Peptide mapping of innovator /biosimilar pair using HPLC coupled to Time-of-Flight Mass spectrometry

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Agilent Technologies

HPLC 2016
Poster

Introduction

Monoclonal antibodies (mAbs) are becoming one of the most important classes of biomolecules for the treatment of various cancers(1). Biosimilar mAbs, which are the replicas of licensed innovator products in the market, are also gaining significant attention. The development of these biosimilars is expanding due to patent expiry of innovator biotherapeutics(2). In order to show comparability between innovator and biosimilar mAbs, LC/MS characterizations are proving to be essential.

To ensure the quality of biosimilar mAbs and to show molecular similarity with their innovators, amino acid sequence confirmation is of crucial importance in the biopharmaceutical industry. Peptide mapping is one of the vital steps to show similarity in the sequence and modifications between the innovator and biosimilar pair. Peptide mapping involves protease digestion of proteins/mAbs followed by LC/MS analysis. In this work, commercial Rituximab - a chimeric mouse/human monoclonal antibody from innovator and biosimilar sources was subjected to trypsin digestion followed by peptide separation and mass determination on LC/Q-TOF. In this presentation, we have compared the innovator and biosimilar mAbs for sequence similarity, oxidation and deamidation status.

Experimental

Materials

Rituximab biosimilar and innovator were purchased from a local pharmacy and stored according to manufacturer's instructions. DL-Dithiothreitol (DTT), idoacetamide, Trisbase, LC/MS grade solvents were purchased from Sigma-Aldrich. High quality sequence grade Trypsin was acquired from Promega.

Trypsin Digestion

Before the digestion of the mAbs with trypsin, the disulfides were reduced and alkylated under denaturation conditions. This pretreatment was done to ensure that the monoclonal antibody was completely denatured and soluble so that the protease could access the substrate efficiently. The mAbs in solution were lyophilized and equal concentrations of both were reconstituted in 8 M urea in 0.25 M Tris buffer, pH 7.6, containing dithiothreitol (DTT), and the solutions were then incubated at 37°C for 30 min. To these solutions, iodoacetamide in 0.25 M Tris buffer, pH 7.6, was added and the sample was incubated at ambient temperature in the dark for 15 min. The solutions were diluted with 0.25 M Tris buffer, pH 7.6, before digestion with trypsin. To the above pretreated mAb solutions, trypsin at a ratio of 20:1 (protein to protease w/w) was added. The reaction was kept for overnight incubation at 37°C before LC/MS analysis. The enzymatic activity was quenched by adding 1 µL of 10% formic acid solution. The samples were either immediately analyzed or stored at -80 °C until LC/MS/MS analysis.

Instrumentation

LC system

Agilent 1290 Infinity LC System including:

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity Column Compartment (G1316C)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostat (G1330B)

MS system

- Agilent 6550 iFunnel Q-TOF LC/MS System with Agilent JetStream

Experimental

Table 1: LC/MS parameters

Parameter	Agilent 1290 Infinity LC System
Column	Agilent AdvanceBio Peptide Mapping, 2.1 × 250 mm, 2.7 µm (p/n 651750-902)
Sample thermostat	5 °C
Mobile phase A	0.1 % formic acid in water
Mobile phase B	90 % acetonitrile in water with 0.1 % formic acid
Gradient (segmented)	At 0 minutes → 3 %B At 40 minutes → 40 %B At 42 minutes → 95 %B At 44 minutes → 95 %B At 44.1 minutes → 3 %B
Stop time	44.1 minutes
Post time	10 minutes
Column temperature	60 °C
Flow rate	0.3 mL/min

Parameter	Agilent 6550 Q-TOF LC/MS System
Ion mode	Positive ion mode
Source	Agilent Dual JetStream
Drying gas temperature	250 °C
Drying gas flow	12 L/min
Sheath gas temperature	250 °C
Sheath gas flow	10 L/min
Nebulizer	25 psi
Capillary voltage	3,500 V
Nozzle	0 V
MS range	*m/z* 300–1,700
MS/MS range	*m/z* 50–1,700
MS scan rate (spectra/second)	6
MS/MS scan rate (spectra/second)	8
Ramped collision energy	Charge state slope offset 2 3.1 1 3 and >3 3.6 –4.8 1 3.5 6
Data analysis	The data obtained from LC/MS were analyzed using Agilent MassHunter Qualitative Analysis Software B.07 and Agilent MassHunter BioConfirm Software B.07

Results and Discussion

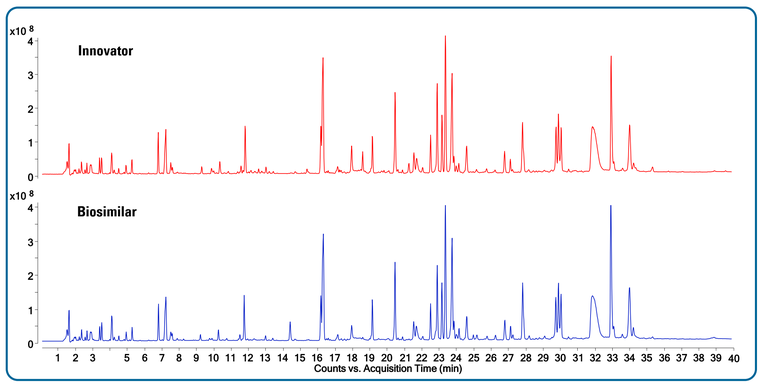

Figure 1. Total ion chromatogram (TIC) of peptide digest from Innovator (A) and Biosimilar (B).

