SOP Title: **Packing Instructions for the BPG Series Columns**
SOP Number: **14100**
Revision: **05**

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1. PURPOSE

The purpose of this procedure describes the method for packing a BPG Series Column.

2. SCOPE

This SOP provides instructions for packing BPG columns used in CGMP Purification within the BDP.

3. RESPONSIBILITIES

3.1 The Manager, Technical Operations, Purification, Biopharmaceutical Development Program (BDP)

- Defines the procedure.

3.2 BDP personnel

- Implements the procedure.

3.3 Biopharmaceutical Quality Assurance (BQA)

- Provides quality oversight.

4. MATERIALS AND REAGENTS

Part Number	Description	BDP Approved Substitution Permitted?
N/A	BPG Series Columns.	☐ YES ☒ NO
N/A	A packing reservoir.	☐ YES ☒ NO
N/A	Water for Injection (WFI) quality water	☐ YES ☒ NO
N/A	A Type 316 stainless steel pressure vessel	☐ YES ☒ NO
20235	High-pressure plastic tubing from Amesil	☐ YES ☒ NO
20244	Pharmed tubing	☐ YES ☒ NO
46109 CL	0.5N NaOH	☐ YES ☒ NO
46205CL	2M NaCl	☐ YES ☒ NO

5. EQUIPMENT

- A Type 316 stainless steel pressure vessel
    - Calibrated pressure gauge (1).
    - Vent relief valve (1)
    - Elbow fittings (2).
    - Ball valve (1).
- 4-port, 4-way valve or 4-port, 2-way valve
- Chromatography Skids: Bio Process (6 mm) BDP 75640, AKTA pilot BDP 80060 or BDP approved equivalent.

6. PROCEDURE OR USE

6.1 Assembling the Column and Support Nets.

6.1.1 Verify columns were cleaned as per **SOP 14146, Cleaning Chromatography Columns for GMP Processes** and fill out the release testing information.

6.1.2 Carefully assemble the column with new support nets and mesh screens on the top and bottom adaptors appropriate for the resin to be used, following the BPG Column Instruction Manual and/or **SOP 21529, Equipment Interproduct Cleaning and Clearance.**

Appropriate torque wrench settings are described in the table below.

BPG Column				
	100	140	200	300
Moment Units: Nm	4	5	5	Rod nuts (2) - 6 Nm Top Plate Clamp Nuts (4) - 6.5 Nm

6.2 Test For Leakage Across the Adaptor

6.2.1 Fill the Column with WFI. No air space should be present in the column tube. There is a danger of explosion if air is present in the column glass tube during the leakage test. Fill the pressure canister with WFI and attach it to the nitrogen tank equipment with high pressure tubing. Pressurize the tank to 5 PSI ± 2 PSI. (See Attachment 4).

6.2.2 Purge air from the outlet line of the pressure canister by opening the ball valve. Close the ball valve and attach the pressure canister outlet line to the column's top adapter. Close the valve on the bottom adapter of the column. Open the ball valve and slowly increase pressure in the column to 50% - 80% of the maximum acceptable pressure for the column. (Maximum acceptable pressure is usually found on the glass tube of the column. If not, refer to the BPG Column Instruction Manual.)

6.2.3 Maintain pressure on the column for 5 - 10 minutes. After the pressure test, if no leaks are present, the column is ready to be stored until packing. If leaks are present during the pressure test, disassemble the column, replace O-rings, and reassemble. Repeat Sections 6.2.1 - 6.2.3. If the column passes the leakage test, depressurize the column and pressure vessel. Verification of successful pressure test is to be recorded on **Form 14100-01.**

6.3 Packing Preparations

NOTE: Refer to Batch Production Record (BPR) for packing flow rates.

6.3.1 Attach a 4-way valve to the top and a 2 or 4 way valve to the bottom adaptors of the column.

6.3.2 If needed, mount the packing reservoir to the top of the column.

6.3.3 Prior to packing, fill the column with 0.5N NaOH and allow the column a minimum of one hour exposure. Record the exposure time in **Form 14100-01**. Drain the 0.5N NaOH from the column, rinse the column with WFI and level the column.

