Unified Workflow for Monoclonal Antibody Charge Heterogeneity, Purity, and Molecular Weight Analysis

Separation and Online Detection of Intact mAb Variants and Impurities using CESI-MS

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Overview

Biopharmaceutical development and manufacturing of monoclonal antibody (mAb) therapies requires routine analyses and monitoring of various physiochemical properties. Here, we describe a unified capillary electrophoresis and electrospray ionization with mass spectrometry (CESI-MS) workflow for characterization of mAb samples. The workflow combines the unique separation capabilities of capillary zone electrophoresis (CZE) with the advantages of an MS-based detector to provide high resolution structural information and accurate molecular weight information. Small amounts (~10 ng) of samples containing intact (non reduced) or reduced mAbs can be analyzed to determine charge heterogeneity (including charge variants, glycoforms, and clipped species), purity, and molecular weight.

Who Should Read This: Senior Scientists and Lab Directors

Focus: Analytical testing of mAb samples during drug development or manufacturing. Potential applications of the workflow include mAb characterization and purity testing during mAb candidate screening, manufacturing process development, and commercial production quality control.

Goal: Determine charge heterogeneity, purity, and molecular weight of therapeutic mAb samples using a single CESI-MS workflow that has improved performance and utility compared with existing industry-accepted methods such as CE-SDS and capillary isoelectric focusing (cIEF)

Problem: mAbs are susceptible to modification and degradation events that can change their physicochemical properties and ultimately impact the stability, efficacy, and safety of mAb therapies. Impurities and excipients introduced during development and manufacturing can also have similar effects. Because of this, thorough characterization at all stages of development and manufacturing is required. Currently, industry relies on multiple methods to obtain charge heterogeneity, purity, and molecular weight information. Together, these methods can consume relatively large quantities of expensive and mass-limited samples. Further, because most methods rely on the use of reduced mAb samples instead of intact mAb samples, identification of clipping events is challenging. Missed clipping events may lead to incorrect assumptions regarding sample handling, storage conditions, and stability information.

CESI 8000 Plus High Performance Separation Module coupled to a TripleTOF® 6600 System via a Nanospray III source, CESI adapter, and neutral-coated OptiMS capillary cartridge

Results: A single CESI MS workflow was developed and tested for determination of charge heterogeneity, purity, and molecular weight information in intact and reduced mAb samples. Identified species in mAb samples included the intact mAb, variants (eg, charge variants, clipped species, glycoforms), impurities, and excipient components. Results were similar to those determined using a combination of industry-accepted methods (ie, CE-SDS and cIEF). Notably, the CESI-MS results provided additional structural information of species which allowed for identification of potential mAb clipping events that may have been missed by traditional methods.

Key Benefits:

- Simplified workflow with a single CESI-MS analysis for multiple mAbs characterization insights
- Glycoforms, clipped species, impurities, and excipients all separated and identified by the same method with accurate mass detection

Introduction

Immunoglobulin gammas (IgGs) are the most common molecules used for generation of therapeutic monoclonal antibodies (mAbs). The ability to characterize these molecules with high sensitivity and comprehensiveness is essential to their development and for regulation of their efficacy, bioavailability, and biosafety. Charge heterogeneity, purity, and molecular weight analyses are powerful CE- and MS-based methods for mAb characterization. Intact and reduced analysis of mAbs by CE are used in the mAb screening, analytical development, and quality control processes. Combining a similar CE separation with mass spectrometric (MS) detection of intact mAbs allows for unification of these three methods into one. Additionally, the high resolution and mass accuracy MS detection can facilitate identification of unknown CE peaks and may also provide more accurate and sensitive purity and molecular weight measurements than with optical detection alone.

The integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into one process (CESI) provides these possibilities while also lowering the sample mass analysis requirements. We present the analysis of intact and reduced IgGs using a single CESI-MS method which provides charge heterogeneity, purity, and molecular weight information similar to the multiple current industry-accepted CE-SDS, capillary isoelectric focusing (cIEF), and capillary zone electrophoresis (CZE) analysis methods combined.[1-3] Notably, the intact and reduced IgG analyses by CESI-MS demonstrate the capabilities for quickly screening and characterizing candidate or developed therapeutic IgG molecules which may have either charge and/or size heterogeneity, particularly due to stress or instability.

