A PATsmart™ ZipChip® System Based CZE-MS Analysis for Quick Assessment of Biotherapeutics

Application Note

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Introduction

Monoclonal antibodies (mAbs) are an important class of biologics for the treatment of critical diseases. Assessment of the heterogeneity of mAbs is essential for product quality control, especially for biosimilars. A biosimilar is a recombinant protein that is therapeutically similar to the reference biologic or "innovator" molecule but is manufactured with a different bioprocess. It is important to investigate structural similarities and differences between the proposed biosimilar and the innovator molecule. In fact, a critical step in the approval process of biosimilars is to assess their functional and structural similarities with the innovator. Before in-depth characterization, a quick study of a mAb for fast assessment can help save time and reduce bottlenecks with mass spectrometer usage for busy analytical laboratories.

Capillary Zone Electrophoresis (CZE) coupled with mass spectrometry (MS) is well suited for the assessment of mAb heterogeneity with the ability to assess important product quality attributes, such as molecular weight, N-glycan profiling and C -terminal lysine variant differences between samples. While capable of in-depth mAb characterization as reported by Fussl *et al.* from NIBRT,[1] CZE-MS can also be used to quickly assess the quality and authenticity of a mAb.

A microfluidic CZE device, PATsmart™ ZipChip® System enables seamless integration of CZE with a variety of commercial mass spectrometers (For example, instruments from ThermoFisher Scientific, Sciex, and Bruker). For this work, a Thermo Scientific™ Orbitrap Exploris™ 240 hybrid quadrupole Orbitrap mass spectrometer (Exploris 240 brochure) was used in conjunction with the ZipChip System as shown in Figure 1.

Charge variant analysis of Cetuximab was used as an application example. Cetuximab samples were analyzed to compare structural similarity and heterogeneity (an innovator Cetuximab, and two biosimilars). Rapid assessment was accomplished with minimum method development for both the CE separation and MS detection methods.

Reagents and Methods

Reagents and ZipChip System Consumables. The Native Antibodies Kit* (Repligen) was used for all analyses. The background electrolyte (BGE) was modified with 4% DMSO (Alfa Aesar p/n 22914) following the ZipChip "Intact Charge Variant Analysis" protocol. The "high resolution native" (HRN) (Repligen) chip was used for sample analysis by the ZipChip Systems. The Cetuximab innovator and biosimilar 1 were obtained from LGM Pharma, the biosimilar 2 was obtained from Absolute Antibody.

Sample Preparation. Biosimilar 1 was directly diluted with the BGE to a final concentration of 1 mg/mL. Cetuximab and biosimilar 2 were buffer exchanged into Native Antibodies BGE using Bio-gel P-6 molecular weight cut-off filters (Bio-Rad). After buffer exchanging, Cetixumab and biosimilar 2 were further diluted to 2 mg/mL and 1 mg/mL respectively using the Native Antibodies BGE.

Figure 1. ZipChip System mounted to a Thermo Scientific™ Orbitrap 240 mass spectrometer.

*The Native Antibodies Kit has since been replaced by the Charge Variant Analysis Kit (p/n: 850-00052)

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Instruments. A ZipChip System (Repligen) was used as the microfluidic CZE inlet. The Mass Spectrometer used was the Orbitrap Exploris 240 MS with BioPharma Option (Thermo Fisher Scientific). It was operated with Thermo Scientific™ Xcalibur™ 4.2 SP1 software and controlled by Orbitrap Exploris Series 2.0. instrument control software.

ZipChip System Method Settings.

ZipChip protocol: Intact Charge Variant Analysis[2]
Field Strength: 500 V/cm
Injection volume: 1 nL
Pressure Assist Start Time: 0.5 min
Analysis time: 20 min

MS Method Settings.

Scan Range (m/z): 2,500–8,000
Resolution setting: 30,000 at m/z 200
Sheath gas: 2
In source-CID (V): 125
Normalized AGC Target (%): 300
RF Lens (%): 60
Microscans: 5

Data Processing. Charge variant separations were visualized using Thermo Scientific Xcalibur Qual Browser software. Data were processed using Thermo Scientific Biopharma Finder 4.1 software.

Results and Discussion

Cetuximab is a highly complex mAb due to the presence of four glycosylation sites—two in the Fc region and two in the Fab region. Unlike Fc glycans that are biantennary, the Fab glycans on Cetuximab are larger, more complex and sialylated. The presence of several sialic acid groups on the N-glycans, together with other modifications of the primary sequence are responsible for a very complex charge variant profile of Cetuximab. This structural heterogeneity makes it challenging to characterize.

