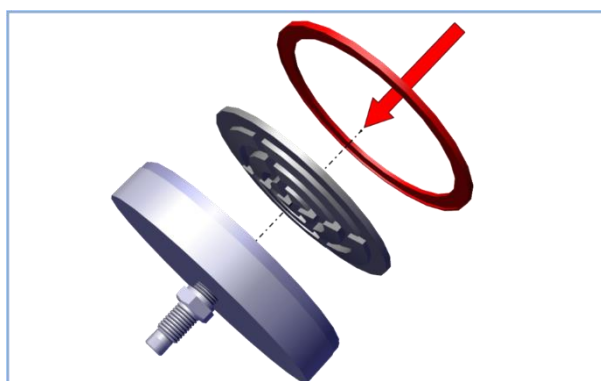
3. Remove the union nut of the connector.



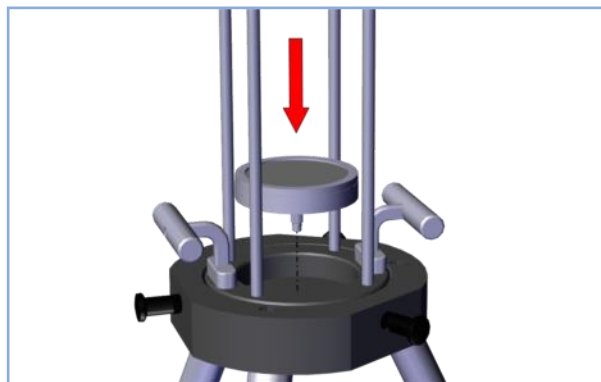
4. Press the fixed end piece with the connector out of the closing disc (CB).



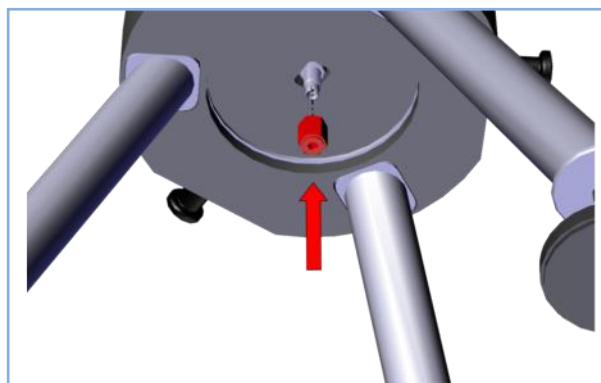
5. Remove the ring gasket A.
6. Remove the frit.
7. Insert the frit. The distribution profile faces the plunger.



8. Reattach the ring gasket A and press firmly in position.



9. Replace the fixed end piece into the closing disc (CB).



10. Fix the union nut at the connector.
11. Assemble the glass body ▶ Chapter 7.4.2, S. 37.

8 Troubleshooting

WARNING

Incorrect maintenance

Severe injuries during operation after incorrect maintenance possible if the column or column parts do not withstand the operating pressure.

- ▶ Only authorised and qualified personnel should carry out the maintenance.

WARNING

Incorrect replacement of spare parts

Severe injuries due to breakage possible, if not authorised spare parts are used.

- ▶ Use only the manufacturer authorised spare parts.

Fault description	Possible cause	Solution
Dead volume occurred	Exceeding the maximum allowable pressure of the column packing	▶ If dead volume occurs, proceed as follows: 1. Stop the pump. 3. Readjust the piston of the adjustable end piece.
	Stressed column packing	
Peak shape of the eluted substances deteriorates	Dead volume at the column inlet	See fault description "Dead volume occurred"
	Partially clogged inlet frit	Frit replacement ▶ Chapter 7.4.1, S. 36
	Partially clogged outlet frit	Frit replacement ▶ Chapter 7.4.3, S. 40
	Separation performance of the stationary phase altered by impurities	Wash the stationary phase. Contact also the manufacturer of the packing material ▶ Chapter 7.1, S. 31
	Stationary phase mechanically destroyed	Replace packing material
Air in the column	Degassing or evaporation of solvent during storage	New column conditioning. Possibly new packing material is necessary.
Exceptional pressure rise during operation	Valve circuit incorrect	Check valve positions
	Clogged frit	Frit replacement ▶ Chapter 7.4, S. 36
	Over-tightened fixing screws	Replace fixing screws and clamping tubing, cut the end of the tubing
Pressure drop during operation	Leakage of tubing or fitting between pump and column	Check tubing and connections
	Depleted solvent reservoir	Refill solvents

Fault description	Possible cause	Solution
Solvent drips from the column	Column body leaks	<p>► If the column body leaks, proceed as follows:</p> <ol style="list-style-type: none">1. Visual check, if solvent leaks between glass body and flat gasket.2. Check the contact surface of the glass body and the sealing surface for impurities.3. Check the torque and the concentric arrangement of the parts.
	Tubing connection leaks	<p>► If the tubing connection leaks, proceed as follows:</p> <ol style="list-style-type: none">1. Tighten the fixing screw.2. Check tightness of tubing connection.3. New column conditioning.



