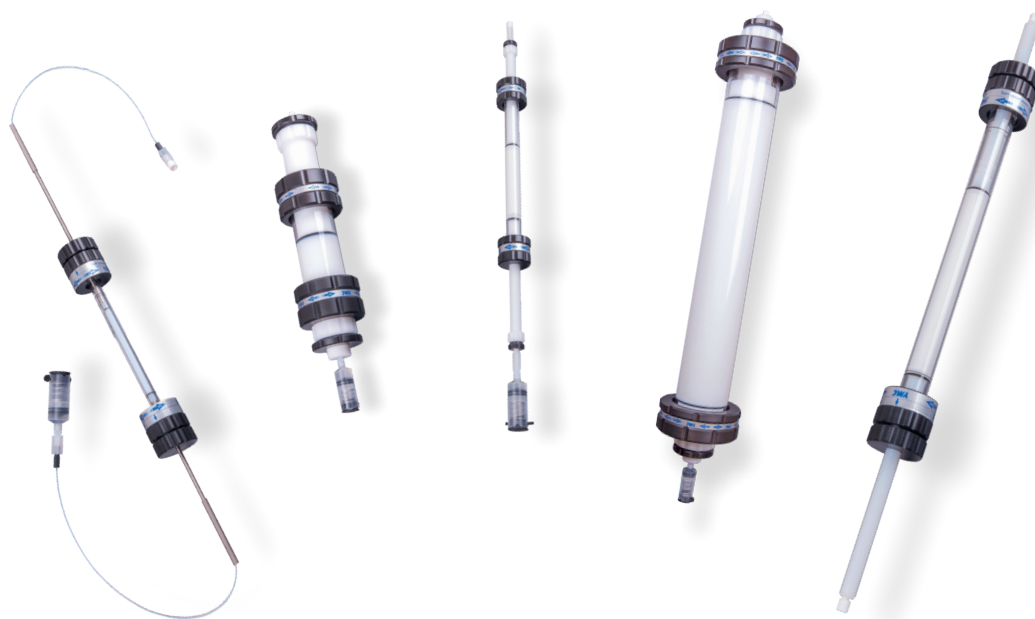


Step-by-Step Guide to packing a Lab Scale Glass Column

Packing of ECO and ECO^{PLUS} columns with soft gels



1 Introduction

In this guide, all steps and relevant parameters for successfully packing lab scale ECO and ECO^{PLUS} columns are presented.

