OPUS® 5 - 80R Pre-packed Chromatography Columns

User Guide

UG-OPUS-5-80R-2

REPLIGEN

The information contained in this document is subject to change without notice.

With respect to documentation accompanying Product Repligen makes no warranty, express or implied. Any and all warranties related to the documentation accompanying Product are expressly disclaimed. Customer shall refer to the terms and conditions of sale governing the transaction for any and all warranties for the Product.

Repligen Corporation shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

No part of this document may be photocopied, reproduced, or translated to another language without the prior written consent of Repligen Corporation.

Products are not intended for diagnostic or therapeutic use or for use in vivo with humans or animals.

For further information, please contact Repligen Corporation at www.repligen.com.

Customer Support

customerserviceUS@repligen.com
+1 (781) 250-0111

Repligen Corporation

41 Seyon Street
Building 1, Suite 100
Waltham, MA 02453
www.repligen.com

Contents

List of tables

List of figures

Abbreviations

µm	micron
ASME BPE	American Society of Mechanical Engineers: Bioprocessing Equipment Standards
BSE	Bovine Spongiform Encephalopathy
C	Celsius
cm	centimeter
CoA	Certificate of Analysis
CoQ	Certificate of Quality
CV	column volume
hr	hour
in	inch
IPA	Isopropyl alcohol
kg	kilogram
L	liter
m	meter
M	molar
mm	millimeter
NaCl	Sodium chloride
OPUS	Open platform user specified
PBS	phosphate buffered saline
PP	polypropylene
QC	Quality Control
RODI	reverse osmosis deionized water
TC	tri-clamp
TSE	Transmissible Spongiform Encephalopathy
w/v	weight/volume

1. Introduction

This user guide provides detailed instructions for the set-up, sanitization, and storage of OPUS® 5 - 80R Pre-packed Chromatography Columns. Hardware specifications and chemical compatibility are also shown. For questions and further information, please contact your Repligen representative.

2. Safety precautions

The uncrating guidance is based on column internal diameter. Please abide by the following movement and handling recommendations.

Note: *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 36R - 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.
- Beware of OPUS Column caster wheel position to avoid trip hazards.
- Follow all local regulations for safe disposal.
- For laboratory and manufacturing production use only.
- Not for administration to humans.

3. Product description

3.1 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 for standard internal diameters.

3.2 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.

3.3 Applications

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.
- Are available in a wide range of bed heights and industry-standard column diameters.
- Are configurable for specific packing procedures, release tests, and storage solutions.

4. Receipt, uncrating, and storage

4.1 Upon receipt

1. Inspect the outside of the heavy-duty cardboard carton (for OPUS 5 - 45 Columns) or wooden crate (for OPUS 60R and 80R Columns) for any unusual signs of damage. If significant damage has occurred, please contact Repligen immediately.
2. Locate the shipping documents attached to the outside of the box.
3. Locate the Certificate of Analysis (CoA) for the column inside the box.
4. Remove the top layer of protective foam to expose the column.
5. For GMP Run Ready OPUS Columns, locate the quality control (QC) resin sample and store as specified by the resin supplier.
6. In some cases, excess resin not used during column packing may also be shipped with the column. This may be found either in the column box/crate or in a separate box.

4.2 Uncrating

Remove the column from the box/crate using the instructions in the guides available at repligen.com.

- For maximum cleanliness, keep the clear plastic bag containing the column intact for this step. Please note there is no bag for the OPUS 36R - 80R Columns.
- Reference Table 1, Table 2, and Table 3 for safe handling guidance and features.
- Do not pull or strain the white inlet and outlet ports protruding from the top of the OPUS column.

