Charge heterogeneity characterization and peak identification of complex protein therapeutics using icIEF-UV/MS

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Abstract

Protein therapeutics are typically produced in heterogeneous forms. Post-translational modifications (PTMs) are a common cause of sample heterogeneity and can yield charge variants, which impact the efficacy, potency and safety of therapeutics.

Capillary isoelectric focusing (cIEF) is a powerful technique for monitoring protein charge heterogeneity. However, direct peak identification from electropherograms is challenging due to low sample abundance and MS compatibility. Here, we use the Intabio ZT system (SCIEX), which offers direct chip-based integration of icIEF-UV with mass spectrometry (on ZenoTOF 7600 system) to characterize the charge variants on the monoclonal antibody (mAb) and antibody-drug conjugate (ADC).

Introduction

- Charge variant characterization is important for therapeutics product manufacturing control, as it can impact the efficacy, potency and safety.
- Capillary isoelectric focusing (cIEF) is a powerful technique to monitor charge heterogeneity, but direct electropherogram peak identification is challenging.
- The Intabio ZT system (SCIEX) is able to comprehensively characterize the charge variants on mAb and ADC molecules and disentangle the ADC payload modifications from antibody.

Methods

Sample preparation
Zeba Spin Desalting Columns, 7K MWCO, 0.5 mL (Thermo Fisher Scientific, P/N 89882) was used to remove formulation excipients. To prepare 200 µL of icIEF-UV/MS samples, 80-200 µg of protein was combined with 1-3% Pharmalyte 8-10.5 pH (Cytiva, PN 17045601), 3% Pharmalyte 5-8 pH (Cytiva, PN 17045501), 7.5-15 mM L-arginine (Sigma-Aldrich, P/N A8094-25G) and peptide pI markers.

Separation conditions
icIEF separation, imaging and electrospray ionization was performed on an Intabio ZT system. The sample was focused using 1% formic acid (anolyte) and 1% diethylamine (catholyte) for 1 min at 1500 V and then 1 min at 3000 V followed by 4.5 min at 4500 V. Mobilization and ESI were performed with 25% acetic acid and 25% acetonitrile at 2.5 µL/min for 6.0 min while 3000 V was applied between the anolyte and mobilizer and 5500 V was applied at the tip.

MS conditions
ZenoTOF 7600 system from SCIEX was used to analyze the mobilized peaks. The MS was set to scan 2,000-6,000 m/z (mAb) or 1,500-6,000 m/z (ADC) at 2 Hz. The interface was set at 100ºC with a curtain gas setting of 25 psi. The declustering potential was set at 275 eV and the collision energy at 55 eV.

Data analysis
The deconvoluted mass spectra was analyzed using Biologics Explorer software. The time resolved deconvolution was used to extract intact base peak electropherograms (BPE).

Results

Figure 1 Complex mAb icIEF-UV and icIEF-MS Profiles

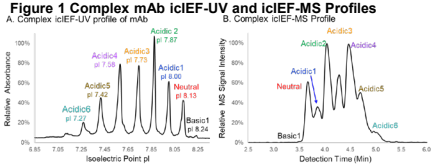

Figure 1. The left Figure 1 (A) is an icIEF-UV profile of the complex mAb charge isoforms separated by icIEF. The right Figure 1(B) is icIEF-MS profile of the same corresponding peaks after mobilization, electrospray and MS detection. The separation profiles generated by icIEF-UV (once inverted) and icIEF-MS on the Intabio ZT system and ZenoTOF 7600 system, respectively, are similar, as basic peaks are analyzed first by MS. Estimated pIs for the charge isoforms were 7.27 to 8.24 pI units.

