use of the highly abundant NMR active ^{1}H nuclei to conduct various forms of one-dimensional (1D) proton (^{1}H) NMR, that employs new NMR procedures to extract HOS information. Such NMR procedures have been able to significantly reduce data acquisition times, while still providing the investigator with a significant amount of HOS information (Frank et al., 2015; Poppe et al., 2013, 2015). Such approaches may create significant opportunities for engaging the use of NMR in assessing HOS characterization information in the biopharmaceutical industry, especially for use in comparability and biosimilarity studies.

2.7 PHYSICOCHEMICAL METHODS THAT CAN PROVIDE BOTH PRIMARY AND HOS FINGERPRINT INFORMATION TO ASSESS BIOSIMILARITY

In characterizing the biochemical primary structure of biopharmaceuticals, it has been noted that the key analytical tool is MS. This dominant role of MS in primary structure analysis is achieved through its ability to assess the nature of the primary structure alterations present in the variant forms of a biopharmaceutical solely on the basis of accurate MW measurements. However, as pointed out in Section 2.6.1, much of the primary structure characterization work on biopharmaceuticals is facilitated by the implementation of additional prior separation techniques (see Figure 2.9A, C, and D). In many cases, these separations are conducted under denaturing conditions (e.g., using reversed phase LC). In other cases, however, separations may be conducted under experimental conditions where the native or native-like HOS of the biopharmaceutical is maintained. Under the latter conditions, the HOS of the different variant forms of the biopharmaceutical can also play an important role in the separations that are achieved. In some cases, the observed separations may not actually be associated with any primary chemical change in the biopharmaceutical's structure, but rather with the change in its HOS (e.g., see Figure 2.5E). As a result, separation methods such as IEC, isoelectric focusing (IEF) electrophoresis, CZE, or hydrophobic interaction chromatography by themselves offer the ability to empirically detect both primary structure and/or HOS differences between samples when these separations are conducted native or native-like experimental conditions (without knowing the details about the exact nature of the change in the biopharmaceutical). Indeed these separation methods on their own offer great opportunities to rapidly generate detailed empirical physicochemical fingerprint information, as shown in Figure 2.4D, to help in assessing consistency, comparability, and biosimilarity studies.

2.8 ESTABLISHING BIOSIMILARITY IN TERMS OF CONCENTRATION AND POTENCY

In establishing dosing equivalence in assessing biosimilarity, two important attributes must be assessed: (1) the physical mass of the API that is present in a biopharmaceutical's commercial product (e.g., vial, syringe) and (2) the amount of biological or functional activity measurement associated with that specific amount of