method, known as LC-MS or liquid chromatography-tandem mass spectroscopy (LC-MS/MS), the individual peptides are analyzed, allowing the identification of PTM sites. In some cases, there are potentially multiple sites in a single peptide that may be modified. Absolute identification of the modified amino acids may require more than one enzyme digest to produce different peptides. Some kinds of modifications that are easily identified by MS include phosphorylation of threonine or serine, sulfation or phosphorylation of tyrosine, deamidation of asparagine or glutamine, *O*- or *N*-linked glycosylation, oxidation of methionine or cysteine, and *N*-terminal modification by formylation or prenylation. Combining enzymatic maps (tryptic mapping) with MS/MS may identify single amino acid variants of the protein that cannot be seen otherwise.

Mass spectroscopy is often used as part of hyphenated methods, such as LC-MS, where the proteins are separated by a chromatographic method and the column eluant is then directed to the mass spectrometer for additional characterization. One of the common confusions experienced when evaluating the results of MS analyses of proteins involves equating the observed size of the ion current peak (for a particular ion species) with the amount of the species present. The size of the peak is sensitive to several things and cannot be used for quantification. For this reason, the use of LC separation and quantification "front-end" for the MS allows the relative amounts to be determined.

9.9.3.4.1 Validation

Synthetic drugs are readily characterized by established analytical methods. Biologics, on the other hand, are complex, high-MW products, and analytical methods have limited abilities to completely characterize them and their impurity profiles. Regulation of biologics includes not only final product characterization but also characterization and controls of raw materials and the manufacturing process. The FDA has defined process validation as "establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes." This involves supporting product and manufacturing process claims with documented scientific studies. Protocols, results with statistical analysis, authorizations, and approvals must be available to regulatory inspectors. Process validation is part of current good manufacturing practices (cGMP) and is required in the United States and Europe for a manufacturing license.

Various types of validation generally required in biopharmaceutical manufacturing include process validation, facility and equipment validation, analytical method validation, software validation, cleaning validation, and expression system characterization. Combined with other elements of cGMP, including lot release testing, raw material testing, vendor quality certifications, and vendor audits, the quality of product can be consistently assured.

9.9.3.5 Process Validation

Process validation involves the identification, monitoring, and control of sources of variation that can contribute to changes in the product. It starts with process characterization studies, using scale-down models for optimization, operating range