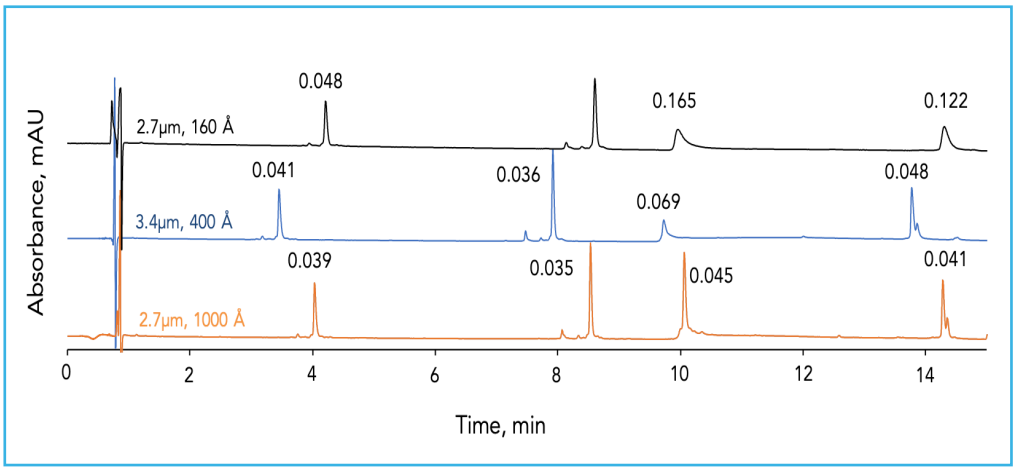
HALO®

BIOPHARMACEUTICALS

Effect of HALO® ES-C18 Pore Size on Protein Peak Shape and Width

Application Note 170-PR



PEAK IDENTITIES:

1. Ribonuclease A (13.8 kDa)
2. Lysozyme (14.4 kDa)
3. SILu™ Lite SigmaMAb Antibody (~150 kDa)
4. Enolase (46.7 kDa)

Pore size can play an important part in HPLC separations. A range of proteins and a monoclonal antibody are separated on HALO® ES-C18 160 Å, 400 Å, and 1000 Å columns. Peak widths decrease as the column's pore size becomes larger, especially for the monoclonal antibody. The 160 Å pore size is recommended for molecules in the range of 100 Da to 15kDa. The 400 Å pore size is recommended for molecules between 2kDa to 500 kDa. The 1000 Å pore size is used for molecules over 50 kDa.

TEST CONDITIONS:

Columns:
1) HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92122-702
2) HALO 400 Å ES-C18, 3.4 µm, 2.1 x 150 mm
Part Number: 93412-702
3) HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-702
Mobile Phase:
A: Water (0.1% TFA)
B: 80/20 acetonitrile/water (0.085% TFA)
Gradient: 27–60% B in 15 min
Flow Rate: 0.4 mL/min
Temperature: 60 °C
Detection: UV 280 nm, PDA
Injection Volume: 4.0 µL
Sample Solvent: Water (0.1% TFA)
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

STRUCTURES:

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