to detect since there is no change in the biopharmaceutical's MW. Changes that do occur in a biopharmaceutical, as a result of physical PTMs, may typically involve just a small portion of a biopharmaceutical's large collection of noncovalent weak secondary bonds, the dominant element in a biopharmaceutical that is responsible for its HOS. Such changes can arise from a range of different conditions that a biopharmaceutical experiences during its production that can physically stress the biopharmaceutical altering or breaking some of its weak secondary bonds (e.g., low pH viral inactivation). When the stress is removed, these weak secondary bonds may not be regenerated properly, resulting in small or potentially even large permanent change(s) in a given biopharmaceutical's HOS and causing changes also in the surface properties of these drugs.

Collectively, the total collection of all the covalent and noncovalent PTMs found on a given biopharmaceutical, their distribution among drug molecules, and variability on a lot-to-lot basis that a biopharmaceutical can experience is the key factor for their high heterogeneity. The unique features of this structural heterogeneity and its associated variability, as evaluated by the innovator over many years of work, defines what will be referred in this chapter as the physicochemical "window of consistency" of an innovator's biopharmaceutical (see Figure 2.3A). This experimental window comprises a large collection of physicochemical characteristics associated with CQAs of a biopharmaceutical, which its innovator used in collaboration with regulators to define the complex boundaries of limits or specifications that each innovator's biopharmaceutical lot must fall within in order to be released for commercial sale. It's this window of consistency that a biosimilar manufacturer must try to successfully uncover about the biopharmaceutical it is trying to copy through its own experimental work on the innovator's commercial biopharmaceutical. Once this task is achieved, the biosimilar manufacturer will use this window of consistency to define the window of biosimilarity that its biosimilar will need to fall within in order to achieve the necessary biosimilarity to the innovator's biopharmaceutical to obtain regulatory approval. The experimental physicochemical window of consistency assessed by the biosimilar manufacturer should ideally be identical to the physicochemical window of consistency that the innovator established and filed with regulators to obtain their drug's approval. In reality, however, small differences may exist, which will be explained in more detail below in various subheadings listed under Section 2.5.1, making this the window of consistency assessed by a biosimilar manufacturer as an *apparent* window of consistency.

2.5 THE PHYSICOCHEMICAL "WINDOW OF CONSISTENCY" OF THE REFERENCE PRODUCT (RP) AND ASSESSING BIOSIMILARITY

The process of assessing a biopharmaceutical's physicochemical window of consistency in building the totality of the evidence data package for a biosimilar is the first major step a biosimilar manufacturer will need to undertake in initiating its biosimilar program (see Figure 2.3B) (Holzmann et al., 2016). It involves the critical analytical process of establishing the fine detail of biochemical and biophysical PTMs present