If faults occur which are not listed here, please contact YMC for support.

9 Storage, packaging and transport

WARNING

Solvents in the pilot column

Dangers possible due to liquids leaking from the column during transportation.

- ▶ Unpack and clean the column before transport.
-

9.1 Storage



For storage of packed columns, the chemical resistance of the wetted parts and of the packed stationary phase (please contact the manufacturer) should be considered.

The pilot column should be stored as follows:

- unpacked
- cleaned
- dry
- empty

9.2 Transport

We recommend the use of the original wooden box for transportation. If you are no longer in possession of the box, please pack the column in a similar container.

- ▶ The column is packaged as follows:
 1. Unpack the pilot column ▶ Chapter 6.4, S. 28.
 2. Clean the pilot column ▶ Chapter 7.3, S. 33.
 3. Insert the adjustable end piece.
 4. Wrap the glass body in a bubble wrap.
 5. If existing, fix the wheels to the base of the wooden box.
 6. Make sure that there is a minimum distance of 100 mm from the outer wall.
 7. Fix the pilot column in the wooden box with bubble wrap.
 8. Attach appropriate warning signs to the box (e.g. “Caution breakable glass” and “Top”).

10 Disposal

For the disposal of the pilot column it has to be disassembled and disposed according to the national and other applicable environmental regulations.

11 Appendix

11.1 Spare parts column stand

Pos.	ID [mm]	Part-No.	Description	Material
1	70 - 100	00128	Sliding disc	Teflon (PTFE)
2	140 - 200	000203	Sliding disc	Teflon (PTFE)

11.2 Spare parts adjustable end piece

Pos.	ID [mm]	Part-No.	Description	Material
1	70	000014	Profile gasket	75 Fluoroprene XP41
2	70	000022	Frit (AEP)	Poroplast UHMW-PE
3	100	000114	Profile gasket	75 Fluoroprene XP41
4	100	000122	Frit (AEP)	Poroplast UHMW-PE
5	140	000146	Profile gasket	75 Fluoroprene XP41
6	140	000142	Frit (AEP)	Poroplast UHMW-PE
7	200	000204	Profile gasket	75 Fluoroprene XP41
8	200	000208	Frit (AEP)	Poroplast UHMW-PE

11.3 Spare parts column body 70 – 100 mm ID

Pos.	ID [mm]	Length glass body [mm]	Part-No.	Description	Material
1	70		000006	Flat gasket	EPDM white (0082E)
2	70	500	000007	Glass body	Borosilicate glass 3.3
3	70		SI15,0LM21,0	Drainage tubing	Silicone
4	70		000024	Ring gasket A	75 Fluoroprene XP41
5	70		000023	Frit (CB)	Poroplast UHMW-PE
6	70		000015	Ring gasket B	PTFE (USP)
7	70	850	000057	Glass body	Borosilicate glass 3.3
8	100		000106	Flat gasket	EPDM white (0082E)
9	100	500	000107	Glass body	Borosilicate glass 3.3
10	100		SI15,0LM21,0	Drainage tubing	Silicone
11	100		000130	Ring gasket A	75 Fluoroprene XP41
12	100		000123	Frit (CB)	Poroplast UHMW-PE
13	100		000115	Ring gasket B	PTFE (USP)
14	100	850	000157	Glass body	Borosilicate glass 3.3

11.4 Spare parts column body 140 – 200 mm ID

Pos.	ID [mm]	Length glass body [mm]	Part-No.	Description	Material
1	140		000141	Flat gasket	EPDM white (0082E)
2	140	500	000147	Glass body	Borosilicate glass 3.3
3	140		000140	Ring gasket A	75 Fluoroprene XP41
4	140		000143	Frit (CB)	Poroplast UHMW-PE
5	140		000162	Ring gasket B	PTFE (USP)
6	140		SI20,0LM24,0	Drainage tubing	Silicone
7	140	850	000148	Glass body	Borosilicate glass 3.3
8	200		000211	Flat gasket	EPDM white (0082E)
9	200	500	000200	Glass body	Borosilicate glass 3.3
10	200		000216	Ring gasket A	75 Fluoroprene XP41
11	200		000213	Frit (CB)	Poroplast UHMW-PE
12	200		000201	Ring gasket B	PTFE (USP)
13	200		SI20,0LM24,0	Drainage tubing	Silicone
14	200	850	000250	Glass body	Borosilicate glass 3.3

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