OPUS® 5 - 80R
Pre-packed Chromatography Columns

USER GUIDE

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

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1 Safety Notices

Please abide by the following movement and handling recommendations according to uncrating guide based on column internal diameter.

Do not pull or strain the white inlet and outlet ports protruding from the top of the OPUS® column.

Table 1. Removal of OPUS® Pre-packed Columns from Packaging

Column Size	Instruction
OPUS® 5 - 14 Columns	• Use two hands to grab the column and lift it from the box • For OPUS® 14 columns, one handle is provided on the top cap with additional hand holds on the bottom cap
OPUS® 20 – 30 Columns	• Use two people to grab one handle each • Lift the column slowly up from the box • Place it carefully onto the floor or cart. LIFT BY THE HANDLES ONLY
OPUS® 45R – 60R Columns	• Roll out the column from the wooden crate with the help of attached casters and ramp
OPUS® 80R Columns	• Roll out the column from the wooden crate with the help of attached casters and ramp • Two or more individuals are required to roll the column out from the crate and about the facility

- Unless otherwise specified by the end-user, OPUS® pre-packed columns are generally shipped in 20% Ethanol solution, a recognized bacteriostatic agent. Consult the Certificate of Analysis (CoA) or Certificate of Quality (CoQ) for confirmation of storage solution
  - Follow all local regulations for safe disposal
  - For laboratory and manufacturing production use only
  - Not for administration to humans

2 Introduction

2.1 What is Open Platform User Specified (OPUS®)?

Open Platform User Specified (OPUS®) Columns are designed to perform chromatography purification of biological molecules in either GMP or non-GMP applications.

The OPUS® Pre-Packed Column platform offers an alternative to conventional “pack in place” glass or stainless steel columns and can be reliably packed with virtually any resin from any source. To accommodate a wide range of biopharmaceutical applications, OPUS® Columns are configurable for nearly any bed height and industry standard internal diameters.

2.2 Column Design

The OPUS® platform has been designed to meet the requirements of GMP manufacturing in the pharmaceutical and biopharmaceutical industries for campaign-use and single-use applications.

2.3 Platformable

OPUS® Columns are designed to be broadly configurable to accommodate a wide range of purification and polishing applications for vaccines, monoclonal antibodies, and recombinant proteins. For example, OPUS® Columns:

- Accept nearly all commercially available bioprocessing resins
- Available in a wide range of bed heights and industry standard column diameters
- Configurable for specific packing procedures, release tests, and storage solutions

Regulatory Support File

For additional details into supporting data for the design of the OPUS® product line and testing of qualified processes, refer to the Regulatory Support File (RSF). The RSF may be accessed via Repligen website.

