ALMAC

Partnering to Advance Human Health

Case study

N- and C-terminal sequence analysis

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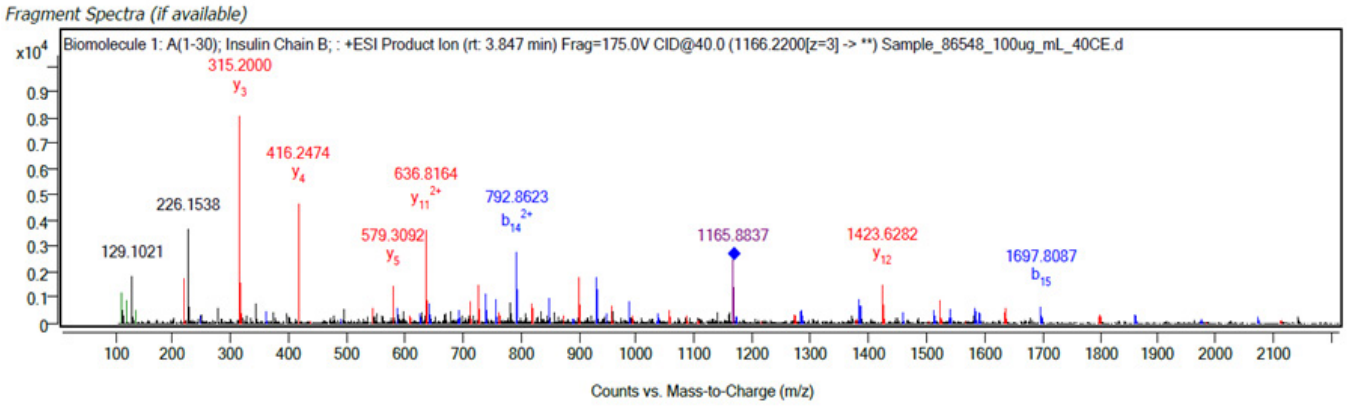
N- and C-terminal sequences play an important role in the structure of proteins and polypeptides and can influence their biological function.

The N-terminal and C-terminal sequences of biopharmaceuticals are important characteristics for licensing applications and form a key part of the ICH Q6B Guidelines for biotechnological/ biological products. The analysis is also necessary to demonstrate comparability and consistency between cGMP batches. Therefore, structural characterisation of a biopharmaceutical product should include assessment of the N- and C-terminal sequence.

This analysis provides confirmation of the consistency and homogeneity between batches for lot release purposes and can reveal if proteins have been truncated or N-terminally blocked. If heterogeneity with respect to terminal amino acid sequences is found, further characterisation analysis should be conducted, and the relative amounts of the variant forms determined.

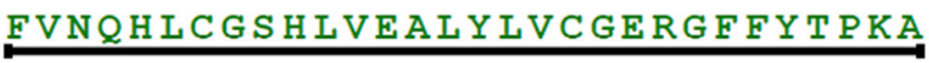
Example Polypeptide Sequencing Analysis

In this case study we will explore the undigested sequencing and modification identification of Insulin Chain B, oxidised from Bovine Pancreas. Insulin Chain B Is a linear polypeptide with 30 Amino Acids and two oxidised Cysteine residues. A sample was analysed by LC-ESI-MS/MS with an Almac developed generic sequencing method using an Agilent 1290 Infinity II LC coupled to an Agilent 6530 Accurate Mass Q-TOF MS. Collision induced disassociation (CID) was employed to fragment the sample to form ‘b’ and ‘y’ ions.



The data acquired was processed using MassHunter BioConfirm software, which allows the user to define the sequence of interest and input known modifications. If the site of modification or the type of modification is unknown, these can be added as changes which the software can then identify from the observed fragments based on the primary sequence. In this instance, the C7 and C19 amino acids were known to be oxidised and these cysteine oxidations were added as modifications to the sequence. A workflow was used to match the observed fragment ions with the theoretical ions based on the inputted sequence. The software determined that 100% sequence coverage was achieved based on the fragment ions observed. A match score is also calculated based on the calculated mass vs. theoretical mass and observed isotopic

distribution vs. theoretical isotopic distribution, Insulin Chain B was matched with a score of 92.51 out of 100 with a difference of only 2.05 ppm. The high score coupled with the low ppm difference gives high confidence that 100% sequence characterisation was attained.



Biomolecule Summary

Biomol	Seq Loc	Mods	Pred Mods	Score (Bio)	RT	Height	Mass	Tgt Mass	Diff (ppm)
1	A(1-30)	Cysteine Oxidation 19, Cysteine Oxidation 7		92.51	3.830	216395	3493.6364	3493.6435	-2.05

At Almac, we have the necessary experience to determine any required enzyme digestion strategies where appropriate and use the subsequent peptide mapping with the sequencing capabilities of LC-ESI-MS/MS to characterise the N- and C-terminal sequence of your products.

Click here

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