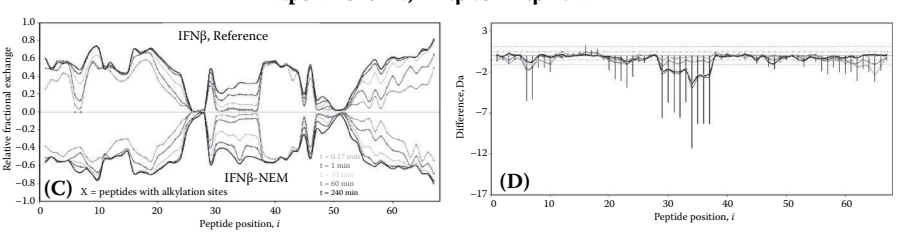

FIGURE 2.12 (CONTINUED) (See color insert.) Results from this experiment are shown in (C), again as a mirror plot where the deuterium uptake data for the unmodified IFNβ Reference (control) are displayed in the positive *y*-axis direction and the deuterium uptake data for IFNβ-NEM (experimental) are displayed in the negative *y*-axis direction. Data in "C" are also shown in (D) as a difference plot as described in "C" as described in "B". In H/XD-MS experiment #1, data show no significant indication of difference in the H/DX-MS data between the two IFNβs, as indicated by the absence of any qualitative visual differences in the mirror plot "A" and the absence of quantitative difference data in plot "B" exceeding its 98% confidence limit (CL) D line (blue) and sum of difference data exceeding its 98% CL SD line (black). These results support the comparability of the HOS of the two IFNβ indicating the absence of any effect on the biophysical properties of IFNβ as a result of the change made in its production cell culture media. In H/XD-MS experiment #2, however, data show that the simple chemical modification of IFNβ's sole free cysteine has a significant impact on its HOS, as indicated by the qualitative visual differences seen in the mirror plot "C" and numerous quantitative difference data exceeding its 98% CL D line (blue) and sum of difference data exceeding its 98% CL SD line (black). These results support the noncomparability of the HOS of these two INFβ. (Figure 2.12A and B reprinted from Houde D et al. 2011. The utility of hydrogen/deuterium exchange mass spectrometry in biopharmaceutical comparability studies. *Journal of Pharmaceutical Sciences* 100(6), 2071–2086. With permission from Elsevier with minor modifications.)

(Kaltashov and Eyles, 2005a; Kaur et al., 2015). As in H/DX-MS, the extent and/or rate of these reactions can be followed and assessed by MS in collaboration with fragmentation methods inside or outside the mass spectrometer to assess changes in the biopharmaceutical HOS and identify which structural elements on the biopharmaceutical are being altered (Deperalta et al., 2013; Madsen et al., 2016). One important difference between covalent labeling and H/DX-MS is the absence of the reversibility of the labeling process in the case of the former, which removes some of the experimental constraints that must be adhered to when doing H/DX-MS, which could offer a significant advantage when using covalent footprinting techniques.

2.6.2.2.2 Using Native MS to Assess HOS

In conducting native MS, one effectively uses the resulting charge state distribution of the native or native-like structure of the injected biopharmaceutical retained under the gas-phase conditions inside the mass spectrometer as an indirect indicator of the biopharmaceutical's HOS (Huber, 2015; Rosati et al., 2012). Since the charge state distribution of a biopharmaceutical is highly dependent on its HOS, samples of