

1. A confirmation of the biopharmaceutical's amino acid sequence.
2. The detection of PTMs.
3. Identification of the specific nature of those PTMs.
4. The localization as to where those PTMs occur (what exact amino acid is modified) within the biopharmaceutical's unique sequence of amino acids.
5. The percent level to which these specific amino acids are modified.

This ability to provide such an array of information leaves little doubt that MS can provide the biopharmaceutical scientist with powerful capabilities for attacking the challenges of characterizing the chemical heterogeneity of a biopharmaceutical.

In addition, as mentioned in Section 2.6.1, in many cases the resulting raw fingerprint output from these MS experiments themselves or in combination with a prior separation method can provide an informative empirical approach for conducting a range of powerful comparisons, without even initially understanding the nature of what each peak corresponds to. Indeed, in head-to-head comparisons conducted in a biosimilarity study, the resulting MS spectrum data from the biosimilar and its corresponding RP can be plotted on the same graph but in opposite directions on the *y*-axis. Such a plot should ideally appear as a highly similar mirror or butterfly plot of the fingerprint pattern of MS peaks having peak areas, *m/z* or MW and even retention or elution time values (generated from the two samples being compared) that are highly similar to each other (see Figure 2.9A and B). Any qualitative observed nonmirrored areas would indicate a difference that should readily be recognized visually. Furthermore, such collected data could also be analyzed quantitatively, if so desired, to achieve a higher level of assessment of biosimilarity using various approaches (e.g., by simply conducting difference measurements between the two plots coupled with appropriate statistical assessment—for example, using the *t*-test with appropriate CL; see section 2.5.1.1.1) to help assess whether the observed difference constitutes a real difference as in the case of analyzing hydrogen/deuterium exchange-MS (H/DX-MS) data (Houde et al., 2011), shown in Section 2.6.2.2.1, or by using local correlation calculation as is done for the case of proton nuclear magnetic resonance (¹H NMR) spectral data comparisons (Amezcuca and Szabo, 2013; Mei-feng et al., 2015).

2.6.1.2 Structural Analysis of Glycosylation

Of all the different types of PTMs identified on biopharmaceuticals [which total well over several hundred (Doll and Burlingame, 2015; Walsh, 2006a)], none can compare in terms of importance, complexity, and level to which they have and are being researched as glycosylation (Moremen et al., 2012; Solá et al., 2007) (see Chapter 4 for a more in-depth discussion). In this type of PTM, a collection of monosaccharides are linked together through a complex sequence of enzymatic reactions (within the cell) to form a structure called a glycan, which is covalently coupled to the polypeptide chain of a biopharmaceutical to yield a glycosylated biopharmaceutical. Although the size of the glycan that can be coupled to a protein can be very large, glycans that consist of only a relatively small number of monosaccharides (e.g., 3–9 monosaccharide units) are referred to as an oligosaccharide (as mentioned earlier in this chapter). Oligosaccharides attached to biopharmaceuticals can in general be put