5.3.3 Effectiveness

5.3.3.1 Bioactivity The biological activity describes the ability or the capacity of the drug substance to achieve a defined biological effect. Examples of procedures used to measure the biological activity include animal-based biological assays, cell culture–based biological assays, biochemical assays, and ligand- and receptor-binding assays. A biologic assay may be replaced by physicochemical tests provided sufficient information and correlation between the bioassay and said tests can be given and there exists a well-established manufacturing history (ICH *Harmonized Tripartite Guideline Specifications: Test Procedures and Acceptance Criteria for Biotechnological Products [Q6B]* on March 10, 1999). In some cases, the specific biological activity may provide additional useful information.

5.3.3.2 Protein content The quantity of the target protein is determined as the total peptide/protein content in a given sample excluding any inactive derivatives such as des-amido forms, oxidized forms, polymeric forms. High-performance chromatographic methods are used to quantitate both the target protein as well as its derivatives using UV detection and determination of peak areas. If the extinction coefficient is not known (e.g., analogues), amino acid or Kjeldahl analysis is used as a primary reference method. Bioassays are often replaced with quantity determination partly because of the usual high costs associated with biological assays and partly because of the much higher accuracy of quantitative assays. The commonly used quantity methods are amino acid analysis, Kjeldahl analysis, UV spectrometry, and high-performance chromatographic procedures.

5.3.4 Purity

Protein purity has been historically linked to the specific biological activity in terms of units of biological activity per mass unit of the product. The purest product was that of the highest specific biological activity. In contrast to drugs based on small molecules, which could be controlled on the drug product level, protein-based pharmaceuticals were closely linked to the process due to the complexity of the active pharmaceutical ingredient and the lack of proper characterization of the final product. With the introduction of recombinant technology and modern analytical methods, a much better drug substance/product characterization became possible resulting in the well-characterized protein concept and the widespread use of comparability studies. The importance of a stronger focus on the presence of adventitious agents and specific impurities was also recognized, as the presence of even minor amounts of toxic, immunogenic, or adventitious compounds proved to have severe side effects. Unless otherwise specified, the level of acceptable impurities depends on the nature of the drug product and the dose.