Table 2. Approximate Column Weights (in kg)

Bed height	OPUS 5	OPUS 8	OPUS 10	OPUS 12.6	OPUS 14	OPUS 20	OPUS 25	OPUS 30	OPUS 36R	OPUS 45R	OPUS 60R	OPUS 80R
5 cm	0.5	1.0	2.0	4.0	4.0	6.0	10	14	43	69	117	235
10 cm	0.5	1.5	2.5	4.5	5.0	8.0	13	18	48	77	131	260
15 cm	1.0	1.5	3.0	5.5	6.0	10	16	22	54	86	145	285
20 cm	1.0	2.0	3.5	6.0	7.0	12	19	26	59	94	159	310
30 cm	1.5	2.5	4.0	8.0	9.0	16	24	34	69	110	187	335

Table 3. Column Handling Features

Feature	OPUS 5	OPUS 8	OPUS 10	OPUS 12.6	OPUS 14	OPUS 20	OPUS 25	OPUS 30	OPUS 36R	OPUS 45R	OPUS 60R	OPUS 80R
Handles	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No	No
Casters	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes
Manually lift	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No

Note: *The OPUS 36R Column design contains an extended footprint based on the caster wheel position on the bottom cap. Beware of tripping hazard in storage and transport of OPUS 36R Columns within your facility.*

4.3 Storage

If you are not ready to use the column, refer to the storage recommendations of the resin supplier for the pre-packed resin. For OPUS 8 - 80R Columns, refer to column label for recommended storage temperature. Be sure to store the column in a proper location to avoid trip hazard from the OPUS Column caster wheels. 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 36R - 80R Columns, so they should be wiped down prior to use with ethanol or isopropyl alcohol solution, if necessary.

- If storing the column at 2 - 8°C, allow the column to equilibrate to room temperature overnight prior to use of the column.
- Using a wire cutter or sharp scissors, remove the white cable-tie on the SaniSure® clamp sealing the inlet port (Figure 1).

Figure 1. SaniSure Clamp Removal

5. Connection and Operation

5.1 Pressure and temperature specifications

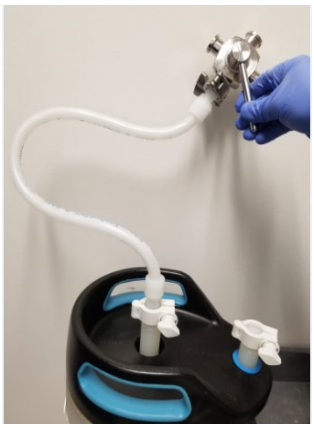
Maximum Packing Pressure: Chromatography skid pressure alarms should be set based on the packing pressure for each column operating temperature: 2° - 40°C.

5.2 Valves

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

- If valves must be used for fluid management controls, first connect tubing to inlet and outlet ports. The other end of that tubing can be connected to valves (Figure 2).
- Tubing length of <50 cm is suggested to minimize hold-up volume.

Figure 2. Stainless Steel valve connected to tubing



- If an OPUS Column needs to be connected to an AKTA™ system, which does not have TC connections, Cytiva offers a suitable connector: Connector, 25mm TC - UNF 5/16" Female; Part Number 18116922.
- 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 Section 10.
- Instructions on how to connect an OPUS Column to chromatography skids can be found in Section 11.

5.3 Connection to skid

1. Connect the column to the chromatography skid while limiting the entry of air to the inlet port connection.
    a. 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 a low salt equilibration buffer (e.g. 0.1 M NaCl) prior to making connections if needed.
    b. With the outlet closed, connect tubing to inlet under low flow (~50 cm/hr) with a tri-clamp connector (Table 4 or Table 5) so that no air is introduced into the column.
    c. Once the inlet has been connected under low flow, immediately stop the flow of liquid into the column. Column outlet may now be opened.
    d. Connect tubing to column outlet with a tri-clamp connector (Table 4 or Table 5).

Note: *When both the inlet and outlet ports are open, the column has the potential to drain and draw air into the column flow path.*

Note: *For additional details on tubing connection and priming, refer to OPUS Pre-packed Chromatography Columns Connection and Priming User Guide on repligen.com.*

2. Flush the storage solution from the column with 3 - 5 column volumes of RODI 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 expected due to the viscosity of the solution. Flow rate must be reduced if pressure reaches >75% of the column packing pressure. Air exiting the column is common during the flush and will dissipate when the ethanol is fully cleared from the column. All the solutions loaded on to the column should be 0.22 or 0.45 µm filtered to reduce column fouling.*

3. Start storage solution flush at a flow rate of 50 cm/hr. Flow rate may be increased while adhering to the resin supplier's pressure recommendations.
4. Equilibrate the column width using 2 - 3 CV of equilibration buffer or mobile phase.
5. To test for chromatographic performance and compare results to the CoA, a short instruction guide can be found in Section 12.

