Charge variant analysis of Cetuximab with ZipChip® on Bruker maXis II UHR-QTOF

Application Note

Yue Ju, Guillaume Tremintin
Bruker Scientific LLC, San Jose, US

Aditya Kulkarni
908 Devices, Boston, MA, US

Phanikumar Nasika[3], Arpana Dutta,
Biofusion Therapeutics Limited, Bangalore, India

Repligen Corp. now owns the life sciences PAT product portfolio of 908 Devices, Inc. Please contact Repligen for further inquiries.

Cetuximab has a complex glycosylation profile because of the presence of an additional sialylated glycan on the heavy chain. Many of these glycoforms have closely related masses making it impossible to perform a complete intact mass analysis with a typical reverse phase LC-MS measurement.

Abstract

A ZipChip® capillary zone electrophoresis system coupled to Bruker maXis II UHR-QTOF offers an alternative selectivity to separate charge variants prior to MS analysis and affords a more complete intact mass characterization without adding complexity or high salt eluents. Comparison between sialidase-treated and non-treated Cetuximab samples analyzed with this workflow confirms the separation of sialylated forms with this method.

Keywords

Charge variants, capillary electrophoresis, monoclonal antibody, glycosylation, sialylation, intact mass analysis, native MS

Figure 1. Comprehensive profiling of proteoforms present in the charge variants of Cetuximab is enabled by coupling ZipChip platform with Bruker's ultra-high resolution maXis II mass spectrometer.

Introduction

Charge variants related to glycosylation, C-terminal processing, deamidation or glycation often occur in mAb-based biotherapeutics. Routine charge variants analysis is conducted throughout manufacturing to evaluate quality attributes that could affect the final drug quality, safety, and potency. Monoclonal antibody Cetuximab has four N-glycan sites with two sites on each heavy chain located in the Fc and CH1 domains. Previous studies have shown that the Fab glycans are complex with multiple sialylated species.[1] The complexity of the glycan profile makes this molecule challenging to characterize in detail by traditional reverse phase LCMS analysis. However, several of the heterogeneities of interest impact the average charge of the molecule, for example incomplete enzymatic lysine truncation and sialic acid containing glycans. This makes methods with charge-based selectivity attractive for the evaluation of Cetuximab.

Traditional methods used for charge variants analysis of mAb-based biotherapeutics are commonly inter- faced with optical detection, with peak collection following off-line MS analysis. Capillary zone electrophoresis (CZE) coupled with high resolution mass spectrometry provides a powerful MS hyphenated alternative to identify charge variants present in complex biopharmaceuticals, which is critical to drug development and production in the BioPharma industry. ZipChip is a microfluidic device integrating CZE with electrospray ionization. In this work, ZipChip is coupled to Bruker's ultra-high resolution QTOF maXis II mass spectrometer for deep profiling of Cetuximab charge variants.