2.4 Materials of Construction

Figure 1. Materials of Construction Summary: OPUS® 5 and 8 Columns

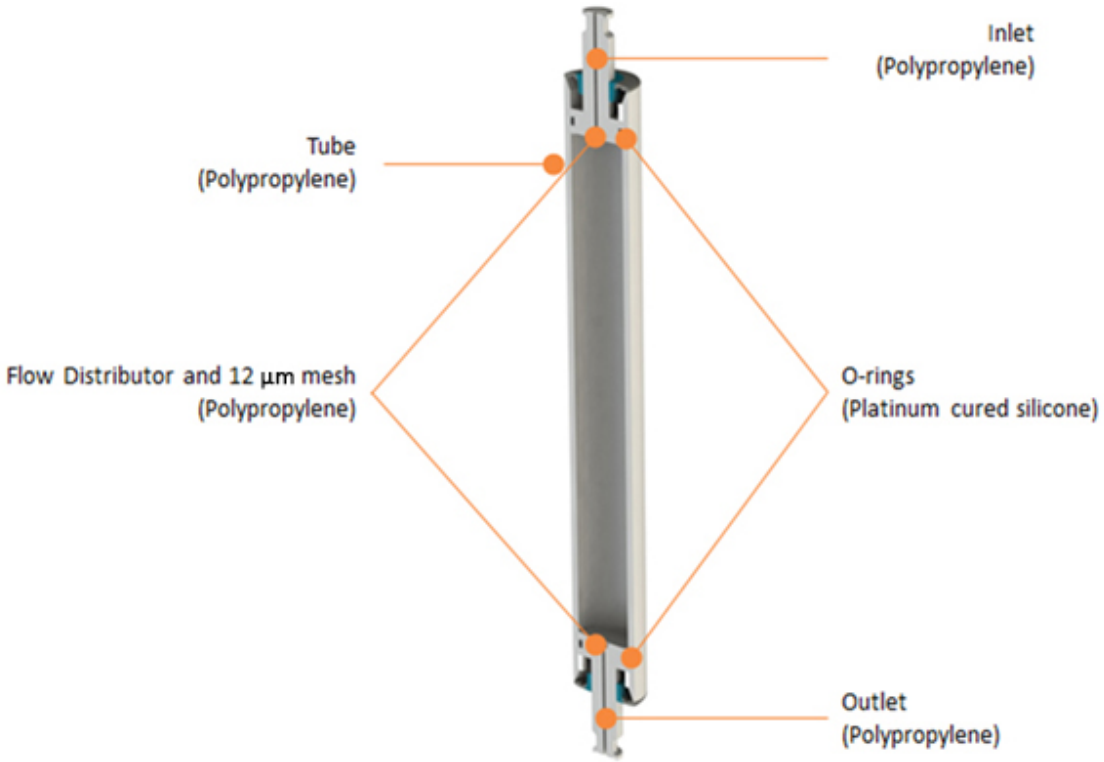


Figure 2. Materials of Construction Summary: OPUS® 10 – 30 Columns

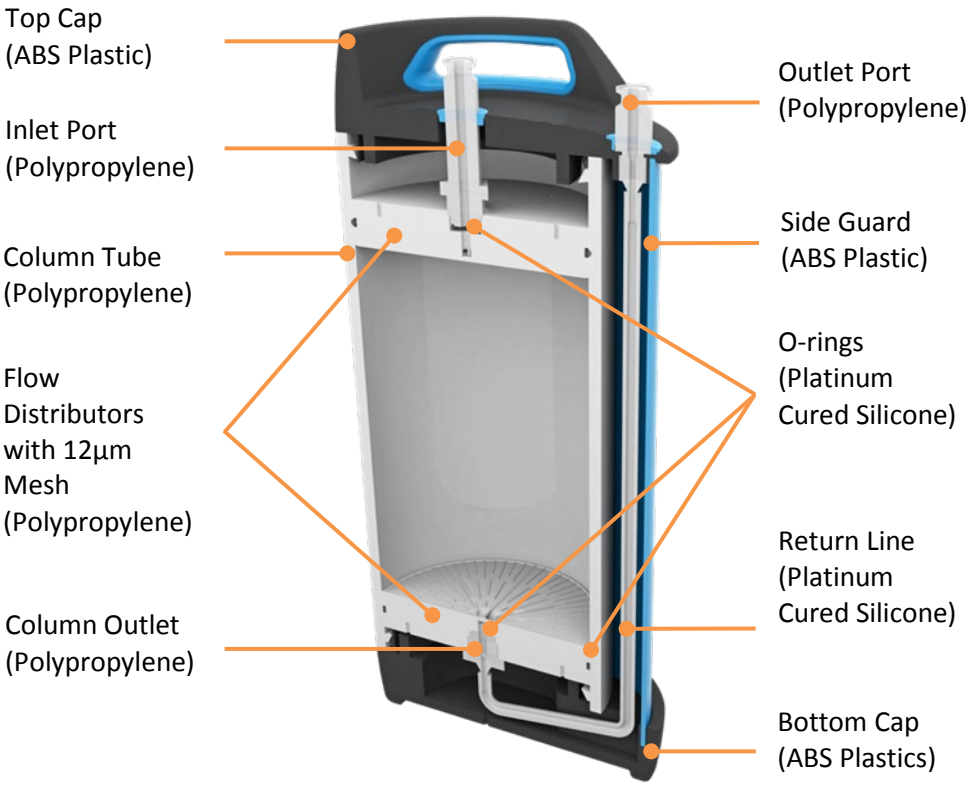
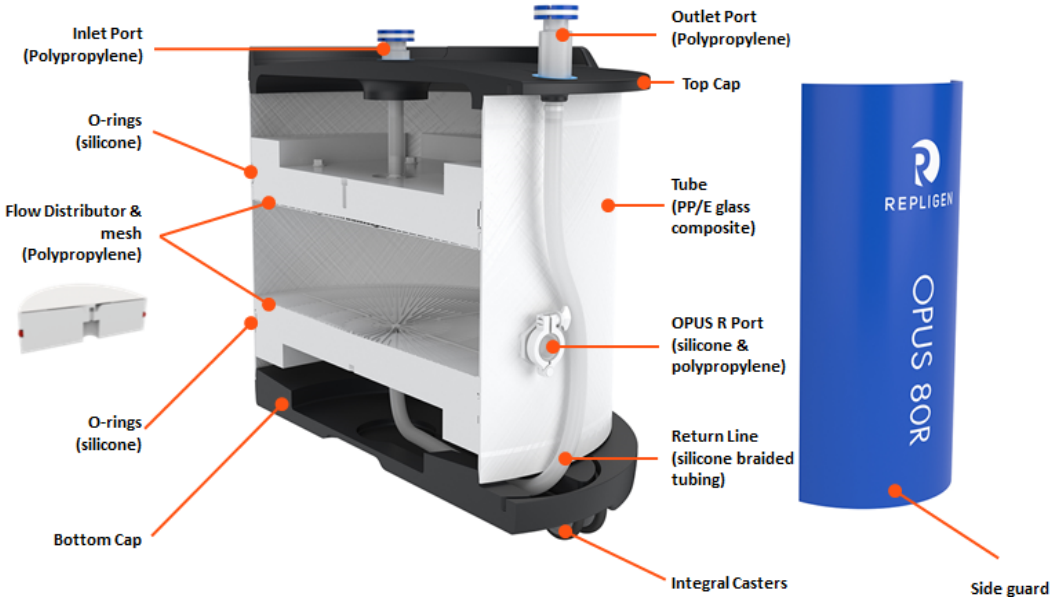


