into two general classes that differ in terms of the specific amino acid they are linked to in the biopharmaceutical's polypeptide chain. These two classes include oligosaccharides linked to the amide nitrogen side chain of an asparagine, called *N*-linked oligosaccharides, and those linked to the hydroxyl side chain on serine or threonine, called *O*-linked oligosaccharides. In the physicochemical characterization of a glycosylated biopharmaceutical, the following are key points of interest concerning these attached oligosaccharides:

1. The monosaccharide composition of each oligosaccharide.
2. The specifics of the covalent linkage of these monosaccharides to each other and their sequential arrangement in each oligosaccharide.
3. The characterization of additional chemical modifications that might be present on a monosaccharide and their specific location on the monosaccharide structure.
4. The number and location of the oligosaccharide's glycosylation site(s) on the polypeptide chain(s) of a given biopharmaceutical.
5. The percent of biopharmaceutical molecules in a given sample that have an oligosaccharide present at each glycosylation site (commonly referred to as the percent occupancy of each glycosylation site) on a biopharmaceutical.
6. The distribution profile of different oligosaccharides' structures that occupy each glycosylation site.

To obtain all this information requires similar analytical approaches used to characterize the protein part of a biopharmaceutical. This includes the collaboration of information extracted from the analysis of the intact biopharmaceutical along with peptide mapping, where separation science together with MS characterization again plays a dominant role in revealing and quantitating the detailed characteristics of the oligosaccharide(s) and their location on the biopharmaceutical (Ayoub et al., 2013; Beck et al., 2012, 2013, 2015; Biacchi et al., 2015; Chen et al., 2013; Dotz et al., 2015; Gahoual et al., 2014a,b; Kaltashov et al., 2012; Klepárník, 2015; Leurs et al., 2015; Reusch et al., 2015b; Sandra et al., 2014; Xie et al., 2010; Zhang et al., 2009). However, an additional mode of oligosaccharide characterization also exists that involves the unique ability of the biopharmaceutical investigator to release quantitatively the oligosaccharides from a biopharmaceutical to assess the average global distribution of all the different oligosaccharides present in a given biopharmaceutical sample. The ability to release these oligosaccharides and analyze them independent of a biopharmaceutical's protein structure can provide a detailed fingerprint of the glycosylation pattern (oligosaccharide profile) of the biopharmaceutical using various types of LC and CE separations in combination with any one of a number of various detectors [e.g., pulse amperometric detection (PAD), fluorescent, or MS]. Such fingerprint information can be used empirically to qualitatively, as shown in Figure 2.10, or quantitatively assess glycosylation lot-to-lot variability of the RP and biosimilar lots to establish their consistency of manufacturing and in the glycosylation biosimilarity assessment of a biosimilar to its RP (Beck et al., 2013; Reusch et al., 2015a,b; Sandra et al., 2014; Suzuki, 2013).