Microheterogeneity assessment of intact pharmaceutical proteins by LC-MS and CE-MS

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The role of mass spectrometry (MS) in quality control and assurance of intact pharmaceutical proteins is steadily growing. However, biopharmaceuticals frequently are heterogeneous presenting isoforms, related impurities and minor degradation products, which cannot be distinguished consistently by MS only. Separation prior to MS detection is essential to achieve reliable assignment of intact protein variants. Unfortunately, traditional liquid chromatographic (LC) protein separation methods often show poor compatibility with MS and/or lack the selectivity to resolve proteoforms. Moreover, LC conditions may be denaturing, precluding assessment of protein conformers and proteoform affinity.

This lecture presents the design and application of new LC-MS and capillary electrophoresis (CE)-MS methods allowing detailed determination of the microheterogeneity of intact proteins of pharmaceutical interest. CE-MS at acidic conditions shows highly useful for integrity monitoring of protein antigens and nanobodies, revealing proteoforms and degradation products. Hydrophilic interaction liquid chromatography (HILIC)-MS appears extremely useful for high-resolution glycoform profiling of therapeutic proteins. We also developed MS-hyphenated methods for the assessment of conformation and affinity of components of pharmaceutical protein samples under conditions that preserve native macromolecular structures. Resolution of biopharmaceutical conformers by CE-MS will be demonstrated. Native ion-exchange (IEX) and affinity LC-MS proved valuable for selectively resolving charge and oxidation variants of monoclonal antibodies under non-denaturing conditions.