N- and C-terminal sequence analysis

The N-terminal and C-terminal sequences of biologics are important characteristics for licensing applications, and form a key part of the ICH Q6B Guidelines for Biotechnological/Biological products. 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.

We can determine an appropriate enzyme digestion strategy, and use peptide mapping with the sequencing capabilities of LC-ESI-MS/MS to characterise the N- and C-terminal sequence of your products. Alternative options for N-terminal sequence analysis include blotting of samples onto inert membranes (such as PVDF) followed by N-terminal Edman degradation sequence analysis, in which the N-terminal amino acid is sequentially cleaved, derivitised, identified and quantified – successive cycles of this procedure can reveal the N-terminal sequence of the test protein.