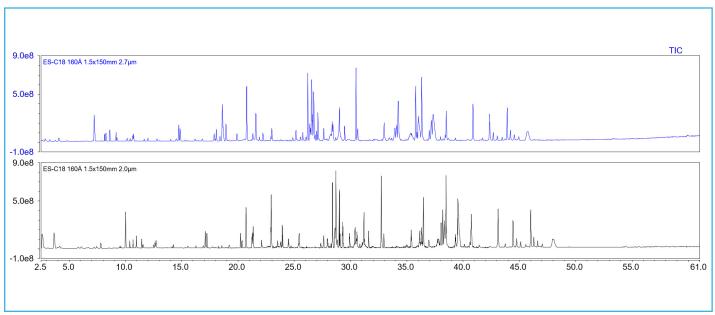
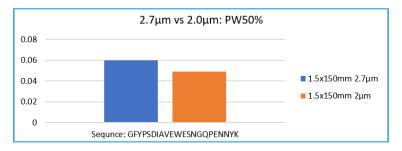


BIOPHARMACEUTICALS

Trastuzumab Digest Sensitivity Improvement Using a 2µm Particle Size in a 1.5 mm ID Format

BIO-326





A full MS scan of a peptide map is separated on two different HALO® columns. This comparison features both the 1.5 mm 2.7 μ m ES-C18 column and the 1.5 mm 2 μ m ES-C18 column. In order to get the best separation of a full peptide map, the sample must be run using a shallow gradient and an efficient column. Column efficiency increases particle size is reduced. While the 1.5 mm 2.7 μ m column gives the benefits of a smaller ID, increased sensitivity and decreased solvent consumption, it does not have the same efficiency of a 2 μ m particle size. By using a HALO® 1.5 mm ID 2 μ m ES-C18 column not only can you gain the benefits of a smaller ID but you can also decrease the peak widths of your separation with the smaller particle size. A graph of the peak widths at 50% height for the sequence GFYPSDIAVEWESNGQPENNYK has been included in order to visualize the efficiency of the 2 μ m column.







BIOPHARMACEUTICALS



TEST CONDITIONS:

Column: HALO 160 Å ES-C18 , 2.0 μm, 1.5 x 150 mm

Part Number: 9112X-702

Column: HALO 160 Å ES-C18 , 2.7 µm, 1.5 x 150 mm

Part Number: 9212X-702

Mobile Phase A: Water, 0.1% DFA Mobile Phase B: Acetonitrile, 0.1% DFA

 Gradient:
 Time
 %B

 0.5
 2

 60.5
 50

 61.0
 70

 65.0
 70

 65.5
 2

 70.0
 2

Flow Rate: 0.2 mL/min Pressure: 372 bar 2µm

240 bar 2.7µm

Temperature: 60 °C Injection Volume: 1 μL

Sample: 1mg/mL Trastuzumab tryptic digest **Sample Solvent:** 1.5M Guanidine HCl/0.5% Formic

Acid/~50mM Tris pH: 7.8

LC System: Shimadzu Nexera X2

TUBING OPTIMIZATION:

 $50\mu m\ x\ 600mm\ Column\ to\ Diverter\ Valve\ 50\mu m\ x\ 350mm\ Diverter\ Valve\ to\ Ground$

50µm x 100mm Ground to Source

MS CONDITIONS:

System: ThermoFisher Q Exactive

Spray Voltage (kV): 3.8

Capillary temperature: 320 °C

Sheath gas: 35 Aux gas: 10 RF lens: 50