BRUKER | REPLIGEN

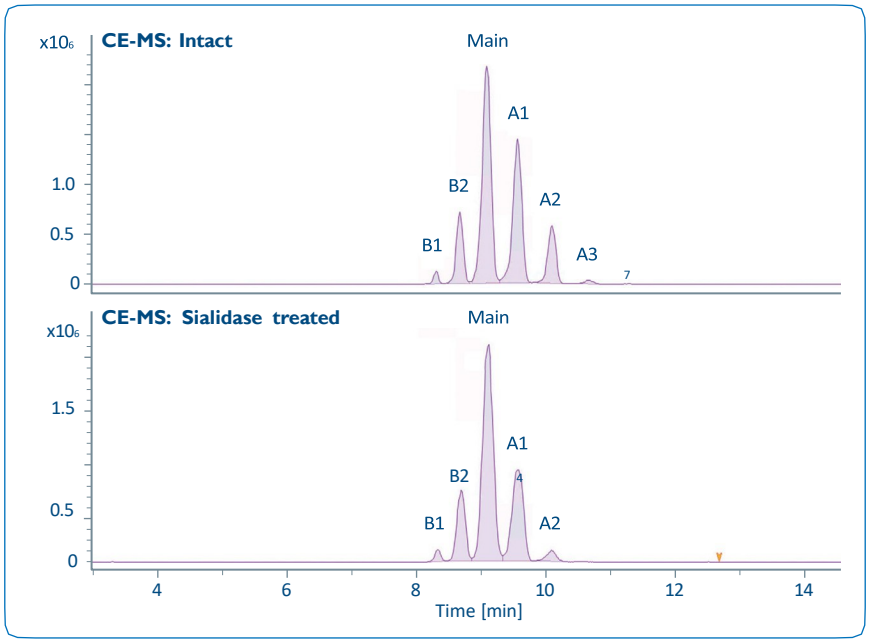

Figure 2. Charge variants separation of Cetuximab (top) and sialidase-treated Cetuximab (bottom). Six main species were separated: Basic 1 (B1), Basic 2 (B2), the main species (Main), and acidic variants acidic 1 (A1), acidic 2 (A2) and acidic 3 (A3). Sialidase treatment clearly reduces the intensities of acidic species including A1, A2, and A3.

Experimental

2 mg/mL Cetuximab was buffer exchanged (Micro Bio-Spin™ 6 Columns, Bio-Rad) into Charge Variant TOF BGE for intact Cetuximab analysis. The same Cetuximab was incubated with sialidase (Neuraminidase Au, QA bio) at 37°C for 1 hour before proceeding to buffer exchange into the same Charge Variant TOF BGE. “High resolution native” (HRN) type chips and the Charge Variant – TOF BGE Kit were used for all analyses. 500 V/cm field strength was applied over the chip and 1 nL sample was injected for each analysis. The whole process lasts for 15 min with other ZipChip parameters set as default for running intact charge variants. Bruker maXis II UHR -QTOF mass spectrometer was employed for the MS detection with settings tuned for high mass analysis. Bruker Data Analysis software was used for data processing and mass deconvolution.

Results and Discussion

Figure 1 and Figure 2 show the major components of this platform and a representative electropherogram of Cetuximab. ZipChip source can be seamlessly coupled to Bruker maXis II UHR-QTOF mass spectrometer for online CZE separation and MS detection with ultra-high resolution. The representative electropherogram shows well separated peaks for acidic and basic variants, which is essential for following in -depth MS characterization of the individual variant.

After incubation with sialidase, treated Cetuximab was analyzed under the same experimental and instrumental conditions as that of non-treated Cetuximab. Comparison of the electropherogram obtained for both treated and non-treated Cetuximab samples is shown in Figure 2. 6 major charge variant peaks were generated in this CZE separation of the non-treated Cetuximab within a ~3 min separation window. The separated peaks have excellent signal-to-noise ratio with minimal background noise, possibly due to the high desolvation efficiency from the low flow rate from the microfluidic ZipChip device. In this CZE-MS experiment, electrophoretic mobility of Cetuximab variant ions, under the positive applied field, is negatively correlated with increasing negative charge. The net charge for peak B1, B2, Main, A1, A2, and A3 are +2, +1, 0, -1, -2 and -3 in respect to the main variant. Relative intensities for A1, A2, and A3 over the main peak from intact Cetuximab CZE are clearly decreased compared to those from sialidase treated Cetuximab, which is coherent with past findings where the acidic variants are caused by sialylation.[2]

Figure 3 shows the averaged deconvoluted mass spectra for each of the 6 variants. Excellent mass accuracy was obtained for these variants, with 0.6 ppm, 1.3 ppm and -13 ppm for B1, B2, and Main, respectively, on the top panel, and -9 ppm, -7 ppm, and-20 ppm for A1, A2, and A3, respectively, on the bottom panel in Figure 3 (the most abundant peak in each spectrum was selected for mass error calculation). A positive 128 Da mass shift was observed from B1 to B2 with highly