6.4 Resin Preparation

6.4.1 The Manufacturer may recommend removal of the storage buffer for packing. This can be done by suspending the resin to homogeneity, allowing it to settle, then pouring off the storage buffer and replacing it with the proper volume of desired packing buffer. Repeat this step 1 to 3 times.

6.4.2 Determining the % slurry. Slurry the resin and pour 10 mL resin into a 15 mL conical tube or a graduated cylinder and allow standing until settling of the resin stops. Divide the settled height of the resin by the total height of the resin plus the buffer and multiply it by 100, i.e., settled resin height 7 cm. Total height (Resin + Liquid) 10 cm; (7 ÷ 10) x 100 = 70%. Once the slurry settles again, add or remove buffer to the desired amount. Record % slurry and calculations on **Form 14100-01**.

For chromatography resins that are already prepared for use by the manufacturer, the height of the settled resin and total height of the resin plus the storage buffer, can be obtained using a ruler measuring both parameters from the outside of the manufacturers' container.

6.4.3 Determine the volume of slurred resin to pour. (Target Packed bed volume x compression factor) ÷ (% Slurry ÷ 100) = Volume of Slurred Resin to Pour. Record on **Form 14100-01.**

NOTE: Compression factor is stated by the manufacturer and varies for different types of Resin.

6.5 Pouring the Resin

6.5.1 Suspend the resin to homogeneity by gently inverting and rocking. Pour the suspension into the column in one movement down the inside of the column to avoid the generation of air bubbles. Stir gently with a plastic paddle for 1-5 minute(s) to remove any air that may have been trapped in the slurry.

6.5.2 Proceed to Section 6.6 if packing with a chromatography skid, or Section 6.7 if packing with a pressure canister.

6.6 Packing with a Chromatography Skid

NOTE: A peristaltic pump should not be used.

6.6.1 Connect the chromatography skid col1 top/inlet to the column adaptor. With the valve on the column adapter bypassing the net, run fluid through the tubing to remove air. Once air is removed from the lines, direct flow through the adapter and remove any air trapped in the net. Remove trapped air using either a pipette or syringe. Attach the top column adaptor to the column. Lower the top adaptor just into the liquid level in the column. Remove air bubbles trapped below the top adaptor by wiggling the top adapter while moving it down.

6.6.2 With the top adaptor valve in the waste position, tighten the top adaptor O-ring and lower the adaptor until fluid comes out of the top adaptor valve to remove any remaining air.

6.6.3 Open the column effluent tubing on the lower adapter and tighten the top adaptor O-ring. Put the top adaptor valve in line with the column.

6.6.4 Start the flow of the packing buffer at a constant linear flow rate equal to the recommended Phase I packing flow rate. See Section 6.3 for recommended flow rate.

6.6.5 When the resin bed is stable, simultaneously stop flow to the column and turn the adapter valve to the waste position and close the bottom valve. Loosen the top plunger O-ring. Lower the top adaptor ≤ 2 cm above the resin bed surface, without disturbing the resin bed. Tighten the top adapter O-ring and open both the top and bottom valves simultaneously.

6.6.6 When using a packing reservoir, you may remove the reservoir once the resin bed is below the top of the column and the top adaptor can be lowered without touching the resin bed. Close the bottom column valve, stop flow, and remove the top adaptor followed by the packing reservoir. Connect the top adaptor; lower the top adaptor ≤ 2 cm above the resin bed surface, without disturbing the resin bed. Tighten the O-ring, open the bottom valve and resume phase I flow until resin bed is stable.

NOTE: There may be a large fluid spill in performing this step.

6.6.7 Gradually increase the flow rate to a linear flow rate equal to the recommended Phase II packing flow rate. See Section 6.3 for Phase II recommended packing flow rate. Continue Phase II packing until the resin bed is stable.

