An Approach for the Characterisation of Biologic Products by Accurate Mass Orbitrap LC-MS

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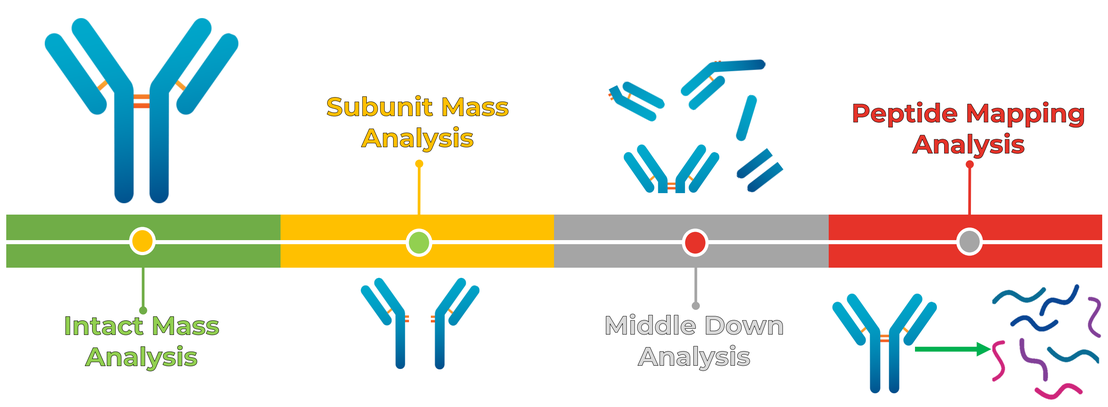
Background

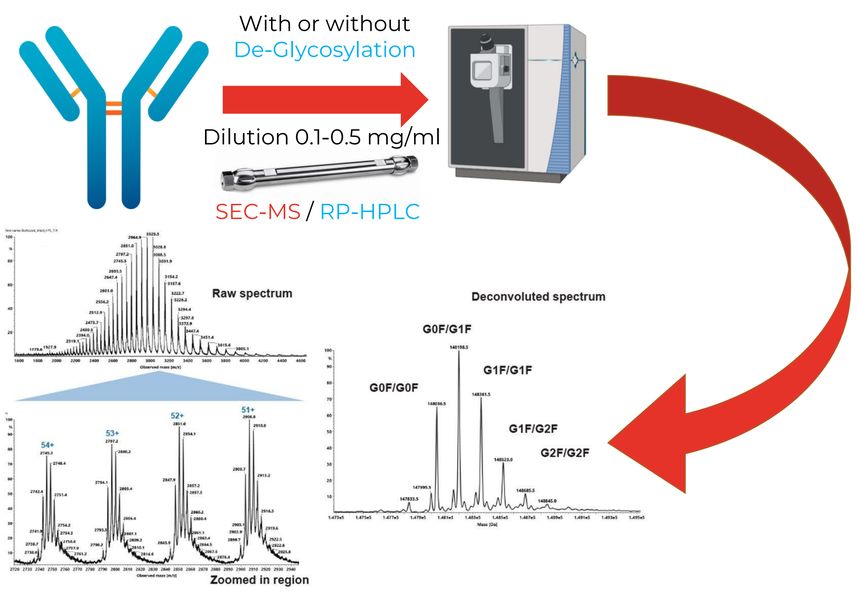
Biologic Products such as Monoclonal Antibodies and Therapeutic Proteins are a rapidly growing area of interest in the Pharmaceutical industry. As a result, the technology, methodology and regulations required to support this new emerging area have had to catch up to demand. High Resolution Mass Spectrometry is a key component in the characterisation of such molecules. This poster discusses the approach taken to establish the test methods for key analyses required for characterisation using a Thermo Q-Exactive Plus BioPharma High Resolution Mass Spectrometer. Almac provides the necessary skills and equipment to successfully deliver the required analysis to support GMP characterisation of Biologic Products.

