BIO_23 - Characterization of an IgG1 monoclonal antibody oxidation variants at intact, subunit and peptide levels by High Resolution Accurate Mass (HRAM) mass spectrometry

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Introduction: During production of biotherapeutics, oxidation must be assessed and monitored because it can have an impact on the stability, safety and efficacy of the final drug product. Samples of ipilimumab were assessed at the intact protein, subunit and peptide level to pinpoint the locations of oxidation hotspots within the primary sequence.

Objectives: We demonstrate the utilization of High Resolution Accurate Mass mass spectrometry for the identification and localization of methionine oxidation of biotherapeutics via LC-MS analysis.

Methodology: Chromatography: Thermo Scientific Vanquish Duo, Solvent A: Water with 0.1% FA, solvent B: Acetonitrile with 0.1% FA, flow 0.3 mL/min. 10 µL injected. HRAM: Orbitrap Exploris 240 mass spectrometer, using application-specific MS tune acquisition settings. Xcalibur and BioPharma Finder for data acquisition and processing. Intact protein: MAbPac RP, 5-min linear gradient. Ipilimumab was exposed to varying levels of hydrogen peroxide for 24 hours to induce oxidation. Subunits: MAbPac RP, 16-min linear gradient. Control and stressed samples of ipilimumab were digested using IdeS protease, denatured and reduced using guanidine hydrochloride and TCEP. Peptide mapping: Acclaim C18 column, 45-min linear gradient. Control and forced degraded samples of ipilimumab were digested using the SMART Digest kits.

Results: We observed the full charge envelope of the intact mAb control and stressed samples. Data were acquired with a resolution setting of 30,000 which provided mass accuracies below 4 ppm for the three most abundant glycoforms of ipilimumab. A mass shift of +64 Da was observed for the stressed ipilimumab sample, indicating potential oxidation at four methionine residues at the intact mAb level. Results obtained upon deconvolution of the entire subunit charge envelope including all Fc/2 subunit glycoforms using Xtract algorithm, obtaining accurate monoisotopic masses of control and stressed samples presenting singly and doubly oxidized subunits. Comparing the peptide mapping total ion chromatogram of the control and stressed ipilimumab after digestion, we observed mass shifts indicating methionine oxidation. Peptide identification is supported by low mass accuracy (<1 ppm) and confident assignment of HCD MS/MS spectra, showing related series of fragment ions, with indicative shifts for the oxidized peptides.

Conclusion: HRAM delivers confident tracking of PTMs in mAbs at intact, subunit and peptide level with operational simplicity, simplified spectral interpretation and exceptional mass accuracy.

Keywords: Mass spectrometry; Monoclonal Antibodies; Biopharmaceuticals