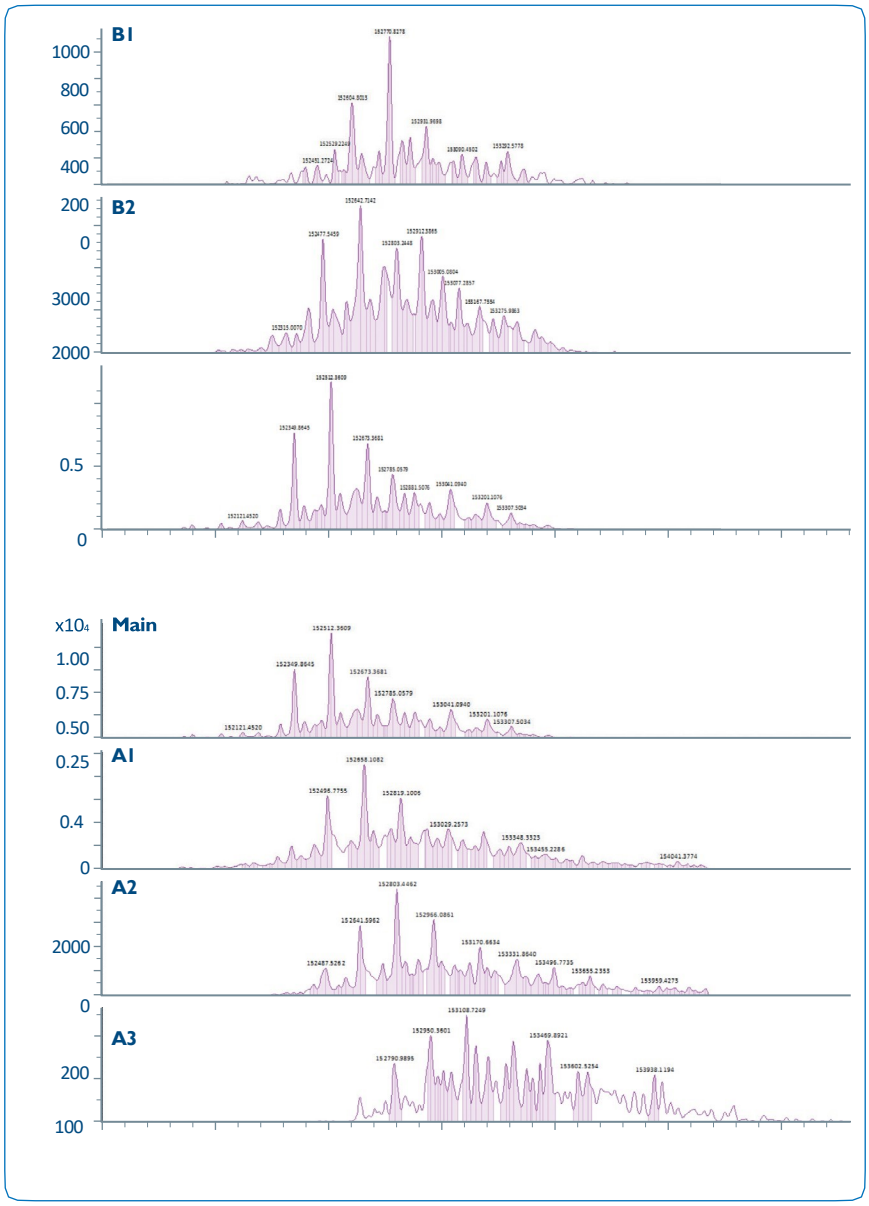

Figure 3. Deconvoluted mass spectra from corresponding charge variant peaks in the electropherogram as shown in Figure 2. High resolution mass measurements allow the glycoforms of Cetuximab to be measured. Basic variants related to incomplete lysine processing and acidic variants related to sialic acid addition are confirmed.

conserved MS pattern. A similar situation applied to B2 compared to Main MS. Incomplete C-terminal lysine processing was suggested to be the cause for basic variants. For acidic variants, compared to Main MS, positive mass shifts of multiple 145 Da was observed for A1, A2, and A3. Sialylation explains this shift with 145 Da being the mass difference between a galactose and a N-glycolyl neuraminic acid. Deconvoluted mass spectra of acidic variants in sialidase -treated Cetuximab indicate incomplete sialidase digestion (data not shown).

The robustness of this integrated platform is investigated by conducting multiple injections of Cetuximab-related samples from ZipChip source into the MS. Those injections were independent runs with a new injection from sample reservoir from individual sample vials. BGE refresh was adopted between each sample injection. This procedure maximizes the reproducibility by avoiding sample dilution and keeping the BGE pH constant.

