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1.0 Purpose

This procedure describes the method for packing FineLine Columns.

2.0 Scope

This SOP applies to all purification personnel who will be packing FineLine columns to be used for purification.

3.0 Authority and Responsibility

3.1 The Manager, Purification, Biopharmaceutical Development Program (BDP) has the authority to define this procedure.

3.2 BDP personnel are responsible for the implementation of this procedure.

3.3 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

4.0 Materials and Equipment

4.1 A suitable FineLine Series Column.

4.2 WFI quality water.

4.3 Appropriate chromatography resin (Source 15 or 30, or similar matrix resin).

4.4 Suitable tubing such as Amesil (BDP PN 20235) tubing, or BDP approved equivalent.

4.5 Akta Pilot (BDP 80060), BioProcessor System (BDP PN 75640) or BDP approved equivalent.

4.6 Pressure canister (BDP PN 71260) with pressure gauge (BDP PN 71261) or BDP approved equivalent.

4.7 0.5N NaOH (BDP PN 46109) and 0.05N NaOH (BDP PN 46102).

4.8 20% Ethanol (BDP PN 46202).

4.9 1M NaCl (BDP PN 46212), 2 M NaCl (BDP 46205CL) or BDP equivalent.

5.0 Procedure

5.1 Packing Preparations

5.1.1 Verify the column was cleaned and released as per ***SOP 14146, Cleaning Chromatography Columns for GMP Process***.

5.1.2 Verify the valve attached to the bottom of the Fineline column is closed. Fill the bottom of the Fineline column with a 1-2 cm layer of WFI. Purge air from the bottom mesh screen of the column by opening the valve on the bottom of the column. When the WFI starts to drain from the column, close the bottom valve.

5.1.3 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 14121-01. Drain the 0.5N NaOH from the column and rinse with WFI.

5.1.4 Attach a 2-way valve to the top adaptor and a 4-way valve to the bottom adaptor to columns sized Fineline 70 and greater.

5.2 Resin Preparation

5.2.1 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.

5.2.2 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 14121-01.

5.2.3 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. The calculation for % slurry would be the height of settled resin, divided by, the total height of the resin plus the storage buffer, and multiplied by 100.

5.2.4 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 14121-01.

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

5.2.5 Calculate and add the amount of appropriate packing buffer, as per manufacturer recommendation or the MPR that will result in a 30% – 50% slurry. Initial volume x initial concentration = final volume x final concentration.

For example: 2800 mL x 75% slurry = 4200 mL x 50% slurry; 4200 mL – 2800 mL = 1400 mL. Therefore, adding 1400 mL of WFI will result in 50% slurry. Make sure the entire slurry volume can be poured into the column at once with the slurry level at or below the seated

5.2.6 Pouring the resin- 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. To remove trapped air bubbles, use a long plastic rod to stir the media for 10 – 30 seconds (avoid scraping the bottom screen).

5.3 **Column Packing with a Pressure Canister**

5.3.1 Fill the pressure vessel with at least 10L of WFI, as per manufacturer recommendation or the MPR. Make sure the column shutoff valve

(#1, Attachment 4) is closed to the pressure vessel.

5.3.2 Pressurize the vessel to 60 – 63 psi, using in-house pressurized gas or individual pressurized air containers.

5.3.3 Bolt the adaptor and lid in place, and thread one of the locking pins (with washer) just through the top column flange.

5.3.4 Open the shutoff valve (#2) on the column lid that goes to waste. Push down firmly on the adapter into the column tube, close the top adaptor valve.

5.3.5 Partially open the shutoff valve (#1) to the pressure vessel allowing liquid to fill the hydraulic chamber.

5.3.6 When the liquid comes out the shutoff valve (#2) to waste, close this valve and open the top adaptor valve.

5.3.7 Regulate the liquid entering the hydraulic chamber (via shutoff valve #1) such that the adapter slowly descends at 1 – 2 cm/minute.

5.3.8 When the adapter enters the slurry, liquid will exit up through the adapter rod displacing the air.

5.3.9 Close the top adaptor valve as soon as the air is cleared.

5.3.10 Fully open the shutoff valve (#1) to the pressure vessel, and immediately open the bottom adaptor valve of the column to waste.