6.6.8 Once the bed is stable, simultaneously stop flow to the column and turn the adapter valve to the waste position and close the bottom valve. Loosen the top plunger O-ring and lower the top adaptor quickly to a Level 3 mm into the Phase II packed resin bed surface. Switch the top adaptor valve from inline with waste to inline with packing pump. Allow the resin to stabilize for ≥ 5 minutes.

6.6.9 Restart the chromatography skid at the Phase II packing flow rate. The resin bed should not compress away from the top adaptor. If 6 compression occurs, a second and third adapter set may be made. If after a third adapter compression occurs consult Purification Manager.

6.6.10 After the column is successfully packed, shut off the chromatography skid, shut off the bottom and top adaptor valves, and siphon off the liquid above the top adaptor.

6.6.11 Record all column packing data on **Form 14100-01**. Attach **Form 14100-01** to the batch production record for which the column was packed. Proceed to Section 6.8 in this SOP.

6.7 Packing with a Pressure Canister.

6.7.1 Fill the pressure canister with WFI.

6.7.2 Connect the tubing from the pressure canister inlet valve to the nitrogen tank regulator valve.

6.7.3 Connect the tubing from the column top adaptor to the pressure tank outlet valve.

6.7.4 With the valve on the column adapter bypassing the net, run fluid through the tubing to remove air. Once air is removed from the lines, direct flow through the adapter and remove any air trapped in the net. Remove trapped air using either a pipette or syringe. Attach the top column adaptor to the column. Lower the top adaptor just into the liquid level in the column. Remove air bubbles trapped below the top adaptor by wiggling the top adapter while moving it down.

6.7.5 With the top adaptor valve in the waste position tighten the top adaptor O-ring and lower the adaptor until fluid comes out of the top adaptor once air is removed turn the valve to a closed position.

6.7.6 With the ball valve closed on the pressure tank, slightly open the regulator valve on the nitrogen tank to apply approximately 1 - 3 psi pressure.

6.7.7 With the top adaptor valve inline with pressure canister outlet and waste, open the ball valve on the pressure canister and remove air from tubing. Once air is removed close the ball valve. Put the top adaptor valve inline with the column and the pressure canister.

6.7.8 Open the column effluent tubing on the lower adapter and tighten the top adaptor O-ring.

6.7.9 Open the regulator valve on the nitrogen tank to 3 ± 2 psig. Slowly open the ball valve on the pressure canister. Adjust the pressure to correspond to a flow equal to the recommended Phase I packing linear flow rate.

6.7.10 When the resin bed is stable, loosen the top plunger O-ring and lower the top adaptor ≤ 2 cm above the resin bed but not touching the resin bed. Tighten the top adaptor O-ring.

6.7.11 When using a packing reservoir, you may remove the reservoir once the resin bed is below the top of the column and the top adaptor can be lowered without touching the resin bed. Close the bottom column valve, stop flow, and remove the top adaptor followed by the packing reservoir. Connect the top adaptor; lower the top adaptor ≤ 2 cm above the resin bed surface, without disturbing the resin bed. Tighten the O-ring, open the bottom valve and resume phase I flow until resin bed is stable.

NOTE: There may be a large fluid spill in performing this step.

6.7.12 Increase the pressure slowly to correspond to a flow equal to the recommended Phase II packing linear flow rate for the column effluent. See BPR for Phase II packing flow rate. Continue Phase II packing until the resin bed is stable.

6.7.13 Once the bed is stable, simultaneously close the ball valve on the pressure canister, close the bottom adaptor valve, and switch the top adaptor valve from being in line with the pressure canister to in line with a waste line. Lower the top adaptor quickly to a Level 3 mm into the Phase II packed resin bed surface, switch the top adaptor valve from in line with waste to in line with the pressure canister. Allow the resin to stabilize for >5 minutes.

