HALO®

BIOPHARMACEUTICALS

LC/MS/MS Analysis of Desamido Glucagons via HALO® PCS C18

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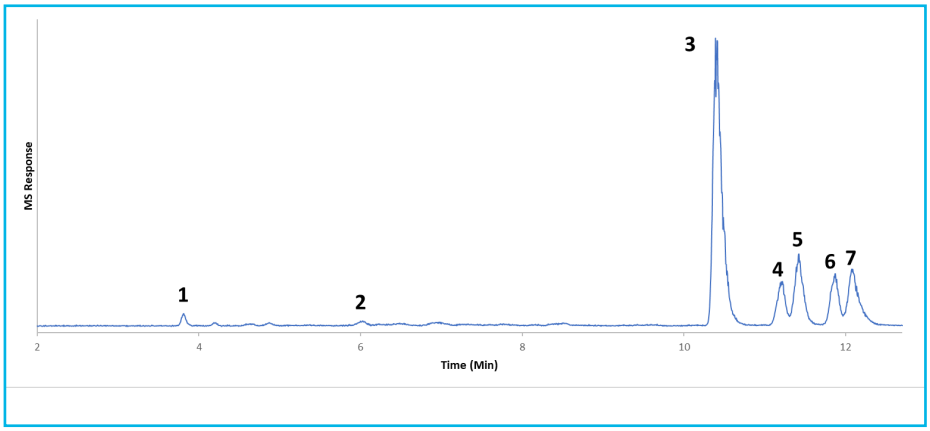

PEAK IDENTITIES

1. Glucagon Fragment [SRRAQDFVQWLMNT]
2. Glucagon Fragment [YSKYLDSRRAQD-FVQWLMNT]
3. Glucagon
4. Asp^{28}-Glucagon
5. Glu^{3}-Glucagon
6. Glu^{20}-Glucagon
7. Glu^{24}-Glucagon

TEST CONDITIONS:

Column: HALO 160 Å PCS C18 , 2.7µm, 2.1 x 150 mm
Part Number: 92112-717
Mobile Phase A: Water with 0.1% Formic Acid
Mobile Phase B: Acetonitrile with 0.1% Formic Acid
Gradient:

Time	%B
0.0	20
15.0	25
15.5	80
17.0	80
17.1	20
22.0	20

Flow Rate: 0.4 ml/min
Pressure: 330 bar
Temperature: 60 °C
Detection: High Resolution MS
Injection Volume: 2 µl (Sample diluted 1:10 in Mobile phase A; 100ug injected
Sample Solvent: Glucagon (0.5mg/ml) in 0.01N HCl incubated at 50°C for 48 hrs
(For system suitability study)
LC System: Shimadzu Nexera X2

MS CONDITIONS:

Mass Spectrometer: Thermo Q-Exactive HF
Ion mode: Positive Electrospray
Sheath Gas Flow Rate: 35
Aux Gas Flow Rate: 10
Sweep Gas Flow Rate: 1
Spray Voltage: 4000 V
Capillary Temp: 320 °C
S-Lens RF: 50 V
Aux Gas Heater Temp: 300 °C
MS1 Resolution: 120,000
AGC Target: 3.00E+06
Max IT: 100ms
Scan Range: *m/z* 350-2000
MS2 Resolution: 45,000
AGC Target: 3.00E+05
Max IT: 50 ms
Isolation Window: *m/z* 4.0
Scan Range *m/z* 200-2000
NCE: 30

Glucagon is a peptide hormone belonging to the secretin family which increases the concentration of glucose in the bloodstream by binding to glucagon receptors in hepatocytes and stimulating the conversion of stored glycogen into glucose via glycogenolysis.

Glucagon is an important medicaton for the treatment of low blood sugar in medical conditions such as Diabetes Mellitus. Pharmaceutical grade glucagon is commonly manufactured via recombinant methods and the USP monograph for Glucagon stipulates that acceptance criteria for pharmaceutical grade glucagon contain NLT 90% and NMT 105% Glucagon from recombinant origins. Recombinant Glucagon is required to contain NMT 6% total impurities and NMT 2% of 4 characterized desamido glucagons.

The 4 described desamido glucagons, (Glu^{3})-Glucagon, (Asp^{28})-Glucagon, (Glu^{24})-Glucagon, and (Glu^{20})-Glucagon must be clearly resolved via chromatography according to the USP monograph. The monograph describes a separation method requiring 45 minutes using an L1 column with 3mmx150mm dimensions and 3µm particle size, at a flow rate of 0.5ml/min that will resolve all 4 desamido-glucagons.

Here we describe a separation method in 12.5 minutes using a HALO 160 Å PCS C18 2.1x150mm, 2.7µm L1 column that achieves baseline separation of all 4 desamido glucagons prepared via USP system suitability solution method and confirmed via LC/MS/MS as well as characterization of 2 additional impurities. This demonstrates the capabilities of the HALO® PCS C18 column for rapid characterization of pharmaceutical biologics.

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