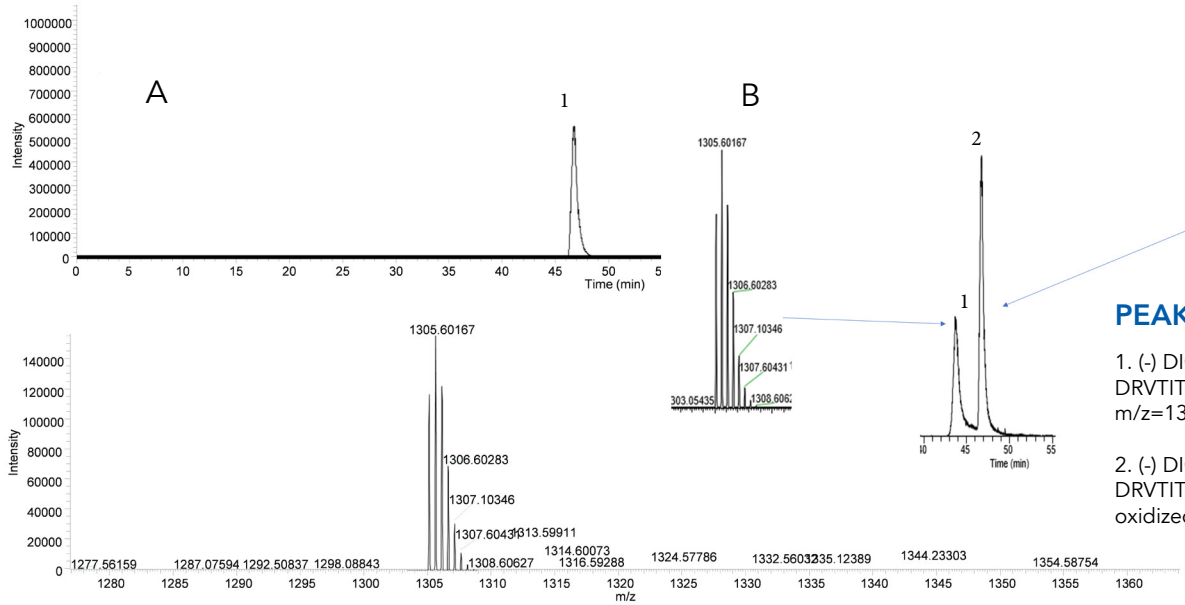
HALO®

BIOPHARMACEUTICALS

Oxidation of NIST mAb Fragment

274-PE



PEAK IDENTITIES

1. (-) DIQMTQSPSSLSASVG-DRVTITC(Carbamidomethyl)R(A) m/z=1305.60167

2. (-) DIQMTQSPSSLSASVG-DRVTITC(Carbamidomethyl)R(A) oxidized m/z= 1313.59911

(A) Extracted ion chromatogram of (-) DIQMTQSPSSLSASVGDRVTITC(Carbamidomethyl)R(A)
(B) Extracted ion chromatogram oxidized (-) DIQMTQSPSSLSASVGDRVTITC(Carbamidomethyl)R(A) showing a mass shift of 8, as expected for an oxidized doubly charged peptide

TEST CONDITIONS:

Column: HALO® 90 Å Penta-HILIC, 2.7 µm, 0.5 x 150 mm
Part Number: 98215-705
Mobile Phase A: 50 mM Ammonium formate, pH 4.4
Mobile Phase B: 0.1% formic acid in acetonitrile
Gradient:

Time	%B
0.0	80
4.0	80
55	48
59	48
63	80
70	end

Flow Rate: 50 µL/min
Pressure: 158 bar
Temperature: 60 °C (standard)
80 °C (oxidized)
Detection: +ESI
Injection Volume: 5.0 µL
Sample Solvent: 70% ACN, 30% Water
LC System: Shimadzu Nexera X2
MS System: Thermo LTQ VELOS PRO

Post-translational modifications (PTMs), such as oxidation, are a critical variable that must be accounted for during protein analysis. Often times the minor mass shifts associated with these modifications are too small to be resolved during intact protein analysis, due to the charge envelope produced by large proteins, such as monoclonal antibodies (mAbs). However, chromatographically, these compounds will have a difference in retention time relative to the native, and can be separated before getting to the detector. Peptide analysis is an important method of characterization for mAbs because, in addition to revealing modifications such as oxidation, it can provide valuable insight into additional post-translational modifications, which may not be evident during intact mass analysis. In this experiment, the digested NIST mAb was exposed to high temperature in order to induce oxidation, and then analyzed using the HALO® Penta-HILIC capillary column, demonstrating it is an ideal choice for peptide oxidation analysis of mAbs.

MS CONDITIONS:

Ion mode: Positive
Aux gas: 2 arbitrary units
Sheath gas: 4 arbitrary units
Sweep gas: 0 arbitrary units
Rf lens: 55 V
Heater temp: 225°C
Ion transfer tube: 275°C
Capillary Voltage: 3.5 kV

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