Analytical method development for low- and high-molecular pharmaceuticals: two different worlds?

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Very stringent safety regulations by the health authorities mandate elaborate analytical schemes for comprehensive characterization of the efficacy and safety of pharmaceutical products. High-performance liquid chromatography (HPLC) was developed in the mid 1960-ies mainly for the separation of small molecules. It took at least another decade until chromatographers were able to appreciate the peculiarities of biological macromolecules to tailor suitable separation systems in order to enable their efficient chromatographic separation. In the past decade, the development of stationary phase configurations enabling very high efficiency such as sub-2 μm particles, superficially porous particles, or monolithic phases have significantly enhanced the separation performance of HPLC for biological macromolecules.

Likewise, progress in mass spectrometry (MS) technologies, especially electrospray ionization (ESI) for biopolymers, as well as high-resolution mass analyzers such as time-of-flight or Orbitrap mass analyzers have significantly contributed to the success of bioanalytical methods in pharmaceutical and biomedical research. This presentation reviews the potential, challenges, and achievements of HPLC-ESI-MS methods in the analysis of low- and high-molecular pharmaceutical compounds with emphasis on confirming the identity and purity of the drug compounds. Examples of application will include the impurity profiling of synthetic drugs such as levothyroxine or liothyronine, as well as structural confirmation and analysis of posttranslational modifications in therapeutic proteins produced by recombinant biotechnology, such as monoclonal antibodies, growth factors, and fusion proteins.