# Charge variant analysis of Cetuximab with Chip Based CZE-MS

ASMS 2021

**Yue Ju[1], Guillaume Tremintin[1], Aditya Kulkarni[2]**

[1]Bruker Scientific LLC., San Jose, CA, US
[2]908 Devices, Boston, MA, US

## Introduction

Charge variants related to glycosylation, C-terminal processing, deamidation or glycation often occur in mAb based biotherapeutics. Routine charge variant 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 domain. Capillary zone electrophoresis (CZE) coupled with high resolution mass spectrometry provides a powerful MS hyphenated alternative to identify charge variants present in complex biopharmaceuticals. 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.

## Methods

2 mg/mL Cetuximab was buffer exchanged (Micro Bio-Spin™ 6 Columns, Bio-Rad) into Charge Variant BGE (908 Devices Inc.) 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 BGE.

"High resolution native" (HRN) type chips and the Charge Variant - TOF BGE Kit were used for all analyses. The ZipChip parameters were set as default for running intact charge variants. Bruker Data Analysis software was used for data processing and mass deconvolution.

## Results

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. 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.

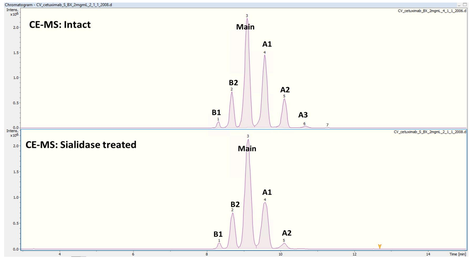


**Figure 1**. Charge variant separation of Cetuximab (***top***) and sialidase treated Cetuximab (***bottom***). Six main species were separated: Basic 1 (B1), Basic 2 (B2), the main species (Main) and acidic variant acidic 1 (A1), acidic 2 (A2) and acidic 3 (A3). Sialidase treatment clearly reduces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

Excellent mass accuracy was obtained for these variants (Figure 2), with 0.6 ppm, 1.3 ppm and -13 ppm for B1, B2 and Main respectively (left), and -9 ppm, -7 ppm and -20 ppm for A1, A2 and A3 respectively (right). The most abundant peak in each spectrum was selected for mass error calculation.

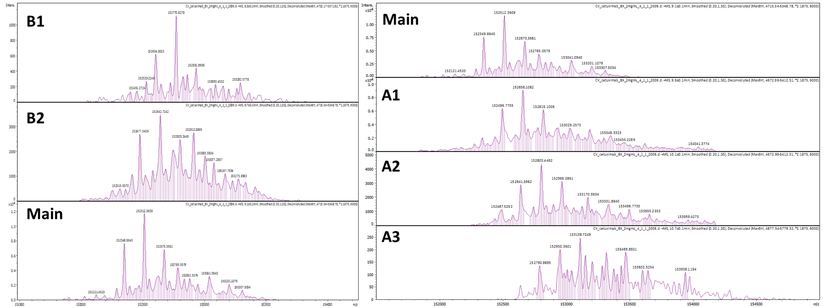


**Figure 2.** Deconvoluted mass spectra from corresponding charge variant peaks in the electropherogram as shown in Figure 1. High resolution mass measurements allow the glycoforms of Cetuximab to be measured. Basic variants related to incomplete lysine processing and acidic variants related to sialic acid addition are confirmed.

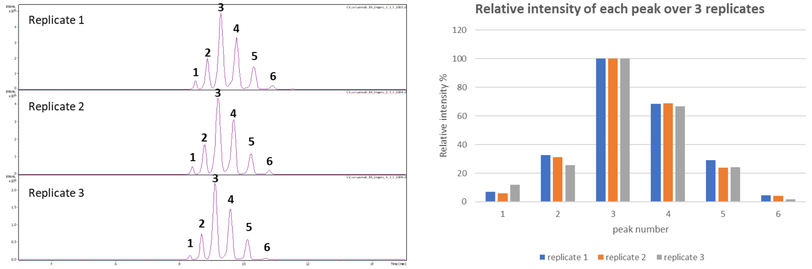


**Figure 3**. Electropherograms of triplicate injections of Cetuximab analyzed. The reproducibility of this workflow is accessed by comparing the relative intensities of peak 1 to peak 6 shown in the electropherogram over three independent injections. Good agreement in relative intensities are found in peak2, 3, 4 and 5 with higher abundancies while lower abundant peak 1 and 6 show higher variations among runs.

A positive 128 Da mass shift was observed from B1 to B2 with highly conserved MS pattern. 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. 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.

## Conclusions

- 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

BioPharma