In a recent study, the use of ZipChip-MS was demonstrated for an in-depth characterization of Cetuximab charge variants.[1] The authors identified over two hundred proteoforms with relative abundance values as low as 0.1% with respect to the main proteoforms, and noted that ninety-two of those proteoforms were isobaric or had a mass shift of less than 1 Da. These proteoforms would be extremely difficult to detect at the intact level without the ZipChip System charge variant separation. ZipChip-MS uses a standardized generic method that can be easily adopted for direct comparison of charge variant profiles between the innovator molecules and their biosimilars without having to spend time on optimizing method parameters.

The charge variant profile for innovator Cetuximab is shown in Figure 2. Similar to what has been reported,[1] eight different baseline resolved charge variants were detected in the base peak electropherogram. This charge-based front-end separation reduced the complexity of the MS spectra significantly, resulting in confident identification for various proteoforms.

Inspection of the mass spectra averaged across charge variant peaks revealed the presence of several different glycoforms belonging to each charge variant. The two basic variants were the result of C-terminal lysine truncation (+128 Da shift) on one or both heavy chains. The relative abundance of the basic species was ~15–20% of the main peak. The acidic variants were attributed to the presence of up to five N-glycolyl neuraminic acid (NGNA) residues (+145 Da shift) on the Fab glycans and were detected in relative abundances varying between ~3–50% with respect to the main variant peak in the base peak electropherogram.

