HALO®

BIOPHARMACEUTICALS

Ultrafast Screening Assay for Semaglutide Impurities using 2.0 µm 160 Å PCS C18

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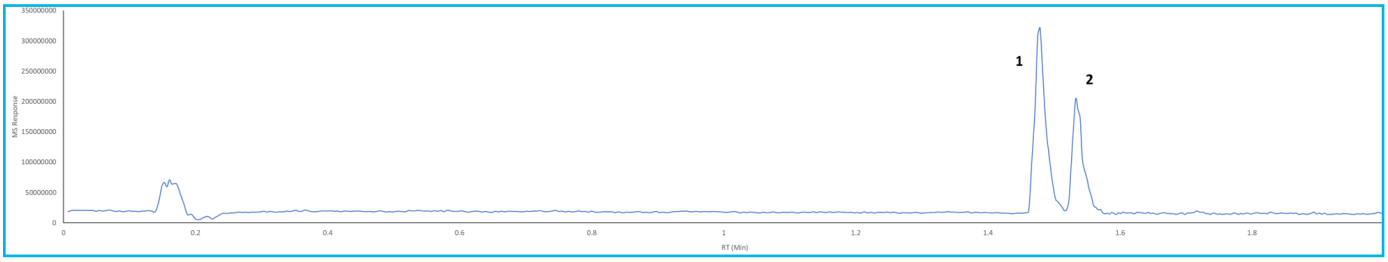

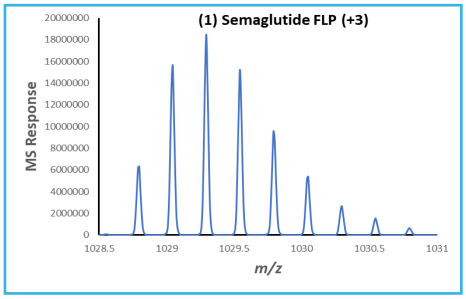

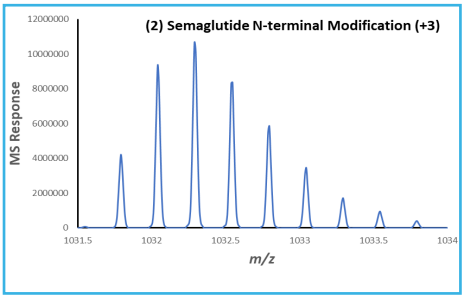

TEST CONDITIONS:

Column: HALO 160 Å PCS C18, 2.0 µm, 2.1 x 50 mm
HALO 160 Å PCS C18 2.7 µm, 2.1 x 50 mm
Part Number: 91182-417
Part Number: 92112-417
Mobile Phase A: Water + 0.1% Formic Acid
Mobile Phase B: ACN + 0.1% Formic Acid
Gradient:

Time	%B
0.0	20
2.0	55
3.0	90
4.0	90

Flow Rate: 0.7 mL/min.
Back Pressure: 2.0 µm - 340 bar
2.7 µm - 200 bar
Temperature: 60 °C
Injection: 1µL of 20ng Semaglutide modified with 10mM Tris pH 8.0
Sample Solvent: H_2O
LC System: Shimadzu Nexera X2
MS System: Thermo Orbitrap QE-HF

MS CONDITIONS:

Polarity: Positive
Resolution: 60k
AGC Target: 3e6
Max IT: 200ms
Scan Range: 300-2000 *m/z*
Sheath Gas Flow Rate: 35
Aux Gas Flow Rate: 15
Sweep Gas Flow Rate: 1

Spray Voltage: 4.0kv
Capillary Temp: 375 °C
Aux Gas Heater Temp: 350 °C
S-Lens RF level: 60
In-Source CID: 10 eV

Column Type/Sample	Retention Time (min)	50% Peak Width (sec)	Tailing Factor (EP)
2.0 µm PCS Semaglutide FLP	1.476	0.72	1.34
2.0 µm PCS N-terminal Mod	1.533	0.96	1.77
2.7 µm PCS Semaglutide FLP	1.457	1.14	1.4
2.7 µm PCS N-terminal Mod	1.513	1.26	1.69

GLP-1 targeted therapeutics are a rapidly growing business. This in turn has driven demand for versions that are produced by compounding pharmacies at a lower price point. We have previously demonstrated the risk for the generation of a specific impurity of Semaglutide during the compounding process likely caused by exposure to trace levels of formaldehyde. This exposure causes cyclization of the N-terminal histidine, creating a 12 dalton shift in molecular weight. Currently, the clinical risk of this impurity is unknown.

Here we demonstrate an ultrafast assay for separation of the Semaglutide full-length product from the N-terminal modified impurity on our 2.0 µm 160 Å PCS C18 column in a 2.1x 50 mm format. The PCS C18 bonding phase contains a positively charged surface ligand in acidic conditions which improves peak shapes in weak ion pairing conditions required for LCMS. Compared to 2.7 µm 160 Å PCS C18 in ballistic gradient conditions, peak widths are reduced by approximately 30%, generating peak widths at 50% to less than 1 second.

This assay demonstrates the ability to perform high-throughput screening for potential contamination in compounded GLP-1 samples to determine patient risk.

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