An example of the difference may be in the glycosylation pattern. Knowing that differences in fucosylation and high mannose can impact ADCC function; these differences are significant and must be resolved before the biosimilar product development moves forward. The causes of these variations can be traced back to the choice of the host cell to the bioprocessing conditions. Since the biosimilar product developer is likely to claim extrapolation of indications, which means asserting similarity for all MOAs, the matching of glycosylation pattern, even if it is not totally relevant, will always be required to remove any residual uncertainty of biosimilarity. The reference product developers know this well and have provided broad protection to their process that results in a particular glycan pattern. This is one good example to demonstrate the need for the biosimilar product developer to engage early in the IP evaluation of the proposed biosimilar product.

For the nonglycosylated proteins, the structural challenges may be less onerous, yet there remain process-related impurity profiles that will require optimization of upstream processing to match these profiles early in the development stage.

5.3.1.2 Sequencing Protein sequencing is a technique to determine the amino acid sequence of a protein, as well as which conformation the protein adopts and the extent to which it is complexed with any nonpeptide molecules. The two major direct methods of protein sequencing are MS and the Edman degradation reaction (N-terminal as well). It is also possible to generate an amino acid sequence from the DNA or mRNA sequence encoding the protein if this is known. However, there are many other reactions that can be used to gain more limited information about protein sequences, and can be used as preliminaries to the aforementioned methods of sequencing or to overcome specific inadequacies within them. The mass of the intact met-GCSF protein is determined by MS (ESI MS and MALDI-MS).

5.3.1.3 Extinction coefficient The extinction coefficient method is based on the absorbance of tyrosine, tryptophan, and phenylalanine residues at 275–280 nm (UV region). Phenylalanine is only weakly absorbing and is usually neglected for most purposes. The protein structure is not affected by the method making on-line measurement a possibility. Pigments, organic cofactors, and phenolic compounds interfere with the assay. The UV absorbance method is used for determining the total protein in semipurified and purified samples. The assay may be used as an on-line in-process control method, for determining the total protein in the intermediary samples and in the drug substance/product.

The extinction coefficient can be determined by two methods: amino acid analysis and UV light absorption at 280 nm. This is in line with ICH Q6B, which recommends determining the coefficient (sometimes referred to as molar absorptivity) by a combination of the two. This method is important