6. Sanitization, storage, and disposal

6.1 Cleaning and sanitization

Notes: *Please consult resin supplier for recommended cleaning and storage protocols, including resin chemical compatibility guidance. OPUS Columns can be cleaned with any sanitization agent that is compatible with the materials of construction (Section 9.2).*

1. Once the chromatography process is completed, the column should be prepped for disposal or storage.
    a. Disposal: Clean and sanitize the column prior to disposal according to local government regulations.
    b. Storage: Clean, flush, and prepare the column for storage per the recommendations of the resin supplier or other validated procedure.
2. Post-storage reuse: Start with the general usage instructions (Section 5).

7. Physical specifications

Table 4. Physical specifications (OPUS 5 - 30 Columns)

Physical attributes		OPUS 5	OPUS 8	OPUS 10	OPUS 12.6	OPUS 14	OPUS 20	OPUS 25	OPUS 30
Diameter		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	0.2 L	0.5 L	0.8 L	1.3 L	1.5 L	3.1 L	4.9 L	7.1 L
	20 cm bed height	0.4 L	1 L	1.6 L	2.5 L	3.1 L	6.3 L	9.8 L	14.1 L
	30 cm bed height	0.6 L	1.5 L	2.4 L	3.8 L	4.6 L	9.4 L	14.7 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
Outer diameter (cm, 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 in)	4.57 mm (3/16 in)	6.35 mm (1/4 in)	6.35 mm (1/4) in	6.35 mm (1/4 in)	6.35 mm (1/4 cm)	9.53 mm (3/8 in)	9.53 cm (3/8 in)
Inlet/outlet port connectors		3/4 in mini tri-clamps	3/4 in mini tri-clamps	3/4 in mini tri-clamps	3/4 in mini tri-clamps	3/4 in mini tri-clamps	3/4 in mini tri-clamps	3/4 in mini tri-clamps	3/4 in mini tri-clamps

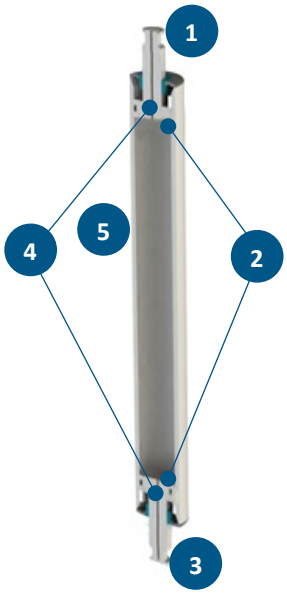
Table 5. Physical specifications (OPUS 36R - 80R Columns)

Physical attributes		OPUS 36R	OPUS 45R	OPUS 60R	OPUS 80R
Diameter		36 cm	45.7 cm	59.9 cm	79.9 cm
Internal cross section		1020 cm^2	1640 cm^2	2818 cm^2	5014 cm^2
Column body pressure rating		3 bar	3 bar	3 bar	3 bar
Bed height range		10 - 30 cm	10 - 30 cm	10 - 30 cm	10 - 30 cm
Column volumes	10 cm bed height	10 L	16 L	28 L	50 L
	20 cm bed height	20 L	33 L	56 L	100 L
	30 cm bed height	31 L	49 L	84 L	150 L
Assembled column height[1]	≤22 cm bed height	~87 cm	~90 cm	~93 cm	~97 cm
	>22 cm bed height	~113 cm	~116 cm	~120 cm	~123 cm
Outer diameter (including caps)		45 cm	54 cm	68 cm	91 cm
Inlet/outlet flow path internal diameter		12.7 mm (1/2 in)	12.7 mm (1/2 in)	19.1 mm (3/4 in)	19.1 mm (3/4 in)
Inlet/outlet port connectors		3/4 in mini tri-clamps	3/4 in mini tri-clamps	1 in tri-clamps	1 in tri-clamps

[1]Estimates based on the target bed height.

