identification after electrophoreses, such as 1D or 2D PAGE. When using multiple enzymes, peptide mapping is applied for the confirmation of a complete amino acid sequence, for example, when confirming the amino acid sequence of a biosimilar and comparing it with the originator molecule. Depending on the experimental setup, peptide mapping can also be used to determine the N- and C-termini of a protein. This is sometimes crucial information for our clients, for example, in the case of monoclonal antibodies, where the truncation level of the C-terminal lysine can be monitored. Proteins and peptides are ionized and fragmented in the mass spectrometer. The resulting MS/MS spectra are used to sequence the individual amino acids in order of their appearance in a protein or peptide. In combination with additional sample preparation, peptide mapping can also be used to determine the degree of deamidation or oxidation for each affected amino acid, glycosylation sites and structure, N-glycosylation sites, and disulfide linkages.

5.3.1.6 Terminal sequence The N- and C-terminal sequencing can reveal a great deal of information about proteins and antibodies at any stage of the drug development process. It is also performed to demonstrate consistency and comparability between lots. It can determine the N-terminal blockage or signal peptide cleavage. The level of C-terminal lysine truncation of monoclonal antibodies is a critical quality attribute and, therefore, needs to be closely monitored. When done by MALDI-in source decay (ISD), N- and C-terminal sequencings can be specifically used to monitor terminal amino acids for modifications such as glycosylation, disulfide bridging, and oxidation. With the digestion strategy, we can use peptide mapping with LC-ESI-MS/MS to identify and quantify the N- and C-terminal sequence variants.

5.3.1.7 Disulfide link The disulfide linkages are an important structural feature of many proteins. Intrachain bonds form or stabilize the tertiary structure of a protein (for example, the zinc-binding domain of zinc-finger proteins) and interchain disulfide bonds covalently link protein subunits together, (for example, the insulin A and B chains). The processes for identifying disulfide linkages include investigating the differential appearance of the linked peptides before and after reduction of the disulfide bond and analyzing the fragment spectra of the linked peptides using MS. Peptide mapping may be helpful to confirm the primary structure and aid in determining the most appropriate enzymatic digestion strategy for the disulfide bridge analysis. N- and C-terminal sequencing using MALDI-ISD can be used to determine the presence of free sulfhydryl groups or disulfide linkages within these regions. Finally, electrophoresis, such as reducing and nonreducing SDS-PAGE to identify disulfide-linked subunits of a protein, may also be undertaken.

5.3.1.8 Glycosylation MAbs, having high selectivity and specificity, constitute a large and growing portion of the biosimilars. The majority of marketed mAbs belong to the IgG class. IgGs, which consist of two heavy