6.7.14 Restart flow at the Phase II packing flow rate. The resin bed should not compress away from the top adaptor. If compression occurs, a second and third adapter set may be made. If after a third adapter set compression occurs, consult Purification Manager.

6.7.15 After the column is successfully packed, close the ball valve, shut off the bottom and top adaptor valves, close the regulator valve on the nitrogen tank, and depressurize the pressure canister. Siphon off the liquid above the top adaptor.

6.7.16 Record all column packing data on **Form 14100-01**. Attach **Form 14100-01** to the batch production record for which the column was packed.

6.8 Testing Column Packing Efficiency

6.8.1 The packing efficiency can be determined by the number of theoretical plates per meter and the asymmetry of the salt peak. This can be accomplished with a mobile phase of 50 mM NaCl and an additional NaCl solution with a concentration ≥ 500 mM.

6.8.2 Column Qualification is to be performed at flow rates not to exceed Phase II packing flow rates. Equilibrate the column with the mobile phase buffer, 50 mM NaCl. Using a superloop, syringe or chromatography skid inject the test slug (≥500 mM NaCl) equal to 1 to 3% of the packed bed volume.

6.8.3 Column efficiency is measured by the conductivity trace and calculated according to the following formula (See Attachment 5). This calculation can be performed by the Unicorn Software. If software is not available, document all calculations in the comments section of **Form 14100-01**.

- HETP = L/N.
- N = 5.54 (Ve/Wh)2.
- Ve = Peak elution volume (mm).
- Wh =Peak width at half peak height (mm).
- L = Bed height.
- N = Number of Theoretical plates.
- Ve and Wh must be in same units.

6.8.4 Peak symmetry is calculated by the formula As = b/a. This calculation can be performed by the Unicorn Software. (See Attachment 5). If software is not available, document all calculations in the comments section of **Form 14100-01**.

6.8.5 Acceptance Criteria

- Salt test elution profiles must be consistent.
- Salt test elution profiles of packed columns must contain no split peaks. A split peak is defined as adjacent peaks that have a deflection from baseline between them.
- Salt test elution profiles must be visually symmetrical.

6.9 Resin Cleaning in Place and Storage

6.9.1 Clean and store the packed column according to the BPR.

6.9.2 Document the cleaning in the BPR.

6.9.3 Label the columns per **SOP 14150, Labeling of cGMP Purification Equipment for Cleaning Status**.

6.9.4 The column should be charged, where appropriate, and equilibrated as close as practicable to the time when it is planned to be used in a process. However, a column may be charged and/or initially equilibrated up to seven days prior to the actual start of purification where it is logistically necessary. Though an initial equilibration may be performed more than 1 day in advance of actual use, a final equilibration will be performed <1 day prior to actual use.

7. REFERENCES AND RELATED DOCUMENTS

Document Number	Title
N/A	BPG Columns Instruction for use Manual
N/A	Gel Filtration: Principles and Methods
N/A	Ion Exchange: Principles and Methods
N/A	Column Packing Course Literature

Document Number	Title
14146	Cleaning Chromatography Columns for GMP Processes
14150	Labeling of cGMP Purification Equipment for Cleaning Status
21529	Equipment Interproduct Cleaning and Clearance
14100-01	BPG Column Packing
14100-02	BPG column Use Log