The CESI-MS results are compared to existing industry-accepted, CE-based charge heterogeneity, purity, and molecular weight analyses. Charge heterogeneity separations by CESI-MS using a capillary zone electrophoresis (CZE)-based separation mechanism showed similar profiles to a cIEF-based method which has been previously demonstrated using optical detection methods.[3-6] With the MS-based detection, molecular changes that cause charge heterogeneity, such as glycoforms, were associated with CE migration shifts. Other peaks within the charge heterogeneity separation could also be primarily attributed to potential clipped IgG impurities within the samples. Collectively, the results demonstrate the advantages of using MS as the detector for a CZE-based charge heterogeneity analysis since it also provides molecular weight and purity information.

Materials and Methods

Sample Preparation: For CESI-MS experiments, IgG1, IgG2, and IgG4 molecules (20 mg/mL) were desalted and buffer exchanged into 50 mM ammonium acetate, pH 4 using Zeba spin columns (Thermo Fisher Scientific). For reduced analysis, IgG molecules were incubated for 45 min at 60° C in 10 mM DTT and 0.1% Rapigest SF Surfactant (Waters). Rapigest was cleaved (0.5% formic acid, 37° C, 10 min) and spun down with precipitants (14K x g). Concentrated (2 M) ammonium acetate, pH 4 was added for a 50 mM final concentration. For stand-alone CE experiments, IgG molecules were either diluted into a urea-gel-ampholyte solution (cIEF-based analysis) or SDS gel solution (CE-SDS analysis). Reductions in SDS gel solutions were performed at 60° C for 10 min in 10 mM DTT.

CESI 8000 Plus MS Mode Conditions: CESI experiments were carried out with a SCIEX CESI 8000 Plus system (P/N A98089) equipped with a temperature controlled auto sampler and a power supply with the ability to deliver up to 30 kV. An OptiMS Neutral Surface Cartridge (P/N B07368) with a porous tip was used. Solutions of 3% and 10% acetic acid were employed as background electrolytes (BGE) and conductive liquids, respectively. Pressure (5 psi) was applied for 10 sec to generate ~7.5 nL sample injections. Sample stacking after pressure injection was performed by transient isotachophoresis (tITP) due to the 50 mM ammonium acetate sample buffer. CESI separations were performed at 30 kV with 2 psi for 7 min (pre-separation), then 10 psi for 10 min (separation and ESI).

MS Conditions: A SCIEX TripleTOF® 6600 system with a NanoSpray III source and CESI adapter (P/N B07363) were used. Intact protein detection was performed with MS scans from 400 – 4500 *m/z*.

Data Analysis: High resolution MS spectra were analyzed using SCIEX PeakView® and BioPharmaView™ software.

CESI 8000 Plus Stand-Alone CE Mode Conditions: CE-SDS and cIEF experiments were performed using SCIEX PA 800 Plus detectors (P/N B68372), kits (P/Ns A10663 & A80976), and protocols.