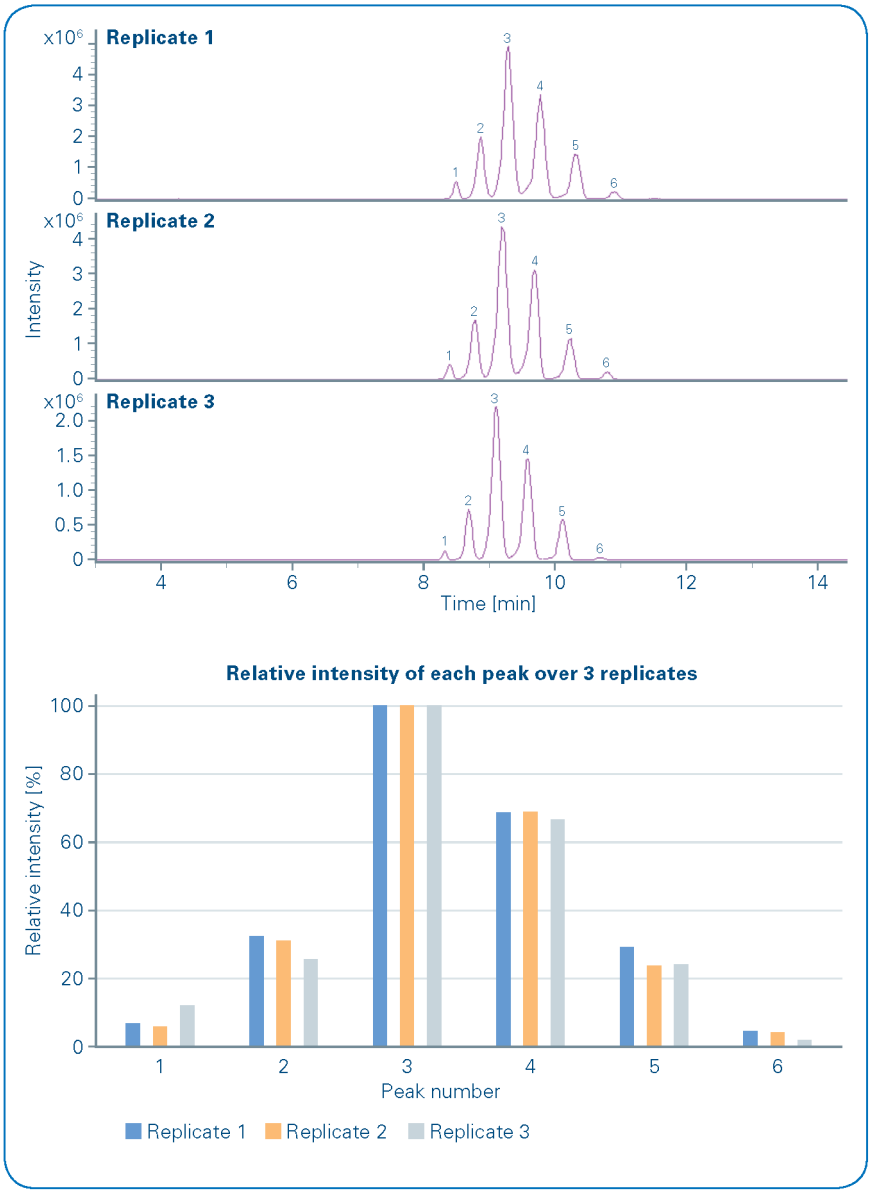

Figure 4. Electropherograms of triplicate injections of Cetuximab analyzed. The reproducibility of this workflow is assessed by comparing the relative intensities of peak 1 to peak 6 shown in the electropherogram over three independent injections. Good agreement in relative intensities are found in peak 2, 3, 4, and 5 with higher abundancies while lower abundant peak 1 and 6 show higher variations among runs.

Figure 4 shows the electropherograms of triplicate injections of intact Cetuximab. The reproducibility of this workflow is evaluated by comparing the relative intensities of peak 1 to peak 6 shown in the electropherogram over triplicate runs. Good agreement in relative intensities are found in peak 2, 3, 4, and 5 with higher abundancies while lower abundant peak 1 and 6 show higher variations among runs.

A similar evaluation of sialidase treated Cetuximab is shown in Figure 5. Overall variations among runs are higher in sialidase-treated runs compared to those in intact Cetuximab, possibly due to solution changes brought in by addition of the sialidase enzyme and buffer.