8. Materials of Construction

Figure 3. Materials of Construction: OPUS 5 and 8 Columns

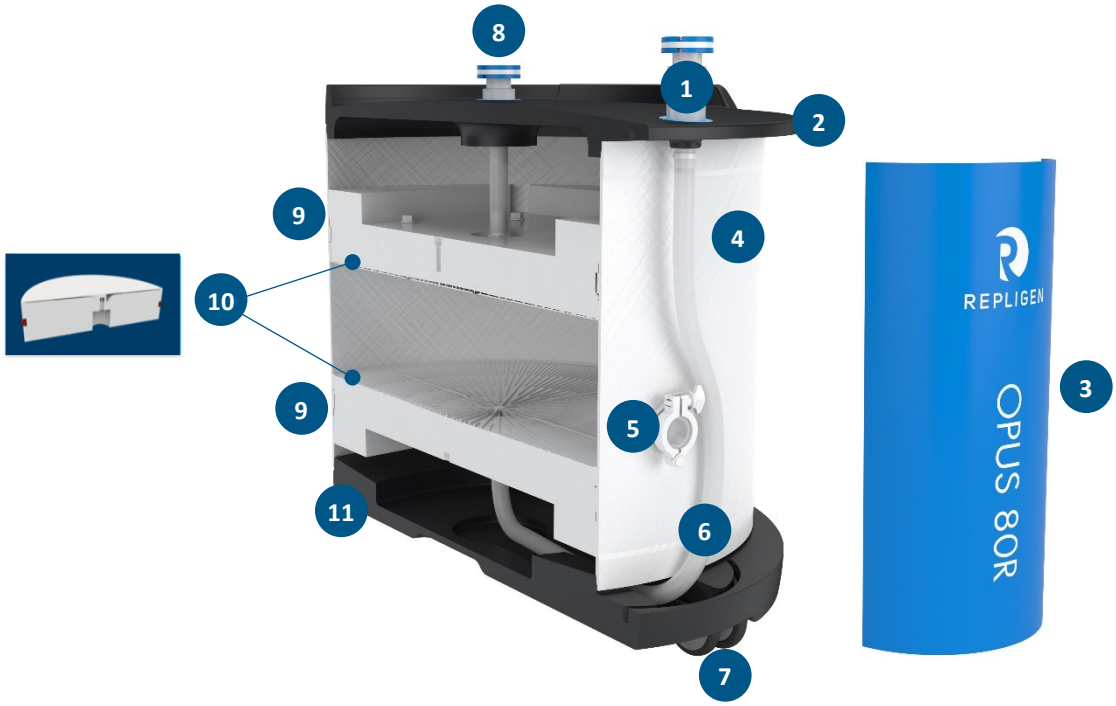


1. Inlet (Polypropylene)
2. O-rings (Platinum-cured silicone)
3. Outlet (Polypropylene)
4. Flow distributor and 12 um mesh (Polypropylene)
5. Tube (Polypropylene)

Figure 4. Materials of Construction: OPUS 10 - 30 Columns

1. Top cap (ABS plastic)
2. Outlet (Propylene)
3. Side-guard (ABS plastic)
4. O-rings (Platinum-cured silicone)
5. Bottom cap (ABS plastic)
6. Inlet (Polypropylene)
7. Tube (10 - 30 cm IDs: Polypropylene)
   (45 and 60 cm IDs: PP/E glass composite)
8. Flow distributor and 12 µm mesh (Polypropylene)
9. Return line (Platinum-cured silicone braided tubing)

Figure 5. Materials of construction: OPUS 36R - 80R Columns



1. Inlet (Polypropylene)
2. Top cap (ABS plastic)
3. Side-guard (ABS plastic)
4. Tube (PP/E glass composite)
5. OPUS R port (Silicone and Propylene)
6. Return line (Platinum-cured silicone braided tubing)
7. Casters (Polyamide hub, Polyurethane tires)
8. Inlet (Polypropylene)
9. O-rings (Platinum-cured silicone)
10. Flow distributor and mesh (Polypropylene)
11. Bottom cap (ABS plastic)

Note: *The OPUS 36R Column design contains an extended footprint based on the caster wheel position on the bottom cap. Beware of tripping hazard in storage and transport of OPUS 36R Columns within your facility.*

8.1 Product contact materials

OPUS 5 - 80R Columns are designed using high grade polymers for downstream processing applications (Table 6).