Figure 3. Materials of Construction Summary: OPUS® 45R, 60R, and 80R Columns



2.5 OPUS® Physical Specifications

Table 2. Physical Specifications Summary (OPUS® 5 – 30 Columns)

Column Diameter								
Physical Attributes	5.1 cm	8.1 cm	10 cm	12.6 cm	14 cm	20 cm	25 cm	30 cm
Internal Cross Section	20.4 cm^2	51.5 cm^2	78.5 cm^2	125 cm^2	154 cm^2	314 cm^2	491 cm^2	707 cm^2
Column Body Pressure Rating	4 Bar	4 Bar	4 Bar	4 Bar	4 Bar	4 Bar	4 Bar	4 Bar
Bed Height Range	5 – 30 cm	5 – 30 cm	5 – 30 cm	5 – 30 cm	5 – 30 cm	5 – 30 cm	5 – 30 cm	5 – 30 cm
Column Volumes **10 cm Bed Height** **20 cm Bed Height** **30 cm Bed Height**	 0.2 L 0.4 L 0.6 L	 0.5 L 1.0 L 1.5 L	 0.8 L 1.6 L 2.4 L	 1.3 L 2.5 L 3.8 L	 1.5 L 3.1 L 4.6 L	 3.1 L 6.3 L 9.4 L	 4.9 L 9.8 L 14.7 L	 7.1 L 14.1 L 21.2 L
Assembled Column Height	~20 cm + bed height	~20 cm + bed height	~20 cm + bed height	~28 cm + bed height	~30 cm + bed height	~30 cm + bed height	~33 cm + bed height	~35 cm + bed height

Column Diameter								
Outer Diameter (including caps)	7 cm	10 cm	16 cm	20 cm	21 cm	27 cm	33 cm	38 cm
Inlet/Outlet Flow Path Internal Diameter	3.45 mm 5/32 inch	4.57 mm 3/16 inch	6.35 mm 1/4 inch	6.35 mm 1/4 inch	6.35 mm 1/4 inch	6.35 mm 1/4 inch	9.53 mm 3/8 inch	9.53 mm 3/8 inch
Inlet and Outlet Port Connectors	3/4 inch mini Tri-Clamp	3/4 inch mini Tri-Clamp	3/4 inch mini Tri-Clamp	3/4 inch mini Tri-Clamp	3/4 inch mini Tri-Clamp	3/4 inch mini Tri-Clamp	3/4 inch mini Tri-Clamp	3/4 inch mini Tri-Clamp

Table 3. Physical Specifications Summary (OPUS® 5 – 30 Columns)

