Table 4.4 Product-Related Impurities of Filgrastim

Impurity	Method of Testing
Oxidized species	RP-HPLC; LC–MS
Covalent dimers	LC–MS
Partially reduced species	LC–MS
Sequence variants: His → Gln, Asp → Glu, and Thr → Asp	LC–MS
fMet1 species	LC–MS
Succinimide species	LC–MS
Phosphoglucunoylation	LC–MS
Acetylated species	LC–MS
N-terminal truncated species	LC–MS

wavelength of the fluorophore. The quencher absorbs the energy emitted by the fluorophore only as long as they are in close proximity, connected by the peptide chain. Enzymatic hydrolysis causes an increase of fluorescence irrespective of the cleavage position, provided that donor and acceptor are disconnected.

Table 4.4 lists some of the product-related impurities listed for filgrastim products.

4.10.2 Process-related impurities

The category of process-related impurities creates a large challenge for a biosimilar product developer since these impurities might have a significant impact on all of the three concerns: purity, potency, and safety of the product. Some impurities come from the host cells and others from the downstream process; the final packaging may itself add to these impurities such as tungsten or silicon. Common tests used include host cell protein (HCP) using 2D SDS–polyacrylamide gel electrophoresis (PAGE), Protein A, DNA, 2D SDS-PAGE, and HCP using LC/MS/MS. However, the FDA does not expect the process-related impurities (such as HCPs) present in the biosimilar product to match those observed in the reference product. However, process-related impurities in biosimilar product should be assessed side by side with the reference product. The FDA recommends performing a risk-based assessment regarding any differences in process-related impurities identified between the biosimilar product and the reference product. If the manufacturing process used to produce biosimilar product introduces impurities different from or levels of impurities higher than those present in the reference product, additional pharmacological/toxicological or other studies may be necessary to evaluate the potential risk of any differences, and any differences should be justified. The adequacy of the risk-based assessment will be a review issue.

Regarding the HCP assay, the sponsor must provide a summary description of the source (in-house or commercial) of the antiserum used for the detection of HCP impurities. The FDA recommends developing a