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

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Key Features

- Capillary zone electrophoresis (CZE) separation and Mass spectrometric (MS) identification of charge variants in a single analysis
- Methodology for separation of basic and acidic infliximab charge variants using neutral coated OptiMS cartridge on a SCIEX CESI 8000 system
- High sensitivity CE-MS (Capillary electrophoresis-Mass Spectrometry) analysis to identify low level intact mAb (monoclonal antibody) variants
- Identification of charge variants and major glycan species using SCIEX TripleTOF® 6600 system

Introduction

Monoclonal antibodies (mAbs) are one of the most dominant biotherapeutics. Unlike chemically synthesized drugs, these biotherapeutics are cell originated and are subjected to many different post translational modifications (PTMs) during the process of manufacturing and storage. The most common PTMs include C-terminal lysine truncation, deamidation, glycation and methionine or tryptophan oxidation. These modifications usually lead to changes in the protein's isoelectric point (pI), which presents as multiple charge variants in charge based 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

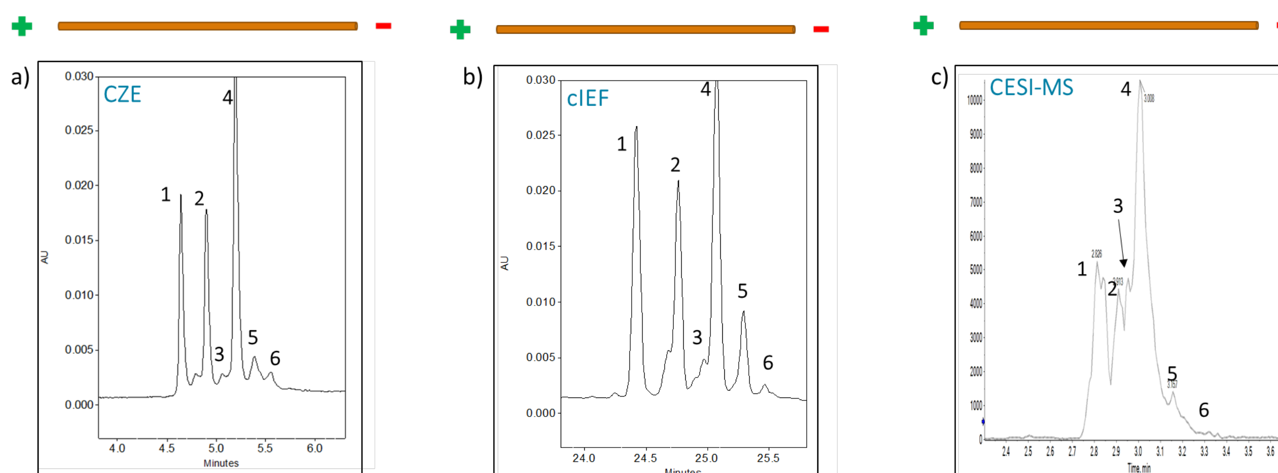

Figure 2.Three different approaches to charge variants analysis of Infliximab: a) UV based platform CZE method; b) UV based Capillary Isoelectric Focusing and c) CESI-MS (CZE) with neutral OptiMS cartridge.

Many different charged based separation techniques can be employed to resolve charge variants in the mAb population. Capillary Electrophoresis (CE) techniques has been widely applied in the entire lifecycle of biotherapeutic development and manufacture. There are two platform CE separation mechanisms with optical detection that are routinely used to monitor changes in charge variants which impact product quality. The first one, Capillary Isoelectric Focusing (cIEF) separates charge variants on established pH gradient based on their pI. The second approach, Capillary Zone Electrophoresis (CZE) separates based on the charge to hydrodynamic size/shape of the molecule. However, while the charge variants can be separated easily with these methods, the characterization of these peaks remains challenging and time consuming. The process typically involves peak fractionation, buffer exchange, fraction confirmation with original method, followed by a RP-LC-MS identification of each fraction. In addition, peak fractionation typically involves an orthogonal technology, which may require additional method development.

The SCIEX CESI 8000 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. Infliximab is a commercial antibody drug which is used for treatment of autoimmune diseases. It has a neutral pI value for the main species. This technote showcases the charge variant analysis of unstressed and stressed infliximab using CESI-MS system.

