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Characterization and Comparison of Neulasta and its Biosimilar at Intact Level on a Quadrupole-Orbitrap Mass Spectrometer

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ABSTRACT

Purpose: Assessment of the similarity and differences between Neulasta and its biosimilar product.

Methods: Apply high resolution LC/MS analysis to measure the intact mass and profile PEG distribution.

Results: Successfully revealed difference in PEG distribution of two products.

INTRODUCTION

Pegfilgrastim is marketed by Amgen under the trade name Neulasta™. It is a prescription medicine used to help reduce the chance of infection due to a low white blood cell count, in people with certain types of cancer (non-myeloid), who receive anti-cancer treatment (chemotherapy) that can cause fever and low white blood cell count.

In China, there are several biosimilars of Neulasta approved to the market. To meet regulatory guidelines, the manufacturers of biosimilars need to prove the similarity of their products to the innovator. In this study, we used a Thermo Scientific™ Q Exactive HF-X™ Biopharma Mass Spectrometer to measure the molecular weight and PEG polydispersity of both innovator and biosimilar to evaluate potential differences.

MATERIALS AND METHODS

Sample Preparation

The innovator and biosimilar were diluted to 1 mg/mL with pure water respectively for LC/MS analysis.

Liquid Chromatography

The innovator and biosimilar samples were separated on a Thermo Scientific™ UltiMate3000™ HPLC System (A: 50 % ACN + 0.1 % TFA, B: 95 % ACN + 0.1 % TFA). Post column addition: 400 mM TEA in water, 5 μL/min; a Thermo Scientific ™ Hypersil GOLD™ C18 column (150 mm × 2.1 mm, 1.9 μm) was employed.

Mass Spectrometry

A Q Exactive HF-X Biopharma Mass Spectrometer was used for all analysis.

Data Analysis

Data analysis was performed using Thermo Scientific™ BioPharma Finder™ Software. Parameters shows in Figure 1.

Figure 1. Data processing parameters used in BioPharma Finder.

RESULTS

Molecular weight determination of Neulasta and its biosimilar

In this study, we measured the molecular weights of Neulasta and a domestic biosimilar. Neulasta is a PEGylated recombinant human granulocyte colony-stimulating factor, containing 174 amino acids (18-20 kDa) and around 20 kDa PEG chains in continuous distributions.

For PEGylated proteins, a multiplicity of signals is generated because of the presence of ladders of multiply charged protein ions and the overlaying polydispersity of the attached PEG. This results in spectra containing overlapping signals that are difficult to deconvolute and interpret. We chose TFA as solvent addition for charge reduction and TEA as post column addition to avoid dePEGylation.

Hundreds of components can be baseline separated and detected with great S/N ratio in this extremely complex mixture, benefiting from the high resolution and sensitivity of the Orbitrap analyzer (Figure 2). MS parameters are shown in Table1.

Table 1. MS parameters.

Full MS	
Runtime	0 to 10 min
Polarity	positive
In-source CID	70.0 eV
Microscans	10/2
Resolution	15,000/120,000
AGC target	3e6
Maximum IT	200 ms
Scan range	1000 to 6000 m/z
Spray Voltage	4000V
Probe Heater Temp.	70℃
S-Lens RF Level	50

