

Sample Preparation for LC/MS (C18 desalting and concentrating)

Materials

Solutions:

100% Acetonitrile (ACN)

0.1% Trifluoroacetic acid (TFA) in H₂O

80% Acetonitrile, 0.1% TFA

Column:

3M Empore High Performance Extraction Disk Cartridges

SPE on Empore C18HD (1 ml)

Procedure:

1. **Adjust the sample to pH 2~3 before loading;**
2. Dilute sample with 1:1 (V/V) 0.1% TFA (for small volume only);
3. Wet column with 1 ml of 100% Acetonitrile to the column;
4. Wash column with 1 ml of 0.1% TFA in 80% Acetonitrile;
5. Equilibrate column with 1ml 0.1% TFA;
6. Load sample slowly to allow peptides binding to column;
7. Wash column with 1 ml 0.1 %TFA. Repeat 3 times if sample contain high concentrate salt, detergent or chaotropic reagent.
8. Insert column to a new 1.5ml tube and eluting peptides slowly with 0.3 ml 0.1% TFA in 80% Acetonitrile.
9. Concentrate elution by Speed Vac at room temperature
10. Store at -20°C until analysis

Resuspend sample in 0.1% TFA in 5% Acetonitrile for LC-MS analysis.