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 deionized (ddi) water daily
2. Background Electrolyte(BGE): 0.3% Acetic Acid prepared from BGE stock by diluting 1.5 mL of 10% Acetic Acid stock into 50mL daily
3. Sample Buffer Stock: 50 mM Ammonium Acetate pH 6.0, It is also used to perform buffer exchange for protein sample
4. Sample Buffer: 5 mM Ammonium Acetate with 20% methanol prepared by mixing 100 µL of sample buffer stock(prepared in #3) with 200 µL of methanol and 700 µL of ddi water
5. Capillary Cleaning Solution: 0.1N HCl

Stressed Infliximab Preparation

Infliximab was diluted into 50mM ammonium bicarbonate pH 8.0 and stored in an incubator (maintained for 35-40ºC) for three days. Then the sample was buffer exchanged into 50 mM ammonium acetate pH 6.0 and aliquoted for storage at -20ºC before analysis.

Sample Preparation

Infliximab mAb (10 mg/mL) 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 before use.

Both unstressed and stressed infliximab was then diluted to a final concentration of 0.3 -0.5 mg/mL with sample buffer before CESI analysis.

CESI Separation Conditions

Intact Infliximab 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 (both capillary) for 10 min. The MS acquisition was triggered at 10min of separation. A 5 min ramp down was included at the end to lower the CE voltage to 1 kV with a 50 psi rinse on both separation and conductive liquid capillary.

MS Conditions

A SCIEX TripleTOF 6600 system with a NanoSpray III source and CESI adapter (P/N B07363) was used. The curtain gas was 5 psi and the temperature of the interface was 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. The MS deconvolution of each peak was performed using a MS range of 140 kDa to 160 kDa.

Results and Discussions

In this technote, Infliximab was employed to demonstrate the capabilities of this CESI-MS method in simultaneously separating and identification of charge variants. The method uses a sample buffer of 5mM ammonium acetate pH 6 with 20% methanol and mAb concentration at around 0.5 mg/mL. The sample buffer pH is about 1 unit lower than the main peak pI, designed to have the charge variants species in the sample presenting different surface charge, which is used to drive their separation under the applied electrical field. 0.3% acetic acid in water was used as BGE for the separation.

Figure 2a, b and c illustrate three different approaches to achieve charge variant separation of Infliximab. Figure 2a employs CZE method using BGE containing 400 mM ε– amino caproic acid (EACA) 2mM triethylenetetramine (TETA) and 0.2% HPMC pH 5.7 whereas Figure 2b employs cIEF a more traditional charge heterogeneity separation scheme using SCIEX cIEF kit. Both approaches use UV detection at 214 and 280 nm respectively on PA800 plus platform.[1, 2,3] In both figures, two major and minor basic variants (Peak 1, 2 and 3) and two major groups of acidic peaks (Peak 5 and 6) in addition to the main peak (Peak 4) are separated. The separation profile shown in Figure 2c was achieved using the CESI-MS method described here. Results indicate that similar number of peaks along with their relative distributions were observed for all three approaches. The deconvoluted spectra of each peak is shown in Figure 3. Caption in blue at upper left corner of each pectra indicates the MS sift compared to Main Peak calculated based on the G0F/G1F species. Caption in green above each spectra indicates the associated glycan pattern.

Peak 4 matches the reported MS of infliximab and presented the highest intensity, therefore identified as the Main Peak.

Compared to the major form, basic variants in the solution has higher pI, therefore have more surfaces charges, while the lower pI acidic variants would have less surface charges in the same buffer environment. The CE separation conditions used in the CESI-MS method allowed for species with higher charges migrates faster and therefore smaller migration time. The deconvoluted MS spectra shown in Figure 3 correspond to each charge variant separated in the electropherogram on Figure 2c. This data reveals that Peak 1 and 2 have 258 and 129 Da mass shift relative to the main peak and were identified as Infliximab with two and one C-terminal lysine residues respectively . The migration time of Peak 3 indicates they are likely a group of species that's slightly acidic than the single lysine variant but more basic than the main peak. Additionally, deconvoluted spectra reveals that peak 3 has a 131 Da mass shift relative to peak 2. The higher migration time combined with mass shift indicate that peak 3 is the deamidated form of the single lysine variant. Peak 5 and Peak 6 show minor sequential increases in mass, consistent with increased acidity possibly due to increasing number of deamidation. In addition, three major glycoform patterns can be assigned for each charge variant and the most abundant glycoform is G0F/G1F (Figure 3).

