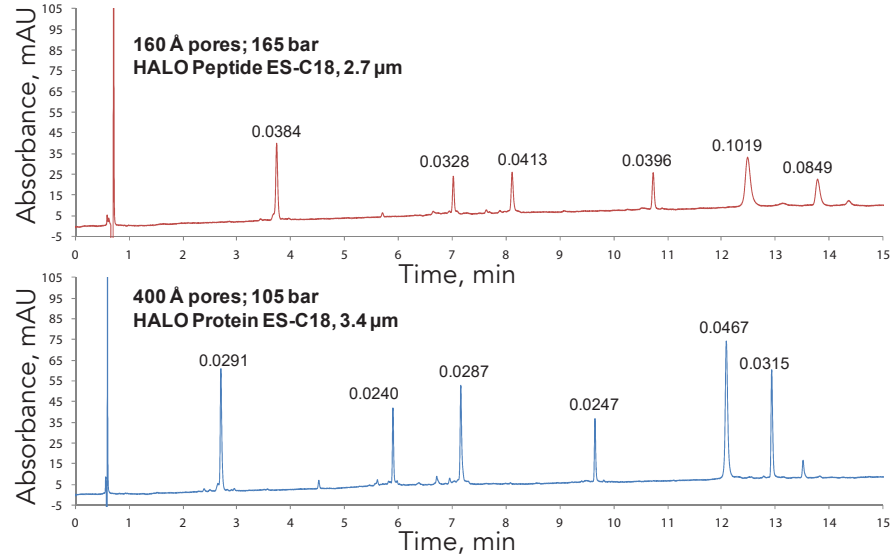
HALO®

BIOPHARMACEUTICALS

Effect of Silica Pore Size on Protein Separations

Application Note 130-PR



PEAK IDENTITIES:

1. Ribonuclease A (13.7 kDa)
2. Cytochrome C (12.4 kDa)
3. Lysozyme (14.3 kDa)
4. α-Lactalbumin (14.2 kDa)
5. Catalase (tetramer of ~60 kDa each)
6. Enolase (46.7 kDa)

Sharper, taller peaks are observed using the HALO 400 Å ES-C18 column because the larger pore size allows unrestricted diffusion for these biomolecules into and out of the porous shell. The half height peak widths above each protein peak are significantly smaller with the HALO 400 Å column despite the larger particle size of the packing material, emphasizing the importance of larger pores when separating proteins.

TEST CONDITIONS:

Columns:
1) HALO 160 Å ES-C18, 2.7 µm, 4.6 x 100 mm
Part Number: 92124-602
2) HALO 400 Å ES-C18, 3.4 µm, 4.6 x 100 mm
Part Number: 93414-602
Mobile Phase:
A: 0.1% trifluoroacetic acid in water
B: 0.1% trifluoroacetic acid in acetonitrile
Gradient: 23% B to 50% B in 15 min
Flow Rate: 1.5 mL/min
Initial Pressure: See chart
Temperature: 60 °C
Detection: UV 215 nm, VWD
Injection Volume: 5.0 µL
Sample Solvent: Mobile phase A
Response Time: 0.12 sec
Flow Cell: 5.0 µL semi-micro
Data Rate: 14 Hz
LC System: Agilent 1100 Quaternary