HALO®

BIOPHARMACEUTICALS

GLP-1 Agonist Separation on HALO® PCS C18

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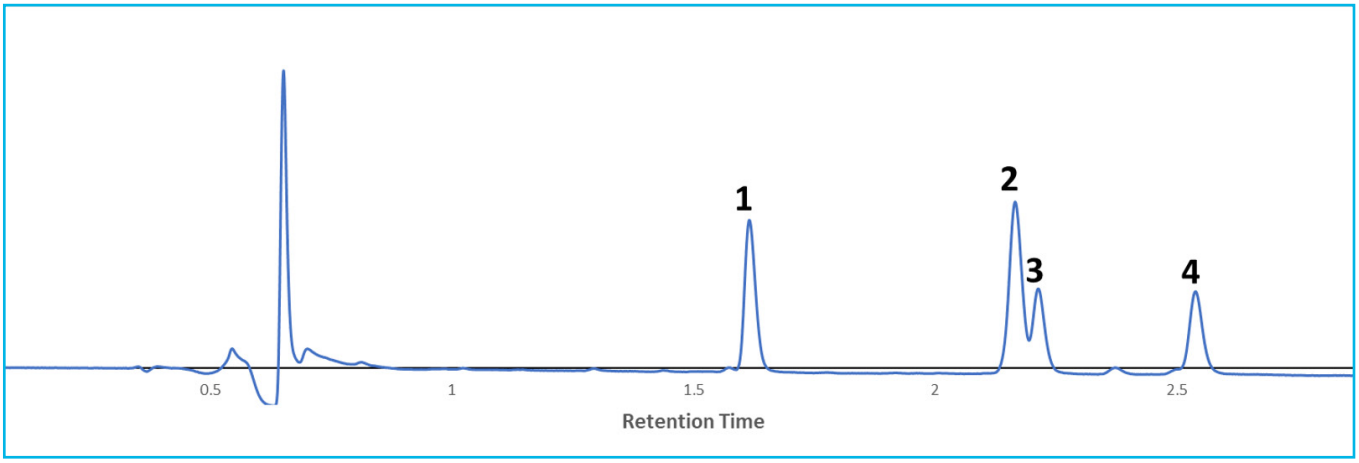

TEST CONDITIONS:

Column: HALO 160 Å PCS C18 1.5 x 150 mm; 2.7 µm
Part Number: 9211x-717
Mobile Phase A: H_2O + 0.1% Formic Acid
Mobile Phase B: Acetonitrile + 0.1% Formic Acid
Gradient:

Time	%B
0	35
3	95
3.01	35
4.5	35

Flow Rate: 0.3 ml/min
Pressure:
Temperature: 60 °C
Detection: 220 nm
Injection Volume: 2 µl
Sample Solvent: 10mM Tris pH 8.0 in H_2O
Data Rate: 40 Hz
LC System: Shimadzu Nexera X2

PEAK IDENTITIES

1. Semaglutide
2. Liraglutide
3. Retatrutide
4. Tirzepatide

MS CONDITIONS:

Ion mode: Positive Electrospray
Scan Mode: MS1
Resolution: 120,000
Sheath Gas Flow Rate: 35
Aux Gas Flow Rate: 10
Sweep Gas Flow Rate: 2
Spray Voltage: 4kV
Capillary Temp: 320 °C
Aux Gas Heater Temp: 275 °C
S-Lens RF Level: 60V
Mass Spectrometer: Thermo Q-Exactive HF

GLP-1/GIP agonists are a rapidly growing class of medications used to manage diabetes and obesity. Recent studies have also shown benefits in cardiovascular disease, cancer, and many other health concerns. The GLP-1 market is expected to grow to over $50 Billion by 2030. Most GLP-1 medications are modified peptides of ~40AA length. Here we demonstrate a rapid separation of 4 GLP-1 peptides, 3 of which are currently commercially available, the fourth in Phase 3 clinical trials as of this writing. Each is lipidated at a lysine residue increasing their hydrophobicity. Additionally Retatrutide and Tirzepatide are modified with Alpha-amino butyric acid.

Using the HALO® PCS C18, 2.7µm 1.5 x 150 mm column, we are able to separate all 4 peptides in less than 3 minutes. Their identification was confirmed via High Resolution MS.