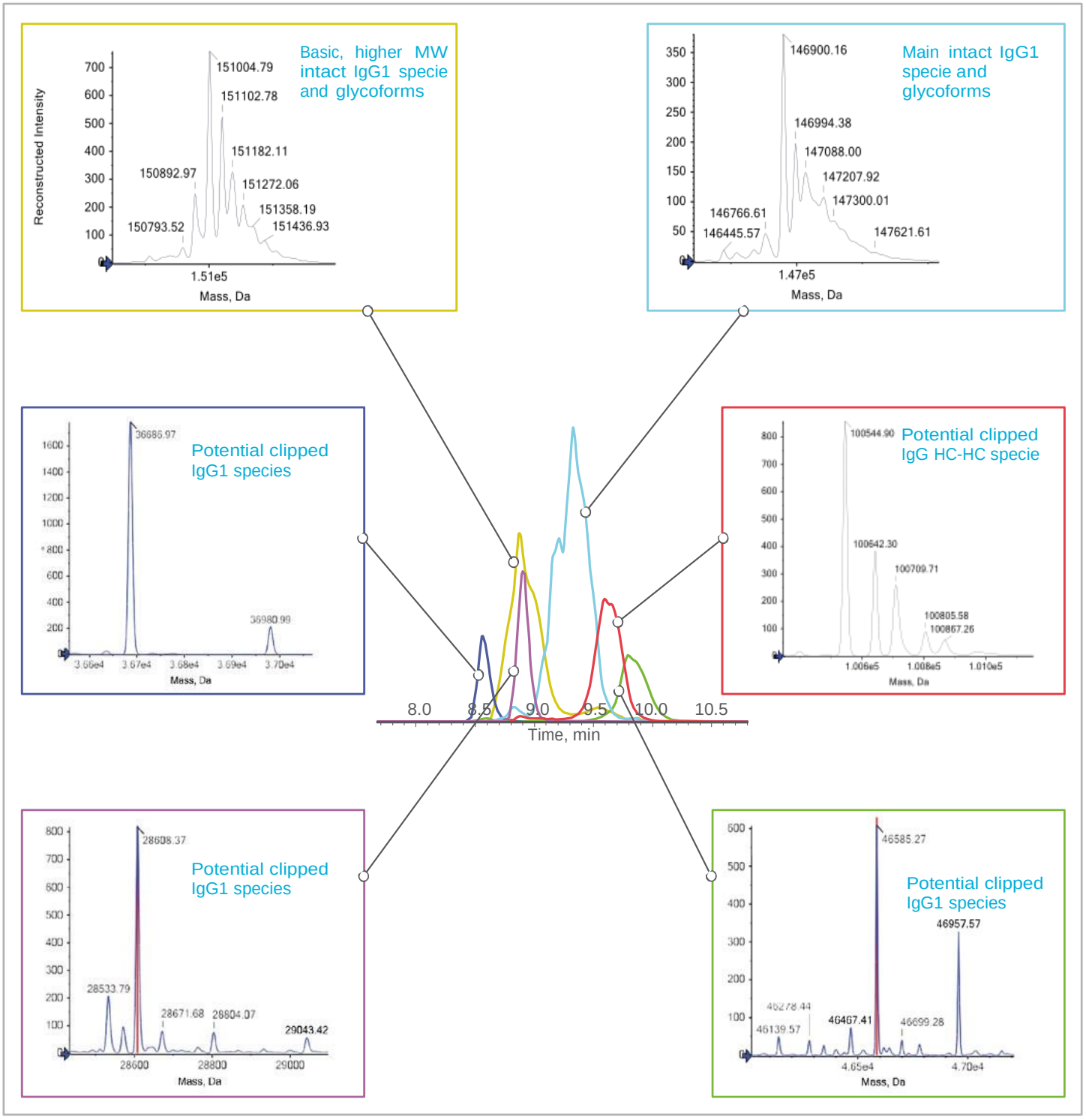

Figure 1: Charge heterogeneity separation of IgG1 with MS detection. Extraction and deconvolution of non-reduced IgG1 species MS spectra from the CESI separation. The extracted ion electropherograms (XIEs) from the most abundant charge states (~3 – 6) from the same charge state envelopes were summed. Spectra were integrated using 15 – 30 sec from each electrophoretic peak using their full width at half maximum height as the integration window.

