Monitoring oxidative stress of biologics

Forced degradation of Trastuzumab and Trastuzumab-Vedotin ADC using a SCIEX X500B mass spectrometer

Biologics, particularly those based on monoclonal antibodies, are highly heterogenous with a variety of post-translational modifications (PTMs) including glycosylation, c-terminal clipping and n-terminal blocking. Some of these PTMs, such as oxidation or deamidation, could be induced via stress or storage conditions and may be critical quality attributes (CQAs) affecting efficacy and patient safety. Oxidation of proteins can be induced through dissolved or free radicals which could be generated via UV exposure or non-organic impurities. Methionine oxidation is a known and well characterised degradation of proteins, though antibody drug conjugates open up the potential for modifications on drugs and linkers. Oxidation of proteins can result in downstream losses if it occurs in a Protein A binding site or could reduce efficacy if the site is in the complementary determining region (CDR).

This study was designed to monitor the oxidatve stress on a monoclonal antibody and an antibody drug conjugate. Samples were incubated in 0.2% hydrogen peroxide in 50 mM Tris pH 7.5 at 37°C. Intact and reduced proteins were considered to track various oxidative sites. All data was collected on a SCIEX X500B mass spectrometer with an EXION HPLC. The chromatography was performed with a bioZen XB-C8 Column (2.6 µm, 50 x 2.1 mm, 50 x 2.1 mm).

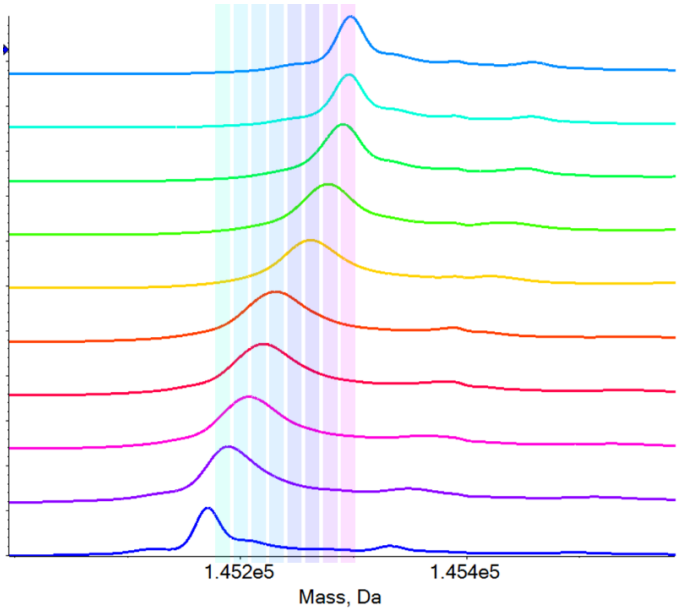

Figure 1: Oxidation of intact deglycosylated heavy chain over T0, 1, 2, 3, 4, 8, 12, 24, 36 and 48 hours (ascending). Bars across spectra indicate mass shift of oxidations from 0-8 additions of +16 Da.

Antibodies generally undergo oxidation of methionines with the conversion to methionine sulfoxide and then sulfone. Two particular conserved sites are known to be the major sites of oxidation and Met-252 (DTLMISR) and Met-428 (SVMHEA) both present in the heavy chain.

We observed rapid formation of four oxidation sites on the intact protein over the first four hours of incubation (Figure 1). This is likely the sites on the heavy chain as mentioned previously. Over the next 44 hours, oxidation progresses more slowly on other potential sites which could be sulfur containing or aromatic. After 48 hours, the sample appears to reach a maximum of eight oxidations across the protein. This is supported on the reduced sample in Figure 2, where we observe two rapid oxidations on the heavy chain in the first two hours with a much slower oxidation on the light chain.

Antibody drug conjugates (ADCs) present the added potential of changes on the drug-linker as well as on the protein. A conjugate of Trastuzmab with vc-PAB-MMAE was analysed under the same conditions as the antibody. Under oxidising conditions, the DAR of the conjugate fell with loss of high-DAR species.

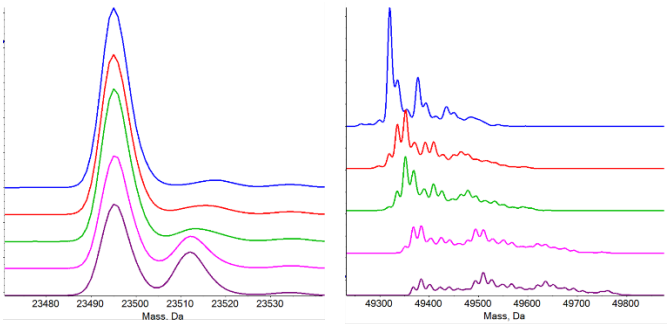

Figure 2: Oxidation of light (left) and heavy (right) chains of Trastuzumab across T0, 2, 4, 12, and 24 hours (descending). The heavy chain of Trastuzmab rapidly oxidises in two locations between two and four hours with very little unoxidised still present. The light chain progresses more slowly with about 30% oxidation after 24 hours.