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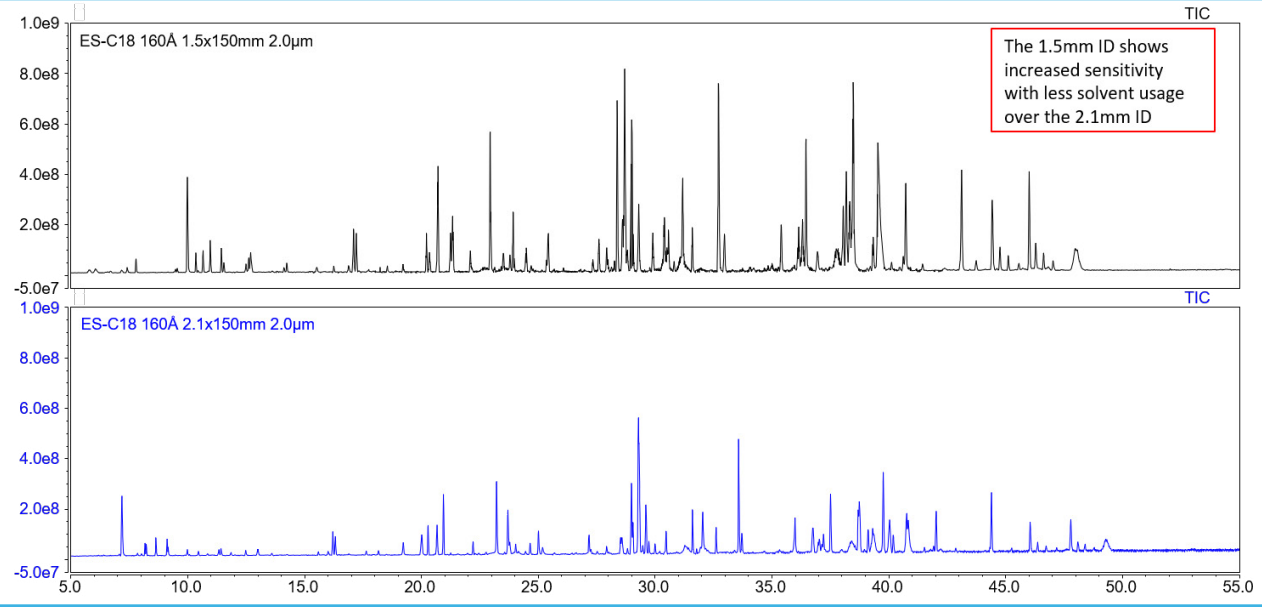
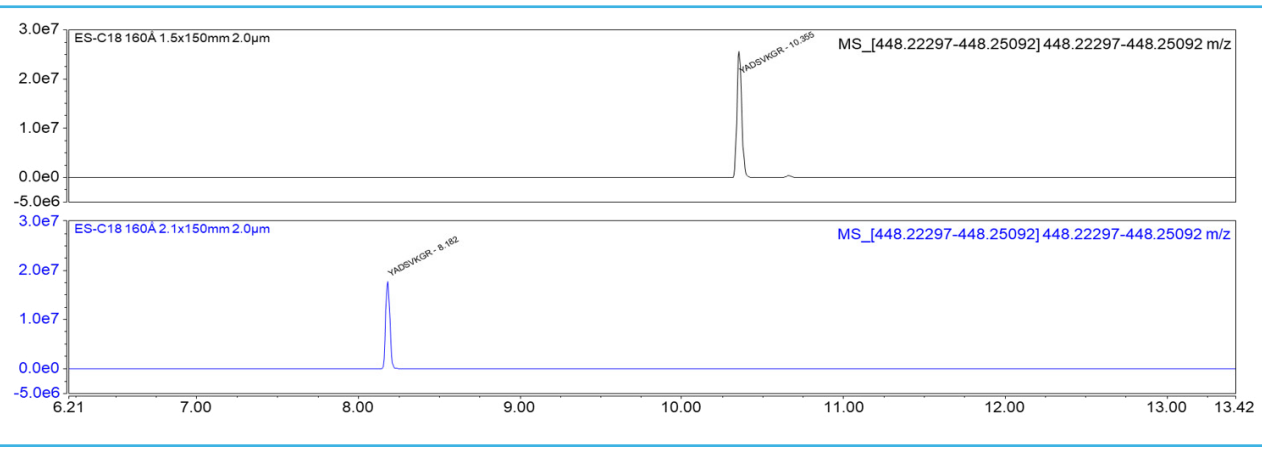
BIOPHARMACEUTICALS

Improved Signal Intensity for Trastuzumab Peptides Using a HALO® 1.5 mm ID Column

BIO-325





This app note features the full MS scan of a sample of Trastuzumab that underwent trypsin digestion to produce peptide fragments of varying length that can then be used for peptide mapping. With the help of a computer program, an extracted ion chromatogram (XIC) of the sequence YADSVKGR is also featured. This XIC is to give a closer look at the benefits of a 2µm 1.5 mm ID peptide column. Samples with similar complexity require the increased efficiency from a smaller particle size and long shallow gradients. By switching from a 2.1 mm ID to a 1.5 mm ID not only can solvent usage be cut in half, but sensitivity can also be increased.
This all can be achieved by using the HALO® 1.5mm ID 2µm ES-C18 product.

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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 , 2.0 µm, 2.1 x 150 mm
Part Number: 91122-702
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	Stop

Flow Rate: 0.2 mL/min for 1.5 mm
0.4 mL/min for 2.1 mm
Pressure: 372 bar 1.5 mm
670 bar 2.1 mm
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:

50µm x 600mm Column to Diverter Valve
50µm 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

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