Column Diameter			
Physical Attributes	45.7 cm	59.9 cm	79.9 cm
Internal Cross Section	1,640 cm^2	2,818 cm^2	5,014 cm^2
Column Body Pressure Rating	3 Bar	3 Bar	3 Bar
Bed Height Range*	5 – 30 cm	5 – 30 cm	10 – 30 cm
Column Volumes **10 cm Bed Height** **20 cm Bed Height** **30 cm Bed Height**	 16 L 33 L 49 L	 28 L 56 L 84 L	 50 L 100 L 150 L
Assembled Column Height (cm)^	≤ 22 cm BH: ~90 cm >22 cm BH: ~116 cm	≤ 22 cm BH: ~93 cm >22 cm BH: ~120 cm	≤ 20 cm BH: ~97 cm >20 cm BH: ~123 cm
Outer Diameter (including caps)	54 cm	68 cm	91 cm
Inlet/Outlet Flow Path Internal Diameter	12.7 mm 1/2 inch	19.1 mm 3/4 inch	19.1 mm 3/4 inch
Inlet and Outlet Port Connectors Per ASME BPE Standards, Current Edition	3/4 inch mini Tri-Clamp	1 inch Tri-Clamp	1 inch Tri-Clamp

*Resin Recovery design columns have a minimum bed height of 10 cm.
^These values are estimates based on the target bed height (BH) of the column.

2.6 Product Contact Materials

OPUS® 5 – 80R Columns are designed using polymers which are best suited to downstream processing applications (Table 4).

Table 4. Product Materials and Quality Standards

Component	Material	USP	CFR 21 177	BSE/TSE
OPUS® 45R, 60R, 80R cm IDs	Composite tube 70% w/w E-Glass / PP* Homopolymer	Class VI USP <88>	177.1520	Animal Free
Column Tubes OPUS® 5-30 cm IDs	Extruded PP Homopolymer	Class VI USP <88>	177.1520	Animal Free
Flow Distributors OPUS® 5 – 80R Inlet and Outlet Ports, OPUS® R Plug, OPUS® R Inside Port	Compression Molded PP* Homopolymer	Class VI USP <88>	177.1520	Animal Free
Bed Support Screens OPUS® 5-80R	PP* Woven Mesh	Class VI USP<88>	177.1520	EMA 410/01
Flow Distributor O-Rings, OPUS® R Plug O-Ring, OPUS® R Inner/Outer Gaskets	Platinum Cured Silicone (PCS^ O-rings)	Class VI	177.2600	Animal Free
Return Line Tube, OPUS® 10-80R	Platinum Cured Silicone (PCS^ braided tubing)	Class VI	177.2600	Animal Free

*PP = Polypropylene
^PCS = Platinum Cured Silicone

2.7 Solvent Compatibility

The product contact materials of construction consist of polypropylene and silicone parts. Table 5 lists common solutions with excellent compatibility with both polypropylene and silicone.

Table 5. Solvent Compatibility for OPUS® Columns

Solvent Compatibility	
Water	Citric Acid
20% Acetic Acid	Methanol
20% Ethanol	Phosphoric Acid
10% Acetone	Hydrochloric Acid (<20%)
2% (w/v) Detergents	Sulfuric Acid (<50%)
8M Urea or 6M Guanidine HCl	2M Sodium Hydroxide
Potassium Hydroxide	Benzyl Alcohol
Isopropyl Alcohol (IPA)	

2.8 Column Mass Table

Table 6. Approximate Column Weight

Column Diameter											
Bed Height	**5.1 cm**	**8.1 cm**	**10 cm**	**12.6 cm**	**14 cm**	**20 cm**	**25 cm**	**30 cm**	**45.7 cm**	**59.9 cm**	**79.9 cm**
5 cm	0.5 kg	1.0 kg	2.0 kg	4.0 kg	4.0 kg	6.0 kg	10 kg	14 kg	69 kg	117 kg	235 kg
10 cm	0.5 kg	1.5 kg	2.5 kg	4.5 kg	5.0 kg	8.0 kg	13 kg	18 kg	77 kg	131 kg	260 kg
15 cm	1.0 kg	1.5 kg	3.0 kg	5.5 kg	6.0 kg	10 kg	16 kg	22 kg	86 kg	145 kg	285 kg
20 cm	1.0 kg	2.0 kg	3.5 kg	6.0 kg	7.0 kg	12 kg	19 kg	26 kg	94 kg	159 kg	310 kg
30 cm	1.5 kg	2.5 kg	4.0 kg	8.0 kg	9.0 kg	16 kg	24 kg	34 kg	110 kg	187 kg	335 kg