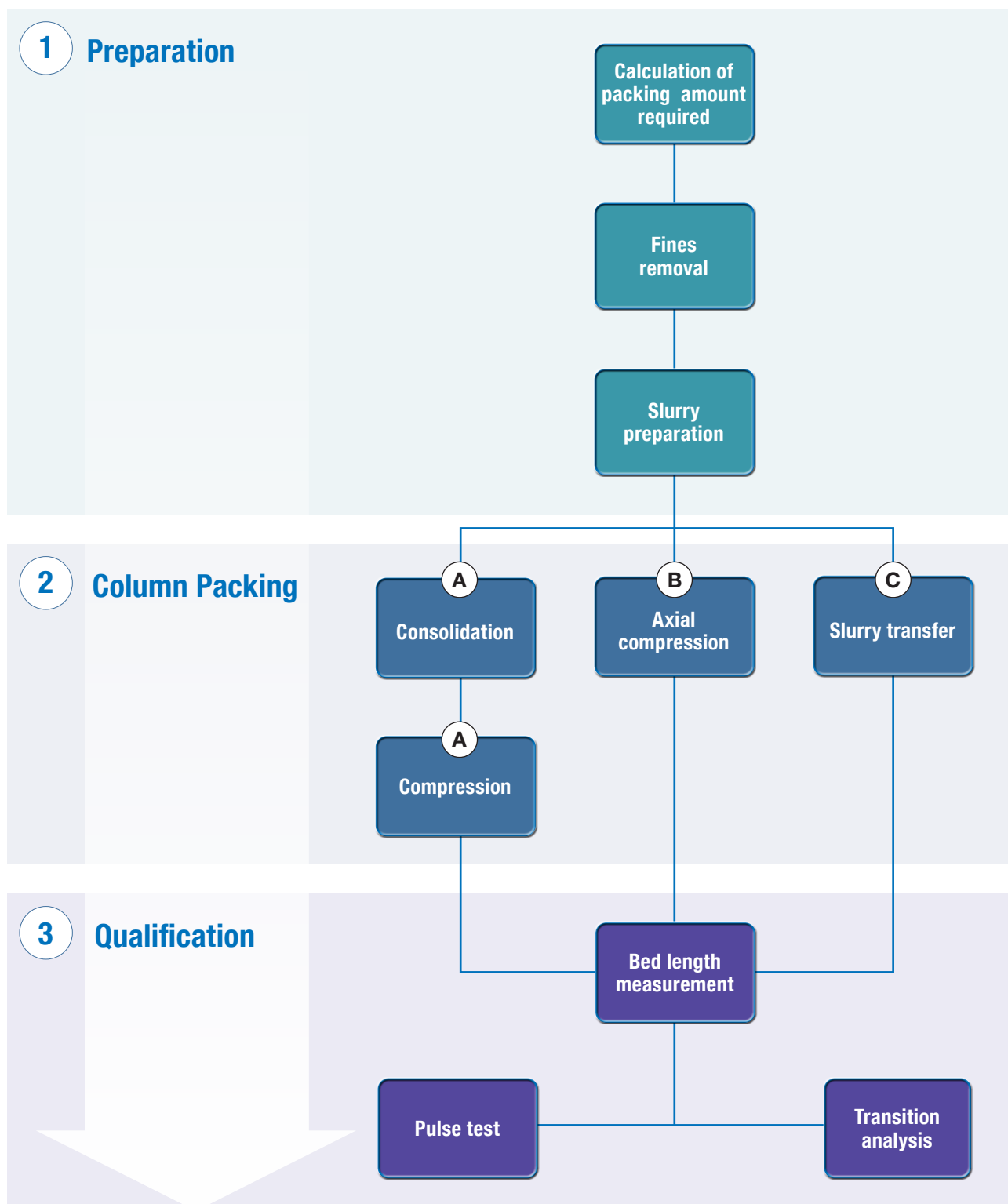
When packing a glass column, different methods can be used. The selection of the most appropriate packing method depends on the column hardware, the packing material and the available system.

Apart from the selection of the resin, there are three consecutive steps which are relevant for column packing:

- ① **In the first step, the column, the system and the resin are prepared.**
- ② **The second step is the actual column packing.**
- ③ **The final step is the testing and qualification.**

In this guide, the flow-packing method is described step by step for packing a pilot scale column. Flow packing is shown in the figure on page 3 in channel A. It consists of the consolidation and compression step during packing.

The three successive processes are shown as a flow diagram in the following figure:



- Ⓐ = Flow-packing
- Ⓑ = Axial compression
- Ⓒ = Qualification

All other terms are described on page 14.

What impact does the packing quality have on the chromatographic results?

The basis for every chromatographic separation is the correct choice of stationary phase. Only this way, can an optimum resolution be achieved. Of course, the packing material needs to be packed into the column as efficiently as possible for robust and reproducible results.

The importance of an ideal packed column bed results from the van Deemter equation:

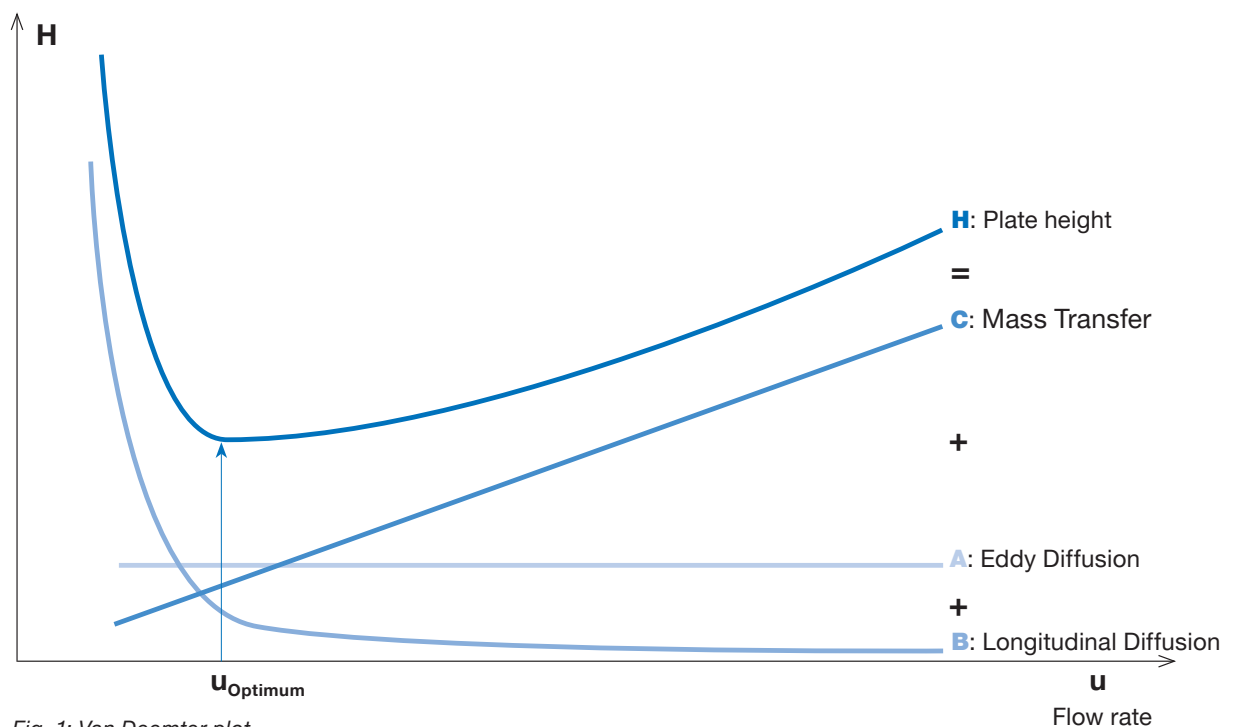


Fig. 1: Van Deemter plot

$$H = A + \frac{B}{u} + C \cdot u$$

Where

H = Plate height

A = Eddy Diffusion

B = Longitudinal Diffusion

C = Mass Transfer

u = Flow rate

The **A term** represents the so-called Eddy Diffusion/spread diffusion effect. It describes the correlation between the plate height (H) and the particle diameter. The analytes take different paths through the column and this requires a different time. This results in peak broadening.

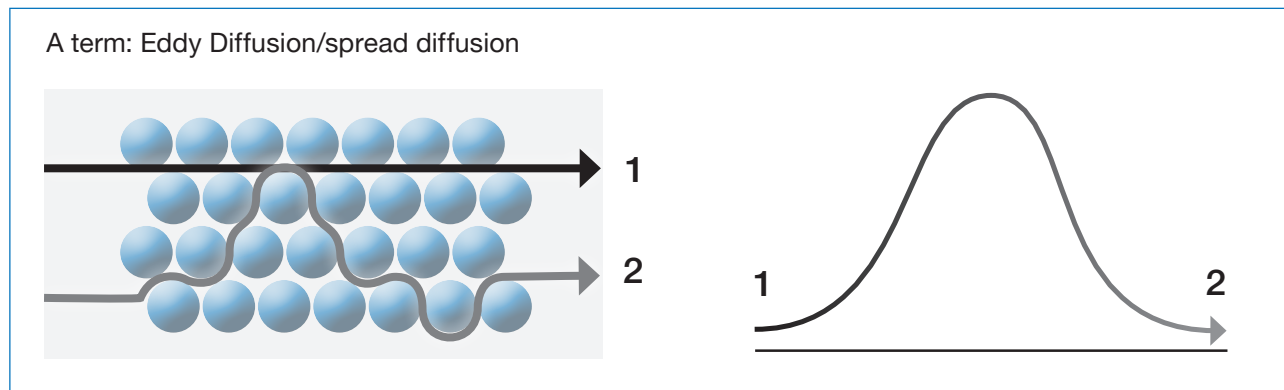


Fig. 2: Graphic description of the A term Eddy Diffusion

Through a homogenous column packing, the peak broadening is reduced. Therefore, the Eddy Diffusion is a direct measure for the packing quality of a column.

