Development of a Sensitive, High-Resolution LCMS HILIC Method of the Oxidation of NIST mAb as an Alternative to Reverse Phase

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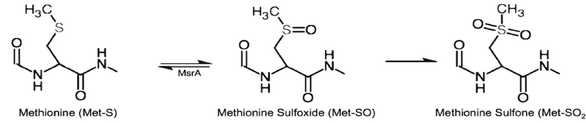
Introduction

Methionine oxidation, a post-translational modification (PTM), is a critical variable that must be accounted for during protein analysis, especially for therapeutic proteins such as Monoclonal antibodies (mAbs). Oxidation in mAbs has been linked to function loss, folding stability decrease, and the increase in the propensity for the mAb to aggregate. Methionine (Met-S) oxidizes to form methionine sulfoxide (Met-SO). Methionine sulfoxide (Met-SO) can further oxidize to form methionine sulfone (Met-SO_2). Met oxidation is possible at various development stages, including production, formulation, and storage. For this reason, the presence of oxidation must be monitored at each development stage in order to determine the condition of the mAb.



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. However, chromatographically, these compounds will have a difference in retention time relative to the native, and can be separated.
Hydrophilic interaction chromatography (HILIC) is an ideal choice for this analysis because polar compounds can be retained while using MS friendly mobile phases and buffers. Whereas RPLC elutes from low to high organic, HILIC elutes from high to low organic, allowing a higher percentage of organic into the ion source during the elution than RPLC, and will enhance the desolvation of the analyte and contribute to better spray stability, which will therefore lead to an increase in sensitivity.

Experimental

Trypsin digestion of the NIST mAb
The NIST mAb sample was denatured and alkylated using 50 mM Tris-HCl (pH 7.8)/1.5M Guanidine-HCl, and 2-iodoacetamide (Sigma Aldrich). Trypsin (Promega) was added in a ratio of 1:30 (w:w; Trypsin:mAb) followed by an incubation at 37 °C overnight.

Instrumentation
Samples were analyzed on a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). Mass spectra were acquired using a Thermo Scientific Velos Pro LTQ Orbitrap mass spectrometer (Bremen, Germany) using a heated electrospray (HESI-II) probe on the Ion Max source.

Column
A 90 Å 2.7 µm, (0.5 mm ID x 150mm)
HALO® Penta-HILIC column was used (Advanced Materials Technology, Inc. Wilmington, DE). Flow rate was 50 µL/min, and 60 °C for initial analysis. In the oxidation experiments the temperature was raised to 80 °C

LC Gradient

Time	%B
0	80
4	80
55	48
59	48
63	80
70	end

Sample Solvent: 70% ACN 30% water
Mobile phase A: 50 mM ammonium formate pH 4.4
Mobile phase B: 0.1% formic acid in acetonitrile.

MS Conditions
Voltage 3.5 kV Aux gas 4 arbitrary units
Sheath gas 2 arbitrary units
Sweep gas 0 arbitrary units
Rf lens 45 v
Heater temp 225 °C