2.9 OPUS® Column Handling

Table 7. Column Handling Features

Column Diameter											
Feature	**5.1 cm**	**8.1 cm**	**10 cm**	**12.6 cm**	**14 cm**	**20 cm**	**25 cm**	**30 cm**	**45.7 cm**	**59.9 cm**	**79.9 cm**
Handles	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No
Casters	No	No	No	No	No	No	No	No	Yes	Yes	Yes
Manually Lifted	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No

3 Preparing to Use Your OPUS® Column

1. Reference the technical specifications below when using your OPUS® Column during processing:
   a. Minimum Packing Pressure
      i. Chromatography skid pressure alarms should be set based on the packing pressure for your particular OPUS® column
   b. Temperature:
      i. Column construction is designed to support a working temperature range of 2°- 40°C.
2. Upon receipt of the box/crate:
   a. Inspect the outside of the heavy-duty cardboard carton (for OPUS® 5 – 30 Columns) or wooden crate (for OPUS® 45R, 60R and 80R Columns) for any unusual signs of damage. If significant damage has occurred, please contact Repligen immediately.
   b. Locate the shipping delivery documents attached to the outside of the box.
   c. Locate the Certificate of Analysis (CoA) for the column inside the box.
   d. Remove the top layer of protective foam to expose the column.
   e. For GMP Run Ready OPUS® Columns, locate the QC resin sample and store as specified by the resin supplier.
   f. In some cases, excess resin not used during column packing may also be shipped with the column. This may be found in the column box/crate, or in a separate box.
3. Remove the column from the box/crate using the instructions in the guides as summarized below:
   a. For maximum cleanliness, keep the clear plastic bag containing the column intact for this step. Please note there is no bag for the OPUS® 45R, 60R and 80R Columns.

b. Reference Table 1 and Table 7 in this User Guide for handling suggestions and features.
c. Do not pull or strain the white inlet and outlet ports protruding from the top of the OPUS® column.

4. If you are not ready to use the column, refer to the storage recommendations of the resin supplier for the pre-packed resin.
   a. OPUS® 8-80R Columns, refer to column label for recommended storage temperature.
   b. OPUS® 5 Column label does not report recommended storage temperature.
5. When you are ready to use your OPUS® Column, remove the clear plastic bag containing the OPUS® 5 – 30 Column. There is no bag for OPUS®45R, 60R and 80R Columns, so they should be wiped down prior to use with Ethanol or Isopropyl Alcohol solution if necessary.
   a. If storing the column in 2-8°C, allow the column to equilibrate to room temperature overnight prior to use of the column.
   b. Using a wire cutter or sharp scissors, remove the white cable-tie on the SaniSure® clamp sealing the inlet port. See Figure 4

Figure 4. SaniSure® Clamp Removal

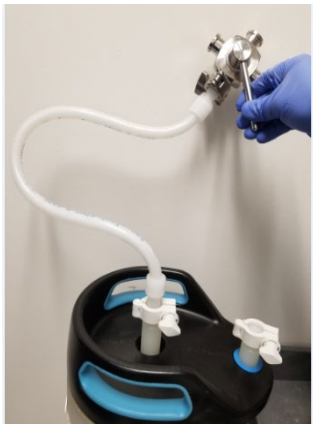
Connecting and Operating Your OPUS® Column

Notes:

Use of stainless steel valves connected directly to the inlet and/or the outlet ports is not recommended. The additional weight to the top of the column will increase the risk of damage to the column hardware.

