Table 4.2 Methods for Quality Safety and Efficacy Assessment of Biosimilars

Attribute	Method
Primary sequence (peptide map and amino acid sequence analysis), immunogenicity (immunoassay), other identity indicators	IE, HPLC, gel electrophoresis
Potency	Cell-based bioassay, expression gene bioassay, ADCC, CDC
Conformation	Near/far-ultraviolet circular dichroism spectroscopy, Fourier transform infrared spectroscopy, x-ray crystallography, and differential scanning calorimetry
Glycosylation	Monosaccharide composition analysis, oligosaccharide profile, CE, LC-MS, MS/MS, ESI, MALDI-TOF
Phosphorylation	Peptide mapping with MS
Truncation	SE-HPLC, gel electrophoresis, AUC, peptide mapping with MS, RP-HPLC
Glycation	Peptide mapping with (MS, HPLC), methylation, isomerization (RP-HPLC)
PEGylation	HPLC, CE
Aggregation	SE-HPLC, gel electrophoresis, light scattering, and AUC
Oxidation	Peptide mapping with MS
Deamidation	Capillary IEF, peptide mapping with MS, and CEX-HPLC, C-terminal lysine
	(Capillary IEF, peptide mapping with MS, and CEX-HPLC), misfolds (RP-HPLC)
Host cell proteins	ELISA, DNA, endotoxin (limulus amebocyte lysate assay)
Binding	Cell assays, spectroscopy, ELISA
Biological activity	Cell assays, animal models

Note: ADCC, antibody-dependent cell-mediated cytotoxicity; AUC, analytical ultracentrifugation; CDC, complement-dependent cytotoxicity; CE, capillary electrophoresis; CEX, cation exchange; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; HPLC, high-performance liquid chromatography; IE, ion exchange; IEF, isoelectric focusing; LC-MS, liquid chromatography–mass spectroscopy; MALDI-TOF, matrix-assisted laser desorption/ionization time of flight mass spectrometry; MS/MS, tandem mass spectrometry; RP-HPLC, reverse phase HPLC; SE, size exclusion.

while more tests are more desirable, the choice of tests to demonstrate biosimilarity must be based on an evaluation of safety, purity, and potency. And that depends a great deal on the mode of action (MoA). For example, when developing simpler molecules like filgrastim, a set of CQAs is easy to select as shown in Table 4.3.

The primary structure must be identical, while secondary and tertiary structures must be highly similar, just like purity and stability profiles as well as the receptor binding and biological activity.