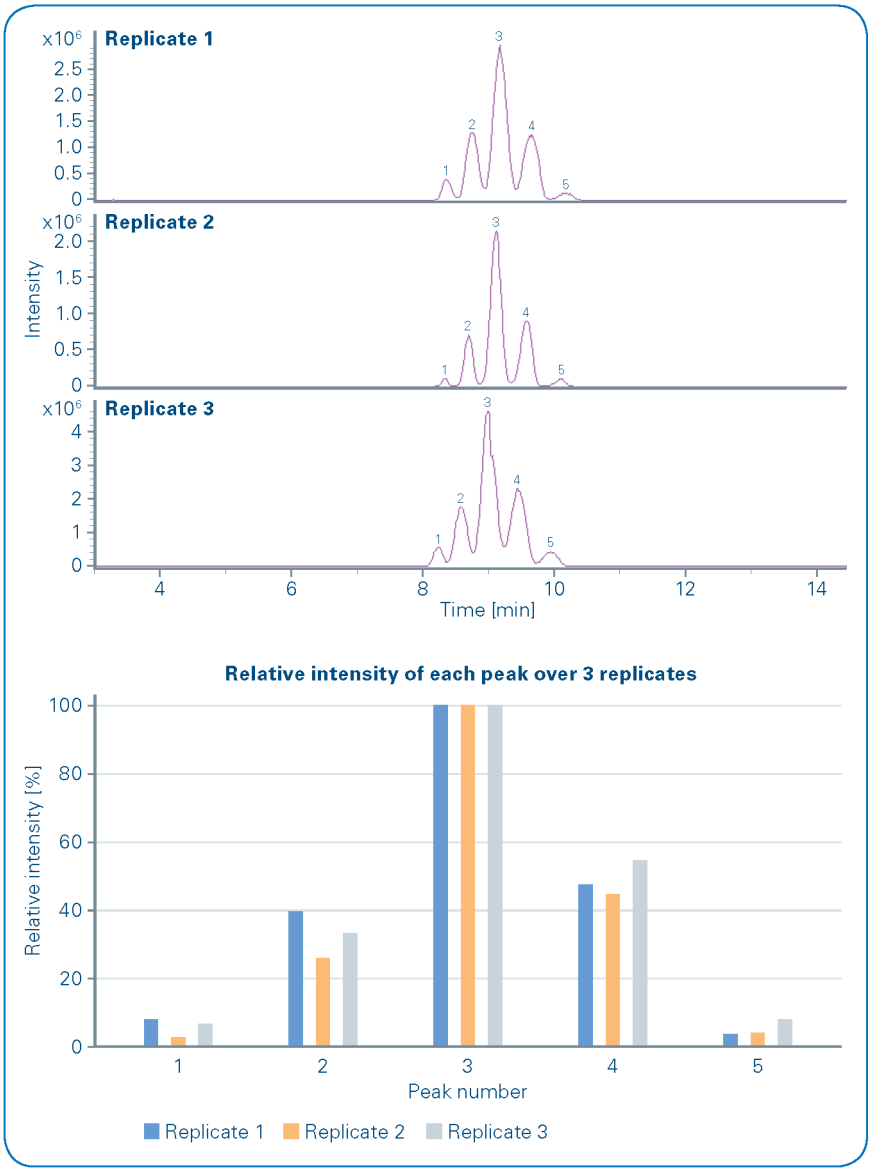

Figure 5. Electropherograms of triplicate injections of sialidase-treated Cetuximab analyzed. The reproducibility of this workflow is assessed by comparing the relative intensities of peak 1 to peak 5 shown in the electropherogram over three independent injections. Overall variations among runs are higher in sialidase-treated runs, possibly due to solution changes brought in by addition of the enzyme and buffer.

Conclusion

- ZipChip combined with maXis II offers a highly selective platform for charge variants analysis of mAbs and glycoproteins.
- Hyphenation of CZE and high-resolution MS provide an easy tool to directly identify sequence variants by intact mass without requiring sample enrichment or a complex separation scheme.
- The selectivity offered by CZE offers a more comprehensive intact mass characterization by resolving heterogeneities with otherwise overlapping mass.

Learn More

Looking for more information? Click the link or scan the QR code below.

www.bruker.com/maxis

References

1. Ayoub D et al. (2013). Correct primary structure assessment and extensive glyco-profiling of cetuximab by a combination of intact, middle-up, middle-down and bottom-up ESI and MALDI mass spectrometry techniques, mAbs, 5:5, 699-710, DOI: 10.4161/mabs.25423
2. Füssl F et al. (2020). Comparative Elucidation of Cetuximab Heterogeneity on the Intact Protein Level by Cation Exchange Chromatography and Capillary Electrophoresis Coupled to Mass Spectrometry, Anal. Chem. 92, 5431−5438

For Research Use Only. Not for Use in Clinical Diagnostic Procedures.

Contact

Repligen Corporation
685 Route 202/206
Bridgewater, NJ, USA 08807

analytics-support@repligen.com

(908) 707-1009

REPLIGEN