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Sepax Monomix Core Column Packing Manual

I. Aims

Instructions for FPLC column packing and QC characterization method for Monomix Core resin.

II. Applicable Scope

Suitable for packing of glass chromatography columns with inner diameter of 6.6-50 mm and the QC characterization after packing completion.

III. Column Packing

1.1 Preparations before packing columns

1) Packing area: clean and dust-free; temperature: 18 ℃ - 35 ℃; humidity: 45% - 65%.
2) Column packing equipment and tubing: the FPLC instrument and column attachment tubing should be rinsed clean. After connecting the tubing, verify there are no leaks, and the pressure is displayed normally.
3) FPLC glass chromatography column: the components of the column are separated and soaked in 20% ethanol for 30 minutes, and then washed with deionized water (DI water) for later use.
4) QC characterization equipment: FPLC instrument; flow rate: 0-100 mL/min；UV detector/conductivity; and corresponding chromatography software.
5) Other： 1.0 M NaCl + 20% EtOH aqueous solution; deionized water; balance (range: 0-1000 g, accuracy: 1 g); measuring cylinder (1000 mL); and beaker (1000mL).

1.2 Column Packing

1) Calculate column volume (CV): CV = column cross-sectional area (πr^2) x bed height (h), r is the column radius.
2) Using a balance, weigh 1.4-2 times more of the desired column volume.
3) Transfer gel into a beaker, and add 4 CV of EtOH-H_2O mixture (1:4, v:v).

Then stir solution and gel gently until the gel is completely dispersed to form a uniform slurry.

4) Let slurry settle, then decant and discard the supernatant (containing fines).
5) Add 3 CV of DI water into the beaker. Stir gently and then let settle naturally for about 30 minutes. Then discard the supernatant. Repeat this procedure 3 times.
6) After decanting the upper layer of DI water, make a slurry of 60-70% with column packing buffer (1.0 M NaCl + 20% EtOH aqueous solution),
7) Stir it well and leave it for more than 12 hours (overnight).
8) Secure the pre-washed glass column next to the FPLC instrument with a clamp and ring stand. Ensure both column endpieces contain the appropriate size frit and attach the bottom endpiece to the column. Attach the top end piece to the instrument and run packing solution through until all the air is removed, and it begins to drip.
9) Calculate about 1.06 times the column volume and then divide by the slurry concentration (ex. 70% slurry and desire a 100mL column (100mL CV x 1.06 / (70/100)). Note: if the volume calculated exceeds the total volume of the empty column, a packing adapter may be needed.
10) Mix slurry until homogenous and using a graduated cylinder, measure the calculated volume above. Immediately pour into the column using a glass rod to extend onto the inner wall of the column tube and fill the column tube up to the brim forming a convex liquid surface ensuring there is no air gap as the top end piece is placed into the liquid at an angle and then straightened to begin tightening. Bring down the top endpiece (distributor) to ~1cm from the top of the resin.
11) Use 2-3 CV of column packing buffer to flush the resin bed. Note: do not exceed pressures above 0.3 to 0.4 MPa.
12) To compress the resin bed, continue flushing using 3x the normal working flowrate. The distributor position can be adjusted during the compressing process to ensure tightness of the resin bed. Column flushing is stopped when the column bed no longer compresses away from the distributor (i.e. no gap between the resin and distributor). Record the height of the column bed.

Packing solution	1.0 M NaCl + 20% EtOH aqueous solution
Packing Pressure	0.3 MPa to 0.4 MPa
% Slurry	60-70%
Compression Factor	1.04-1.08
Flowrate	60 cm/hr to 360 cm/hr

Note: It is not recommended to use suction or gravity-only sedimentation to pack a column, especially for columns with a bed height of more than 10 cm.

Note: When installing a glass chromatography column with an inner diameter of less than 10 mm, first flush it with a low linear flow rate of 30-50 cm/hr, which can make the gel spread more evenly.

13) Evaluation of column packing quality is carried out using a low molecular weight, unretained compound. The specific operating parameters are as follows:

Sample	1.0 M NaCl
Sample Volume	1.0–2.0% of bed volume
Mobile Phase	0.4 M NaCl solution
Flow Rate	60 cm/hr
Detector	Conductivity
Specifications	60 μm resin column efficiency: ⩾3,000/m Tailing factor: 0.8 – 1.5

14) In case of non-ideal result, such as peak tailing, solutions include:
- Reduce the concentration of slurry
- Increase packing flow rate
- Extend packing time

If peak fronting occurs, solutions are the opposite of the above.