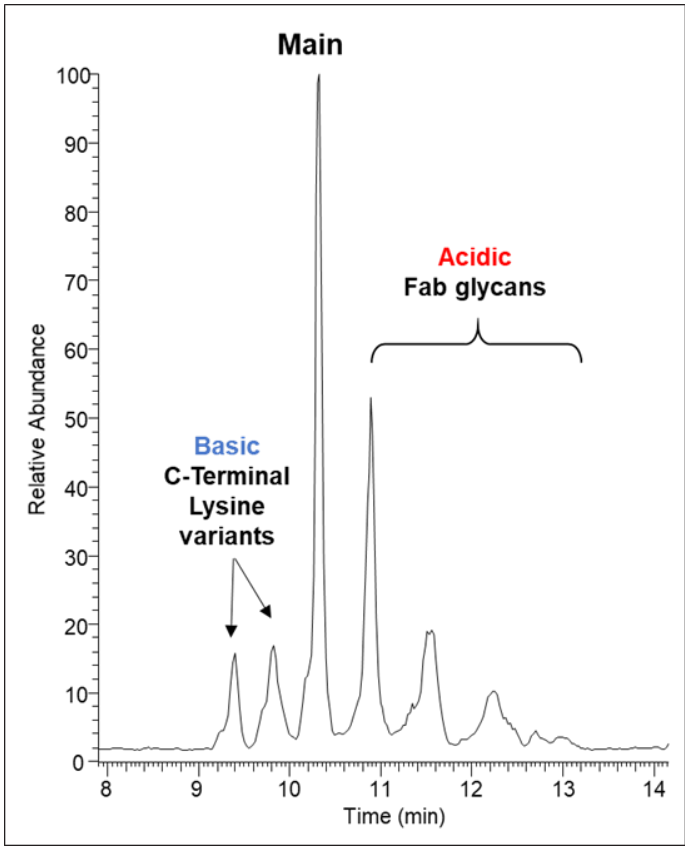

Figure 2. Charge variant profile of innovator Cetuximab

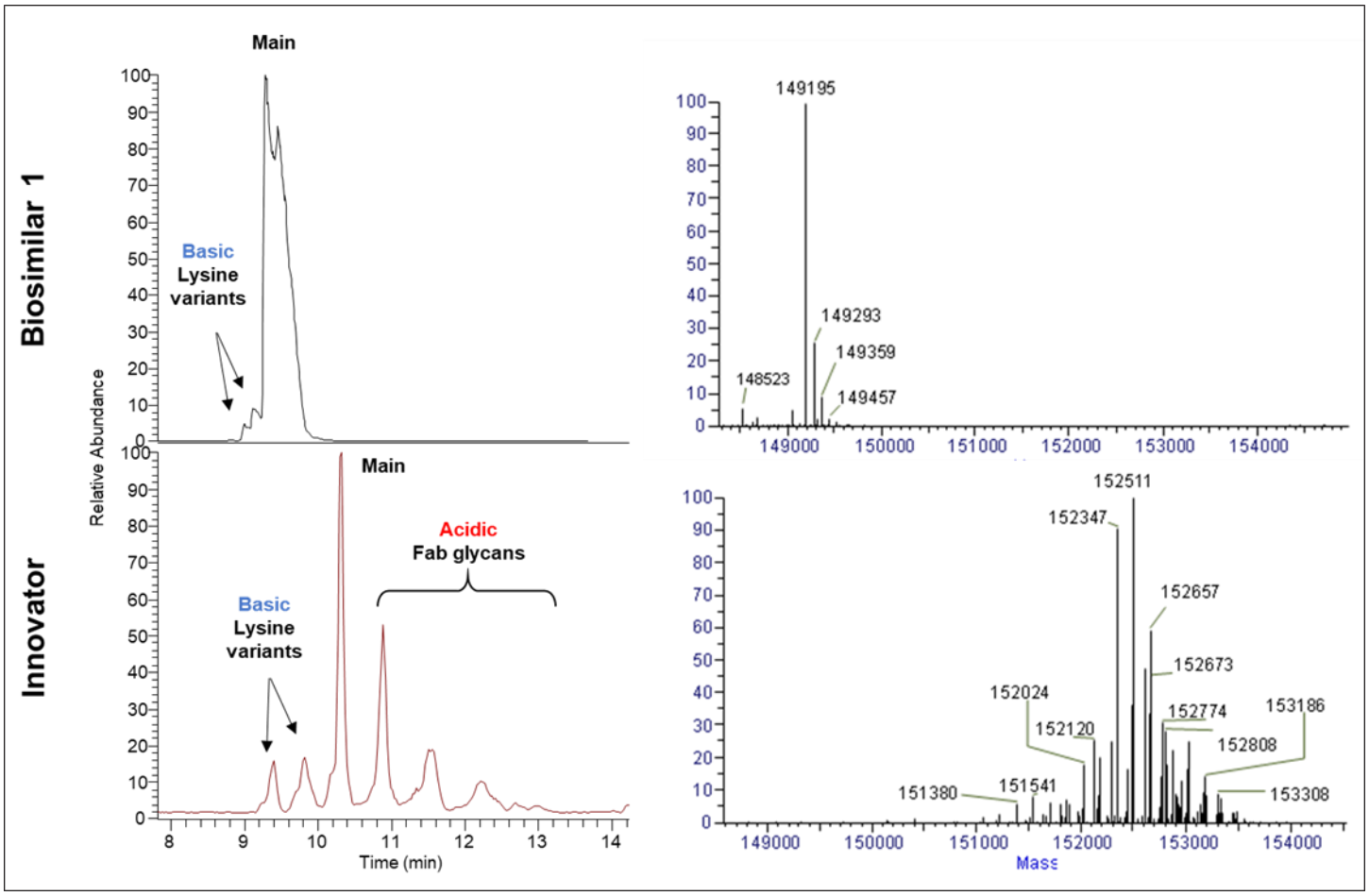

Figure 3. Comparison of charge variants profiles of Cetuximab biosimilar 1 (Top Left) vs Cetuximab innovator (Bottom left) and their respective deconvoluted mass spectra, Cetuximab biosimilar 1 (Top right), Cetuximab innovator (Bottom right).

A comparison of charge variant profiles and deconvoluted mass spectra for biosimilar 1 and Cetuximab is shown in Figure 3. The differences between the two samples were readily apparent from the separation profile as well as the deconvoluted mass spectra. Biosimilar 1 showed three charge variants, compared to the eight that were present in the innovator Cetuximab. Acidic peaks were not detected for biosimilar 1. The MS data indicated that similar to the innovator Cetuximab, the basic variants in biosimilar 1 were C -terminal lysine variants. The charge variants profile and reduced complexity in the MS data indicated that the Fab glycans present in the innovator were likely absent in biosimilar 1. Furthermore, the dissimilarity in the molecular weights of both samples provided an indication of differences in their amino acid sequence.

Figure 4 shows a comparison of charge variants profiles and deconvoluted mass spectra for biosimilar 2 and the innovator Cetuximab. Based on the charge variant profile alone, biosimilar 2 appeared to be similar to the innovator – in that the molecule showed both acidic and basic species in the base peak electropherogram. Six baseline-resolved variants were observed as opposed to eight variants in the innovator. There was a single basic variant with a relative abundance (<5% in comparison with the main variant) that was much lower than the relative abundance of the basic variants (~15–20%) for the innovator Cetuximab. The profile also showed four acidic variants with varying abundances. The deconvoluted mass spectral data revealed that there were differences between the two samples. For example, the molecular weight of the main variant was less than that of the innovator by 1541 Da indicating that they were different species. Biosimilar 2 shared only 30% of all the detected and deconvoluted masses with the innovator. These differences indicated that the two molecules had different glycosylation profiles and possibly different amino acid sequences as well.

