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Comparability study of Rituximab originator and follow-on biopharmaceutical

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Highlights

- Establishment of various mass spectrometry-based methods to investigate intact mass, PTMs and higher order structure of biopharmaceuticals
- Establishment of a comparability study between candidate originator and follow-on biopharmaceutical
- Monitoring of batch-to-batch changes in glycosylation as a critical quality attribute (CQA)
- Functional properties control of three biopharmaceutical batches to investigate the impact of glycosylation on the potency

Abstract

Immunglobolin G (IgG)-based biopharmaceuticals are emerging on the pharmaceuticals market due to their high target selectivity in different diseases. In parallel, a growing interest by other companies to produce similar or highly similar follow-on biologics exits, once the patent of blockbuster biotherapeutics is about to expire. In correlation to their complex structure, an analytical challenge is facing the approval of these biosimilars. Health authorities (e.g. FDA and EMA) have issued several guidelines to define critical quality attributes during manufacturing process changes. In the current study, physicochemical characterization using state-of-the-art analytics were applied to analyse intact mass, post-translational modifications (PTMs) and higher order structure of Rituximab and one of its biosimilars. Intact mass analysis, middle-up approach as well as subunit analysis revealed similar glycoforms but additional lysine variants in the biosimilar. The N-glycosylation site was confirmed for both, the originator and the biosimilar. PTMs and higher order structure were confirmed to be similar. A special focus was given to N-glycosylation due to its potential to monitor the batch-to-batch consistency and alteration during the production bioprocess. Comparison of the N-glycosylation profiles obtained from three batches of the biosimilar and the reference product showed quantitative variations, although the N-glycans were qualitatively similar. Furthermore, a head-to-head comparability of functional properties was performed to investigate the impact of glycosylation alteration and PTMs on potency within the