5.3.11 Allow the adapter to descend unhindered while monitoring the pressure gauge at the top of the column. This should stabilize at around 50 (± 2) psi, and then begin to drop as the column packs.

5.3.12 As soon as the pressure drops to 5 psi, simultaneously close the bottom adaptor valve on the column and the shutoff valve (#1). Place the locking bar over the adapter rod and secured in place with both locking pins. Hand-tighten the pins snugly.

5.3.13 Close the pressure vessel regulator valve. Briefly open shutoff valve (#2) to waste to depressurize column hydraulic chamber.

5.3.14 Gradually release the pressure in the pressure vessel and disconnect the column.

5.3.15 Measure and record the bed height (Attachment 3) and calculate the packed bed volume (PBV).

5.4 Column Equilibration

NOTE: 50 mM NaCL, and ≥ 500 mM NaCL can all be made by diluting the appropriate concentrated solution (2M NaCL, etc.) with WFI.

5.4.1 It is recommended that immediately following the packing, the column be flushed with WFI, or 50 mM NaCL in an **UPWARD** flow direction at the recommended flow rate, as per the MPR or manufacturer's recommendation, for 1-2 PBV followed by **DOWNWARD** flow at the same flow rate for 1-2 PBV.

5.5 Testing Column Packing Efficiency (Salt Test)

5.5.1 The packing efficiency can be tested by determining the number of theoretical plates per meter and 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 NaCl.

5.5.2 Equilibrate the column per Section 5.4. Using a syringe or chromatography skid inject the test solution (≥500 mM NaCl) equal to 1 to 2% of the packed bed volume.

5.5.3 Column efficiency is measured by the conductivity trace and calculated according to the following formula (see Attachment 1). This calculation can be performed by the unicorn software. If software is not available, document all calculations in the comments section.

HETP = L/N

$N = 5.54 (V_e/W_h)^2$

V_e = Peak Elution volume (mm)

W_h = Peak width at half peak height (mm)

L = Bed height

N = Number of theoretical plates

V_e and W_h must be in same units.

5.5.4 Peak symmetry is calculated by the formula: As = b/a. This calculation can be performed by the AKTA Unicorn software. (See Attachment 1). If software is not available, document all calculations in the comments section.

5.6 **Acceptance Criteria**

5.6.1 Salt test elution profiles must be consistent.

5.6.2 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.

5.6.2 Salt test elution profiles must be visually symmetrical.

5.7 **Resin Cleaning in Place and Storage.**

5.6.3 Clean and store the packed column according to the BPR or the resin manufacturer's instruction.

5.6.4 Document the cleaning in the BPR or storage on Form 14121-01.

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

5.7 The column should be charged, where appropriate, and equilibrated as close as practical 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 7 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.

6.0 References and related Documents

6.1 Pharmacia: Source 15Q and 15S, Instruction Manual.

6.2 Pharmacia: Ion Exchange Chromatography Principles and Methods, Edition AA.

6.3 FineLine Column Instruction Manual.

6.4 **SOP 14146** **Cleaning Chromatography Columns for GMP Processes**

6.5 **SOP 14150** **Labeling of cGMP Purification Equipment for Cleaning Status**

7.0 Attachments

7.1 **Attachment 1** Calculations and Definitions

7.2 **Attachment 2** FineLine Column Packing Systems

7.3 **Attachment 3** Measuring Bed Height of Fineline Column

8.0 Change Summary

Section Changed	Summary of Change	Justification for Revision
[redacted]	[redacted]	[redacted]