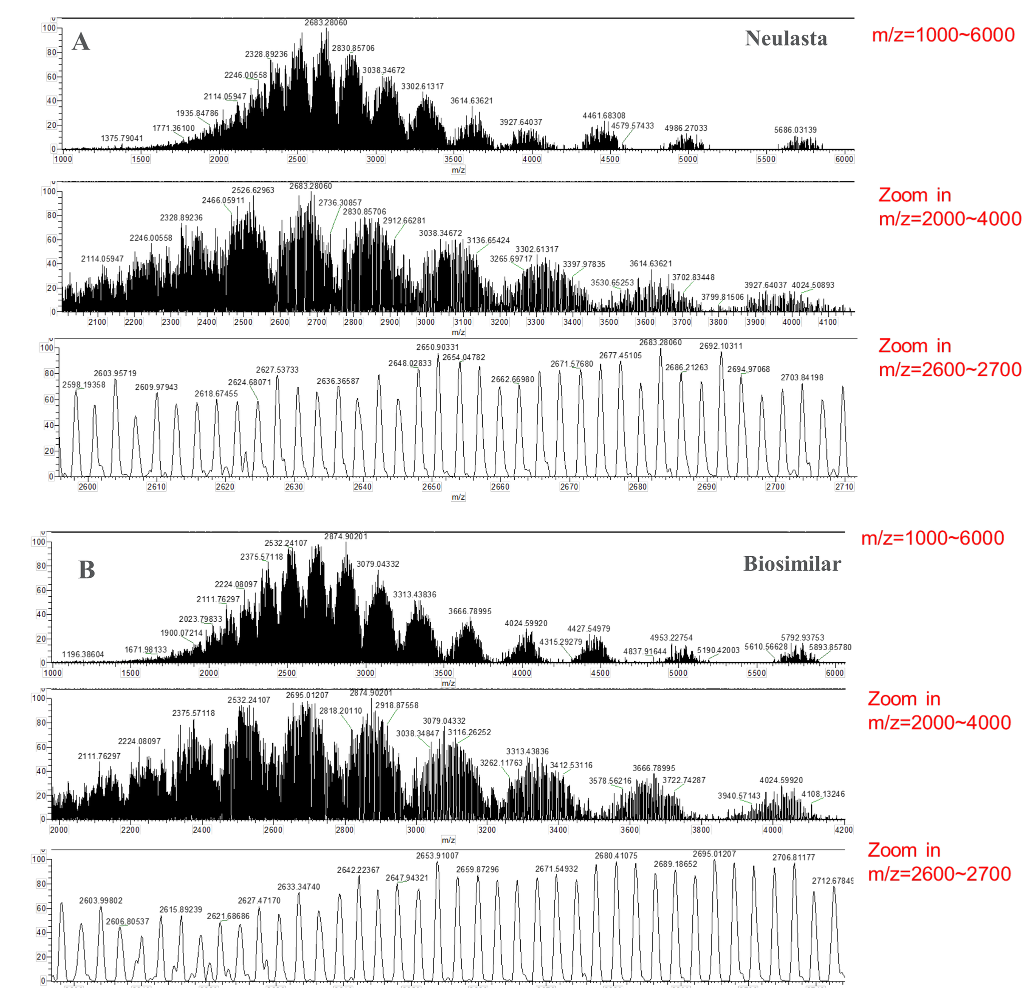


Figure 2. Comparison of combined raw spectra of Neulasta (A) and it's biosimilar (B)

Deconvolution results

The PEG heterogeneity makes molecular weight measurement a real challenge. On the Orbitrap platform, we could use high resolution to separate adjacent peaks in MS spectrum without sensitivity loss.

As an example, we determined the intact molecular mass of the molecule that contains a PEG modification having a degree of polymerization of n=483 (theoretical average molecular mass of 40,148.7594 Da). For the innovator, Neulasta, as the resolution changes from 15,000 to 120,000, the delta mass between theoretical and experimental masses was reduced from 2.26 Da to 0.35 Da, showcasing the power of high resolution (Figure 3). Figure 4 shows the deconvolution results of biosimilar using 15,000 resolution.

Figure 3. Deconvolution results of Neulasta. A, R=15,000. B, R=120,000, enlarged view of component nPEG=483.

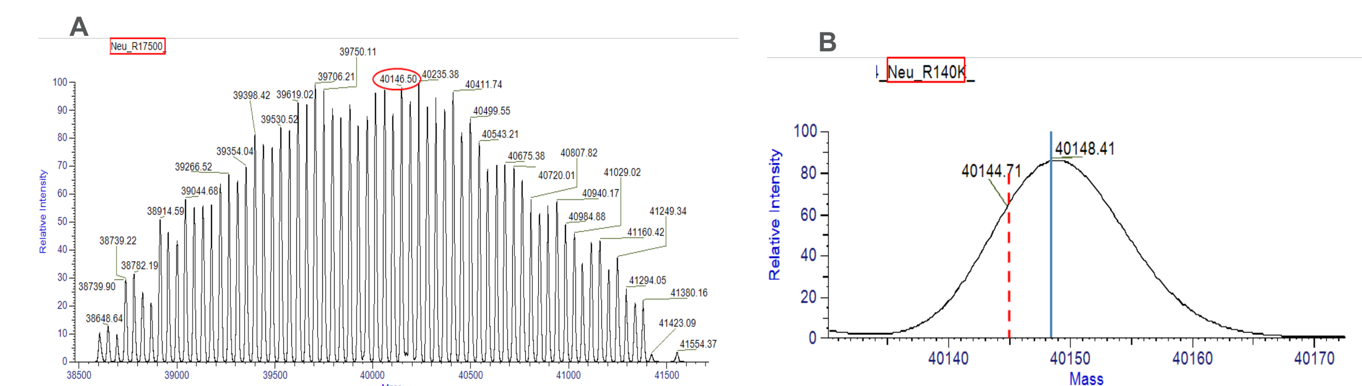


Figure 4. Deconvolution results of biosimilar (R=15,000).