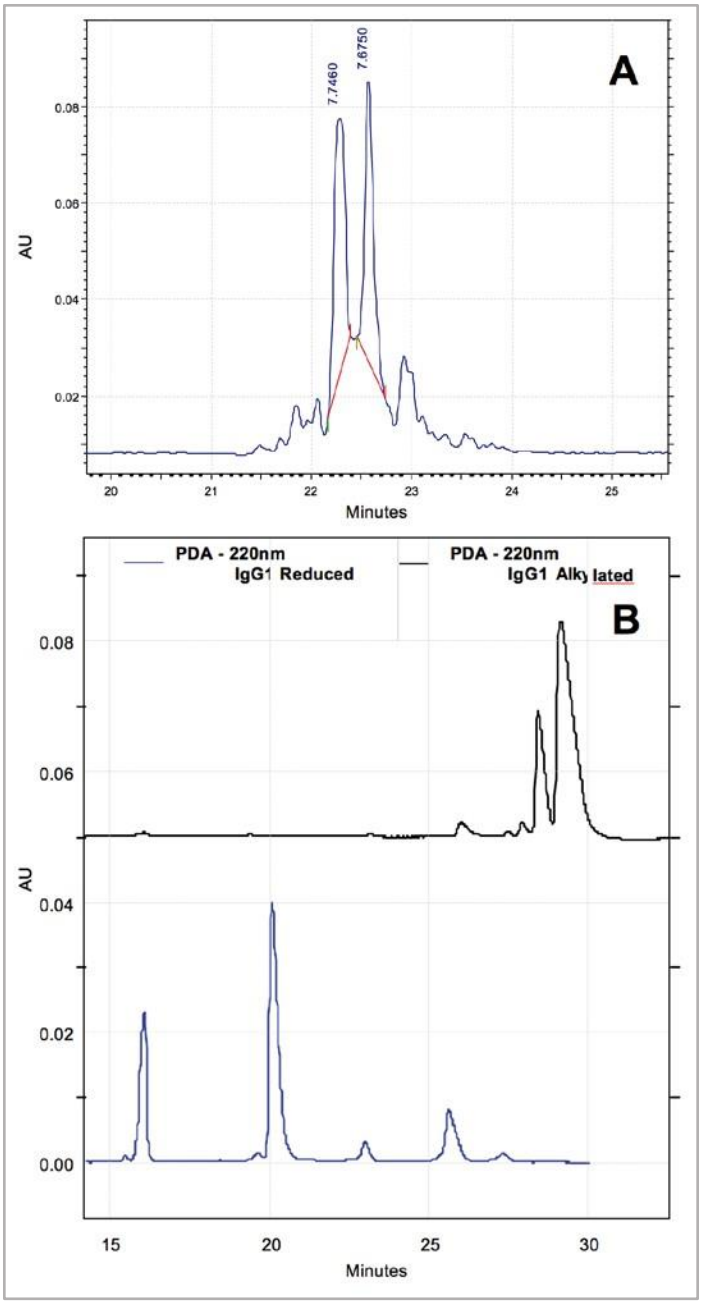

Figure 2: (A) cIEF analysis of non-reduced IgG1 molecules with UV detection. (B) CE-SDS analysis of non-reduced, alkylated (black trace) and reduced (blue trace) IgG1 molecules with PDA detection.

Results and Discussion

CESI-MS based analyses were performed at the non-reduced (intact) and reduced levels using the neutral-coated CESI capillary. The CZE-based separation of non-reduced IgG1 (Figure 1) generated a charge heterogeneity separation and facilitated accurate molecular weight determination of IgG1 species and impurities through spectral deconvolution. Two intact IgG1 species were detected. The main charge variant had an average molecular weight of 146,900 Da while the basic IgG1 charge variant was 151,005 Da. In addition to average molecular weight differences, their glycoform profiles were different. Further data analysis using the high mass accuracy deconvoluted molecular weights would facilitate identification of candidate sequence and glycoform matching. While two main IgG1 variants were detected as electrophoretic peaks and intact MS spectra, their glycoform profiles and partially split electrophoretic peaks also imply that additional variants are also present in the sample. Additional deconvolution analysis indicates a mixture of spectra from due deamidations, disulfide breaks, and glycosylation variants (data not shown). Four other IgG1 species were also detected by MS which appeared to be impurities from potential in-solution clipping events. The IgG1 impurities included abundant molecular weights of 28,608, 36,687, and 46,585 Da. The highest molecular weight impurity was 100,545 Da which had a molecular weight and glycosylation profile consistent with a heavy chain dimer (HC-HC) impurity. Notably, the CESI-MS-based charge heterogeneity analysis provided results consistent with a cIEF (Figure 2A) and CE-SDS (Figure 2B) analysis of the same IgG1 sample. For example, a similar separation profile and number of species were detected between the cIEF and CESI-MS charge heterogeneity separations. Additionally, the number of molecular weight impurities from CE-SDS analysis is also consistent with the number of species detected with unique molecular weights by the CESI-MS based analysis. Thus, the CESI-MS analysis combines both aspects of the cIEF and CE-SDS analyses through a CZE-based charge heterogeneity separation and molecular weight determination by MS. Ultimately, the CESI-MS analysis allows for direct detection and candidate identification of potential IgG1 clipping events that might be otherwise be misinterpreted as intact IgG1 charge isoforms or difficult to identify by UV detection alone. Representative raw MS data used for spectral deconvolution is shown for each electrophoretic peak in Figure 3.

The CZE-based method can also be used for reduced mAb analysis. Figure 4 shows the separation and detection of reduced IgG1 molecules. One IgG1 heavy chain was detected while two IgG1 light chain species were detect. This was also consistent with the three main reduced IgG1 species detected by the CE-SDS analysis. The molecular weight measurement of the two IgG1 light chain species contributes to the understanding of the molecular weight differences of the intact IgG1 forms.

That is, the higher molecular weight IgG1 light chain is likely present in the acidic, higher molecular weight intact IgG1 charge variant. In combination with the high mass accuracy

measurement by the MS, further data analyses could be used to confirm the different intact IgG1 forms using the reduced IgG1 analysis.

The same CESI-MS intact charge heterogeneity analyses were also performed on representative IgG2 and IgG4 molecules (Figures 5 and 6, respectively). Just as with the IgG1 analyses, multiple intact and clipped IgG2 and IgG4 species were detected in each analysis. Even though the intact IgG2 and IgG4 co-migrate with their respective forms, the spectral deconvolution process allows for identification of two different glycoforms of each in both cases. Similarly, potential clipped IgG2 and IgG4 species both migrate before their intact forms, allowing for sensitive identification of the impurities. In the case of the IgG2 analysis, a potential formulation excipient (e.g. polysorbate 80) was well resolved from IgG2 that might otherwise detrimentally affect MS spectral quality by nanoESI- or LC-MS-based analyses. Similar results with other non-ionic detergents would be expected since they will have the same electrophoretic mobilities and migration times.

