Disulfide bond mapping of a monoclonal therapeutic antibody

MS of a trastuzumab biosimilar non-reducing digestion using an IDA MSMS methodology

Monoclonal antibodies are held together by a series of covalent and non-covalent interactions that ensure the antigen binding domain is in the correct orientation. The connectivity of both inter- and intra-chain disulfide bonds of proteins is essential to its three-dimensional structure and therefore its activity. When expressing a therapeutic protein its connectivity can be a critical quality attribute (CQA) that needs to be monitored to ensure its efficacy. Non-reducing digests can be used to localise which peptides are linked via disulfide bonds. Due to the peptides being larger than in reduced digests some MS conditions may have to be optimised to improve their transmission. Here we outline an analysis strategy to confirm the disulfide bond connectivity of a trastuzumab biosimilar after a non-reducing trypsin/Lys-C (Promega) digestion. MS conditions were optimised for transmission of large peptides. All data was collected on a SCIEX X500B mass spectrometer with an EXION HPLC. The chromatography was performed with a bioZen XB-C18 Column (1.7 µm, 150 x 2.1 mm).

Figure 1: Peptide connectivity of trastuzumab with peptides assigned based upon MSMS coverage with an FDR of less than 2% and a peptide coverage of >40%. Based upon IDA MSMS an overall 99.8 % of the sequence was confirmed. Individual peptides are not shown, however expected disulfide bonds are shown as pink lines connecting cysteine residues.

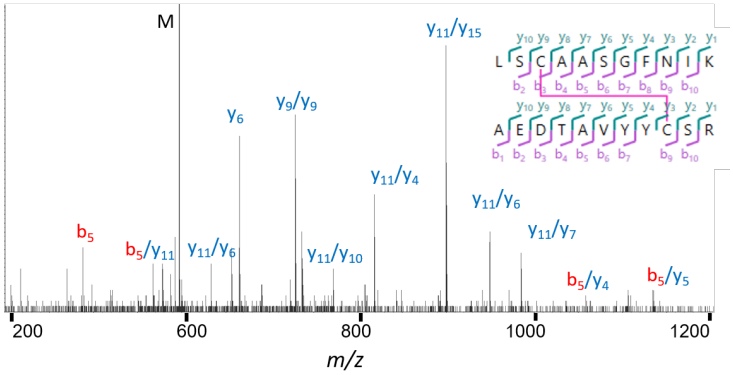

Figure 2: MSMS data from 4+ peptide LSCAASGFNIK AEDTAVYYCSR. Eluting at 29.6 minutes in the 2+ through 4+ charge states, fragmentation provides 100% peptide sequence coverage with ions observed from both peptide chains.

The sample was separated on a 70-minute gradient and analysed using BioPharmaView Flex. Figure 1 shows which expected disulfide bond connections were observed. Most peptides were observed in multiple charge states and the digestion was not run to completion resulting in a variety of missed cleavages.

Figure 2 shows MSMS data of an example disulfide linked peptide LSCAASGFNIK AEDTAVYYCSR. During fragmentation we observe ions containing partial sequences of both chains linked via the disulfide allowing us to confirm its position. All assigned peptides were required to have sequence coverage greater than 40% to exclude poor matches. In addition, a scoring algorithm was applied with an acceptance criteria of 2% false discovery rate. This methodology can be used to identify scrambled disulfides as well as those expected to occur.