Several MS-based footprinting approaches using reversible hydrogen/deuterium exchange (H/DX-MS) or irreversible covalent labeling (e.g., with hydroxyl radicals), along with native MS, are showing signs of being capable of making their way into more critical assessments of comparability and biosimilarity of biopharmaceuticals (Beck et al., 2012, 2013; Bobst and Kaltashov, 2011; Edgeworth et al., 2015; Houde and Berkowitz, 2016; Houde et al., 2011; Zhang et al., 2014). In addition, advancements in NMR are attracting attention (Arbogast et al., 2015a,b; Aubin et al., 2008, 2014; Poppe et al., 2013, 2015; Wishart, 2013), and even new tools such as antibody arrays (Davies et al., 2015; Wang et al., 2013) are showing signs that they can effectively be used to interrogate large portions or even the entire biopharmaceutical molecules to assess the HOS of these molecules with high resolution that can detect small changes in a biopharmaceutical's HOS on a somewhat more practical level than what was possible a few years ago.

Although these analytical biophysical tools in most cases require a high level of expertise, very expensive instrumentation, or critical reagents that are only commercially available (via the vulnerable situation) from a single source, the fact remains that in assessing biosimilarity, the need to perform these types of high-resolution measurements can be contained to a fairly limited number of specific key samples and experiments that when performed can make a significant contribution in successfully demonstrating biosimilarity. As a result, the investment of resources into conducting such analytical biophysical measurements can pay big dividends in facilitating efforts in picking the best biosimilar to work on and/or in acquiring critical data for a BLA filing of a biosimilar to support its approval. Given the potential limited needs for using these advanced high-resolution technologies, it may not be necessary to bring these capabilities in-house (especially in the early phase of biosimilar work). Rather, key collaborations with specific facilities (analytical contract research organization, CRO, or cutting-edge academic laboratories) can offer opportunities for undertaking these types of measurements. Such an approach would allow for the initial evaluation of these tools to determine whether efforts should be made for their acquisition and development in-house, or access to these techniques can should be better pursued scientifically and/or economically by appropriate contract or collaboration approaches.

2.6.2.2.1 MS Footprinting to Assess HOS: H/DX-MS and Covalent Labeling MS

Although H/DX-MS has been around for more than two decades, only in the last decade has the biopharmaceutical industry begun to realize the potential of this technique to greatly improve the industry's ability to provide useful indirect detailed information about the HOS of biopharmaceuticals and its dynamics in a very practical way (Engen and Wales, 2015; Houde and Berkowitz, 2016; Houde et al., 2011; Iacob and Engen, 2012; Kaltashov et al., 2010; Wei et al., 2014). Much of the delay in realizing H/DX-MS's capabilities can be traced to the intense amount of time and effort initially required to obtain useful data from these measurements and the availability of only home-built instrumentation to carry out these measurements. However, with the great advances in MS instrumentation, which includes the commercialization of a turnkey H/DX-MS system by Waters