models (cell culture) and in vivo (animal models), various chromatography techniques (HPLC peptide mapping), electrophoresis methods (sodium dodecyl sulfate [SDS]–PAGE, IEF, capillary zone electrophoresis [CZE]), static and dynamic light diffusion mass spectrometries, x-ray techniques, etc. How the main analytical methods provide information on different orthogonal testing is listed in Table 5.1.

5.1.2 Orthogonal approach

Given that different testing methodologies can yield similar information, the FDA insists on using an orthogonal approach to confirming structural and functional attributes. A good example is that of protein content. The advisory meeting that the FDA held prior to approving the first biosimilar filgrastim product presented the data showing variability in the protein content of the filed product and suggested that the content issue be handled by controlling the manufacturing process; later, more data were added to support that the protein content was within the acceptable range. Generally, an orthogonal approach using both RP-HPLC and ultraviolet (UV) A280 methods to confirm protein content will be desirable.

The basis of using multiple approaches comes from *ICH Q6B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*. The manufacturer should define the pattern of heterogeneity of the desired product and demonstrate consistency with that of lots used in preclinical and clinical studies.

While the monoclonal antibodies can be thoroughly characterized using the available methods (Figure 5.1), the use of orthogonal strategy proves useful in removing what the FDA calls "residual uncertainty" (Figure 5.2).

5.2 Key methodologies

5.2.1 Mass spectrometry (MS)

MS is used to measure molar mass, molecular structure, and sample purity. As an analytical technique, it has distinct advantages such as increased sensitivity over most other analytical techniques because the analyzer, as a mass-charge filter

- Reduces background interference;
- Has excellent specificity from characteristic fragmentation patterns to identify unknowns;
- Confirms the presence of suspected compounds;
- Gives information about molecular weight;
- Provides information about the isotopic abundance of elements; and
- Gives temporally resolved chemical data.