Attachment 1

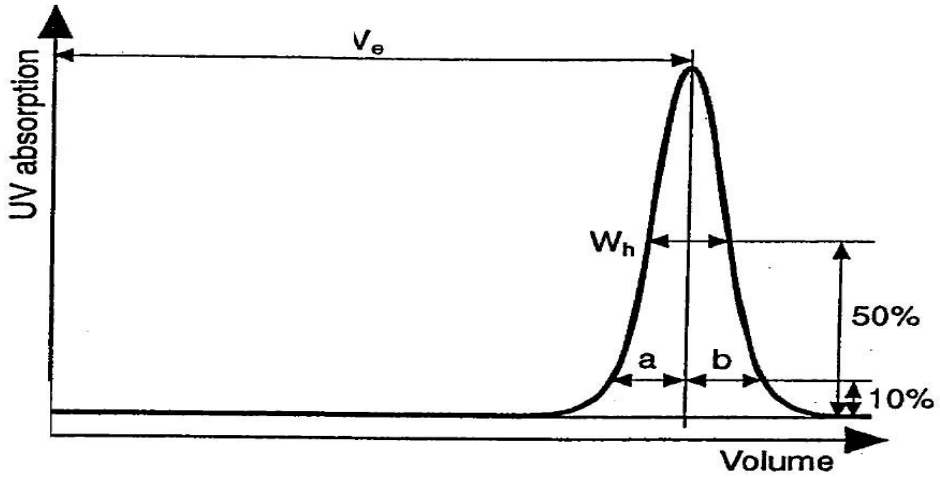
CALCULATIONS AND DEFINITIONS

1. Cross-sectional area: $3.14r^2$, where r = the radius of the column.
2. Bed volume: bed volume = cross-sectional area x height = $3{\cdot}14r^2{\cdot}h$, where h = height of the resin bed.
3. Linear flow rate or fluid velocity is obtained by dividing the volumetric flow rate (mL/hour) by the column cross-section area (cm^2).
4. Linear flow rate (cm/hour) = volume flow rate ÷area
   = cm^3/hour) ÷ cm^2
   cm/hour = [mL/minutes x 60 minutes/hour] ÷ $3.14r^2$
   Conversely, mL/minute = $\frac{\text{(cm/hour) x } 3.14r^2}{\text{60 minute/hour}}$
5. Calculating the volumetric flow rate from the desired linear flow rate.
   Example: to obtain a 10 cm/hour flow rate on a 5 cm diameter column:

   $$\text{mL/minute} = \frac{\text{10 cm/hour x } 3.14(2.5\text{cm})^2}{\text{60 minute/hour}} = 3.3 \text{ mL/minute}$$
6. Measurements for column packing efficiency.

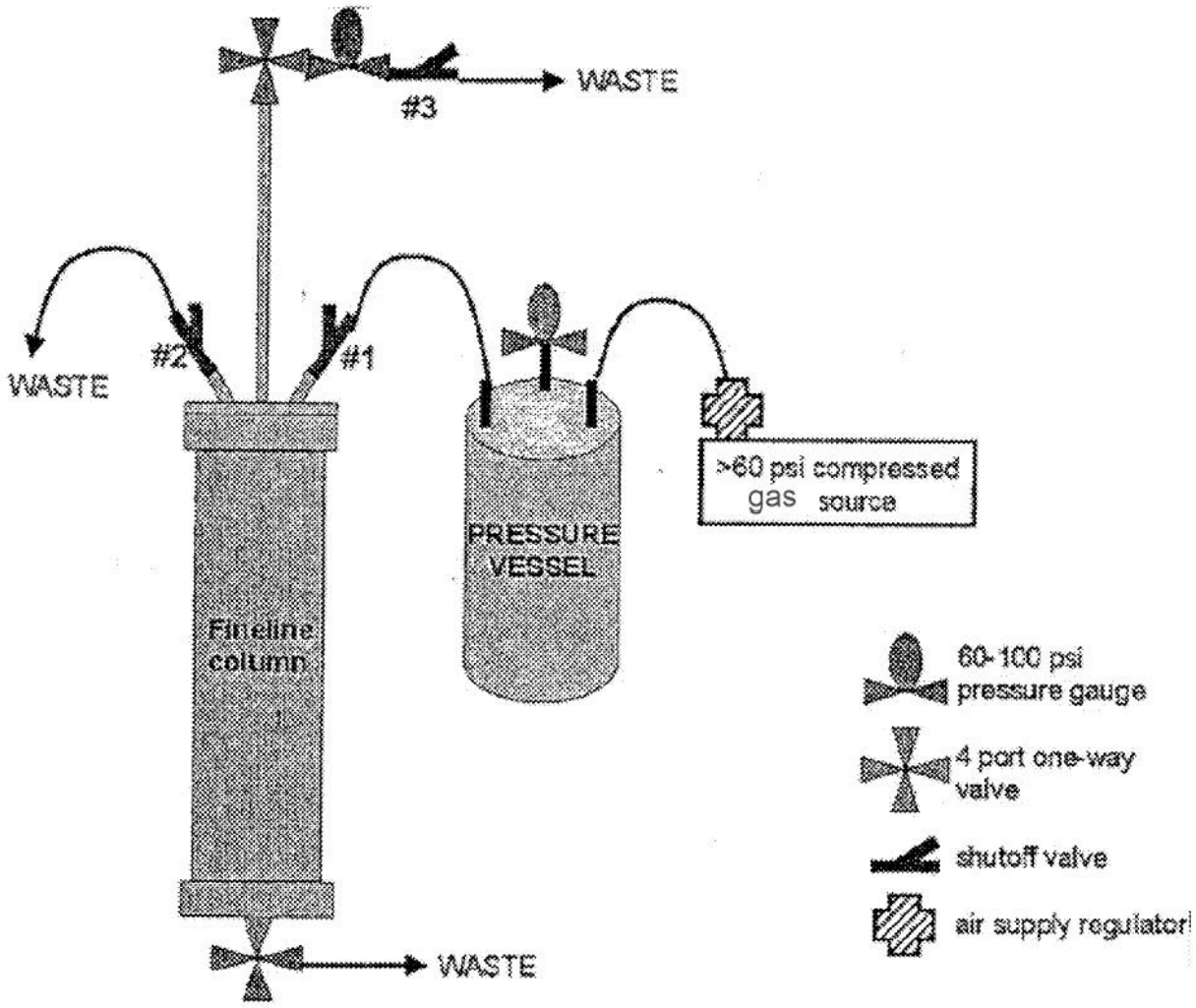
$N = 5.54 (Ve / W_{1/2})^2$	$HETP = L / N$	$A_f = b / a$
N = Number of theoretical plates	HETP = Height Equivalent	A_f = Asymmetry
V_e= Elution Volume	to a theoretical plate	
$W_{1/2}$ = Width at half peak's height	L = Bed height in cm	