We took this method one step further and applied this workflow to pH and temperature stressed mAbs, in order to evaluate its comparability to CZE-UV based method and to further confirm the identity of Peak 3 (Figure 2c). Figure 4 a and b show the profiles of stressed mabs obtained by both CZE-UV and CESI-MS respectively. Deconvoluted spectra (not shown) of the two marked peaks between 5 and 5.3 min, revealed an increased mass of 1-3 Da relative to the main basic variant (~5.4 min), indicating they are likely the deamidated forms of the two lysine and one lysine variants respectively. This is consistent with identification of peak 3 in the unstressed sample (Figure 3) Additionally the increased height observed for the group of peaks migrating between 5.6 and 6.2 min, correspond to fragments with mass around 47 kD (deconvoluted spectra not shown). Similar fragmentation was observed with in NIST mAb in our previous work.[4] As expected, this workflow has shown an overall increase in deamidation and degradation products for antibody stressed samples.[5]

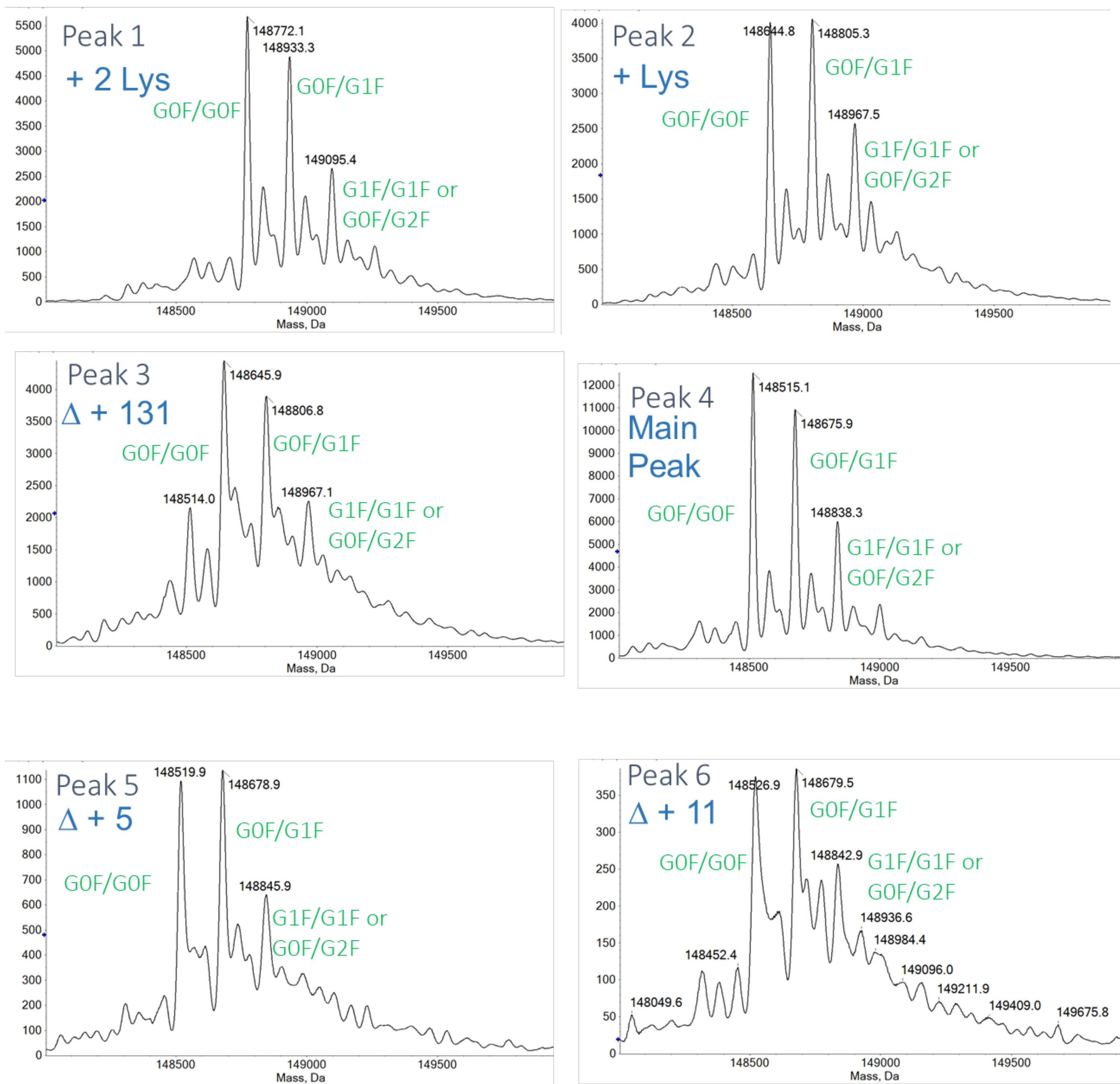

Figure 3. Deconvoluted MS spectra of Peak 1, 2, 3, 4, 5 and 6 labeled in CESI-MS profile shown in Figure 2c with proposed identification labeled. Peak 3 matches the reported MS of infliximab and presented the highest amount, therefore identified as the Main Peak. Caption in blue at upper left corner 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.

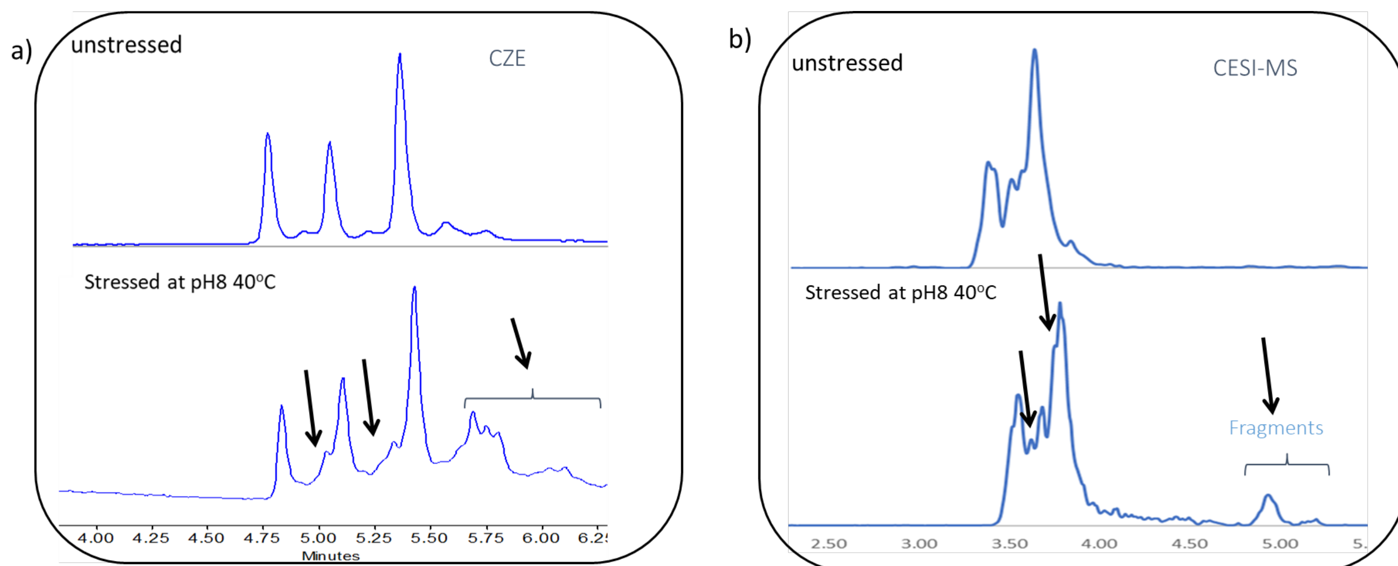

Figure 4. a) Charge variants analysis of unstressed and stressed Infliximab on a PA800 Plus using platform CZE method; b) Charge variants analysis of unstressed and stressed Infliximab using CESI-MS (CZE) with neutral OptiMS cartridge.

Conclusion

Using Infliximab 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
- Direct detection and identification of new charge variants peaks in samples

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Document number: RUO-MKT-02-9497-A

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