9.10 Physiochemical Characterization Tests

9.10.1 Structural Characterization and Confirmation

9.10.1.1 Amino Acid Sequence

The amino acid sequence of the desired product should be determined to the extent possible by using customary approaches and then compared with the sequence of the amino acids deduced from the gene sequence of the desired product.

9.10.1.2 Amino Acid Composition

The overall amino acid composition is determined using various hydrolytic and analytical procedures and compared with the amino acid composition deduced from the gene sequence for the desired product, or the natural counterpart, if considered necessary. In many cases, the analysis of amino acid composition provides some useful structural information for peptides and small proteins, but such data are generally less definitive for large proteins. Quantitative amino acid analysis data can also be used to determine protein content in many cases.

9.10.1.3 Terminal Amino Acid Sequence

Terminal amino acid sequence analysis is performed to identify the nature and homogeneity of the amino- and carboxy-terminal amino acids. If the desired product is found to be heterogeneous with respect to the terminal amino acids, the relative amounts of the variant forms should be determined using an appropriate analytical procedure. The sequence of these terminal amino acids should be compared with the terminal amino acid sequence deduced from the gene sequence of the desired product.

9.10.1.4 Peptide Map

Selective fragmentation of the product into discrete peptides is performed using suitable enzymes or chemicals, and the resulting peptide fragments are analyzed by HPLC or other appropriate analytical procedures. The peptide fragments should be identified to the extent possible by using techniques such as amino acid compositional analysis, *N*-terminal sequencing, and MS. Peptide mapping of the DS or DP using an appropriately validated procedure is a method that is frequently used to confirm the structure of the desired product for lot release purposes.

9.10.2 Sulfhydryl Group(s) and Disulfide Bridges

If, based on the gene sequence for the desired product, cysteine residues are expected, the number and positions of any free sulfhydryl groups and/or disulfide bridges should be determined, to the extent possible. Peptide mapping (under reducing and nonreducing conditions), MS, or other appropriate techniques may be useful for this evaluation.