INTRODUCTION

It has been well established that glycosylation of therapeutic proteins have profound impact on their stability, solubility, pharmacokinetics and pharmacodynamics. Thus, establishing the comparability of glycosylation patterns is a critical attribute for the documentation of biosimilarity. Currently, different MS-based techniques are used for the investigation of carbohydrates, including MRM-MS, LC-MSMS and MALDI-TOF-MS. Among these techniques, however, for some high therapeutic proteins such as Etanercept with multiple glycosylation sites, this method is less informative. The aim of this study was to develop an accurate and robust method for relative quantitation of glycopeptides in a site-specific manner. Here, we used glycopeptide product ions and MS/MS labeling of C-terminal cysteine group as a strategy to obtain quantitative information on glycopeptides. We used a novel strategy involving multiple-reaction monitoring (MRM) for the quantification of glycopeptides and their labeling. Furthermore, we used Chemical shift assignment to distinguish between different types of glycosylation. Our method is site-specific. We have also demonstrated the benefits of this method by showing the relative quantitation and similarity of Etanercept biosimilar.

RESULTS

For glycopeptide quantification, MRM-MS strategy was used to analyze 18O-labeled and unlabeled biosimilar and innovator samples using 180-water at a constant flow rate of 1.5 ml/min. The innovator and biosimilar samples were spiked with 180-water in a suitable ratio to increase the signal intensity and are thus suitable abundant and fast to observe any change in the MS/MS spectrum. The quantification was performed based on ratios of average areas of labeled and unlabeled product ions.

A correlation was established between the calculated and reported ratio of unlabeled vs labeled precursor and product ions based on 180 ISOs. Excellent correlation, R²=0.99 was observed for 11 different precursors (R²=0.99), 4 different product ions (R²=0.99), 26 different precursors (R²=0.99) and 18 different product ions (R²=0.99). The proposed method is therefore highly specific and accurate.

CONCLUSION

The results showed a very similar site-specific expression of N- and O-glycopeptides between the biosimilar and innovator with minimal variations. As compared to the innovator, the biosimilar samples showed a very similar trends in the quantitation of glycans as compared to the innovator.

ACKNOWLEDGEMENTS

All authors listed on the C-MAMP 2017 for this work.

TRADEMARKS/LICENSING

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