8. ATTACHMENTS

Attachment 1 Flow Rate Calculations and Packing Efficiency Figure

Attachment 2 BPG Column Schematic

Attachment 3 BPG Column Top Adaptor Schematic

Attachment 4 BPG Column with Pressure Canister

Attachment 5 Measurements for Testing Column Packing Efficiency

Attachment 1: Flow Rate Calculations and Packing Efficiency Figure

1. Cross-sectional area: 3.14 r2, where r = the radius of the column.

2. Bed volume: Bed volume = cross-sectional area x height (cm) = 3.14X r2 x h, where h = height of the resin bed in cm.

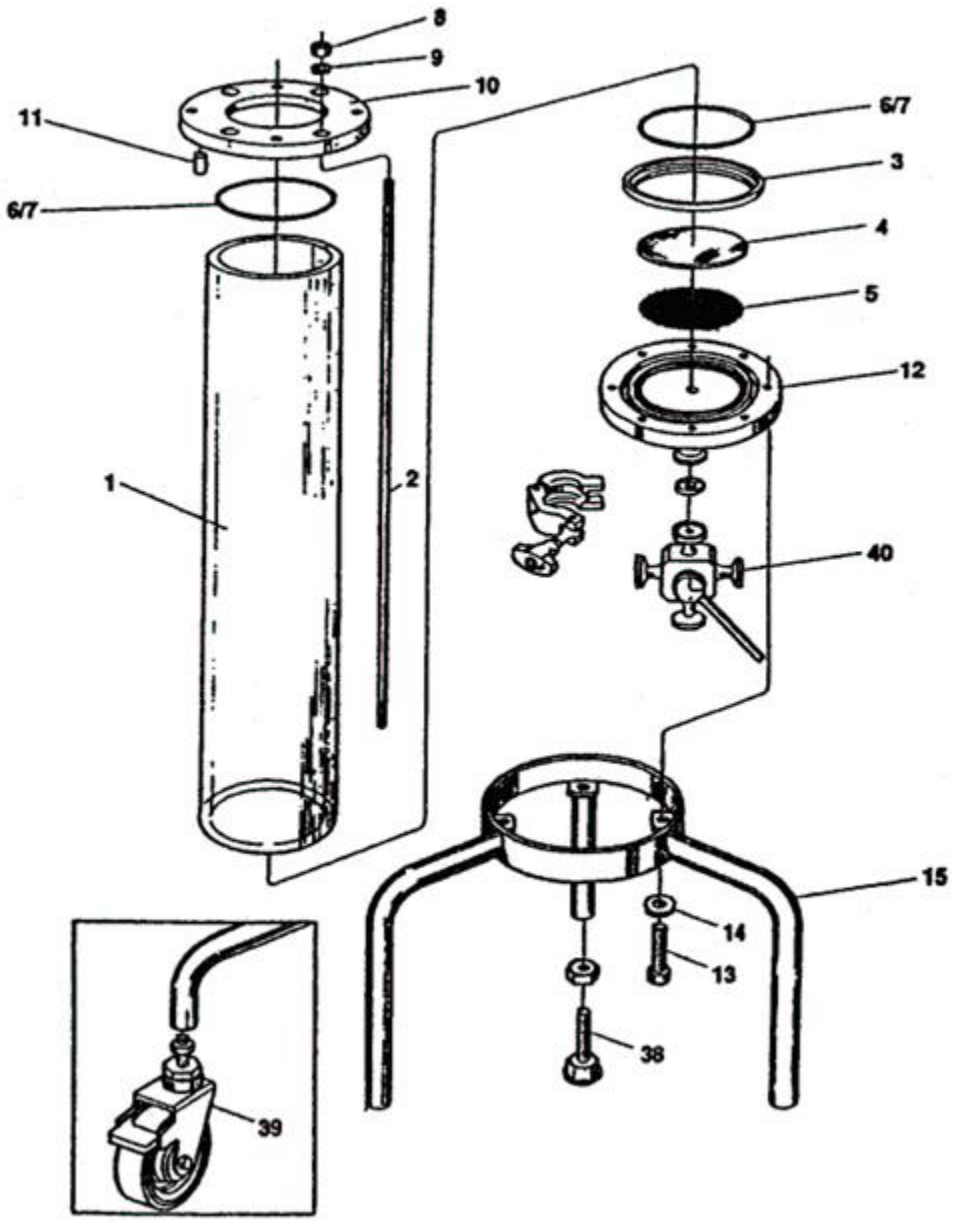
3. Linear flow rate or fluid velocity is obtained by dividing the volumetric flow rate (mL/min) x 60 by the column cross-sectional area.

   $$\text{Linear flow rate (cm/hour)} = \frac{\text{(mL/min) x 60minute/hour}}{3.14\ r2}$$

