## BIOPHARMACEUTICALS

HALO



## **Oxidation of NIST mAb Fragment**



## **TEST CONDITIONS:**

Column: HALO<sup>®</sup> 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 80 4.0 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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