Table 6. Product Materials and Quality Standards

Component	Material	USP	CFR 21 177	BSE/TSE
OPUS 5 - 30 Column tubes	Extruded polypropylene (PP) homopolymer	Class VI USP <88>	177.1520	Animal-free
OPUS 36R - 80R Column tubes	Composite tube 70% w/w E-glass / PP homopolymer	Class VI USP <88>	177.1520	Animal-free
OPUS 5 - 80R Flow Distributors Inlet and Outlet Ports OPUS R Plug OPUS R Inside Port	Compression-molded PP homopolymer	Class VI USP <88>	177.1520	Animal-free
OPUS 5 - 80R Bed Support Screens	12µm 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	Class VI	177.2600	Animal-free
OPUS 10 – 80R Return Line Tube	Platinum Cured Silicone braided tubing	Class VI	177.2600	Animal-free

8.2 Solvent Compatibility

The following chemicals are compatible with both polypropylene and silicone:

- 10% Acetone
- 2 M Sodium hydroxide
- 2% (w/v) detergents
- 20% Acetic acid
- 20% Ethanol
- 6 M Guanidine HCl
- 8 M Urea
- Benzyl alcohol
- Citric acid
- Hydrochloric acid (<20%)
- Isopropyl alcohol (IPA)
- Methanol
- Phosphoric acid
- Potassium hydroxide
- Sulfuric acid (<50%)
- Water

9. Troubleshooting

9.1 Air in the column

Suggested remedies:

- If air entered the inlet port and did not reach 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 CV. 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., Polysorbate 20 or 80) and 20% Ethanol for normal phase resins
    - Contact Repligen Customer Support for troubleshooting tactics specific to column dimension and packed resin

Retest column performance (efficiency, asymmetry) according to instructions in Section 12**.** Results conforming to the provided CoA for your column will help justify release into production.

9.2 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 Remedies

- Refer to Table 4 or Table 5 for flow path sizing
- Check valve position.
- Reduce the flow rate to bring column within pressure limit of the packed resin bed.
- Confirm that high viscosity solutions were not used during pressure evaluation.
- Check solution running at time of pressure excursion. Alcohols can increase pressure.
- Ensure all buffers and the column are equilibrated to ambient temperatures.

9.3 Pressure increase during run

Causes

- Product or precipitates clogging 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 >5 CV 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 on CoA.
- Check valve position.
- Allow all buffers and the column to equilibrate to ambient temperatures.

9.4 Pressure drop during run

Causes

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

Potential Fixes

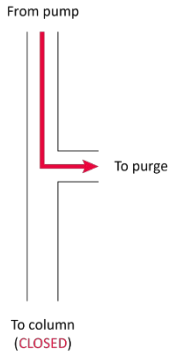
- Check lines and connections.
- Allow all buffers and the column to equilibrate to ambient temperatures.

10. Appendix 1: Use of a 3-way valve

A 3-way valve can be used to purge air when the column is first connected to the chromatography system.

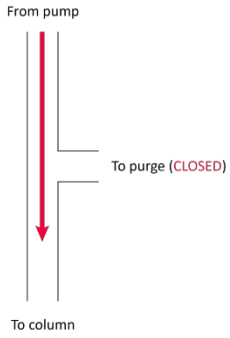
1. Connect one end of the 3-way valve to the column inlet and the other end to the chromatography system pump. Leave the column outlet closed.
2. Configure the 3-way valve flow path (Figure 6).

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



3. Choose next step based on the OPUS Column ID:
    a. 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.
    b. 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.
4. After all the air has been purged from the inlet line, engage the 3-way valve (Figure 7).