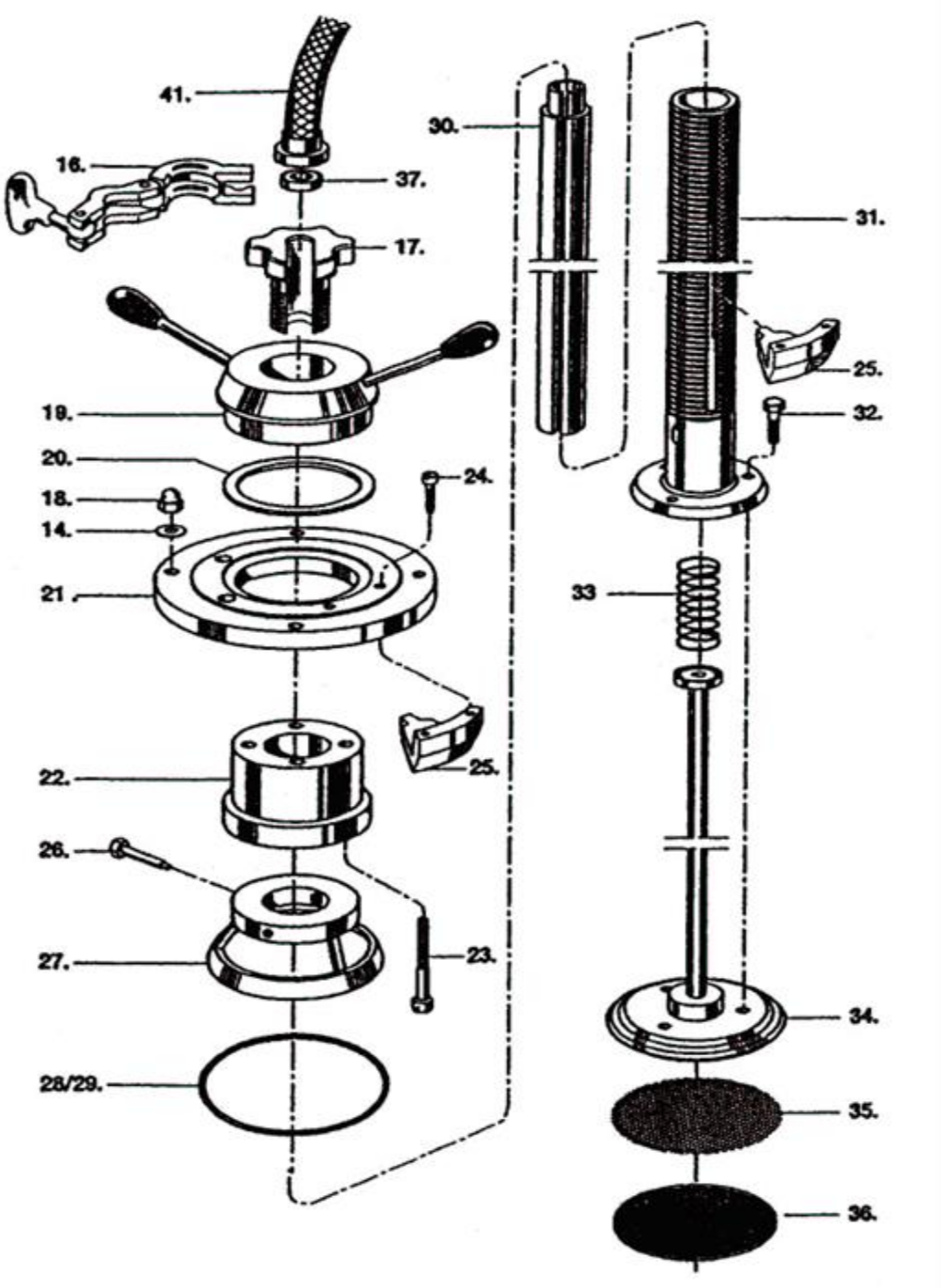
4. Calculating the volumetric flow rate from the desired linear flow rate.

