N-terminal sequence confirmation is an integral requirement of the ICH Q6B guidelines and can be used throughout all stages of drug development, or to demonstrate comparability and consistency between batches for release during manufacturing.

Intertek carry out N-terminal sequence confirmation through application of LC-MS/MS. A strategic approach to the enzyme digestion via an in silico digest selectively optimises the N-terminal peptide fragmentation to achieve suitable peptides of 20-50 amino acid residues. The intact peptide is then deliberately fragmented within a mass spectrometer in order to gain structural information from the fragment ions created.

Applications:
- Confirmation of structure (integral part of protein characterisation package)
- Demonstration of comparability
- Modified terminals (including acetylation and pyroglutamic acids)
- Degradation of proteins (perhaps due to deamidation)
- Determination of enzymatic cleavage specificity

Sample formats:
- SDS-PAGE gel bands
- Solution samples

Truncated Proteins and Blocked termini Possible truncations of the amino-terminus, can be determined by N-termini sequencing as well as to assess the level of chemically blocked termini which generally prevents full sequencing with other approached such as Edman sequencing.

Did you know?

Intertek Analytical Sciences Group (ASG) has been inspected by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) for GLP and GMP compliance and by the US Food and Drug Administration (FDA) for cGMP compliance in relation to our customers’ pharmaceutical manufacturing license.
N-Terminus Sequencing Strategies

Case study: N-terminal sequencing of Murine IgG2b

Intact Molecular weight by MALDI-MS

Reduced and Alkylated material

Intact Molecular Weight of Anti-Ro, determined by MALDI-MS

Light Chain

Heavy Chain

Mass differences of H2O from residues, different glycosylation

Isolate by gel

MALDI Analysis

LC-MSMS data

Related Sequencing Services:
- Confirmatory protein sequencing
- De Novo Sequencing
- N-terminal Sequencing
- C-terminal Sequencing
- Peptide mapping (LC-MS or MALDI-TOF)
- In silico digest to form enzyme strategies
- Sequencing for location of structurally important sites: positions of Post Translational Modification

Get in touch.

Your business knows no boundaries - and neither does ours. Whatever your target market, Intertek’s global network of experts is ready to help you reach it. For more information email ASGenquiries@Intertek.com or call +44 (0)161 721 5247.