- *Level of the attribute*—An attribute of the reference product known to be of high risk but present at a level that is unlikely to have significant clinical impact could potentially be assessed at a lower tier. To justify placing a high risk attribute in a lower tier for this reason, the level of the attribute should be confirmed in both the reference product (as determined by the proposed biosimilar sponsor's analysis of the reference product) and the proposed biosimilar product. The selected limits regarding the level of an attribute should be defined and justified. The justification should also include consideration of how the level of the attribute changes over time.
- *Assays used for assessing the attribute*—Although multiple, orthogonal assays are encouraged for assessing a single attribute, not all assays need to be included in the same tier of assessment. The assay with the best performance characteristics for detecting product differences should be used for testing with the highest tier methods, while other assays should be used for testing with lower tier methods. A justification should be provided for the assays selected for testing at each tier.
- *Types of attributes/assays*—Some attributes or the assays used to assess the attribute will, by their nature, be excluded from certain statistical evaluations. For example, compendia assays, qualitative assays, or limit assays might be excluded from evaluation with Tier 1 and, in some cases, Tier 2 methods. The analytical similarity assessment plan should clearly define the conditions used to exclude assays from evaluation at any tier.

Applicable data and cited literature should be provided in the application to support the use of any additional factors in determining the appropriate tier of statistical assessment.

9.3.1.3 Development of the statistical analysis plan

A detailed statistical analysis plan should be developed and included in the analytical similarity assessment plan because the statistical aspects of the evaluation will impact whether or not the similarity acceptance criteria are ultimately met. The plan for the statistical evaluation of analytical similarity requires the selection of design features from among many possibilities. These design features include the following five factors:

- The choice and risk ranking of attributes
- The statistical approach (tier) for assessing each attribute
- The number of proposed biosimilar and reference product lots to be evaluated for each attribute, and the number of replicates to be evaluated per lot
- For each attribute, a determination of the largest acceptable difference between the proposed biosimilar and reference product that is considered to not have clinical impact
- The methods of statistical analysis for each tier, and the type of assay(s) used to evaluate each attribute