The **B term** describes the longitudinal diffusion. It depends on the viscosity and the temperature of the mobile phase. The B term becomes smaller for higher flow rates.

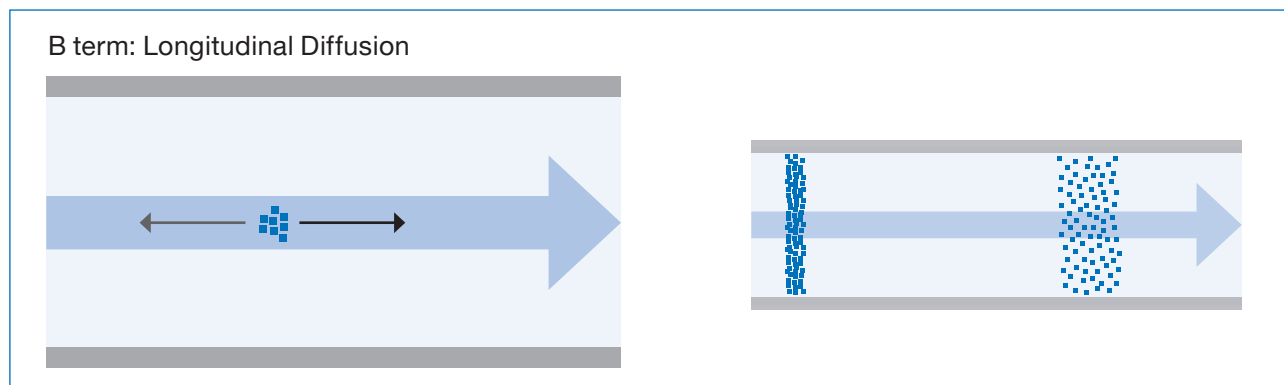


Fig. 3: Graphic description of the B term Longitudinal Diffusion

The **C term** describes the mass transfer of the analyte and therefore the establishment of the equilibrium between the mobile and the stationary phase. This term is affected by the porosity of the stationary phase and the column geometry.

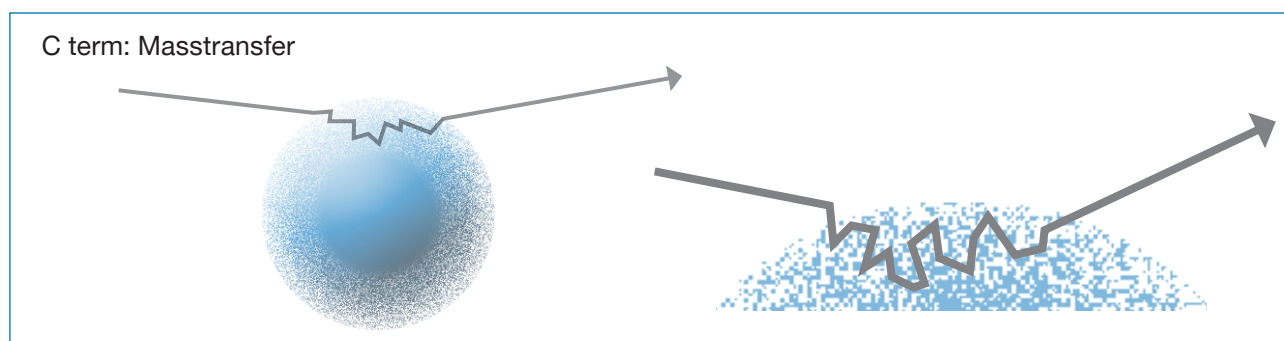


Fig. 3: Graphic description of the C term Mass Transfer

2 Preparation

2.1. Calculation of amount of packing required

1. Calculation of column bed volume:

$$V_{\text{bed}} = \frac{\pi d^2}{4} * l$$

2. Calculation of compressed gel volume:

$$V_{\text{material}} = V_{\text{bed}} * f_{\text{compression}}$$

3. Note the ratio of settled material and supernatant in the storage vessel.
4. Homogenise the material and the solvent in the storage vessel.
5. Transfer the required amount of packing material to a graduated cylinder.
Meet the ratio from step 3.

$$\text{Amount}_{\text{material}} = V_{\text{material}} + \text{solvent}$$

6. Let the material settle overnight.
7. Check the amount of settled material.
If required, add or remove packing material.

