HALO®

BIOPHARMACEUTICALS

Trastuzumab Tryptic Digest Peak Capacity Comparison between 2.7µm and 2µm 160 Å PCS C18

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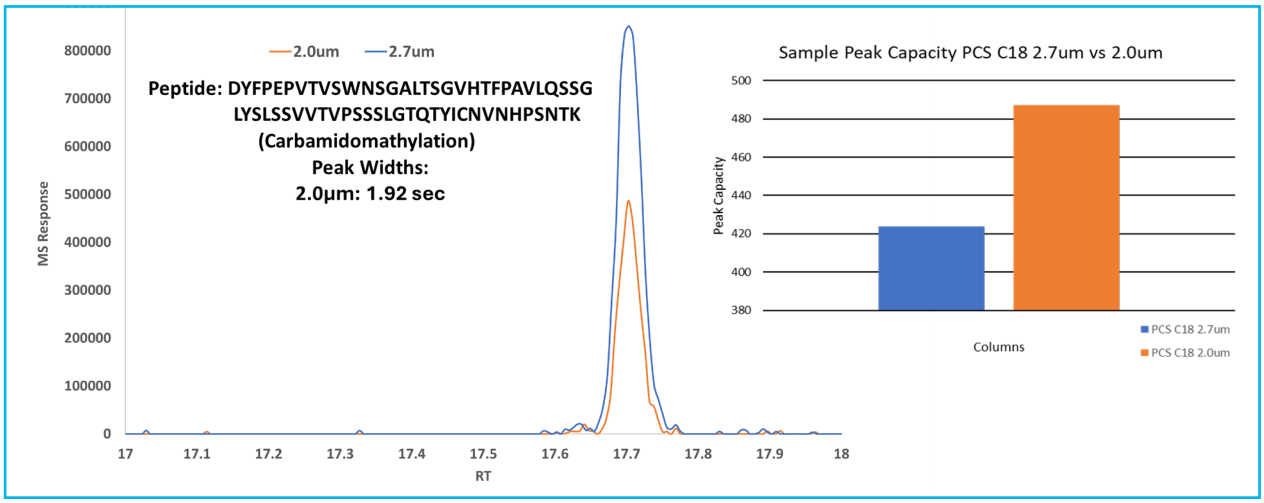

TEST CONDITIONS:

Column: HALO 160 Å PCS C18, 2.0 µm, 2.1 x 100 mm
Part Number: 91182-617
Mobile Phase: A: Water + 0.1% Formic Acid
Mobile Phase B: Acetonitrile + 0.1% Formic Acid
Gradient:

Time	%B
0.0	3
30.0	50
35.0	3

Flow Rate: 0.3 mL/min.
Back Pressure: 330 bar
Temperature: 60 °C
Injection: 1µL of 200 ng/mL Trastuzumab Digest
Sample Solvent: H_2O
LC System: Shimadzu Nexera X2
MS System: Thermo Q-Exactive HF

MS Conditions:

Polarity: Positive
Resolution: 120k
AGC Target: 3e6
Max IT: 200ms
Scan Range: 200-2000 *m/z*
Sheath Gas Flow Rate: 25
Aux Gas Flow Rate: 10
Sweep Gas Flow Rate: 1
Spray Voltage: 4.5kv
Capillary Temp: 350 °C
Aux Gas Heater Temp: 300 °C
S-Lens RF level: 60

This application note compares the performance of two HALO® 160 Å PCS C18 columns with different particle sizes, 2 µm and 2.7 µm, using a trastuzumab digest. Peak capacity was evaluated based on eight selected peptides identified in the digest, with calculations derived from peak width measurements. The 2 µm particle size demonstrated significantly narrower peak widths (~15%), resulting in improved resolution and a substantial increase in peak capacity compared to the 2.7 µm column. The difference in peak capacity between the two columns was 64, highlighting the advantage of smaller particle sizes for complex peptide separations. Additionally, the HALO® PCS C18 phase, featuring a positively charged surface, enhances peak shape under formic acid conditions by reducing secondary interactions and improving peptide peak shape compared to a standard C18 phase. This benefit is particularly valuable for LC-MS workflows where formic acid is commonly used as a mobile phase additive.

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