Characterization of charge heterogeneity of monoclonal antibodies using a high resolution native chip coupled to mass spectrometry

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INTRODUCTION

- Cellular expression and purification processes can lead to charge heterogeneity in a biotherapeutic sample[1]
- Modifications can include charged glycan structures such as sialic acids, differential lysine (K) clipping at the C-terminus, and deamidation events[1]
- Monitoring these product quality attributes (PQA) is often required as part of the characterization to ensure products meet expected criteria
- Traditional approaches use capillary electrophoresis (CE) as a standalone technique with optical detection
- More recently, the focus is on coupling the powerful separation of CE systems with mass spectrometry enabling a better understanding of the nature of charged variants

Key features of the ZipChip system coupled to the TripleTOF® 6600 LC-MS/MS System

- Fast, reproducible and high quality data acquisition with limited optimization of the CE and MS method
- Little sample prep due to on chip desalting and removal of detergents without carry-over issues increasing the throughput
- Great separation of different charged isoforms with MS compatible, ready-to-use buffer systems allowing for an additional dimension of information via MS

MATERIALS AND METHODS

Three monoclonal antibodies (mAb): the NISTmAb reference material 8671, trastuzumab and adalimumab were used. Separation was performed using the ZipChip system (908 Devices Inc.) with a charge variant TOF Kit and an interface for the SCIEX TripleTOF® 6600 System. Data were processed using Sciex OS Software.

Figure 1: Schematic Workflow for Charge Heterogeneity Analysis with the ZipChip and the TripleTOF® 6600 System.

RESULTS

A major challenge for interfacing CE with MS has been the usage of buffers composed of high salt and detergent, which are not compatible with MS analysis. Here, a commercially available kit was used, resulting in great separation power for charge variants of each of the mAb (Fig. 4) while maintaining great raw data quality (Fig. 2).

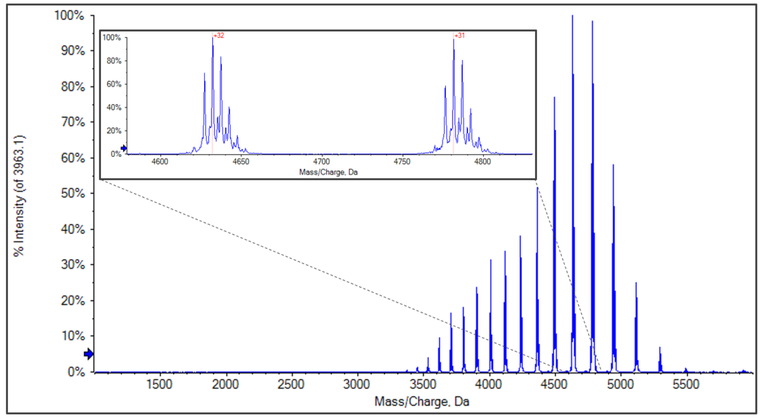

Figure 2: Raw Data From Major Form of NISTmAb (Migration Time = 7.15 min) .

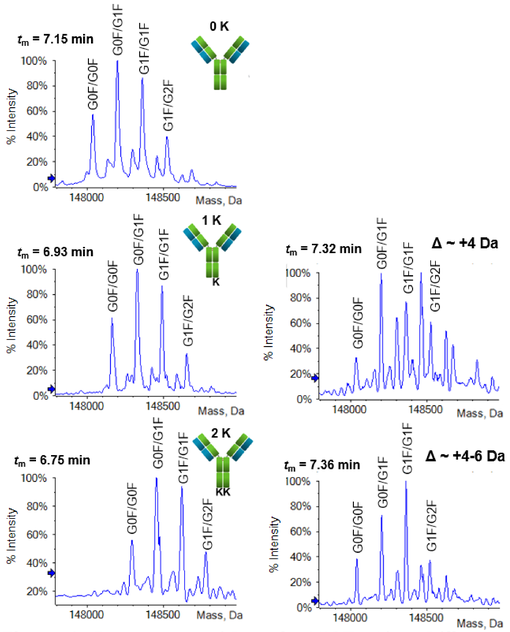

Figure 3: Reconstructed Data for NISTmAb at Different Migration Times.

The raw data for each of the peaks in the total ion electropherogram (TIE) were reconstructed (Fig. 3) and compared against the theoretical molecular weight (MW). For all mAbs the major form could be linked to the loss of two C-terminal K residues (0 K). Basic variants with one C-terminal K residue (1 K) and two C- terminal K residues (2 K) could be identified based on reconstructed masses. In addition, peaks with longer migration times than the main species were observed which were linked to acidic species of the sample (Fig. 3 right).

The reconstructed mass showed small mass shifts for these acidic forms compared to the main form, which can be due modifications. The migration times of the protein forms were in alignment with the MS findings: variants with a higher iP migrate faster than species with a lower iP meaning more basic variants (additional terminal K) will be detected first, followed by variants with less positive charges/lower pI. For the Adalimumab sample an additional peak was observed: The MS information revealed a MW of ~ 47 kDa. It is possible that it is a degradation product of the biotherapeutic.

CONCLUSIONS

- The ZipChip system coupled to the TripleTOF® 6600 System is a turnkey solution providing a great separation power for a variety of antibodies' charge variants
- Excellent raw data quality from the TripleTOF® 6600 System allowed for assignment of different glycoforms, basic and acidic variants
- The high resolution TOF data provides the possibility for detection and characterization of unexpected impurities

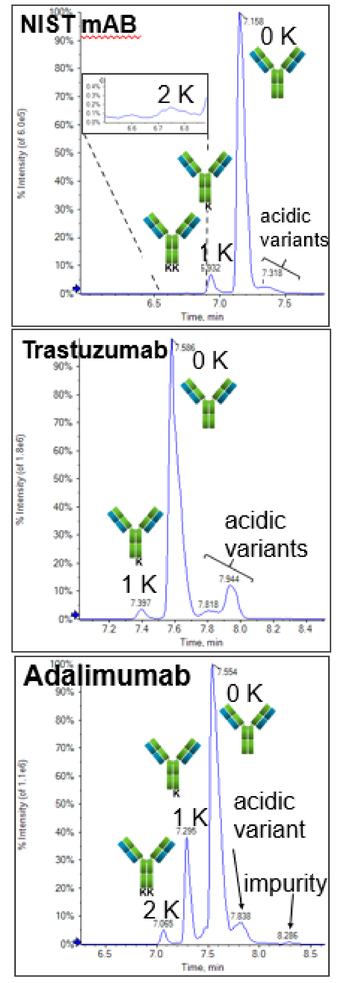

Figure 4: TIE for Three mAbs.

REFERENCES

1 Hutanu A, Kiessig S, Bathke A, et al. Application of affinity capillary electrophoresis for charge heterogeneity profiling of biopharmaceuticals. *Electrophoresis*. 2019;40(22):3014–3022. doi:10.1002/elps.201900233.
2 Characterization of Charge Heterogeneity of Monoclonal Antibodies Using Capillary Electrophoresis (CE) coupled to Mass Spectrometry (MS). SCIEX Technical Note RUO-MKT-02-10949-A.

TRADEMARKS/LICENSING