primary structure characterization for revealing and quantifying the underlying complex chemical heterogeneity of biopharmaceuticals (Ayoub et al., 2013; Beck et al., 2012, 2013, 2015; Chen et al., 2013; Dotz et al., 2015; Fekete et al., 2015; Gahoual et al., 2014a,b; Klepárník, 2015; Reusch et al., 2015b; Sandra et al., 2014; Xie et al., 2010).

This enhanced ability to separate a complex mixture of variant forms of a biopharmaceutical into its individual constituent components, or into far less complex mixtures of variant forms of the biopharmaceutical, can go a long way in enhancing the characterization process by providing a more detailed view of the biochemical fingerprint of the heterogeneity of these drugs to provide a more meaningful biosimilarity assessment. In some cases, even higher multidimensional separation-analysis approaches beyond LC-MS and CE-MS have been employed. Often, this is achieved by carrying out appropriate fractionation of a sample during a given separation (either manually or via automated fraction collection) to create a number of fractions that are further processed individually offline using a second different separation mode that frequently incorporate the additional unique separation and analysis capabilities of MS (Biacchi et al., 2015; Neill et al., 2015). However, preferable automated online coupling of these multiple separations using special automated coupled hardware that ends with the employment of one of several general MS analysis approaches outlined in Figure 2.8 are becoming feasible and more attractive for carrying out higher-level primary structure characterization work (Mellors et al., 2013; Sandra and Sandra, 2015; Stoll, 2015; Stoll et al., 2015; Vanhoenacker et al., 2015).

By using these separation- and multidimensional separation-analysis approaches, complex fingerprint data patterns are frequently generated (see Figure 2.9A and B). The creation of such complex fingerprint patterns, without knowing exactly what each observed peak corresponds to (initially), can in themselves allow for making useful detailed empirical biosimilarity comparisons and consistency assessments between RP lots and between biosimilar lots. Indeed, since a biosimilarity project is a multistep exercise (consisting effectively of the following steps)—(1) experimentally determine and define the RP's window of consistency, (2) find a biosimilar that will adequately match the window of consistency of the RP, (3) tweak various manufacturing conditions to maximize biosimilarity of the biosimilar to its RP and experimentally establish and document this biosimilarity, and (4) assess the consistency of the biosimilar's manufacturing process to make sure that biosimilar lots can adequately meet all the established biosimilarity and consistency criteria—these initial acquired empirical fingerprint patterns can at each of these steps help to make rapid and meaningful assessments to speed up a biosimilar's development. For example, initial empirical fingerprints may just serve as a rapid comparison template for scanning and honing in on finding a feasible collection of biosimilar candidates that could best match the same fingerprint profile observed for the RP, for example, as is commonly done using intact MS (Beck et al., 2015; Xie et al., 2010; see Figure 2.9B). In later work, these same fingerprint assessments can also serve as a key starting point for much more intense, but focused, investigational work into understanding the exact nature of any observed differences between RP lots and biosimilar lots, and eventually between RP and biosimilar lots.