- If valves must be used, first connect the inlet and outlet ports to tubing. Then the other end of that tubing may be connected to valves as seen in Figure 5
- Tubing length of <50 cm is suggested to minimize hold up volume of the system

Figure 5. Stainless Steel Valve Connection to OPUS® Column



- If an OPUS® Column needs to be connected to an AKTA™ system which does not have TC connections, the following item may be purchased from the GE Healthcare (GEHC) website as a converter
  - Part Number: 18116922
  - Connector, 25mm TC – UNF 5/16" Female
  - AKTA™ is a trademark of GE Healthcare
- Instructions for using a 3-way valve to connect the column inlet as well as purge air from the inlet line can be found in Appendix 1
- Instructions on how to connect an OPUS® Column to chromatography skids can be found in Appendix 2

1. Connect the column to the chromatography skid while limiting the entry of air to the inlet port connection. Repligen suggests the following sequence for reducing air introduction to the column:
   1.1. The column may have off-gassed during shipment which leaves the ports dry; however, the packed bed will remain hydrated in its storage solution. Top off the inlet and/or outlet port with low salt equilibration buffer (e.g. 0.1M NaCl) prior to making connections if needed.
   1.2. With the outlet closed, hook up the column inlet under low flow (~50 cm/hr) with a Tri-clamp (check Table 2 or 3 for the inlet and outlet size) so that no air is introduced into the column.
   1.3. Once the inlet has been connected under low flow, immediately stop the flow of liquid into the column. Column outlet may now be opened via same instruction as Step 1.2.
      1.3.1. If both the inlet and outlet port are open, the column may drain out and draw air into the column flow path.
2. Connect outlet tubing to the outlet port and flush the storage solution from the column with 3-5 column volumes of RO/DI water or mild buffer (e.g. PBS).
   Notes:
   - When in contact with Ethanol solutions, high salt concentration buffers may precipitate solids into the packed bed
   - While flushing the Ethanol storage solution out of the column, high pressures are to be expected due to the viscosity of the solution. Flow rate must be reduced if pressures near >75% of the column packing pressure
   - Observations of air exiting the column are common and will dissipate when the Ethanol is fully cleared from the column

   2.1. All the solutions loaded on to the column should be 0.22 or 0.45 µm filtered to reduce column fouling.
   2.2. Start storage solution flush with 50 cm/hr flow.
      2.2.1. Flow rate may be increased as needed while maintaining conformance to the resin supplier's pressure recommendations. The column could yield high pressure during Ethanol removal steps if flow rate is not monitored.
3. Equilibrate your column using 2-3 CVs of your equilibration process buffer or mobile phase.
4. To test for chromatographic performance and compare results to the CoA, a short instruction guide can be found in Appendix 3.

4 OPUS® Column Sanitization, Storage, and Disposal

4.1 Cleaning and Sanitization Notes

- Please consult the resin supplier for recommended cleaning and storage protocols
- OPUS® Columns can be cleaned with any sanitization agent that is compatible with the materials of construction (see Table 5 for Solvent Compatibility Summary)
- Prior to sanitizing your column, check for solvent compatibility with the chromatography resin supplier

1. Once your chromatography process is completed, the column should be prepped for disposal or storage.
   1.1.1. Disposal: Clean and sanitize the column prior to disposal according to local government regulations.
   1.1.2. Storage: Clean, flush, and prepare the column for storage per the recommendations of the resin supplier or other validated procedure.
2. Reuse post storage: Start with the general usage instructions in the “Connecting and Operating Your OPUS® Column” Section.

5 Troubleshooting

5.1 Air in the column

Potential Fixes

- If air entered the inlet port and did not reached the column (to the best assessment of the operator), follow the air purge procedure described in Appendix 1
  If air entered the packed chromatography bed, recondition the column by running a solution with low surface tension in reverse flow for 2-3 CVs. Increased backpressure on the column effluent may aid in forcing air bubbles out from the column
  - Examples of low surface tension solutions include 1% surfactant (e.g. Tween) and 20% Ethanol for normal phase resins
  - Contact Repligen Customer Support for specific troubleshooting tactics specified to column dimension and packed resin
- Retest column performance (Efficiency, Asymmetry) according to instructions in Appendix 3**.** Results conforming to the provided CoA for your column will help justify release into production

5.2 High pressure during first use of column

Causes

- Undersized tubing, fitting, and/or gaskets
- Incorrect column valve position
- Flow path restriction
- Operation under higher flow rate than recommended for the packed resin bed
- Temperature shifts between buffers used in the column

Potential Fixes

- Refer to Table 2 or 3 for flow path sizing
- Check valve position

- Reduce the flow rate of solution through the column to abide by pressure limit of the packed resin bed
- Confirm that high viscosity solutions are not being used during pressure evaluation
- Flow of alcohols through the column is known to increase column pressure
- Allow all buffers and the column to equilibrate to ambient temperatures