a= Leading side of peak at 10% height
b= Tailing side of peak at 10% height



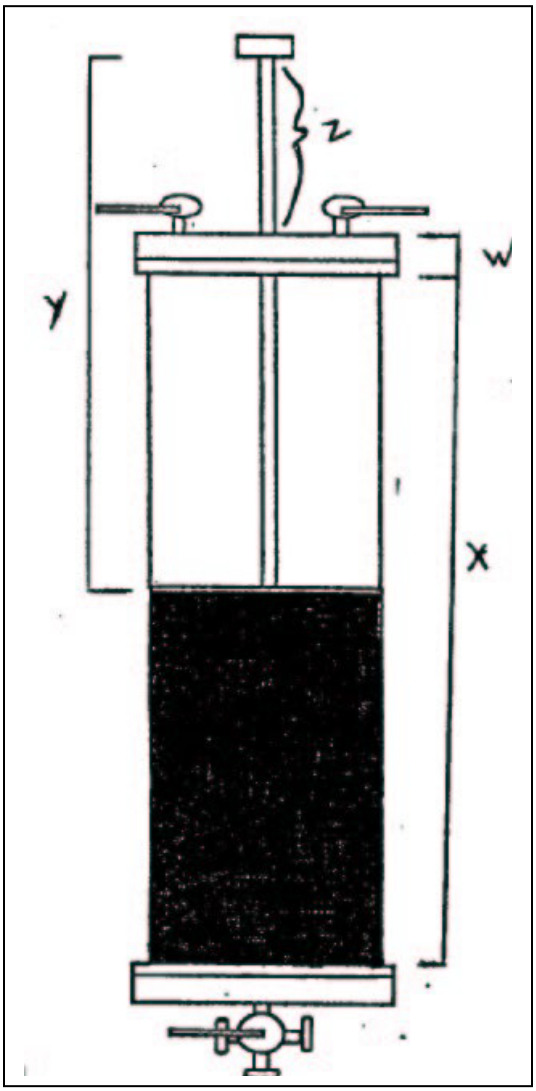
Attachment 2

FineLine Column Packing System



Attachment 3

Measuring Bed Height of FineLine Column



w = height of top lip of column

x = height of column cylinder

y = height of adapter (measure before starting process)

z = height of adapter after packing is complete

h = height of packed bed = (w + x) – (y – z)

For example: w = 1 cm x = 37.5 cm
y = 41.4 cm z = 12 cm

h= (1 + 37.5) – (41.4 – 12) = 38.5 – 29.4 = 9.1 cm