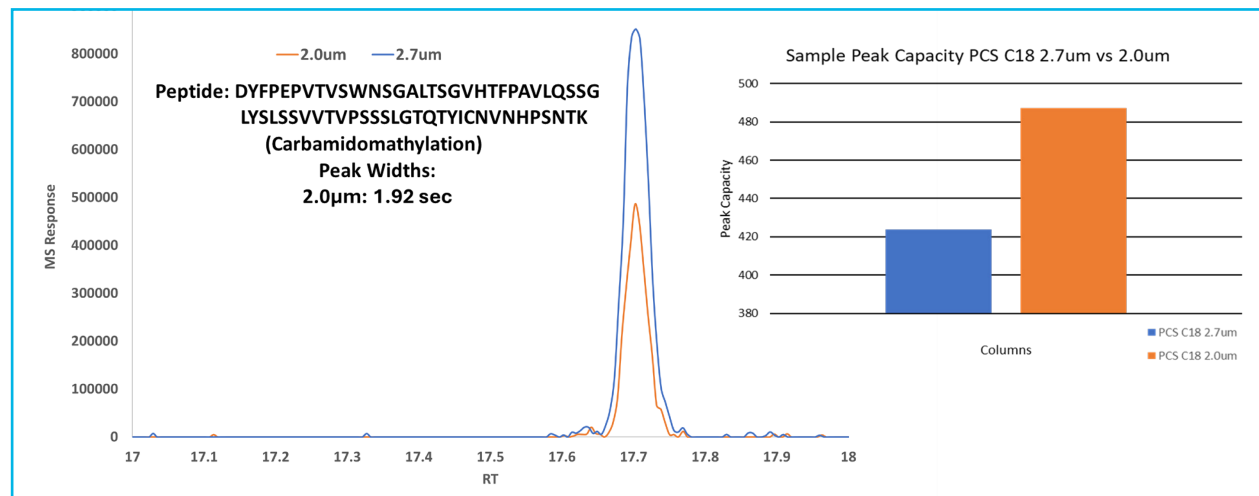




## Trastuzumab Tryptic Digest Peak Capacity Comparison between 2.7 $\mu\text{m}$ and 2 $\mu\text{m}$ 160 Å PCS C18

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### TEST CONDITIONS:

Column: HALO 160 Å PCS C18, 2.0  $\mu\text{m}$ , 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: 1 $\mu\text{L}$  of 200 ng/mL Trastuzumab Digest

Sample Solvent: H<sub>2</sub>O

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  $\mu\text{m}$  and 2.7  $\mu\text{m}$ , 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  $\mu\text{m}$  particle size demonstrated significantly narrower peak widths (~15%), resulting in improved resolution and a substantial increase in peak capacity compared to the 2.7  $\mu\text{m}$  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.

