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Welch Packing Materials Care and Use Manual

Thank you for purchasing and using Packing materials from Welch Materials. To prolong the life time of your packing materials, please read this manual carefully before using.

1. Packing Instructions [for DAC (dynamic axial compression column)]

1.1 Preparation of packing slurry and packing

1.1.1 Preparation of packing slurry

1) Clean the container used for slurry preparation [a 2L container is enough for DAC 50 (300g packing materials) while a 20L container for DAC 200 (4.8kg packing materials)], then blow dry or rinse with isopropanol(isopropanol is routinely used as the packing solvent).
2) The ratio of packing materials to packing solvent (weight g:volume mL) is 1:2. This 1-2 ratio yields good results with particles near 10 μm in diameter, a somewhat more concentrated slurry can be used for larger particles.
3) Measure the required amount of packing solvent and packing materials.
4) Pour a part of the packing solvent into the container first, then pour a part of the packing materials, stir and add the remaining packing materials and the solvent .
5) Manually stir with a glass rod to thoroughly mix the packing materials.
6) Place the container in an ultrasonicator, and stir it manually for more than 10 min while vibrating with ultrasound. Stirring cannot be stopped during the ultrasonic process. Additionally, the treatment of the slurry in an ultrasonic bath will help to homogenize the slurry - but be careful with fragile /sensitive media since they might be damaged by such a sonication.
7) Put a clean stir bar into the container and place it on a magnetic stirrer, stir for more than 30min. Make sure the magnetic stirrer has enough force to agitate the packing materials. If the amount of packing material is large, it is recommended to use stirring paddle to stir, or special homogenate tank.

1.1.2 Packing column

Before packing the column, it is necessary to understand the maximum tolerance pressure of the packing materials and the pressure required when packing the column.
According to the ratio of the sectional area of the hydraulic rod to the upper frit area, calculate the hydraulic gauge pressure according to the following formula.

$$\frac{\text{Hydraulic gauge pressure value}}{\text{Packing pressure}} = \frac{\text{Upper frit area}}{\text{Internal sectional area of hydraulic cylinder}}$$

e.g. the pressure required for packing 10μm packing materials is 8MPa, and the ratio of the sectional area of the hydraulic rod to the upper frit is 1:1.6.

$$\text{Hydraulic gauge pressure Value} = \frac{\text{Upper frit area}}{\text{Internal sectional area of hydraulic cylinder}} \times \text{Packing pressure} = 1.6\times 8 = 12.8\text{MPa}$$

1) Install a stop plug to the end plate's high pressure SS tubing pipe, pour all the homogeneous slurry into the column tube. Turn the direction valve of the hydraulic rod to 'down', turn the emergency stop switch of the air source to "on" and make the hydraulic rod descend.
2) The upper piston will descends with the hydraulic rod, when the upper piston descends to the upper end of the column tube, press the emergency stop switch of the air source, check whether the upper frit is facing the column tube, and adjust it in time if there is any deviation.
3) Open the emergency stop switch, the upper piston continues to descend. At the mean time, spray the upper piston and the outer wall of the sealing ring with IPA to reduce wear.
4) When liquid flows out from upper piston's SS tubing pipe without any air, press the emergency stop switch and install a stop plug to the upper piston's high pressure SS tubing pipe.
5) Remove the stop plug from the end plate's high pressure SS tubing pipe, open the emergency stop switch (the piston is going down), let the IPA comes out from the end plate's high pressure SS tubing pipe.
6) When the liquid is no longer flowing out, check the pressure value and adjust the air source switch to make the pressure reach the required pressure value. It is recommended to adjust the air pressure to a suitable value before the second step if there is a diagram of the relationship between air pressure and hydraulic pressure. This helps to pack a column faster and makes it easier to get a better column.
7) After packing, the column should continue to settle for at least 30 minutes without any operation. Then flush the column with methanol at least 3 times of column volume (the column volume of DAC50 column was about 0.5L).
Note: The flushing flow rate should increase in gradient from low to high. Do not start with a high flow rate. The flow rate is set to keep the pressure of the liquid system low. Then equilibrate the column with testing mobile phase.