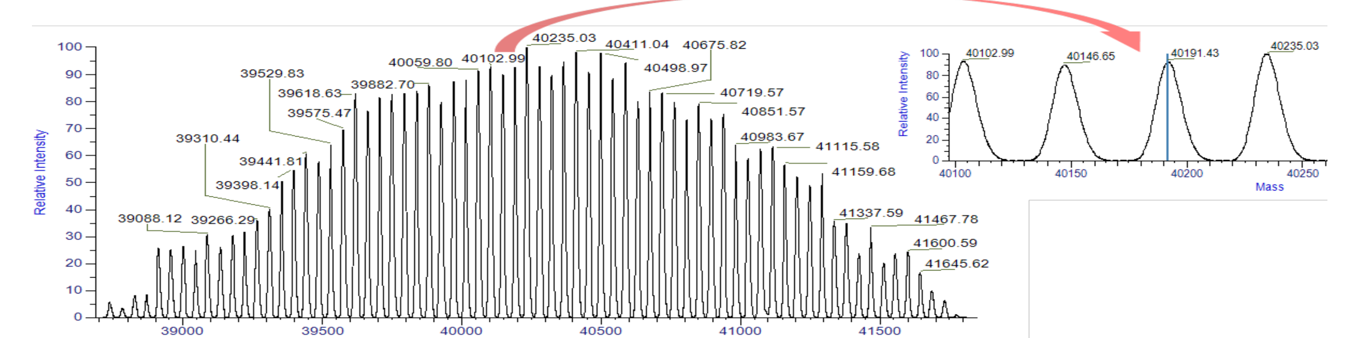
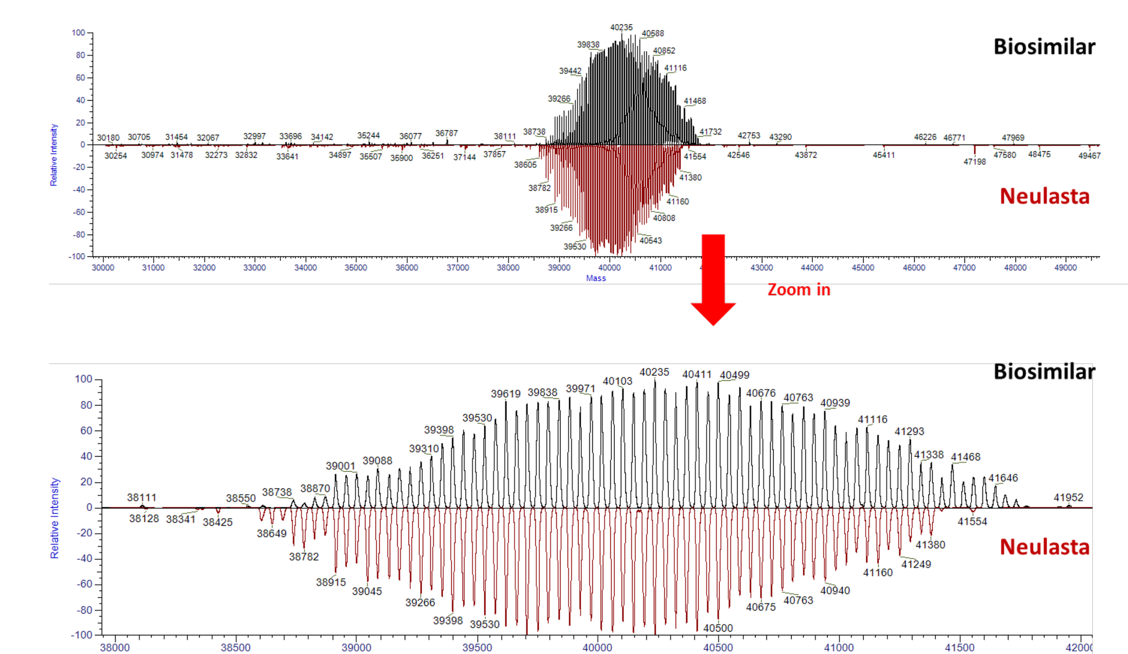


Figure 5. Mirror plot comparison between Neulasta and biosimilar deconvoluted spectra.



PEG polydispersity calculation

The covalent attachment of PEG (PEGylation) is a well-established approach to improve protein stability and solubility, to reduce renal clearance and proteolytic degradation, and to decrease immunogenicity and antigenicity, all of which contribute to an improved clinical efficiency and safety profile. To provide a safe and efficient drug and to meet the regulatory criteria for human use, however, the PEGylated protein needs to be thoroughly characterized.

Using mirror plot comparison, we found that the distributions of PEG modification are different between the innovator and biosimilar (Figure 5) – the distribution of biosimilar shifted to higher m/z range.

Based on the deconvolution results, number-average molecular mass (Mn), mass-average molecular mass (Mm) and PEG polydispersity (PD) were calculated using the formulas below:

Table 2. Mm, Mn and PD of both samples.

Sample	Mm	Mn	PD
Neulasta	39939.32174	39896.98880	1.0011
Biosimilar	40226.91232	40202.11802	1.0006

$$M_n = \frac{\sum M_i N_i}{\sum N_i} \qquad M_m = \frac{\sum M_i^2 N_i}{\sum M_i N_i} \qquad \mathrm{PD} = \frac{M_m}{M_n}$$

The calculated number-average molecular mass (Mn), mass-average molecular mass (Mm) and PEG polydispersity (PD) are shown in table 2.

It's easy to calculate the PD for Neulasta and its biosimilar. While we observe the difference in intact mass measurement, the calculated PD also showed difference between Neulasta and its biosimilar, 1.0011 and 1.0006 respectively, which might lead to potential safety and efficacy differences.

CONCLUSIONS

- Successfully apply the UHPLC-HRAM technology to profile the intact molecular weight and calculate PD of both innovator and biosimilar Neulasta.
- The high resolution and sensitivity of the Orbitrap mass analyzer benefits in complex mixture separation with great S/N ratio.
- PEG distribution difference was observed between these two samples.
- The subsequent peptide mapping analysis could provide more insights and details on PEG modification.

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