2.2. Removal of fines

1. Add four times the volume of solvent to the material.
Check the manufacturer's instructions for the selection of the correct solvent.
2. Homogenise the material and the solvent.
3. Decant the supernatant.
4. Repeat steps 1 to 3 until the supernatant is clear.
If required, add or remove packing material.

2.3. Slurry preparation

1. Wash the packing material with at least three times the volume of packing solvent.
2. Collect the material in a beaker.
3. Add sufficient packing solvent until you meet the required slurry concentration.
You can wash the packing material inside the column.

Preparation

Use the "YMC Glass and Pilot Column Packing Calculator"

!Do not use magnetic stirrers for homogenisation!

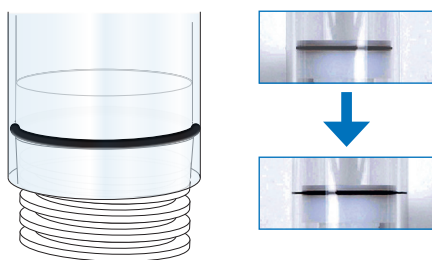
The duration of the sedimentation depends on the particle size: larger particles settle faster than smaller ones.

!Do not use magnetic stirrers for homogenisation!

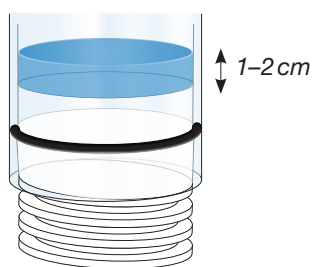
!Do not let the soft gels dry out!

2.4. System and column preparation

1. Flush the system for packing with the packing solvent.
 2. Check the column parts with regards to cleanness and function.
 3. Insert the lower piston into the column body.
Adjust the piston length so that the required bed length can be reached.
- ☐ *Tighten the O-ring (not applicable for ECO^{PLUS} columns).*



4. Fix the assembled column on a packing stand or tripod.
5. Assemble the packing adapter. For shorter bed lengths, a packing adapter is not required.
6. Connect the pump to the lower piston.
7. Pump approx. 1–2 cm of liquid into the column from below.
This way, air in the frits is removed.
Take care that the liquid and the O-ring are free of air bubbles.



8. Stop the pump and disconnect it from the lower piston.
9. Close the lower piston with a plug.
10. Adjust the vertical alignment of the column with a level.
The adjustment of the column is important for a good packing result.
11. Connect the upper piston (outside the column) with the pump.
12. Flush the upper piston with liquid.
This way, air is removed from the upper piston and frit.
13. Stop the pump and disconnect the upper piston from the pump.

Preparation

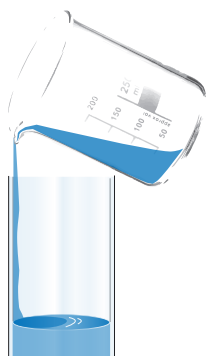
ECO and ECO^{PLUS} column:
O-ring has sealed if a constant,
black line appears in the glass body.

More information on assembly of the
packing adapter are included in the
manual of your column.

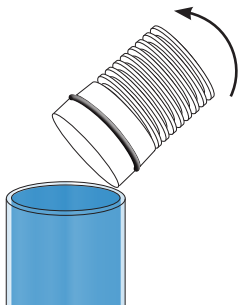
Use K.P316 for this step.