Figure 7. 3-way valve for flow to column



5. With the flow off, open the column outlet and connect it to the chromatography system.
6. 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. Connect a T-line between the column and chromatography system. Use T-line to purge air as above.
2. After the air is purged, clamp the purge line.
3. Connect the column outlet to the chromatography system.

11. Appendix 2: Connecting an OPUS Column to a chromatography skid

1. With the column outlet closed, connect the inlet of the column to the chromatography system.

Note: *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. During this operation, the flow will be split: one portion of the mobile phase will enter the bypass (purge) line and the other portion will enter the inlet line.

Note: *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 through the bypass line.*

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. Open the outlet port and connect the outlet line.
5. Open the outlet valve and run mobile phase through the column to purge the outlet of air.

12. Appendix 3: Column performance testing

Column performance is monitored by measuring the height equivalent to theoretical plate (HETP) and the asymmetry factor (A_s).

Note: *Minor differences (±10% – 20%) in the HETP and asymmetry from the data noted on the column CoA or CoQ are 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

Typically, minimum theoretical plate values are 1000 - 3000 plates/m, and peak asymmetry should be as close to 1 as possible, with acceptable values from 0.8 to 1.8. However, these estimations are dependent on the resin packed within each individual OPUS Column. Following analysis, HETP and asymmetry measurements can be compared to defined acceptance limits from the initial qualification post-pack (CoA or CoQ QC Release Data). The column is considered fit for purpose if the measurements fall within 10% - 20% of the initial qualification data.

12.1 Process

Note: *Refer to CoA or CoQ for the equilibration buffer, flow rate, injection solution, and injection volume used by Repligen.*

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 the 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. Condition the column with the equilibration buffer for 1 - 2 CV at indicated flow rate (***Note***).
4. Conduct a pulse injection with 1 - 2% CV of the injection solution (***Note***).

5. Continue pumping equilibration buffer for 1 - 2 CV at the same flow rate while monitoring UV (acetone solution) or conductivity (salt solution).

12.2 Calculations

12.2.1 HETP calculation

Calculate HETP using the following calculations:

$$N = 5.54 \times \left(\frac{V_R}{W_{1/2}}\right)^2$$

$$HETP = \frac{L}{N}$$

Where (Figure 8):
L = bed height (cm)
N = number of theoretical plates
V_R = peak retention (volume or time)
$W_{1/2}$ = peak width at half height

Note: *V_R and $W_{1/2}$ are in the same units*

To convert HETP from L/N to N/m, take the inverse of HETP and multiply by 100:

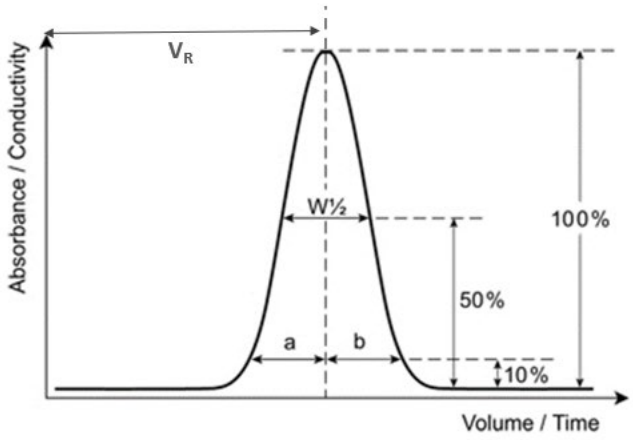
$$\frac{N}{m} = \left(\frac{1}{HETP}\right) \times 100$$

12.2.2 Asymmetry calculation

$$A_S = \frac{b}{a}$$

Where (Figure 8):
a = ascending part of the peak width at 10% of the peak height
b = descending part of the peak width at 10% of the peak height

Figure 8. HETP and A_s calculation parameters



13. Index

Customer Service

Repligen Corporation
41 Seyon Street
Waltham, MA, USA 02453

customerserviceUS@repligen.com

(781) 250-0111

REPLIGEN