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Application of Complementary HRMS Methodologies for a Thorough Biosimilar Comparability Assessment

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OVERVIEW

Purpose

- To develop orthogonal methodologies using HRMS and dedicated software to compare innovator and biosimilar monoclonal antibody drugs.

Method

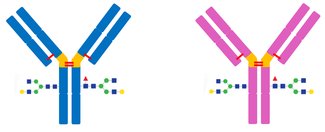
- Enzymatic digestion with IdeS followed by reduction with TCEP was used for middle up approach.
- Alternative LC-MS technique using dimethyl labelling was developed for primary structure comparison in a single LC-MS run.
- TripleTOF™5600 LC-MS/MS and BioPharmaView 1.0 software were used for the biosimilarity assessment.

Results

- Middle-up approach shows an incomplete C-term Lys clipping and N-term pyroglutamate formation of the biosimilar.
- Bottom-up approach using dimethyl labelling confirms the primary structure and PTMs in a single LC-MS run.

INTRODUCTION

Biosimilars or “follow-on biologics” are subsequent versions of innovator biotherapeutics created following patent expiry of the innovator product. The acceptance of biosimilars by regulatory authorities requires extensive characterization to demonstrate appropriate and comparable quality, safety and efficacy with the reference product. In this study, we have developed a robust and fast approach in the comparability of innovator and biosimilar products using high resolution mass spectrometry and BioPharmaView™ software. This approach was used to compare the primary structure and post-translational modifications (PTMs) of the innovator drug Rituximab, a monoclonal mAb used for the treatment of cancer and other autoimmune disorders, and the biosimilar Reditux.



innovator biosimilar

MIDDLE-UP APPROACH

Figure 1: Middle-up analysis with FabRICATOR (IdeS protease)

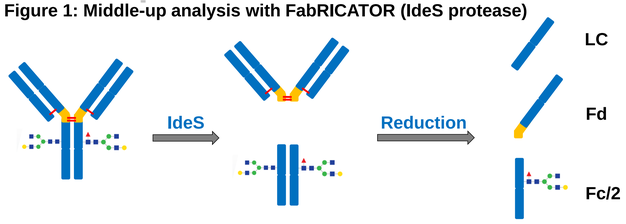
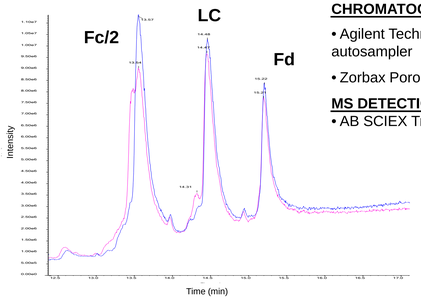


Figure 2: TIC Chromatograms of IdeS digested Rituximab (blue) and Reditux (pink)



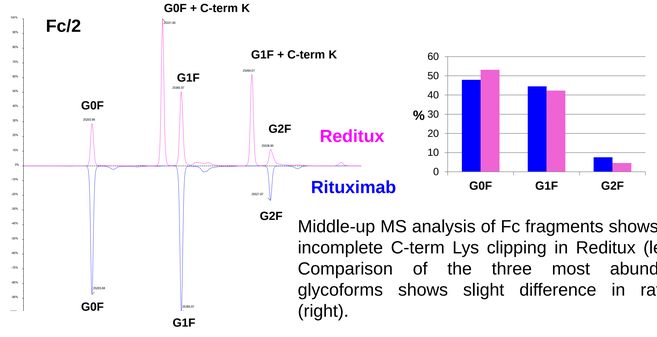
CHROMATOGRAPHY

- Agilent Technologies Series 1100 pumps and autosampler
- Zorbax Poroshell C8, 75mmx2.1mm, 5µm particle size

MS DETECTION

- AB SCIEX TripleTOF™ 5600 in ESI(+) mode

Figure 3: Mirror plot of deconvolved mass graph of IdeS digested Fc fragments of Rituximab and Reditux



Middle-up MS analysis of Fc fragments shows an incomplete C-term Lys clipping in Reditux (left). Comparison of the three most abundant glycoforms shows slight difference in ratios (right).