The Particle/ Penta-HILIC Ligand

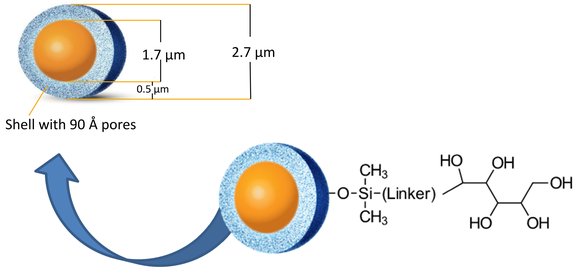


Figure 1. HALO® SPP particle and Penta-HILIC ligand structure

UV Spectra of NIST mAb

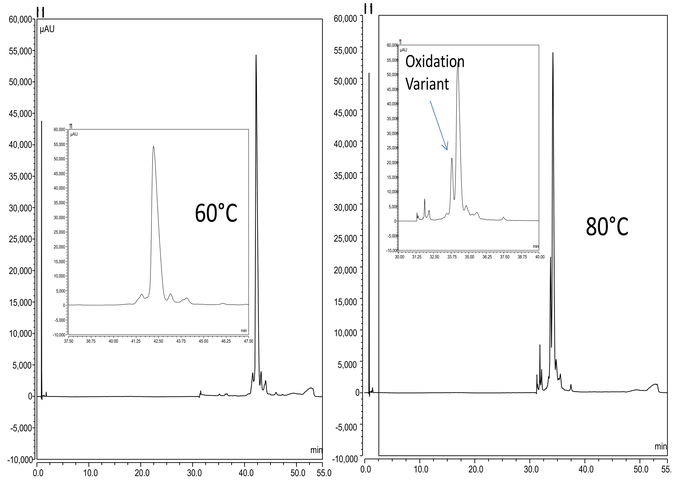


Figure 2. A) UV spectra of NIST mAb run at 60° C. B) UV spectra of NIST mAb run at 80° C showing oxidation artifact

XIC of NIST mAb

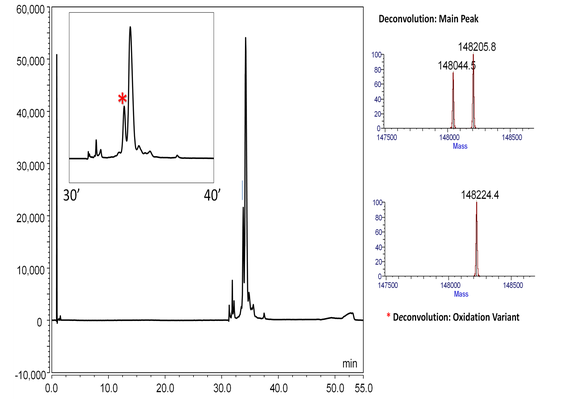


Figure 3. XIC of NIST mAb run at 80° C showing oxidation artifact and deconvoluted spectra

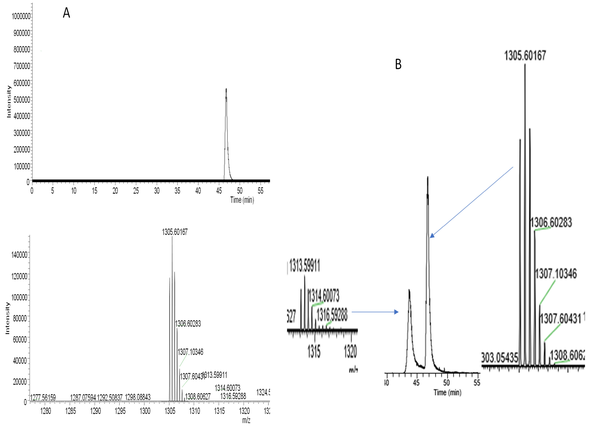


Figure 4. A) XIC of (-) DIQMTQSPSSLSASVGDRVTITC(Carbamidomethyl)R(A), m/z=1305.60167, before the oxidation experiment. **B)** XIC after the oxidation and shows the characteristic mass shift of 8 Da associated with oxidation for a doubly charged peptide species.

XIC of Peptide Fragment

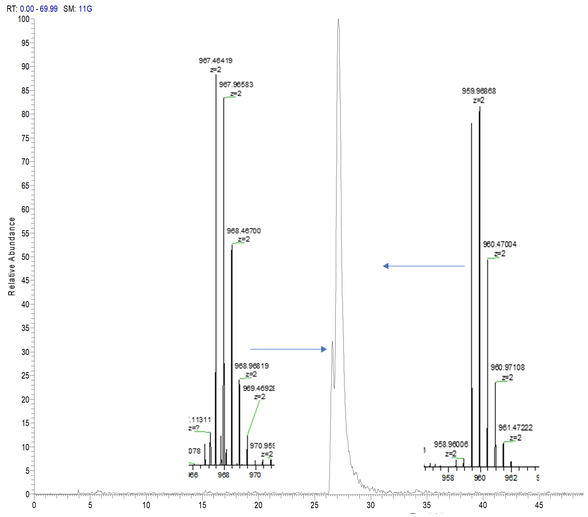


Figure 5. The extracted ion chromatogram of the doubly charged peptide fragment (R)EPQVYTLPPSREEMTK(N) showing a mass shift of 8.

Conclusion

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.

The use of the HILIC phase for the analysis is an ideal choice for this analysis because polar compounds can be retained, and the MS favorable solvent conditions can lead to higher sensitivity.

The HALO® Penta-HILIC capillary column coupled with HRMS delivers the sensitivity and resolution needed to analyze oxidation of mAbs.