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Recent advancements, challenges, and practical considerations in the mass spectrometry-based analytics of protein biotherapeutics: a viewpoint from the biosimilar industry

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Highlights:

- advances of the past ten years in the MS-based characterization of protein biotherapeutics
- how to cope with industrial and regulatory requirements
- how to obtain accurate and reliable analytical data in a time- and cost-efficient way
- new sample preparation approaches, measurement techniques and data evaluation strategies
- increasing role of mass spectrometry

Abstract

The extensive analytical characterization of protein biotherapeutics, especially of biosimilars, is a critical part of the product development and registration. High-resolution mass spectrometry became the primary analytical tool used for the structural characterization of biotherapeutics. Its high instrumental sensitivity and methodological versatility made it possible to use this technique to characterize both the primary and higher-order structure of these proteins. However, even by using high-end instrumentation, analysts face several challenges with regard to how to cope with industrial and regulatory requirements, that is, how to obtain accurate and reliable analytical data in a time- and cost-efficient way. New sample preparation approaches, measurement techniques and data evaluation strategies are available to meet those requirements. The practical considerations of these methods are discussed in the present review article focusing on hot topics, such as reliable and efficient sequencing strategies, minimization of artefact formation during sample preparation, quantitative peptide mapping, the potential of multi-attribute methodology, the increasing role of mass spectrometry in higher-order structure characterization and the challenges of MS-based identification of host cell proteins. On the basis of the opportunities in new instrumental techniques, methodological advancements and software-driven data evaluation approaches, for the future one can envision an even wider application area for mass spectrometry in the biopharmaceutical industry.

Keywords: Mass spectrometry, Biosimilars, Posttranslational modification, Multi-attribute method, Host cell proteins, HDX-MS

Abbreviations: Asp-N, Endoproteinase AspN; BLA, Biologics license application; CDR, Complementarity determining region; CFR, Code of Federal Regulations; CGF, Carboxyl group footprinting; cGMP, Current good manufacturing practice; CHO, Chinese hamster ovary; CID, Collision-induced dissociation; CPMP/BWP, Committee for Proprietary Medicinal Products / Biotechnology Working Party; CQA, Critical quality attribute; Da, Dalton; DDA, Data-dependent acquisition; DIA, Data-independent acquisition; DTT, Dithiothreitol; ECD, Electron-capture dissociation; EIC, extracted ion chromatogram; ELISA, Enzyme-linked immunosorbent assay; EMA, European Medicines Agency; ESI, Electrospray ionization; ETD, Eletron-transfer dissociation; FAB, Fast atom bombardment; Fab, Antigen binding fragment; Fc, Fragment crystallizable; FDA, Food and Drug Administration; FL, Fluorescent; FT-ICR, Fourier-transform ion cyclotron resonance; Glu-C,