and their associated σ and "*i*" σ_i values for their swapped formulations (symbolized as $<_{RS}X>$, ${}_{RS}\sigma$ or $<_{RS}x_i>$, ${}_{RS}\sigma_i$ and $<_{BS}X>$, ${}_{BS}\sigma$ or $<_{BS}x_i>$, ${}_{BS}\sigma_i$, where RS refers to the RP sample swapped or exchanged into the biosimilar formulation and correspondingly BS refers to a biosimilar sample swapped or exchanged into the RP formulation).

Using the above experimental calculated information, if $<_RX> \approx <_{RS}X>$ or all "*i*" $<_Rx_i> \approx <_{RS}x_i>$ are found to be true via statistical testing (e.g., *t*-test) using an appropriate statistical criteria (e.g., 95% or 99% confidence limit, CL, computed from the corresponding experimentally measured uncertainties ${}_R\sigma$ and ${}_{RS}\sigma$ or "*i*" ${}_R\sigma_i$ and ${}_{RS}\sigma_i$ values that go with each appropriate mean comparison) and the same situation holds true for the biosimilar [$<_BX> \approx <_{BS}X>$ or all "*i*" $<_Bx_i> \approx <_{BS}x_i>$], then there is a high level of confidence that method bias due to any matrix differences between the RP and biosimilar is absent. However, if any of these effective equalities between the two different formulations for the RP or the biosimilar is determined to be statistically not true, a possible method bias may exist due to differences in the matrix of the samples being compared. As a result, appropriate steps will need to be taken to overcome this bias in order to achieve a meaningful biosimilarity assessment between the RP and biosimilar.

2.5.1.1.2 Detecting the Possible Effects of Sample Matrix Differences in Biophysical Methods Used for Assessing HOS

The approach outlined in Section 2.5.1.1.1 can also be applied to biophysical methods to assess matrix bias effects between the RP and its biosimilar. However, as already mentioned in Section 2.5.1.1, biophysical measurements display a high sensitivity to a sample's matrix. This is due to the dominant role that the weak secondary chemical bonds play in establishing and maintaining the HOS of biopharmaceuticals and the high sensitivity of these bonds to be altered simply by changing their chemical and physical environment (e.g., buffer, excipients, and pH) of the biopharmaceutical. Hence, in the biophysical assessment of a biosimilar to its corresponding RP, any known difference in the formulation between these two materials will likely lead to a difference in the biophysical measurements between these samples. Consequently, known formulation differences between samples need to be removed in order to conduct valid biophysical biosimilarity assessments. Needless to say, any steps taken to achieve this must be carefully assessed to make sure no sample bias is introduced; see the next section.

2.5.1.2 The Impact of Sample Handling and Processing Steps in Conducting Physicochemical Measurements

As noted previously, for a biosimilar manufacturer the only source of RP material to use to experimentally assess the window of consistency of an RP are the various commercial innovator lots of the RP that are available on the open market. Such material, however, may actually be compounded into a formulation matrix that is different in composition from that used for the biosimilar (e.g., due to patent issues surrounding the RP formulation). This difference in formulation may require the introduction of sample preparation (handling and processing) steps to make sure that all samples are in the same formulation to avoid issues concerning matrix bias