Figure 2 Complex mAb deconvoluted mass spectra and annotation

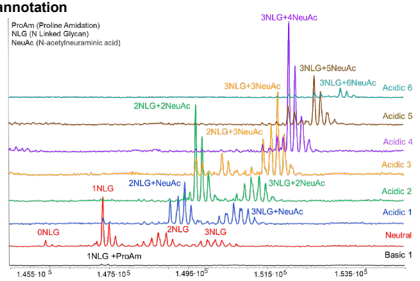

Figure 2. Deconvoluted mass spectra are displayed for each charge variant peak from top to bottom by increasing pI value. Basic peak 1 with the highest pI value of 8.24 comprises proline amidated species. The neutral charge variant with a pI of 8.13 comprises neutral N-linked glycans attached from 0 to 3 glycosylation sites per molecule. As estimated pI decreases from acidic peak 1 (pI 8.00) to acidic peak 3 (pI 7.27) corresponds to the incremental additions of terminal sialic acids. From 0 to up to 6 terminal sialic acids per molecule are detected across the charge profile.

Figure 3 Unconjugated mAb and ADC icIEF-UV and MS Profiles

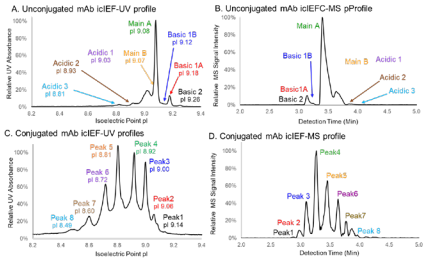

Figure 3. The left side of the Figure 3 (A and C) shows that conjugation of the drug payload results in both an increase in the charge profile heterogeneity and a decrease in pI. The changes observed in the icIEF-UV profiles are also reflected in the icIEF-MS profiles (Figure 3 B and D). All the charge variant peaks detected by icIEF-UV profile have corresponding peaks in the icIEF-MS profile.

Figure 4 Unconjugated mAb charge variant intact mass

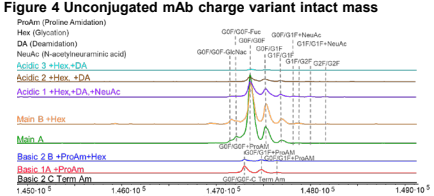

Figure 4. Contains the intact mass for all 7 charge isoforms of the unconjugated mAb. Basic peak 2, pI 9.26, is comprised of 2 C-terminal amidated species. Basic peak 1 A and B (pI 9.06 and 9.00) contain single C-terminal amidated species. Hex addition are present from main peak B (pI 9.07) to acidic peak 3 (pI 8.49). A progressive 1 Da shift in acidic peaks 1 through 3 indicated deamidation. Finally, mass shift consistent with terminally sialylated glycans are observed in acidic peak 1.

Figure 5 ADC deconvoluted mass spectra

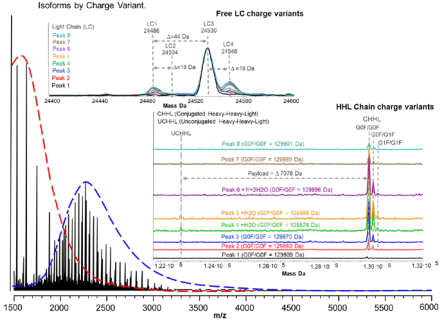

Figure 5. The conjugation of the ADC results in a non-covalently bound LC. During electrospray, the ADC dissociation forms two charge envelopes, (Red trace: free LC) and (Blue trace: HHL). Since the icIEF separation is performed under native conditions, the relationship between intact mass and pI is preserved. As pI decreases from 9.14 (Peak 1) to 8.49 (Peak 8), the relative abundances of negatively charge isoforms LC4 (MW 24549 Da) respectively increase. The observed 18 Da shifts are caused by a ring opening event on the payload resulting in a negative charge reducing pI. These same ring opening events are also observed in peaks 4, pI 8.92 to 6, pI 8.72 of the HHL subunit.

Conclusions

- The Intabio ZT system shows the capability to reduce resources and timelines in the charge heterogeneity characterization of complex AstraZeneca mAb and ADCs biotherapeutics.
- The identification of antibody and payload related charge heterogeneity supports the icIEF method development and a new strategy for icIEF specifications on ADCs.

Conflict of Interest Disclosure
The authors from AstraZeneca declare no competing financial interest.

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