Charge Heterogeneity Analysis of Intact NIST mAb Using CESI-MS and Neutral OptiMS Cartridge

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Key Features

- High sensitivity CE-MS (Capillary electrophoresis-Mass Spectrometry) methodology to identify intact mAb (monoclonal antibody) variants
- Separation of basic and acidic NIST mAb charge variants using neutral coated OptiMS cartridge on a SCIEX CESI 8000
- Charge variants and major glycan species identification using SCIEX TripleTOF® 6600 system
- Method showcases the high resolving power and the high sensitivity of CESI-MS workflows for low level impurity identification in monoclonal antibodies samples

Introduction

Monoclonal antibodies (mAbs) are one of the most dominant biotherapeutics. Unlike chemically synthesized drugs, these biotherapeutics are cell originated and are subject to many different post translational modifications (PTMs) during the process of manufacturing and storage. The most common PTMs such as C-terminal lysine truncation, deamidation, glycation and methionine or tryptophan oxidation usually lead to changes in the protein's isoelectric point (pI), which presents as multiple charge variants in charge purity assays. A comprehensive characterization of the charge variants in the mAb population is crucial, as these variants reportedly affect the safety and efficacy of the biotherapeutics.

Figure 1. CESI 8000 Plus coupled with SCIEX Triple TOF 6600 used in this workflow.

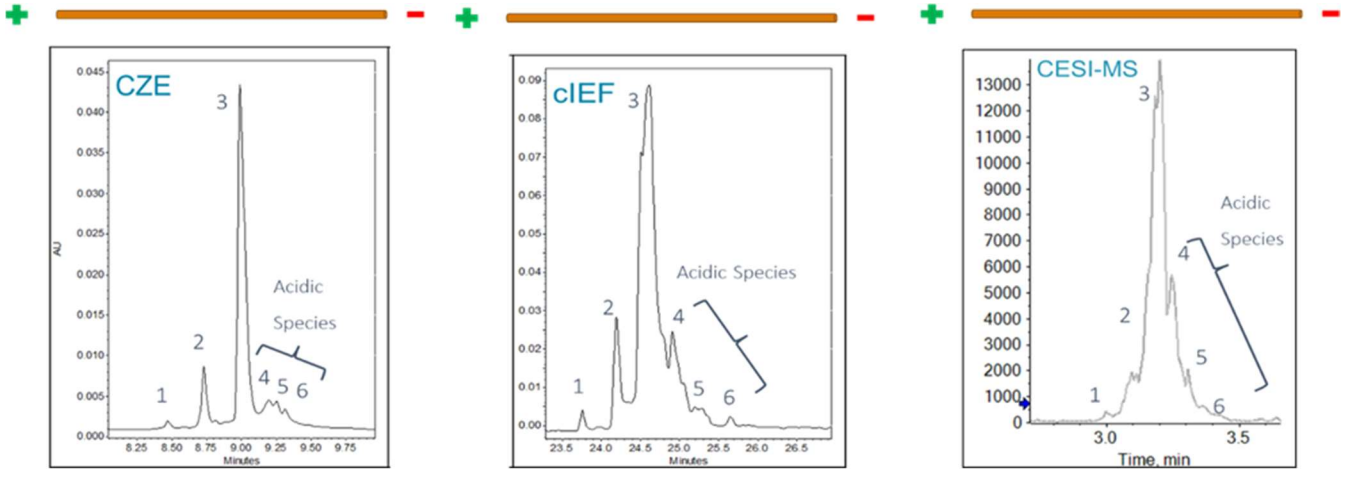

Figure 2. a) Charge variants analysis of NIST mAb on a PA800 Plus using platform CZE method; b) Charge variants analysis of NIST mAb on PA800 plus using SCIEX cIEF kit; c) Charge variants analysis of NIST mAb using CESI-MS (CZE) with neutral OptiMS cartridge.

Intact level characterization of mAbs is gaining popularity in the biopharmaceutical industry. Such analysis procedures can be cost-effective as they save time and labor due to minimal sample preparation. Here, sample material does not need to undergo chemical reduction and/or enzymatic digestion before analysis, which reduces the possibility to induce sample alterations due to less sample preparation. Finally, it allows scientists to evaluate the target molecules in a much earlier stage of the development process.

