what constitutes the required minimum studies. A drug substance is defined by the U.S. FDA as the unformulated active substance that may be subsequently formulated with excipients to produce the drug product.

A clear description of the drug substance should be provided. This description may include, but is not be limited to, any of the following: chemical structure, primary and subunit structure, molecular weight (MV), molecular formula, established U.S. Adapted Names, antibody class/subclass (if appropriate), and so on.

A description and the results of all the analytical testing performed on the manufacturer's reference standard lot and qualifying lots to characterize the drug substance should be included. Information from specific tests regarding the identity, purity, stability, and consistency of the manufacture of the drug substance should be provided. Examples of analyses for which information may be submitted include, but are not necessarily limited to, the following:

- Amino acid analysis
- Amino acid sequencing, entire sequence or amino- and carboxy-terminal sequences
- Peptide mapping
- Determination of disulfide linkage
- Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (reduced and nonreduced)
- Isoelectric focusing
- Conventional chromatography and HPLC, for example, reverse-phase, size-exclusion, and ion-exchange
- Mass spectroscopy (MS)
- Assays to detect product-related proteins, including deamidated, oxidized, cleaved, and aggregated forms and other variants, for example, amino acid substitutions and adducts/derivatives
- Assays to detect residual host proteins, DNA, and reagents
- Immunochemical analyses
- Assays to quantitate bioburden and endotoxin

Additional physicochemical characterization may be required for products undergoing posttranslational modifications, for example, glycosylation, sulfation, phosphorylation, and formylation. Additional physicochemical characterization may also be required for products derivatized with other agents, including other proteins, toxins, drugs, radionuclides, and chemicals. The information submitted should include the degree of derivatization or conjugation, the amount of unmodified product, the removal of free materials (e.g., toxins, radionuclides, linkers, and others), and the stability of the modified product. All test methods should be fully described and the results provided. The application should also include the actual data, such as legible copies of chromatograms, photographs of SDS-PAGE or agarose gel, spectra, and the like.

A description and results of all relevant in vivo and in vitro biological testing performed on the manufacturer's reference standard lot to show the potency and activity(ies) of the drug substance should be provided. Results of relevant testing