5. An understanding of the regulatory constraints concerning the source of RP that can be used in conducting biosimilarity comparisons within a given regulatory jurisdiction and its potential impact.

2.5.1.1 Potential Analytical Method Bias Effects due to Sample Matrix Differences between the RP and Its Biosimilar

In applying an analytical method to measure any physicochemical attribute [parameter *X*, e.g., the sedimentation coefficient, or collection of "*i*" data points (x_i) that gives rise to a graphical pattern or plot of data points; e.g., circular dichroism (CD) spectrum] that characterizes a biopharmaceutical, two basic and important statistical values are extracted as a result of making measurements on a number of different aliquots (*n*) of the same sample to assess the parameter or plot. These statistical values include the *mean* value of the parameter, $\langle X \rangle$, or collection of "*i*" mean values, $\langle x_i \rangle$, involved in generating a given plot, and their associated *uncertainty* (error or variability), σ, or "*i*" uncertainties, σ_i, respectively [which are obtained from standard deviation, SD, calculations computed from a limited population of individual experimental measurements (Beers, 1957; Mandel, 1964)]. For chemical or biochemical (primary structure) analysis methods, the value of these means and their associated uncertainties are likely to be invariant to the nature of a biopharmaceutical's sample matrix. However, for analytical methods that assess or depend on the HOS of a biopharmaceutical (e.g., biophysical and functional analysis methods), a sample matrix difference between the RP and the biosimilar typically have significant impact on the measured mean value of a parameter or plot and may even impact their associated uncertainties (Holzmann et al., 2016; Panjwani et al., 2010) (see Section 2.5.1.1.2 for further discussion of this topic).

2.5.1.1.1 Detecting the Possible Effects of Sample Matrix Differences in Biochemical Methods Used for Assessing Primary Structure

Although differences in a biopharmaceutical sample's matrix typically do not present a major problem of introducing sample bias in biochemical methods used in primary structure characterization, some of these analysis methods could. Consequently, an observed difference(s) in the biochemical primary structure between the biosimilar and RP may not really be due to an actual true chemical difference between the active pharmaceutical ingredient (API, the protein molecule) in the RP and biosimilar. Rather, the difference recorded could be due to some difference between the matrix (formulation or process-related impurities that are present) of the biopharmaceutical samples being compared that influences the data output from a biochemical method that leads to an apparent difference between the RP and biosimilar. To assess this potential problem, especially when samples being compared are known to be in different formulations, the approach discussed below, as well as in Figure 2.6, might prove helpful.

An appropriate amount of sample (which will need to be precalculated) should be taken from a container (e.g., vial, syringe) of the RP and biosimilar and in each case split into two equal parts (note: in some cases, it may be necessary to pool the contents from more than one container from the same lot of RP material and similarly from more than one container from the same lot of biosimilar material if a