5.3 Pressure increase during run

Causes

- Product or precipitates clogging of the polypropylene mesh
- Operation under higher flow rate than recommended for the packed resin
- Residue build up at the top of the column
- Use of high viscosity solutions or high product load concentrations
- Fouled chromatography resin
- Temperature shifts between buffers used in the column

Potential Fixes

- Clean the column with the appropriate cleaning method for the residue that clogged the mesh and/or resin. Running in reverse flow, or up-flow mode is recommended
- Flow >5CV of equilibration buffer through the column in reverse flow. Recheck pressure and column performance (Efficiency, Asymmetry, Pressure vs. Flow) under normal operating conditions for comparison to results for on CoA
- Check valve position
- Allow all buffers and the column to equilibrate to ambient temperatures

5.4 Pressure drop during run

Causes

- Line or fitting leaks
- Temperature shifts during buffer transitions
- Viscosity shifts during buffer transitions

Potential Fixes

- Check lines and connections
- Allow all buffers and the column to equilibrate to ambient temperatures

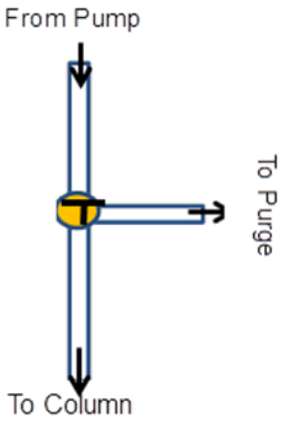
6 Appendices

6.1 Appendix 1: Use of a 3-Way Valve for Inlet Connection and Air Purging

To purge air when the column is first connected to the chromatography system:

1. Connect one end of the 3-way valve to the column inlet and the other end to the chromatography system pump. Leave the column outlet closed.
2. Configure the 3-way valve flow path as shown in Figure 6 below.

Figure 6. Position of 3-way valve for purging air from the column inlet



3. OPUS® 5 – 14 Columns, attach a syringe to the purge line while pumping the mobile phase at low flow rate (i.e. 50 cm/hr), and draw the plunger to create negative pressure. Air bubbles will be drawn into the syringe, and mobile phase will immediately fill the space created.
4. OPUS® 20 – 80R Columns, begin pumping mobile phase at a slow flow rate (i.e. 50 cm/hr). The mobile phase will travel into the column inlet and out of the purge line. This path will engage and dislodge any air bubbles trapped in the column inlet line.
5. After all the air has been purged from the inlet line, engage the 3-way valve as shown in Figure 7 below.

Figure 7. 3-way valve for flow to column

6. With the flow off and the 3-way valve configured as shown in Figure 7, open the column outlet and connect it to the chromatography system.

7. Introduce flow to the column at a low flow rate (i.e. 50 cm/hr) to flush trapped air from the column outlet.

In the absence of a 3-way valve:

1. A "T" line can be connected between the column and chromatography system.
2. The "T" line can be used as described above for purging air.
3. After air has been purged, the purge line can be clamped or closed with a stopper.
4. The column outlet can then be connected to the chromatography system for normal use.

6.2 Appendix 2: Connecting an OPUS® column to Chromatography Skids

1. Connect the inlet of the column to the chromatography system tubing set. At this point, the outlet of the column should be closed and disconnected.

Notes:

- Use of stainless steel valves connected directly to the inlet and/or the outlet ports is not recommended. The additional weight to the top of the column will increase the risk of damage to the column hardware

2. Start flowing the mobile phase through the system at <50% of the recommended operating flow rate for your chromatography resin/process. During this operation, the flow will be split. One portion of the mobile phase will enter the bypass line and other portion will enter the inlet line.

Notes:

- The mobile phase will not enter the column because the column outlet is closed (with the pinch valve, or outlet cap) thus creating a stop barrier for the flow. The fraction of the flow that enters the inlet line will dislodge the trapped air in the tube and connector

3. With the flow split to the bypass and inlet lines, air bubbles will begin to travel upward in the inlet tube and will be evacuated through the column bypass line into the system outlet. To ensure all the air is evacuated, tap and/or shake the inlet tube and inlet connector.
4. After all the air has been removed from the column inlet and connector, close the bypass line pinch valve and connect the outlet line to the column with the outlet pinch valve in the open position.
5. Run mobile phase through the column to purge the outlet of air.