Reversed phase-Liquid chromatography-mass spectrometry (RP-LC-MS) has become a primary technique for antibody characterization and, particularly in early stage development as MS can provide critical insights on the protein integrity and purity. However, the charge variant analysis of intact biopharmaceuticals by conventional RP-LC-MS is not possible. As RP-LC does not routinely separate species of the intact mAb population analysts can only obtain averaged deconvoluted masses of the entire population. The spectra overlap makes it challenging to deconvolute the data and identify the individual species (Figure 3). Unfortunately, this can lead to miss-assignment and missing information of PTMs.

Alternatively charged based separation techniques can be employed to resolve charge variants in the mAb population. As Capillary Electrophoresis (CE) techniques separate based on differential migration of species under an applied field it is well suited towards charge variant analysis. Two platform CE separation mechanisms with optical detection are routinely used in the entire lifecycle of biotherapeutic development to control and monitor charge variants that can impact product quality. The first, Capillary Isoelectric Focusing (cIEF) separates charge variants based on their pI in a pH gradient. The second approach, Capillary Zone Electrophoresis (CZE) separates based on the charge to hydrodynamic size/shape of the molecule. As the size distribution of variants in the mAb population is less than 5% CZE separate solely based on charge.2 However, while the charge variants can be separated the characterization of these peaks remains challenging and time consuming. The process typically involves peak fractionation followed by a RP-LC-MS identification of each fraction.

SCIEX CESI 8000 Plus system allows for the direct coupling of CE and mass spectrometer (MS) providing the possibility of direct identification of peaks separated by CZE. The OptiMS cartridge used on the system achieved the integration of high efficiency and ultra-low flow CE with electrospray ionization (ESI) into a single dynamic process within the same device. In addition, CESI-MS offers a high resolution CE and is known for exquisite sensitivity levels due to the nanoflow regime in which it operates.

This technote demonstrates a short CESI-MS method which provides comparable charge variant separation to the widely used CZE and cIEF platform methods and simultaneously provides information on peak identity and major glycan forms in a single analysis. NIST mAb has been well characterized and detailed information about it is readily available for public. Here, we also used NIST mAb as the test molecule.

