Assessing Biosimilarity by Monitored Multiple Critical Quality Attributes of an Intact Monoclonal Antibody Drug Using Orbitrap Native LC-MS

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ABSTRACT

Purpose: To demonstrate biosimilarity of two proteins by monitoring multiple critical quality attributes (CQAs) in a single high-throughput MS/MS experiment.

Methods: An intact protein-based peptide mapping experiment was performed on the trastuzumab biosimilar and innovator. The ProPac WCX-10 weak cation exchange column, the Vanquish H-Class UHPLC system, Q Exactive Orbitrap mass spectrometer, Chromeleon™ software, and BioPharma Finder™ software were employed. Tryptic digests were analyzed by Hypercube™ software.

RESULTS: 200 µl of trastuzumab 10 mg/mL was injected into the Q Exactive mass spectrometer via an Acclaim PEP mapping trap column, and the theoretical mass difference (ΔM) was determined for the main peak and isomerized peak (RT shift) using a 1ppm mass tolerance. We readily observed these differences between the innovator and biosimilar. We used BioPharma Finder™ software to analyze the mass-based differences between the biosimilar and control. Using the Isoform Multi-Attribute Method (MAM), we were able to determine high abundance species with high precision and sensitivity.

DEAMINATION / ISOMERIZATION PATHWAY DOMINATES TRASTUZUMAB CHARGE VARIANT MS PROFILE

Deamination and tryptic isomerization of the C-terminal lysine residue is the main degradative route, which may lead to variations in the CQAs of the biosimilar. The biosimilar was characterized by a monoisotopic mass of 148,382 Da, while the innovator was characterized by a monoisotopic mass of 148,382 Da. The theoretical mass difference of the biosimilar was 1 Da, while the innovator was characterized by a theoretical mass difference of 0 Da. Using the Isoform Multi-Attribute Method (MAM), we were able to determine high abundance species with high precision and sensitivity.

MODERATE TOLERANCE SLIDING WINDOW DECONVOLUTION (15 ppm) ALLOWS SENSITIVE AND CONFIDENT DETECTION OF LOW LEVEL CQA ISOFORMS

Confident detection of low-level charge variants (CQAs) in intact native mass is a difficult task, as these variants may not be distinguished from noise and background. In this study, we used a moderate tolerance (15 ppm) for the detection of charge variants. We employed a sliding window deconvolution approach to analyze the data. The theoretical mass differences were determined to be within the 15 ppm tolerance. We readily observed these differences between the innovator and biosimilar. We used BioPharma Finder™ software to analyze the mass-based differences between the biosimilar and control. Using the Isoform Multi-Attribute Method (MAM), we were able to determine high abundance species with high precision and sensitivity.

LOW TOLERANCE SLIDING WINDOW DECONVOLUTION (3 ppm) ALLOWS SUB-Da PRECISION FOR INTACT MASS MEASUREMENT

High precision of intact mass measurement is demonstrated by reproducible results. We injected 1 µL (20 µg) of trastuzumab samples in formulation buffer and acquired native MS data. The theoretical mass of the main peak and isomerized peak (RT shift) was determined using a 1ppm mass tolerance. We readily observed these differences between the innovator and biosimilar. We used BioPharma Finder™ software to analyze the mass-based differences between the biosimilar and control. Using the Isoform Multi-Attribute Method (MAM), we were able to determine high abundance species with high precision and sensitivity.

MATERIALS AND METHODS

Trastuzumab was generated by a country with a defined antibody product name. Native mass spectrometry was performed using a Thermo Scientific™ Vanquish™ H-Class UHPLC system. The Q Exactive-Orbitrap mass spectrometer was used to determine the theoretical mass differences of the biosimilar and innovator. The mass spectrometer was operated in the positive ion mode. The mass spectrometer was operated in the positive ion mode. The mass spectrometer was operated in the positive ion mode. The mass spectrometer was operated in the positive ion mode. The mass spectrometer was operated in the positive ion mode.

Figure 1: Platform for the native LC-MS method. Digestion efficiencies for our intact protein were determined using a 1ppm mass tolerance. We used BioPharma Finder™ software to determine the theoretical mass differences of the biosimilar and innovator. The mass spectrometer was operated in the positive ion mode. The mass spectrometer was operated in the positive ion mode. The mass spectrometer was operated in the positive ion mode. The mass spectrometer was operated in the positive ion mode. The mass spectrometer was operated in the positive ion mode.

CONCLUSIONS

We demonstrated that ion exchange native MS provides high precision intact mass measurement, enabling deep protein characterization and further method streamlining for downstream process development. Our method is suitable for the detection of subtle mass shifts and can be readily applied to a variety of other biological samples.

REFERENCES


TRADEMARKS/LICENSES

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