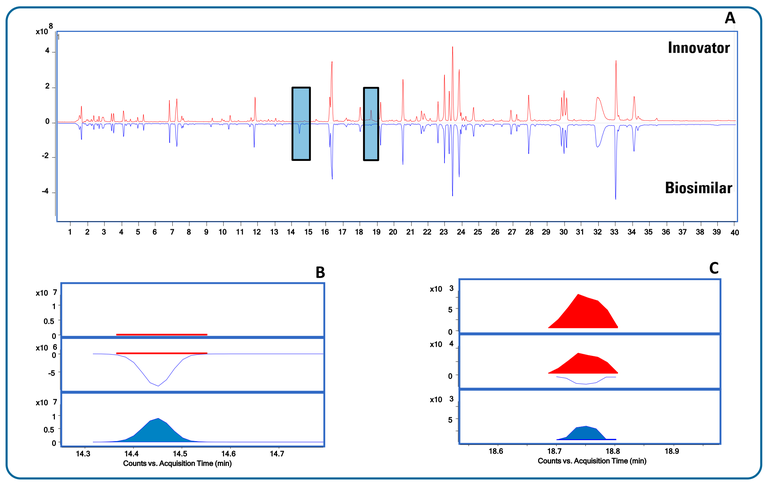

Figure 2. Mirror plot of TIC between innovator (red trace) and biosimilar (blue trace) (A). The region at around 14.4 minutes is highlighted to show the difference of SLSLSPGK peptide. The EIC of SLSLSPGK peptide, shows that this peptide is enriched in biosmiliar (B). Similarly, the peak around 18.7 minutes corresponds to SLSLSPG peptide (lysine truncated). The EIC of SLSLSPG peptide is shown (C), which is enriched in innovator.

Results and Discussion

Peptide mapping showed similar total ion chromatogram profiles with no undigested protein product in either mAb (fig 1). Differential Analysis of TICs showed a prominent peak around 14.4 min in the biosimilar sample not seen in the innovator sample. This peak corresponded to a C- terminal sequence (SLSLSPGK). The higher abundance of the C-terminal peptide can be attributed to charge variants (lysine addition, basic variants) seen in the biosimilar mAb. Similarly, in the innovator peptide map, the C-terminal peptide without lysine (SLSLSPG) has been identified with higher abundance around 18.7 min (fig 2). Peptide maps were also used to quantify the extent of oxidation and deamidation as these are the two most commonly occurring modifications seen during mAb storage, formulation and sample handling (fig 3 & 4).

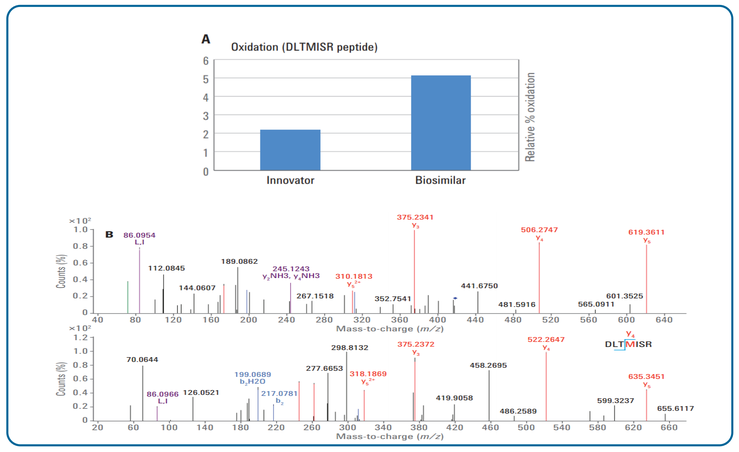

Figure 3. Bar graph between innovator and biosimilar for percentage of oxidation species in the sample (A). MS/MS spectra between unmodified and modified DLTMISR peptide (B).

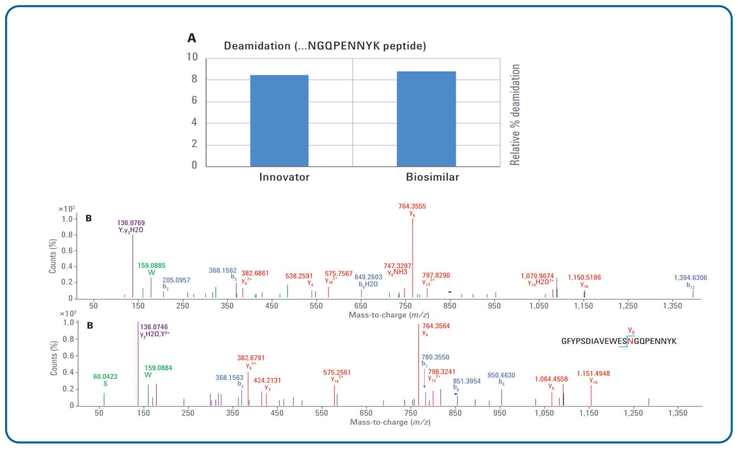

Figure 4. Bar graph between innovator and biosimilar for percentage of deamidation species in the samples (A). MS/MS spectra of unmodified and modified GFYPSDIAVEWESNGQPENNYK peptide (B).

Conclusions

- Effective characterization of innovator/biosimilar pairs with LC/MS/MS peptide mapping and differential software tools.
- Oxidation and deamidation levels between the innovator and biosimilar mAbs were assessed by comparing specific peptides.
- The analyses results showed that the innovator/biosimilar had similar deamidation levels while the biosimilar showed slightly more oxidation as compared to the innovator.

References

1. Nature Reviews Cancer 12, 278-287, (2012)
2. Nature Reviews Drug Discovery 15,13–14, (2016)