cell line–specific HCP detection reagent. For licensure, the anti-HCP antiserum needs to be qualified to detect potential HCP impurities. The data need to include 2D SDS-PAGE gels of the range of HCPs detected by a sensitive protein stain, such as silver stain, compared to the range detected by western blot analysis (or another similarly sensitive assay) using the antiserum employed in the assay. It is the FDA's experience that the analysis of HCP coverage by a one-dimensional SDS-PAGE gel method is not sufficiently sensitive for this purpose.

Process-related impurities, such as HCPs, can be detected using ELISA, 2D gel electrophoresis, western blotting, or mass spectrometry. Examples of process-induced modifications, which may be introduced during sample preparation, downstream processing, or other steps involving the use of chemicals, heat, or light are methylation or acetylation of side chains, fragmentation, and glycation. Biologically induced modifications, which may be introduced into the cell or supernatant by the complex biological system itself, are often crucial to the correct biological function of the protein. Examples include phosphorylation, sulfation, and formylglycine. Investigations for these modifications require the use of RP-HPLC, intact mass determination or peptide mapping.

An ELISA, which is useful when looking at quality control features such as drug purification, lot release, and stability testing, can be applied to detect and quantify specific analytes by applying antibodies to a complex matrix. The major aim is to demonstrate the specificity of the antibodies used in developing the assay. For process-related protein impurities, such as HCPs or column leakage (Protein A), quantitative and qualitative ELISAs are performed in a microtiter plate format using an enzyme-linked detection system with an absorbance or fluorescence readout.

Other significant process-related impurities for biosimilars include tungsten level, leachables and extractables, and visible and invisible particles. While some of these impurities may have a lesser impact on the formulation of small-molecule drugs, these can have a significant effect on the formulation of proteins. The most widely quoted role of process-related impurities is the case of EPO. The incidence of pure red cell aplasia in chronic kidney disease patients treated with epoetins substantially increased in 1998, was shown to be antibody mediated, and was predominantly associated with subcutaneous administration of Eprex®. A technical investigation identified organic compounds that leached from uncoated rubber stoppers in prefilled syringes containing polysorbate 80 as the most probable cause of the increased immunogenicity. The rubber stoppers were switched without conducting the impact on the formulation assuming they have no effect.

4.11 Potency

The potency of the biosimilar products is compared with that of the originator reference product as a definite measure of biosimilarity based