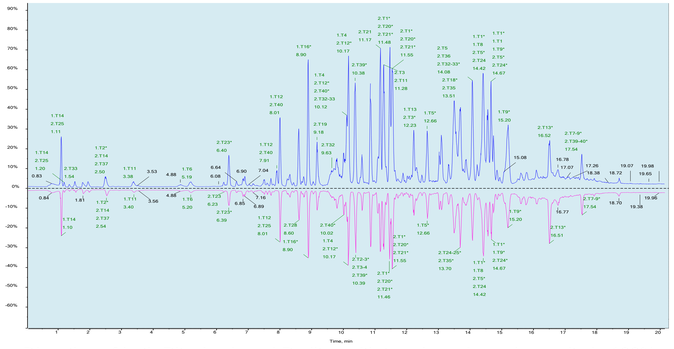
Table 1: Observed masses of innovator and biosimilar fragments after IdeS digestion

Fragment	Rituximab measured mass (Da)	Reditux measured mass (Da)	Theoretical Mass (Da)
*pLC	23039.30	23039.42	23039.69
LC	not observed	23056.01	23056.72
pFd	25327.44	25327.62	25328.49
G0F	25203.68	25203.99	25204.25
G1F	25365.97	25365.97	25366.39
G2F	25527.87	25528.89	25528.54
G0F+C-term K	not observed	25331.90	25332.43
G1F+C-term K	not observed	25494.07	25494.57
G2F+C-term K	not observed	25655.75	25656.71

* p: N-term pyroglutamate formation

BOTTOM-UP APPROACH

Figure 4: Mirror plot showing TIC of Rituximab (blue) and Reditux (pink) tryptic peptides



Digestion of both Rituximab and Reditux with trypsin and pepsin results in 100% sequence coverage, and shows no difference in the primary structure of both antibodies.

DIMETHYL LABELLING APPROACH

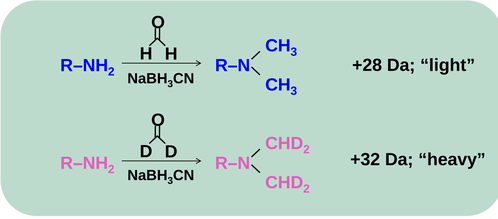
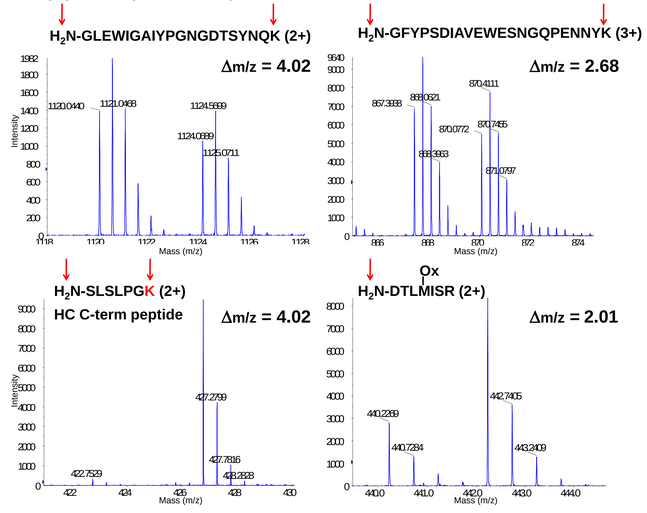


Figure 5: Dimethyl Labelling of tryptic peptides with light (Rituximab) and heavy (Reditux) formaldehyde



CONCLUSION

- Middle-up analysis with IdeS digestion enables fast determination of PTMs, such as glycosylation, N-term pyroglutamate formation and C-term Lys clipping.
- Bottom-up approach using dimethyl labelling technique is an orthogonal approach that permits the determination of primary structure and PTMs, such as oxidation and deamidation in a single LC-MS run.

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