6.3 Appendix 3: Column Performance Testing

Follow the steps below to measure the plate count and asymmetry of your OPUS® Column. Please note, minor differences (plus or minus 10-20%) in the measured plate count and asymmetry noted on the column CoA or CoQ are to be expected. Sources of variation include:

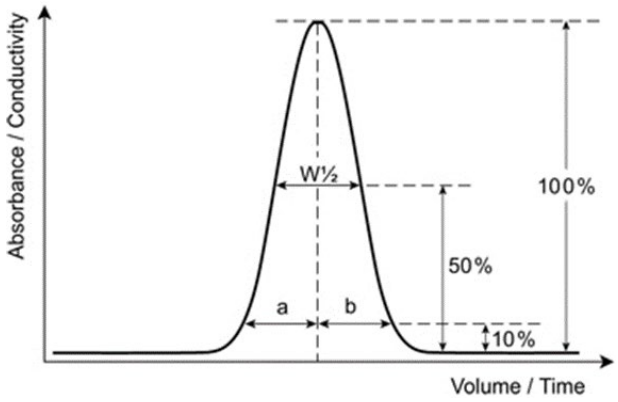
- Chromatography instruments for measurement
- Chromatography system
- Operator variability
- Normal variability within the test methods
  - Flow rate
  - Sample volumes
  - Equilibration/plug solutions
  - Injection method

If the plate count and asymmetry measurements are within defined acceptance limits (reference the column's CoA or CoQ "QC Release Data") then the column should be considered fit for purpose.

1. Remove column storage solution.

2. If column storage solution is alcohol based, run equilibration buffer at low flow rate (i.e. 50 cm/hr) for 2-3 column volumes. Because Ethanol solution is more viscous than water, the flow rate of this step should be chosen such that the pressure drop on the column does not exceed the maximum operating pressure.
3. After the storage solution has been removed, condition the column with the equilibration buffer for 1-2 column volumes at column qualification testing flow rate.
4. Proceed to testing the column:
   4.1. Note: refer to your column's CoA or CoQ for the test "Mobile Phase" and "Testing Flow Rate" used by Repligen.
5. Conduct a pulse injection with 1-2% CV of the Injection Solution.
   5.1. Note: refer to your column's CoA or CoQ for the "Injection Solution" and "Injection Volume" used by Repligen.
6. Elute with mobile phase for 1-2 CV at the same test flow rate while monitoring UV or conductivity depending on the Injection Solution.
   6.1. Salt injection solutions are typically analyzed with a conductivity meter while acetone injection solutions are analyzed with an UV meter.
   6.2. Calculate number of theoretical plates and asymmetry of the eluted peak:

Figure 8. Absorbance / Conductivity Graph



Theoretical Plate

Count: N= 5.54 x $(V_R/W_{1/2})^2$, assuming a Gaussian peak

Where:
N = number of theoretical plates
V_R = peak retention (elution) volume
$W_{1/2}$ = peak width at half height

Asymmetry: As = b/a

Where:
a = partial peak width at 10% of the peak height for the leading part of the peak
b = partial peak width at 10% of the peak height for the tailing part of the peak

If the plate count and asymmetry measurements are within defined acceptance limits (reference the column's CoA or CoQ "QC Release Data") then the column should be considered fit for purpose.

Efficiency Definitions:

Purpose: This section describes the conversion of HETP values expressed in centimeters to Efficiency values expressed in Plates/meter. Both HETP and Efficiency may be used to quantitatively describe the quality of a chromatography column's packed bed.

N: Number of theoretical plates

HETP: Height of an equivalent theoretical plate, expressed in units of cm/N. Smaller values indicate a sharper peak.

Plates/meter (N/m): Number of theoretical plates per meter. Larger values indicate a sharper peak.

Formula: N/m = 100 / HETP

Example:

Given an HETP specification of ≤0.05 cm/N, the conversion follows as below.

$$\frac{1}{0.05\,\frac{cm}{N}} = 20\,\frac{N}{cm}$$

$$20\,\frac{N}{cm} * \frac{100\ cm}{1\ m} = 2000\,\frac{N}{m}$$

Since the formula takes the reciprocal of ≤0.05 cm/N, the inequality inverts to greater than or equal to (≥). Therefore, the converted Efficiency specification is ≥2000 N/m.

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