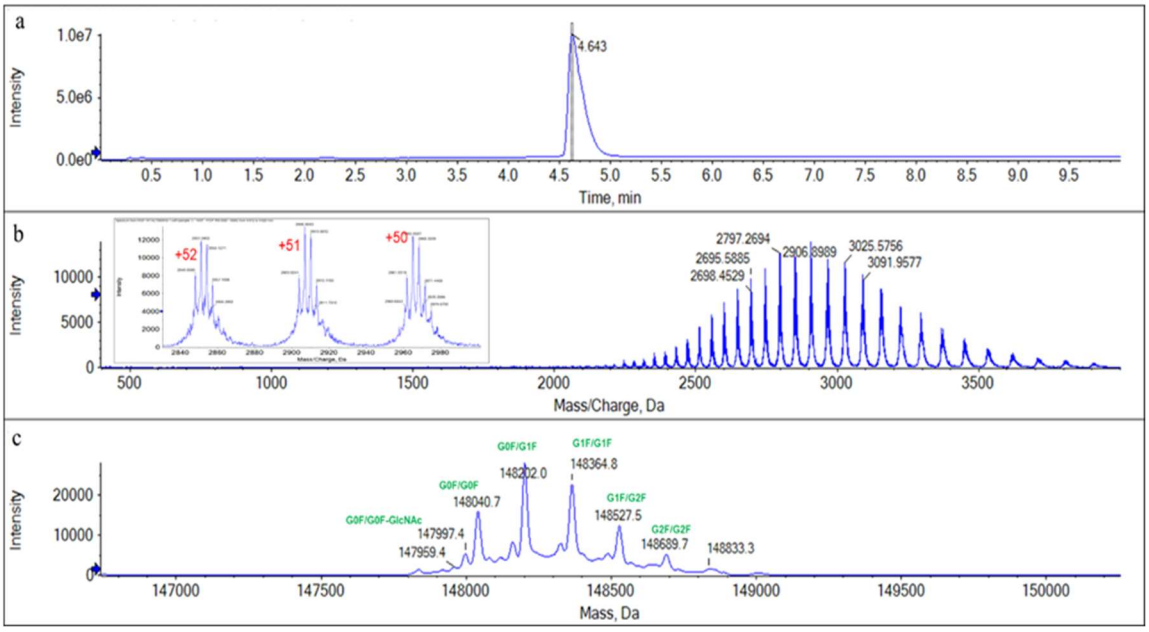

Figure 3. LC-MS intact analysis results of NIST mAb a) Total Ion Chromatograph (TIC); b) MS spectrum of NIST mAb; c) Deconvoluted intact mAb MS and major glycan species detected.

Materials and Methods

Buffers and Reagents

1. Background Electrolyte Stock (BGE stock): 10% Acetic Acid prepared by mixing 1 mL of Acetic Acid with 9 mL of ddi water daily
2. Background Electrolyte(BGE): 0.3% Acetic Acid prepared from BGE stock daily.
3. Sample Cleaning Solution: 50 mM Ammonium Acetate pH 6.0
4. Sample Buffer Stock: 50 mM Ammonium Bicarbonate pH 8.2 (pH adjusted by adding 50 mM acetic acid into 50 mM Ammonium Bicarbonate)
5. Sample Buffer: 5 mM Ammonium Bicarbonate with 20% methanol prepared by mixing 100 µL of sample buffer stock(prepared in #4) with 200 µL of methanol and 700 µL of ddi water.
6. Capillary Cleaning Solution: 0.1N HCl

Sample Preparation

NIST mAb was buffer exchanged into 50 mM Ammonium Acetate pH 6.0 with Amicon 10K filter. The protein concentration should be above 5 mg/mL. The cleanup samples can be stored at 4ºC for up to 2 days. The sample was then diluted to a final concentration of 0.3 mg/mL with sample buffer before CESI injection.

CESI Separation Conditions

Intact NIST mAb was separated using a CESI 8000 High Performance Separation and ESI Module (SCIEX) equipped with a Neutral OptiMS cartridge (P/N B07368) held at a temperature of 20 ºC. The BGE and conductive liquid consisted of 0.3% acetic acid. A sample plug was injected with 1.5 psi for 15 s followed by a BGE injection of 1.0 psi for 10s. The CESI separation was performed at 30.0 kV with 0 psi for 12 min, followed by 30.0kV with 0.5 psi for 10 min. The MS acquisition was triggered at 10min of separation. A 5 min ramp down event was at the end to lower down the voltage to 1 kV.

MS Conditions

A SCIEX TripleTOF 6600 system with a NanoSpray III source and CESI adapter (P/N B07363) was used. The curtain gas was set at 5 psi and the temperature of the interface was set at 70ºC. The ESI voltage was set as 1650 V (calculated as minimum sprayer voltage of the cartridge +150V). The mass range employed was 2000-6000 m/z, the collision energy (CE) was at 70, the declustering potential (DP) was set at 190, accumulation time was 0.5s and time bins to sum was set at 80.

Data Analysis

SCIEX PeakView® 2.2 and BioTool Kit were used for data analysis.

Results and Discussions

In this example, the NIST mAb was employed to demonstrate the capabilities of the CESI-MS separation and identification.

Figure 3 illustrated the challenges using the RP-LC-MS for charge variant analysis of intact mAb. Single peak is observed (Figure 3a) and the MS spectrum (Figure 3b) and deconvoluted MS (Figure 3c) show only the dominant species. Minor variants with critical PTMs cannot be identified in this workflow.

Figure 2a and 1b illustrate three different approaches to achieve a charge variants separation of the NIST mAb. Figure a and b employ CZE and cIEF respectively, both approaches use UV detection (data collected on the PA800 Plus equipped with UV detector see corresponding user guide and technotes for conditions)[1, 2]. In both figures, two basic peaks (Peak 1 and 2) and three groups of acidic peaks (Peak 4, 5 and 6) were observed in addition to the main peak (Peak 3). The separation of Figure 2c was achieved using the CESI-MS workflow, the same number peaks and their relative distributions were observed as the between all three approaches. The deconvoluted spectra of each peak are shown in Figure 4. Known theoretical masses of the NIST mAb was used to assign the identity of the main peak and glycoforms.3 The mass shift between peaks was used identify the PTMs.