Primary Structural Characterisation by LC-MS



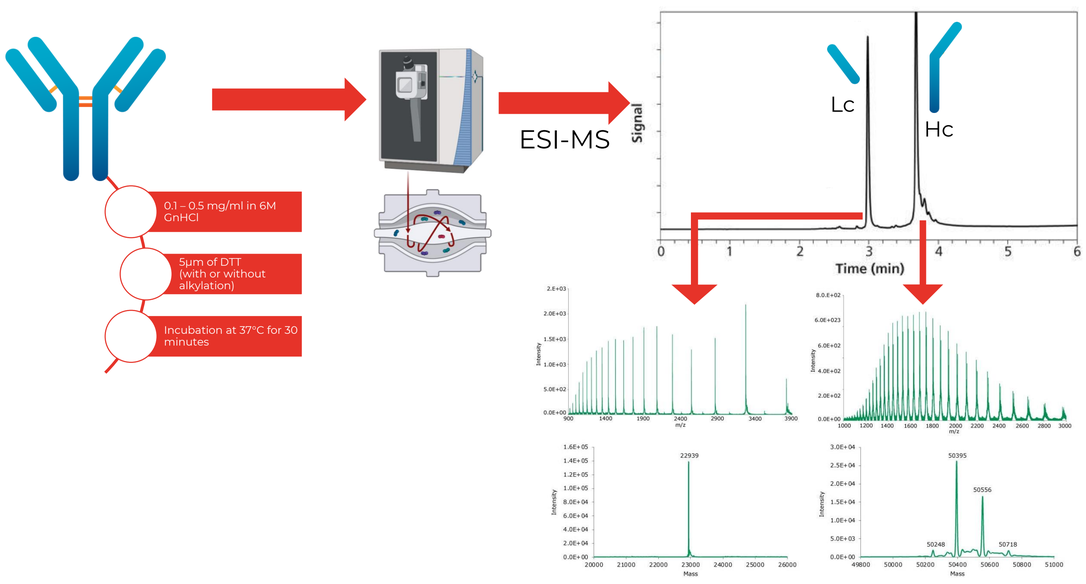
Characterisation of Biologic Products is essential to their safe and effective use. Regulatory guidance prescribes that all Biologic Products are characterised using a selection of interrelated analytical techniques to provide a detailed description of the primary structures, including sequence-based mass confirmation, amino acid sequence confirmation, N- and C-terminal variants as well as disulfide structures and glycoforms. The following sections provide a brief overview of LCMS techniques used to characterise a target Biologic Product.

Intact Mass Analysis



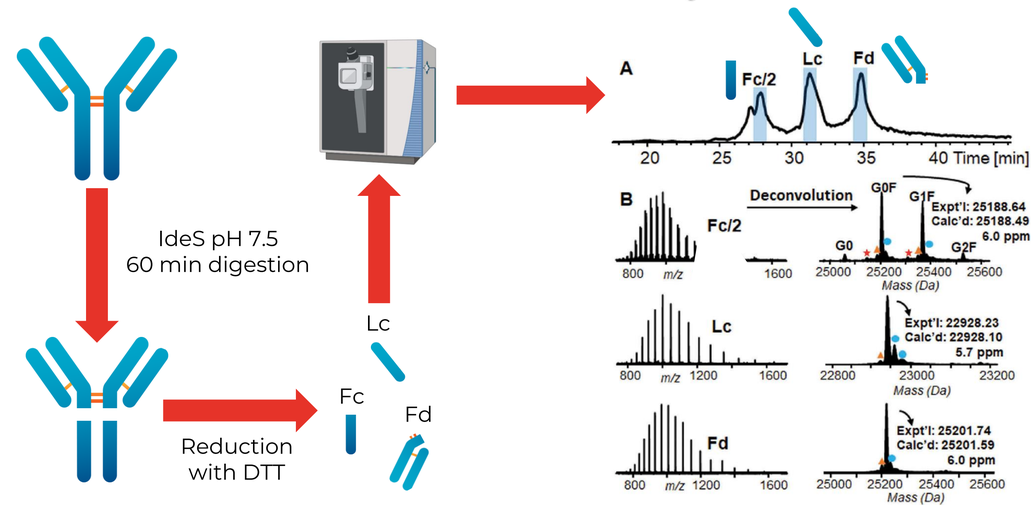
Intact mass analysis is an identification technique for protein analysis involving determination of the mass of the entire molecule. Due to the large masses involved and high number of charges observed. High-resolution accurate-mass (HRAM) mass spectrometry has become increasingly important. Not only is the identity of the molecule confirmed by intact analysis but the various glycoforms, PTMs and protein variants are observed. Intact mass analysis also allows the detection and quantification of the observed isoforms of the parent protein.

