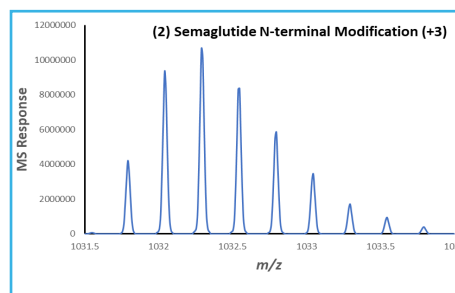
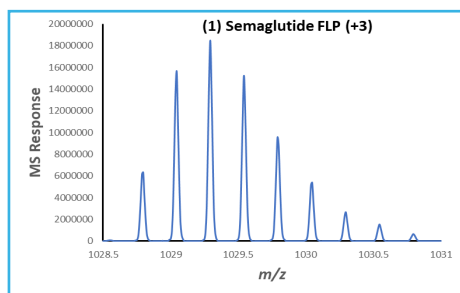
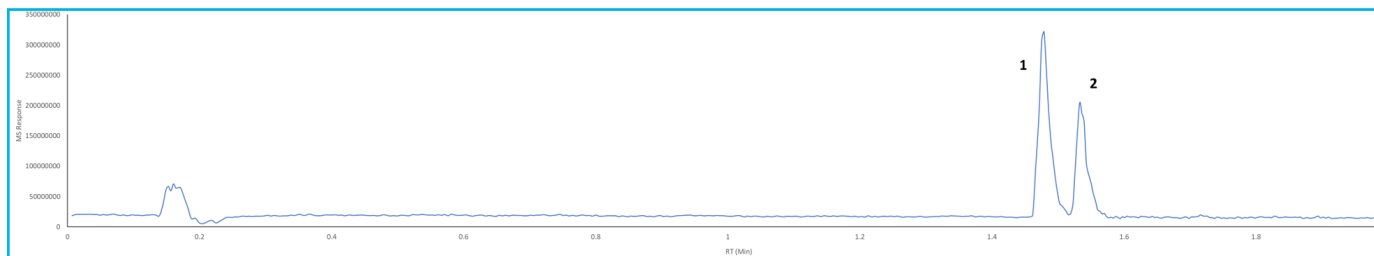




### Ultrafast Screening Assay for Semaglutide Impurities using 2.0 $\mu\text{m}$ 160 $\text{\AA}$ PCS C18

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#### TEST CONDITIONS:

Column: HALO 160  $\text{\AA}$  PCS C18, 2.0  $\mu\text{m}$ , 2.1 x 50 mm  
 HALO 160  $\text{\AA}$  PCS C18 2.7  $\mu\text{m}$ , 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  $\mu\text{m}$  - 340 bar  
 2.7  $\mu\text{m}$  - 200 bar  
 Temperature: 60  $^{\circ}\text{C}$   
 Injection: 1  $\mu\text{L}$  of 20ng Semaglutide modified with 10mM Tris pH 8.0  
 Sample Solvent:  $\text{H}_2\text{O}$   
 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  $^{\circ}\text{C}$   
 Aux Gas Heater Temp: 350  $^{\circ}\text{C}$   
 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 $\mu\text{m}$ PCS Semaglutide FLP	1.476	0.72	1.34
2.0 $\mu\text{m}$ PCS N-terminal Mod	1.533	0.96	1.77
2.7 $\mu\text{m}$ PCS Semaglutide FLP	1.457	1.14	1.4
2.7 $\mu\text{m}$ 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  $\mu\text{m}$  160  $\text{\AA}$  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  $\mu\text{m}$  160  $\text{\AA}$  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.