3 Packing

- Homogenise the slurry.
Stir constantly in one direction in order to avoid the entry of air.
A mixing duration of 5 minutes is common.
For air removal, the slurry can be put in an ultrasonic for 5 minutes. Afterwards, the slurry needs to be homogenised again.
- Pour the homogenised slurry into the open column.
Pour the slurry down the opposite side of the glass wall in order to avoid the entry of air.



- When the material is completely transferred into the column, clean the glass wall with packing solvent to remove any remaining particles.
Only in this way can optimum sealing of the O-ring be guaranteed.
- Fill the column completely with packing solvent until it reaches the upper edge.
- Put the upper piston in a slight angle on the glass edge of the packing adapter.



- Bring the piston slowly into a vertical position.
This way it ensures that no air is trapped below the piston.
- Close the counter screw and tighten the O-ring.
Not applicable for ECO^{PLUS} columns.

Packing

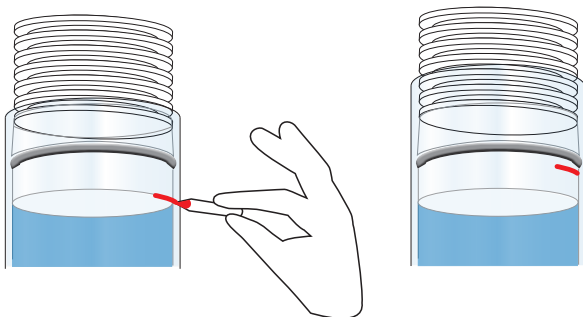
Avoid the entry of air.
Carry out steps 1–3 as quickly as possible in order to avoid resettlement of the material.

Very important for columns with permanent sealing.

O-ring has sealed if a constant, black line appears in the glass body

Packing

8. Lower the upper piston until it is filled completely with liquid.
Liquid comes out of the upper piston.
9. Remove the air from the tubing.
10. Connect the tubing to the upper piston.
11. Remove the plug at the lower piston.
12. Connect the tubing to the lower piston.
The lower tubing can be put into the storage vessel of the packing solvent. This way the packing solvent can be recycled.
13. Increase the flow rate stepwise until you reach the required flow rate and packing pressure.
Wait after every step until the pressure is constant and only increase the flow rate once the pressure has stabilised.
14. Wait until the column bed has formed and the bed length does not change anymore.
For checking, mark the bed length under flow.



15. Decrease the flow rate stepwise until the pump can be stopped.
16. Remove the tubing from the lower piston.
17. Close the lower piston with a plug.
18. Remove the upper piston from the packing adapter.
19. Remove the liquid in the packing adapter with a syringe.
20. Remove the packing adapter.
21. Fill the column with packing solvent up to the upper edge.
22. Insert the upper piston at an angle to the glass edge of the glass column.

Use K.P316.

Not applicable, if no packing adapter is used.

Packing

23. Bring the piston slowly into a vertical position.

This way will ensure that no air remains below the piston.

24. Close the counter screw and tighten the O-ring.

Not applicable for ECO^{PLUS} columns.

25. Lower the upper piston until you reach the column bed.

Take care that the upper piston is filled with packing solvent completely and that liquid comes out.

26. Remove the air in the tubing of the system.

27. Connect the tubing to the upper piston.

28. Remove the plug from the lower piston.

29. Connect the tubing to the lower piston.

30. Increase the flow rate stepwise until you reach the required flow rate and packing pressure.

31. Wait until the bed length does not change anymore.

If the packed bed compresses further, mark the new position of the bed under flow. Continue with steps 32–42.

If the packed bed does not compress further, continue with steps 32–35 and step 43 afterwards.

32. Decrease the flow rate stepwise until you can stop the pump.

33. Remove the tubing from the lower piston.

34. Close the lower piston with a plug.

35. Disconnect the pump from the upper piston.

36. Lower the upper piston until you reach the column bed.

- ☐ *Take care that the upper piston is completely filled with liquid and solvent comes out.*

37. Remove the air from the tubing of the system.

38. Connect the tubing to the upper piston.

39. Remove the plug from the lower piston.

O-ring has sealed if a constant, black line appears in the glass body

Use K.P316.

