

## **Protocol for Packing a Reversed-Phase Microcapillary Column**

Version Number 1

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### **A. Preparation of Capillary tubing**

1. Carefully cut ~ 40 cm of polyimide-coated fused silica capillary (75  $\mu\text{m}$  I.D.). The ends of the capillary must be clean and square.
2. Wash the capillary by forcing MeOH through the capillary for 10 to 15 s by using the pressure bomb.
3. Dry the capillary by forcing the helium through the capillary for 20 s by using the pressure bomb.

### **B. Preparation of Column Frit**

1. **Pull-out tip for classic LCQ users:** Small-diameter tip can be generated by attaching a weight (usually a paper clamp) to an end of the capillary and heating a desirable point (5~10 cm from the capillary end) of the capillary with a microflame torch. The capillary should be held vertically, and the torch should be perpendicular to the capillary length. As the capillary melts, the pull of gravity on the weight stretches the fused-silica capillary to a fine point. The end of the tip should be trimmed. Examine the linearity of the tip by the microscope. If the inside channel of the tip is curved, discard it and try again.
2. **Sintered frit for LCQ Deca users:**
  - a. Burn off ~3 mm of the polyimide coating at one end of the capillary by using the microflame torch. Care should be taken not to burn off too much of the coating because the uncoated fused silica is extremely brittle. The goal is simply to allow the frit formation to be monitored with the microscope.
  - b. Force the frit material (5  $\mu\text{m}$  silica particles) into the exposed end of the capillary by tapping the column end in a small amount of the frit material. Continue the process until 2~3 mm of frit material can be seen inside the capillary.
  - c. Pass the frit-containing end of the capillary through a flame produced by the microflame torch, fusing the frit material to the inside of the column. This process should require two or three passes through the

edge of the flame. Care must be taken to avoid burning-off additional polyimide. Excessive heat will lead to complete melting of the frit material and blocking the column end. Insufficient heat will not fuse the silica particles.

- d. Test the integrity of the frit by forcing MeOH through the column for 5~10 s. The use of longer testing times should be avoided because of possible accumulation of particulate materials at the frit.

### C. Slurry Packing of C18 packing materials

1. Place the ~ 3 mg of the C18 materials (5  $\mu\text{m}$  diameter, 100  $\text{\AA}$  pore recommended) in a 1.8 mL autosampler vial or a microcentrifuge tube. Add ~1 mL of MeOH and a mini magnetic stirring bar, and suspend the C18 materials on the stir plate.
2. Mark the column length on the capillary (usually 10 cm).
3. Force the C18 slurry through the capillary by using the pressure bomb. The packing of the C18 phase at the frit can be observed by using the microscope. Pressures on the order of 1000 psi will ultimately be needed to maintain a good flow of the slurry through the column.
4. Continue the slurry-packing process until a 9.5 cm column has been formed in the capillary.
5. Replace the slurry with MeOH, and wash the column with MeOH for ~5 min by using the pressure bomb. Residual C18 material in the column will pack during this time to give a total column length of ~ 10 cm.
6. Wash the column with LC solvent A and Store the column until use in the solvent A.