Subunit Mass Analysis



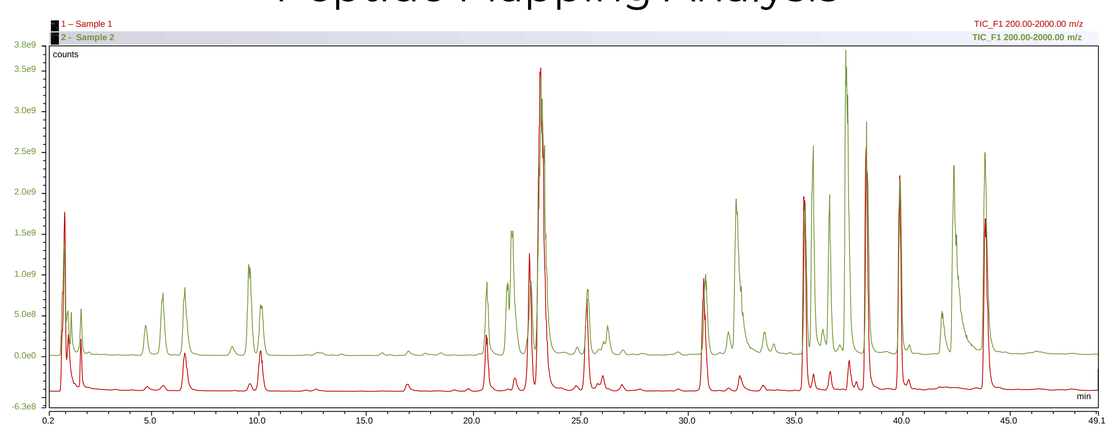
Subunit analysis is the process whereby a monoclonal antibody is separated by proteolytic digestion using DTT into subunit molecules, known as the light and heavy chain. The resulting peptide fragments are then separated and detected by LC-MS. These peptides are quantified to determine the relative amounts present in the Biologic Product. Subunit analysis is essential, as the site where catalytic reactions occur is often composed of specific amino acids located at the interface between subunits. By understanding the compositions. The amino acids involved can be determined. Leading to further understanding of the efficacy.

Middle Down Analysis



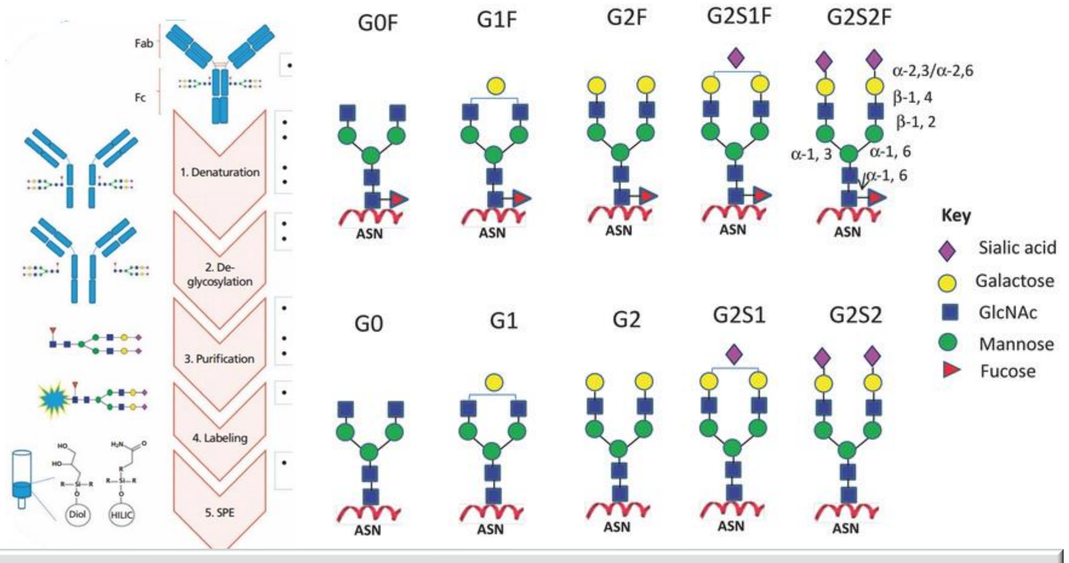
Similar to subunit analysis. Middle down analysis is particularly useful for the determination of Post Translational Modifications (PTM's). These modifications can have a significant effect on the structure and function of the Biologic Product, potentially affecting the affinity, stability, potency and homogeneity. By cleaving the protein into relatively large fragments it is possible to identify regions of the protein that are involved in specific interactions with other proteins. This protein–protein interaction can be used to further develop the therapeutic effects of the Biologic Product.

Peptide Mapping Analysis



Peptide mapping analysis by LC-MS is commonly used for the characterisation of Biologic Products. By digestion of the target molecule into smaller fragments using proteolytic enzymes such as Trypsin or Glu-C. The resulting mixture of peptides can be separated and analysed by accurate mass LC-MS. The profile of proteolytic peptides can be used to fingerprint the identity of protein versus a biosimilar or the entire structural sequence can be determined by LC-MS/MS of the peptides observed.

N-Linked Glycoform Determination



Glycosylation can have significant impact on the protein's function and stability as one of the most common post-translational modifications. Abnormalities in protein glycosylation are correlated with several diseases, such as cancer, inflammatory diseases and congenial disorders. the addition of carbohydrate molecules to a protein can increase its mass, resulting in poor accuracy. Glycosylation analysis can provide insights into protein function, to identify specific glycosylation patterns that are associated with certain protein functions or interactions.

Conclusion

Almac's expertise coupled with Orbitrap High Resolution Mass Spectrometry provides an effective solution for the characterisation of Biologic Products. By incorporating this suite of analysis as part of characterisation testing the identity and suitability of a Biologic Product can be ascertained to GMP standards.