   $$\text{Volumetric flow rate (mL/min)} = \frac{\text{(cm/hour) x 3.14r2}}{\text{60 minute/hour}}$$

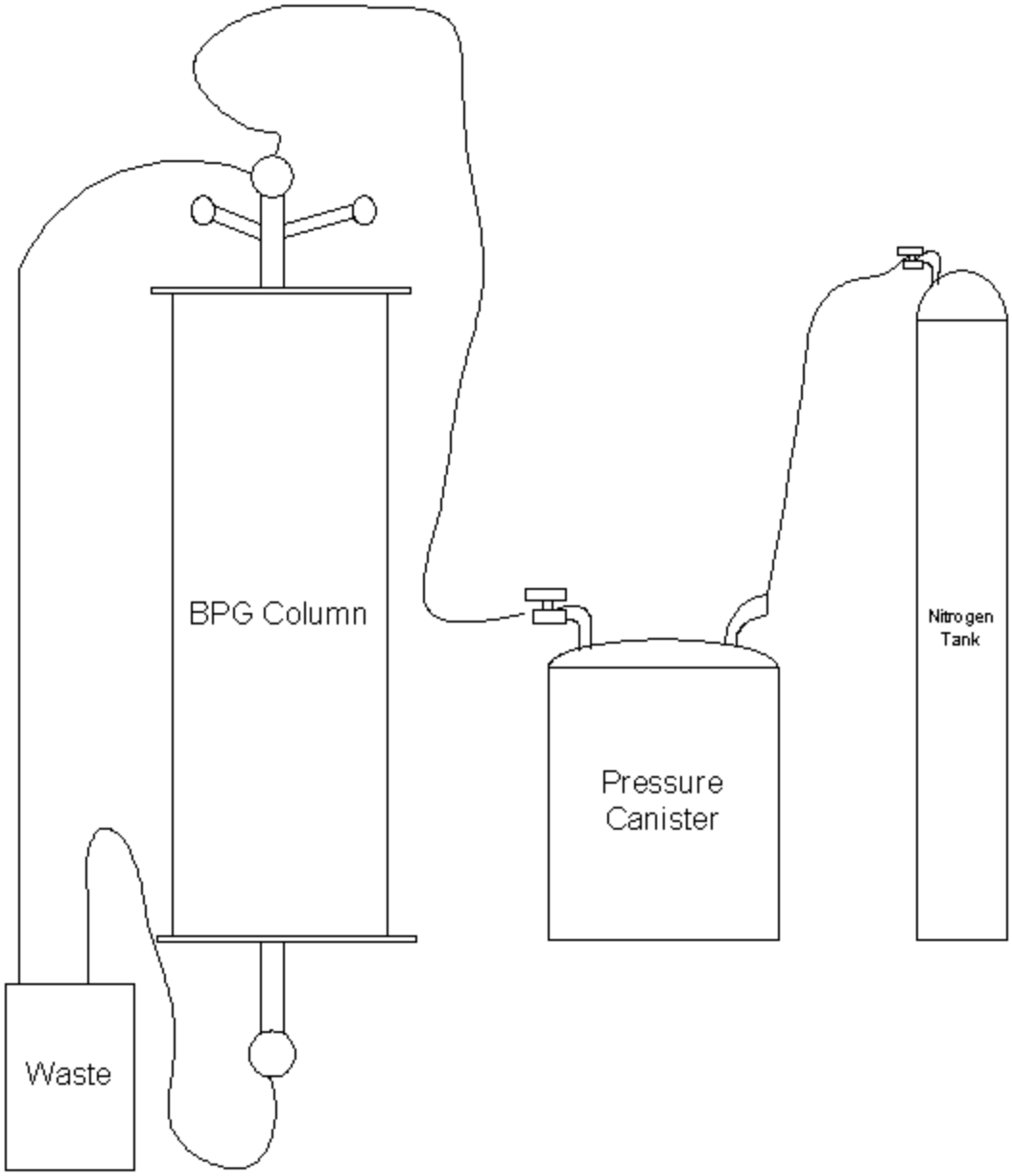
Attachment 2: BPG Column Schematic



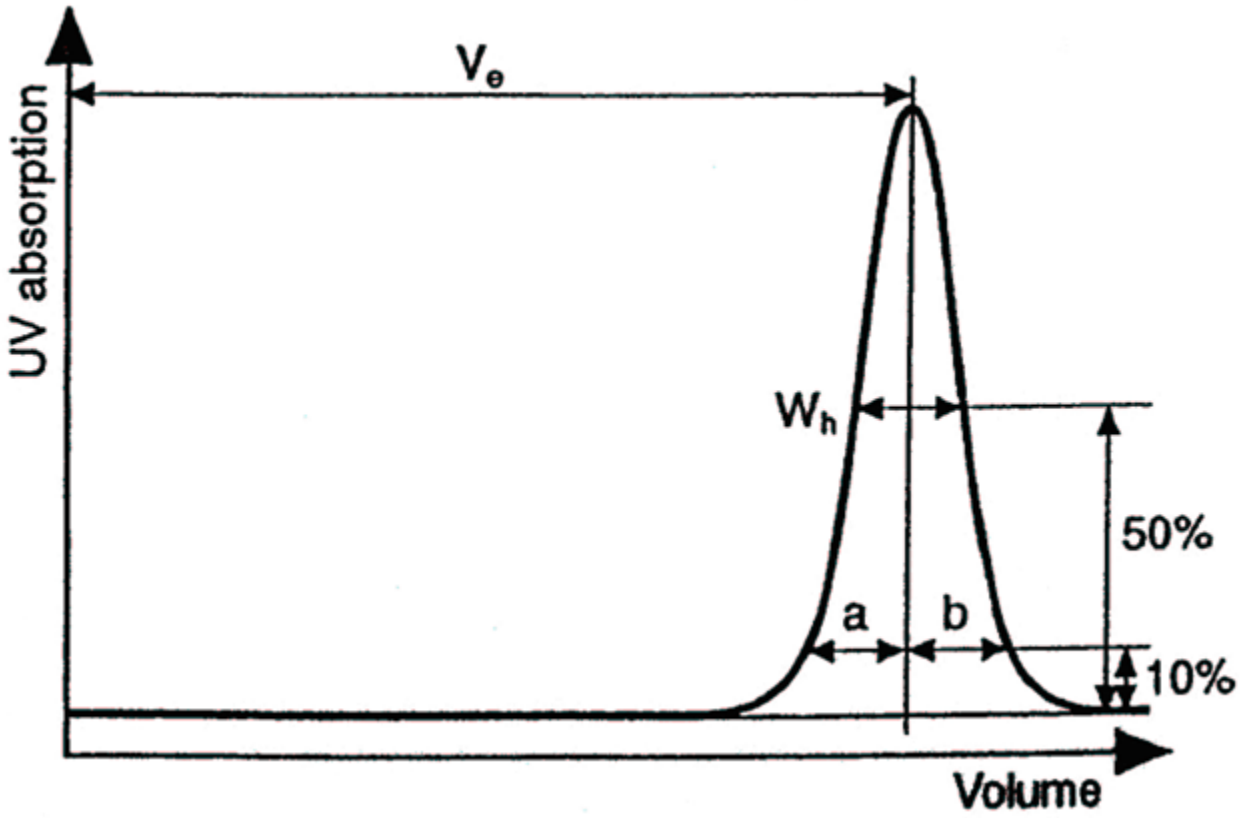
Attachment 3: BPG Column Top Adaptor Schematic



Attachment 4: BPG Column with Pressure Canister



Attachment 5: Measurements for Testing Column Packing Efficiency



HETP = L/N.

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Peak Symmetry: As = b/a