Packing

40. Connect the tubing to the lower piston.
41. Increase the flow rate stepwise until you reach the required flow rate and packing pressure.
42. Go back to step 31.
43. Lower the piston until you reach the last mark (mark under flow).
44. Connect the column to the system for testing and qualification.



4 Testing and Qualification

1. Equilibrate the column with 5 column volumes of the recommended qualification solvent.
 - ☐ *Check the product instructions from the packing material supplier.*
 - ☐ *The flow rate is the same as the flow rate for qualification.*
2. Perform the qualification corresponding to the manufacturer's recommendations or guidelines.
3. Repeat the quality control 3 times.
 - ☐ *This way, the reproducibility of the results is obtained.*
4. Evaluate the column packing and qualification.
 - ☐ *If the required specifications are met, the column is now ready for use.*
 - ☐ *If the required specifications are not met, return the column and the system to the packing solvent, unpack the column and repeat the packing process.*

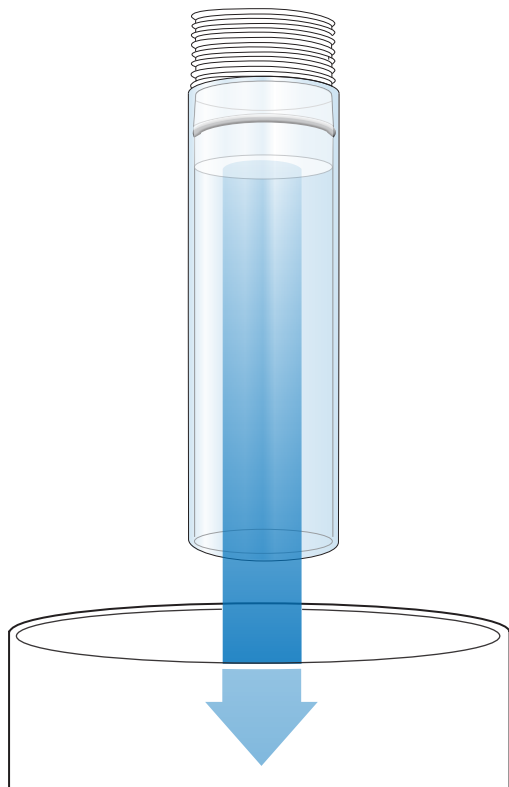
Qualification



5 Unpacking

The column will require unpacking if the required specifications are not met in the qualification run or if the material has to be changed or replaced.

1. Put a collecting vessel below the column.
2. Disconnect the lower piston from the system.
? *The upper piston is still connected with the system.*
3. Loosen the O-ring of the lower piston.
? *Not applicable for ECO^{PLUS} columns.*
4. Remove the lower piston.



5. Increase the flow rate stepwise.
? *The column bed is pushed out of the column.*
6. As soon as all the material is in the collecting vessel, stop the pump.
7. Clean the column for the next packing or storage.
8. Prepare the material as described by the manufacturer's instructions for re-packing or storage.

Unpacking

Wash the O-ring and the frit in order to remove remaining particles.

Terms and definitions

Axial compression	The material is compressed in a one-step process by lowering the piston onto the column bed. The piston movement stops at a defined pressure.
Compressibility	Soft gels are compressible due to their polymeric base particle. This needs to be considered when column packing.
Compression	The consolidated bed is compressed under flow or manually in order to receive the final column packing.
Consolidation	First step in flow packing in which a loose column bed is formed.
Fines	Broken particles which float on the liquid surface of the slurry. These need to be removed from the slurry in order to receive a homogeneously packed column bed.
Pulse test	Evaluation of the detector signal after injection of a defined substance.
Slurry	Suspension of packing/slurry solvent and packing material.
Slurry concentration	Concentration of the packing material in the packing/slurry solvent. Most often, this is a concentration range which is mentioned in the instructions from the packing material supplier.
Slurry transfer	Also known as Pack-In-Place. The piston is already adjusted. The slurry is pumped into the column until a defined pressure is reached.
Transition analysis	Evaluation of the detector signal of a gradient.
Unpacking	Removal of the packing material from the column.