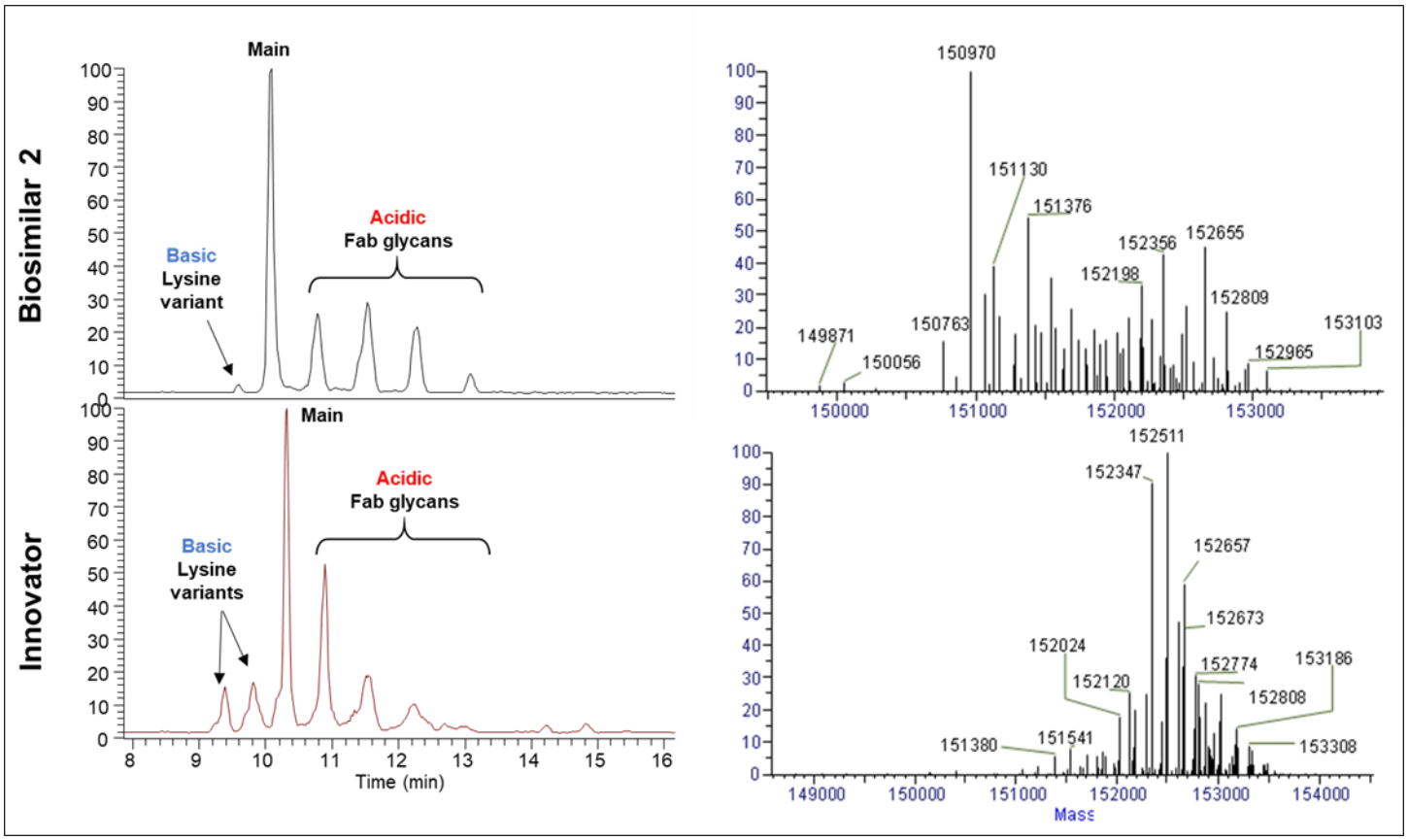

Figure 4. Comparison of charge variants profiles of Cetuximab biosimilar 2 (top left) vs Cetuximab innovator (bottom left) and their respective deconvoluted mass spectra, Cetuximab biosimilar 2 (top right), Cetuximab innovator (bottom right).

Conclusion

A CZE-ESI MS based rapid charge variant profiling of a Cetuximab innovator and two biosimilars under native conditions was performed with ZipChip System - Orbitrap Exploris 240 MS. Quick assessment and comparison of different mAb samples was easily achieved.

Of the 3 samples analyzed in this work, we were able to quickly assess and conclude that neither of the two biosimilars have the same charge variant profiles, or the glycoform profiles compared with the innovator, indicating that both are dissimilar from the Cetuximab innovator. This demonstrates that the ZipChip-MS is an easy and efficient way of quick analysis of biologics for quality control and assessing bio-similarity.

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References

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