future research in this area may eventually allow us to overcome this problem, at present the subtle and unclear knowledge as to what it is that is responsible for immunogenicity effects, pitted against its potential life-threatening repercussions, leaves little justification for taking imprudent risks. Hence, some limited form of clinical assessment will likely continue to be a requirement for some time to deal with this issue (see Chapter 7) until better knowledge is gained concerning what physicochemical attributes of a biopharmaceutical and its aggregates are of key importance in eliciting this type of response in humans (Moussa et al., 2016).

2.11 DETECTING DIFFERENCES DURING BIOSIMILARITY ASSESSMENT: WHAT'S IMPORTANT, WHAT'S NOT IMPORTANT?

The FDA has pointed out in general terms that adequate biosimilarity of a biosimilar to its RP is reached when a biosimilar is found to be "highly similar to the reference product not withstanding minor differences in clinically inactive components and that there are no clinically meaningful difference between the biological product and the reference product in terms of safety, purity and potency of the product" (FDA, 2015b). Since a biosimilar cannot be made identical to its RP, the big question becomes: "How highly similar is highly similar enough?" Given the enormous range of potential structural alterations that can occur to a biopharmaceutical, coupled with the ever-increasing capability of our analytical tools to assess and reveal physicochemical differences (e.g., again see Figure 2.4D), "What's important and what's not important in terms of safety, potency and purity when a physicochemical difference between a biosimilar and its RP is observed?" is unfortunately a very challenging question that needs to be addressed, which Miller (2011) noted would seem to require clinical data to properly answer. Indeed, a small change such as a single amino acid substitution [as seen in the case of hemoglobin (Murayama, 1967)] or the alteration of a PTM on a single amino acid [as seen in the case of the fucosylation of the oligosaccharide on the Fc region of an antibody (Houde et al., 2010)] can alter the structure or/and physicochemical properties of a biopharmaceutical that can translate into an altered biological function. Such a change in the behavior of a protein drug in response to such subtle changes in its structure certainly presents challenges in attempting to answer our posed question. To address this problem, to some extent, the FDA has categorized biosimilarity into several coarse levels: not similar, similar, highly similar, and highly similar with fingerprint-like similarity (FDA, 2014). Nevertheless, we might draw some better insights in attempting to answer our posed question by considering the following two interesting real case situations.

The first case concerns the comparability study carried out by Genzyme in 2008 to scale-up the production of its commercial biopharmaceutical Myozyme (a drug for Pompe disease) from a 160 L reactor to a 2000 L reactor to meet the growing commercial demands for this drug. In carrying out the scale-up, analytical results showed that the levels of an important key phosphorylated monosaccharide [mannose-6-phosphate, which Genzyme researchers and their collaborators had shown earlier to play an important role in the activity of this biopharmaceutical