Whereas most of recombinant product would undergo similar testing protocols, there are specifically recommended tests that may be required for the monoclonal antibodies. Some examples of these tests include the following:

- Heavy chain and light chain molecular weight determination by MS
- Peptide mapping by MS of heavy and light chains
- N- and C-terminal sequencing of heavy and light chains by MALDI-ISD
- Complementarity-determining region sequencing of variable complementary determining regions
- PTM analysis; deamidation, oxidation, pyroglutamate, N-glycosylation
- For most monoclonal antibodies, the degree of Fc- and ADCC-binding profiles will be required for similarity determination even if these do not affect the functional response

It is well known that the terminal functional groups in protein structure may not have a significant impact on the efficacy of the molecule, but it leaves one uncertain whether they would have any potential immunogenic response; the same holds true for disulfide bonding and other intramolecular interactions. In those instances where such changes are determined earlier such as through MS and CD, the sponsor may choose the 351(a) route instead to avoid more extensive analytical comparison.

5.3.2 HOS

The integrity of the secondary, tertiary, and quaternary structures is important to establish similarity. Common methods used include FTIR, near-UV spectroscopy, CD, and DSC. These methods should be sensitive and have high resolution resolving. It should be realized that the reference product extinction coefficient is not always available in public and must be determined. Secondary and tertiary structures are best evaluated using far- and near-UV CD, and 1D and 2D NMR. Far- and near-UV CD spectroscopy provides information about secondary (alpha-helix, beta-sheet, and random coil structures) and tertiary structure, respectively. In addition to similar CD spectra, comparison of transition point (specific ellipticity = 0) and ratio of specific ellipticity ($\theta_{R208}/\theta_{R222}$) data derived from the far-UV CD spectra can be used to demonstrate a high level of similarity. ^{1}H NMR spectroscopy also provides information about the 3D structure of the protein. No significant difference in the NMR spectra of the test product compared to the reference product is required as judged by the number, position, and intensity of peaks. To further support HOS similarity, natural isotope abundance 2D NMR (^{1}H–^{15}N heteronuclear single quantum coherence) spectra can be used. ^{1}H–^{15}N NMR provides better resolution than ^{1}H NMR, and that is considered a structural fingerprint of the protein.