1.2 Column performance test

Conditions (50mm DAC):
Mobile phase: 100% methanol
Detection wavelength: 254nm
Flow rate: 100mL/min
Injection Volume: 2mL
Sample: naphthalene/methanol = 1mg/mL

Note: For DAC200, flow rate is 1200mL/min and injection volume is 30mL.
Evaluation results (WelPacker 50mm DAC):
The number of theoretical plates: ≥ 32000/m, trailing factor ≤ 1.3 . **Note**: This is requirement for C18 (10μm) packing materials.

2.Precautions

2.1 pH range

Each column has its own specific working pH range. Using column under pH out of range may cause irreversible damage like dissolution of silica base and hydrolyzation of bonding phase.
Using column under critical pH may decrease column lifetime, so solvents inside column shall be replaced with eluents which is soluble with mobile phase and suitable for column storage.

2.2 Reagents

High-grade chromatographically pure reagents are suggested for better performance. Each reagent shall be well filtrated before using, to avoid some suspended particles going to the column inlet. Reagents used for mobile phase shall be pre-degassed, to avoid any bubbles into the system.

2.3 Pressure

Column back pressure is concerned with: 1. particle size and distribution; 2. column dimension; 3. reagents viscosity, flow rate and temperature. Change of flow rate shall be slow and steady, avoiding rapid change of pressure, thus to protect the column and extend lifetime
Noted that max. column operating pressure should not be higher than column packing pressure.

2.4 Temperature

Note that operating at or near the maximum temperature will result in shorter column lifetimes. Suggested column temperature range is 30- 50°C. Suitable temperature will decease reagent viscosity, and increase column selectivity and reproducibility.

2.5 Buffer salts

As buffer salts are normally soluble in water and insoluble in organic solvents, high-ratio organic solvents used may cause salting out, and subsequently the salted-out particles will accelerate the wear of system parts, flow into column and block inlet frit and even packing media, causing increase of column pressure and decrease of column efficiency and lifetime. As salted-out buffer salts are difficult to remove, please check the following notes to avoid salting out:

A. Isocratic: Flush column with at least 3 column volumes of transition mobile phase before and after using the column. (**Transition mobile phase:** with same or lower ratio of organic phase and water phase as mobile phase, but without additives like buffer salt, acid and alkali etc.)
B. Gradient: Before using, flush with at least 3 column volumes of mobile phase (same as original mobile phase) in analytical flow rate. After using, flush with at least 3 column volumes of transition mobile phase. Gradient change shall be as steady as possible to avoid salting out.
C. In case of buffer salted out: reversely flush column with 3 column volumes of Methanol/Water (10/90).

3. Chromatographic packing materials flushing

3.1Column rinsing

The accumulation of strong-retention substances and macromolecular compounds inside column is a rather slow process, which will bring strong retention to sample contents, causing peak widening and efficiency decrease. To avoid this, routine column rinsing and maintenance is needed:

A. If mobile phase does not contain buffer salts or salts material, use high-ratio organic phase solvents to flush off strong-retention substances, or use 100% organic phase. For high-liposolubility contents, try adding 10% THF to get better removal efficiency.
B. If mobile phase contains buffer salts or salts material, please refer to Buffer salts precautions (Section 3.5) and flush the column.
C. If macromolecules like proteins and polysaccharides are adsorbed in column, try flushing the column with Acetonitrile/Water/TFA (50/50/0.1). If sample contains such substances or too much impurities, please do sample pretreatment before injection.

4. Packing Materials Storage

4.1 Unused packing materials

Store unused packing materials in dry place at room temperature.

4.2 Used packing materials

The used packing materials should be washed clean of buffer salt while in the column, and filtered, washed with methanol, and then drained. At last, store in dry place at room temperature after vacuum drying at 80 ℃.

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