The CE separation conditions used, allowed for the basic (positively charged) or species with higher pI to migrate faster followed by the main peak and then the acidic species of less charge or lower pI. Indeed, the electropherogram on Figure 1c shows that, Peak 1 is identified as NIST mAb with two C-terminal lysine residues and Peak 2 is identified as NIST mAb with one C-terminal lysine residue. Peak 3 is the main peak, while Peak 4 Peak 5 and Peak 6 show small sequential increases in mass, consistent with increased acidity possibly due to increasing number of deamidation. In addition, four major glycoforms can be assigned for each peak in the electropherogram and the most abundant glycoform is G0F/G1F, consistent with published data on NIST mAb (Figure 4).[3,4]

The CESI-MS workflow not only provides comparable separation and allows direct identity assignment of charge variants peaks,

but also allows detection of low molecular weight (LMW) fragment impurities in the sample. Four different species were separated and identified. [3] This work highlights the resolving power and the high sensitivity of CESI-MS workflow for charge variants analysis of mAb populations and the detection and identification of intact and fragment low level impurities (Figure 5).

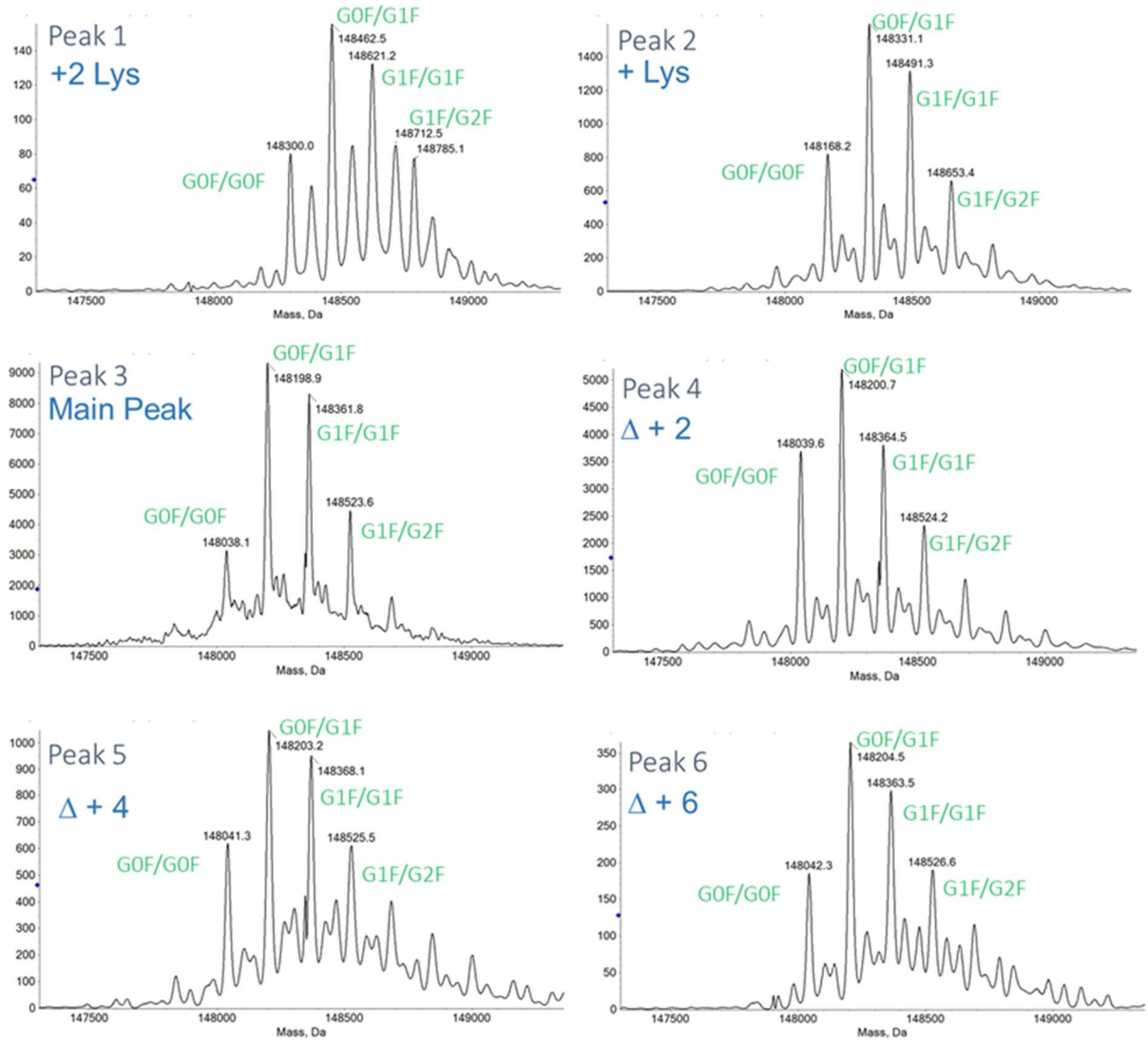

Figure 4. Deconvoluted MS spectra of each peak labeled in CESI-MS profile shown in Figure 2c. Peak 3 matches the reported MS on NIST and presented the highest amount, therefore identified as the Main Peak. Caption in blue at upper left corner of each peak indicates the MS sift compared to Main Peak calculated based on the G0F/G1F species. Caption in green above each deconvoluted MS indicates the associated glycan pattern.

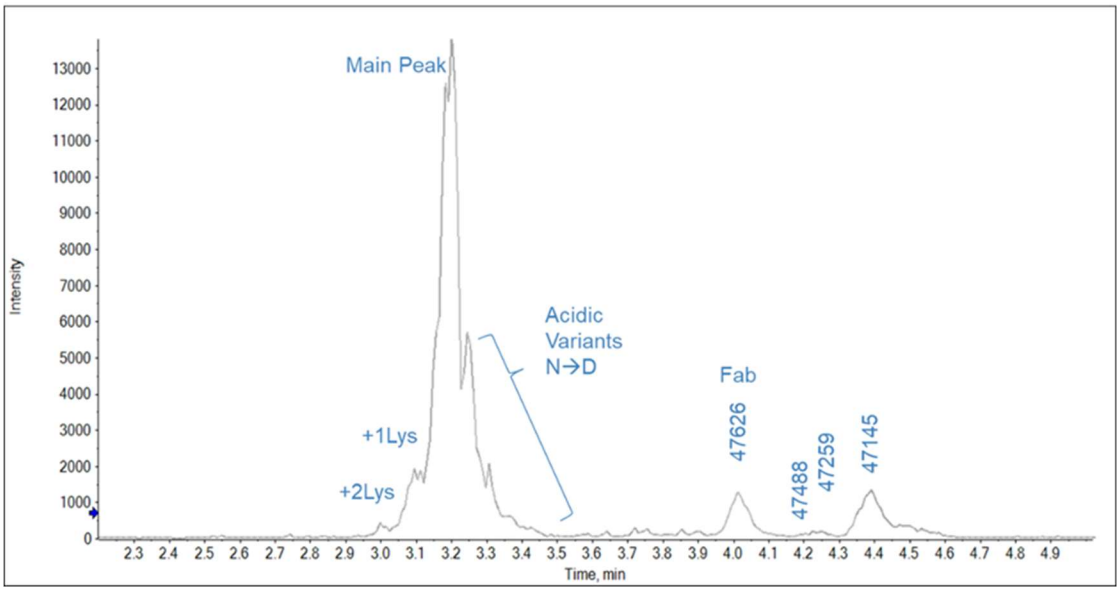

Figure 5. Charge variants profiles and identification of intact NIST mAb and possible fragments impurities.

Conclusion

Using NIST mAb as the test molecule, this technote illustrates an approach for charge variant analysis of intact mAb using CESI-MS and the commercially available neutral OptiMS cartridge. A single assay, it provides:

- Comparable separation of the charge variant with the widely accepted platform methods using optical detection, namely CZE-UV and cIEF
- Mass spectra information allowing the identification of different charge variants and possible impurities in the sample
- Characterization of the major glycosylation pattern for each charge variant peak

References

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