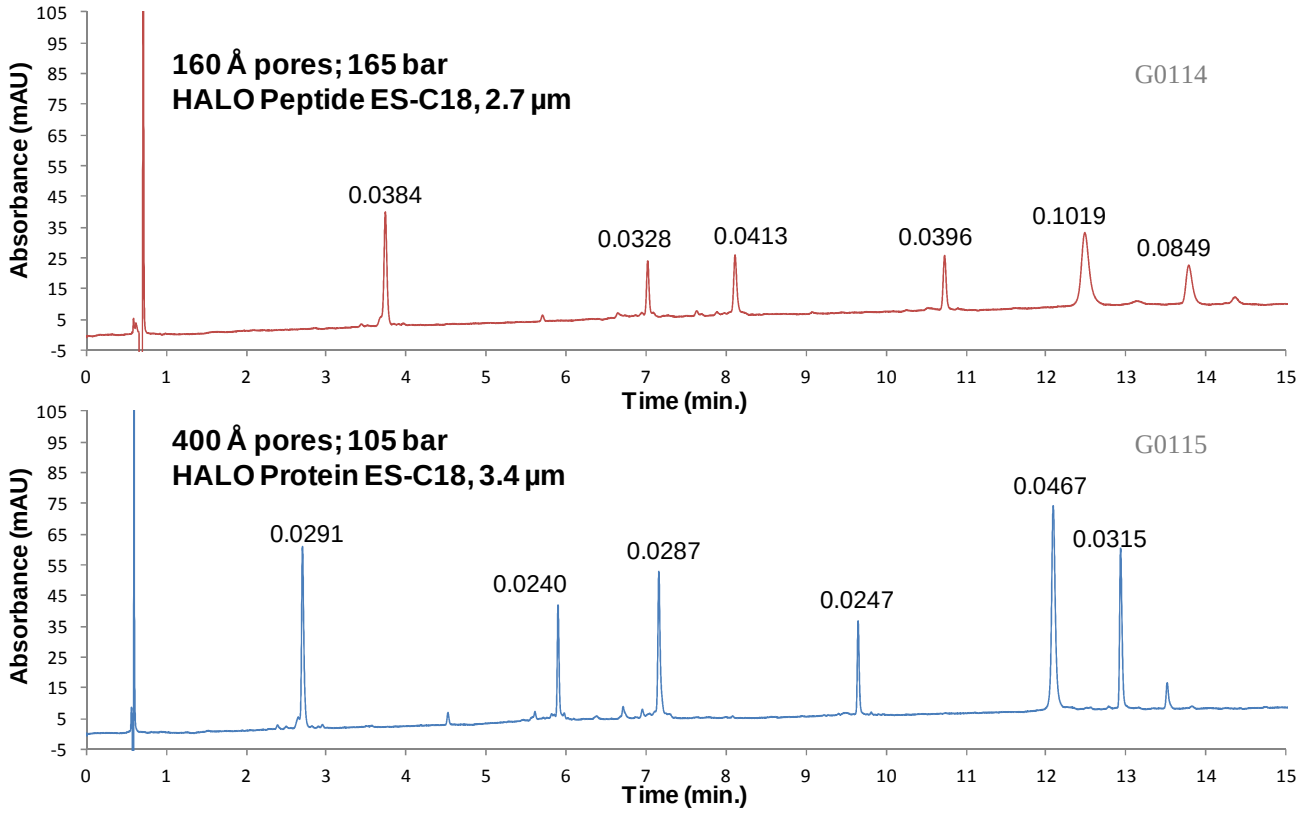
Application Note: 130-PR

Effect of Silica Pore Size on Protein Separations



TEST CONDITIONS:

Columns:
4.6 x100 mm, HALO Peptide ES-C18, 2.7 µm
Part Number: 92124-602
4.6 x 100 mm, HALO Protein ES-C18, 3.4 µm
Part Number: 93414-602
Mobile Phase:
A= 0.1% Trifluoroacetic acid in water
B= 0.1% Trifluoroacetic acid in acetonitrile
Flow Rate: 1.5 mL/min.
Gradient: 23% B to 50% B in 15 minutes
Starting pressure: As indicated on chart
Temperature: 60℃
Detection: UV 215 nm, VWD
Injection Volume: 5 µL
Sample Solvent: mobile phase A
Response Time: 0.12 sec.
Data Rate: 14 Hz
LC System: Agilent 1100 Quaternary
Flow Cell: 5 µL semi-micro

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 Protein 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 Protein column despite the larger particle size of the packing material, emphasizing the importance of larger pores when separating proteins.