Complete amino acid sequence verification of biopharmaceuticals using LC-MS/MS and Edman sequencing

Adrian Moise¹, Natalie Louis¹, Burkhard Fleckenstein¹, Martin Blüggel²

¹Protagen Protein Services GmbH, Heilbronn, Germany, ²Protagen Protein Services GmbH, Dortmund, Germany

The development of recombinant biopharmaceuticals requires intensive investigation of the different levels of structural organization, starting with the primary structure. The confirmation of the amino acid sequence is achieved primarily by mass spectrometry (MS) in combination with liquid chromatography (LC). However, distinction between the isobaric residues leucine (Leu) and isoleucine (Ile) is a major challenge in mass spectrometric peptide sequencing, requiring advanced tandem-MS and ion fragmentation techniques. As an alternative, peptides containing these amino acids can be sequenced by automated Edman degradation in order to unambiguously confirm the identity and position of Leu/Ile residues.

We show here the complete amino acid sequence elucidation including the differentiation of isobaric Leu/Ile amino acids of a biosimilar IgG using a combination of MS-based and Edman sequencing.

The IgG sample was digested using different proteases and each digest was analyzed by RP-UPLC-UV-MS to identify peptide peaks. Digests were then fractionated using RP-UPLC without MS-coupling. Fractions were assigned based on the obtained peak profiles and only those containing Leu/Ile were subsequently analyzed by Edman sequencing. To supplement the sequence data obtained from peptides, N-terminal Edman sequencing of the native sample was carried out.

Using this combined MS/Edman approach the entire amino acid sequence (both light and heavy chain) of the IgG molecule were confirmed. A number of 35 peptides up to 30 amino acids in length were subjected to Edman sequencing where all 61 Leu/Ile residues were unambiguously identified.