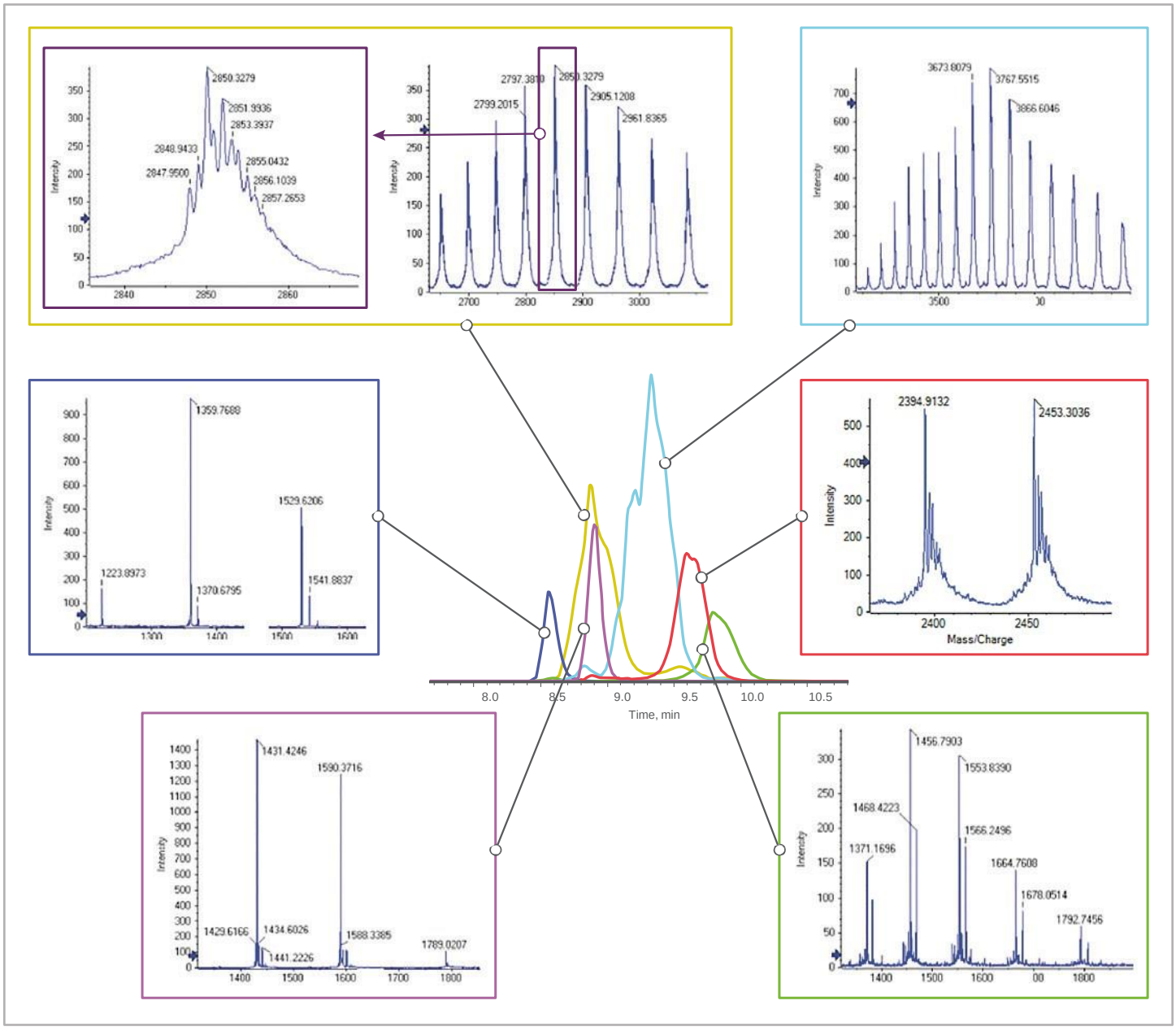

Figure 3: Extraction of representative raw MS spectra from non-reduced IgG1 species from the CESI separation used for generation of deconvoluted spectra in Figure 1. Spectra were integrated 15 – 30 sec from each electrophoretic peak using their full width at half maximum height as the integration window.

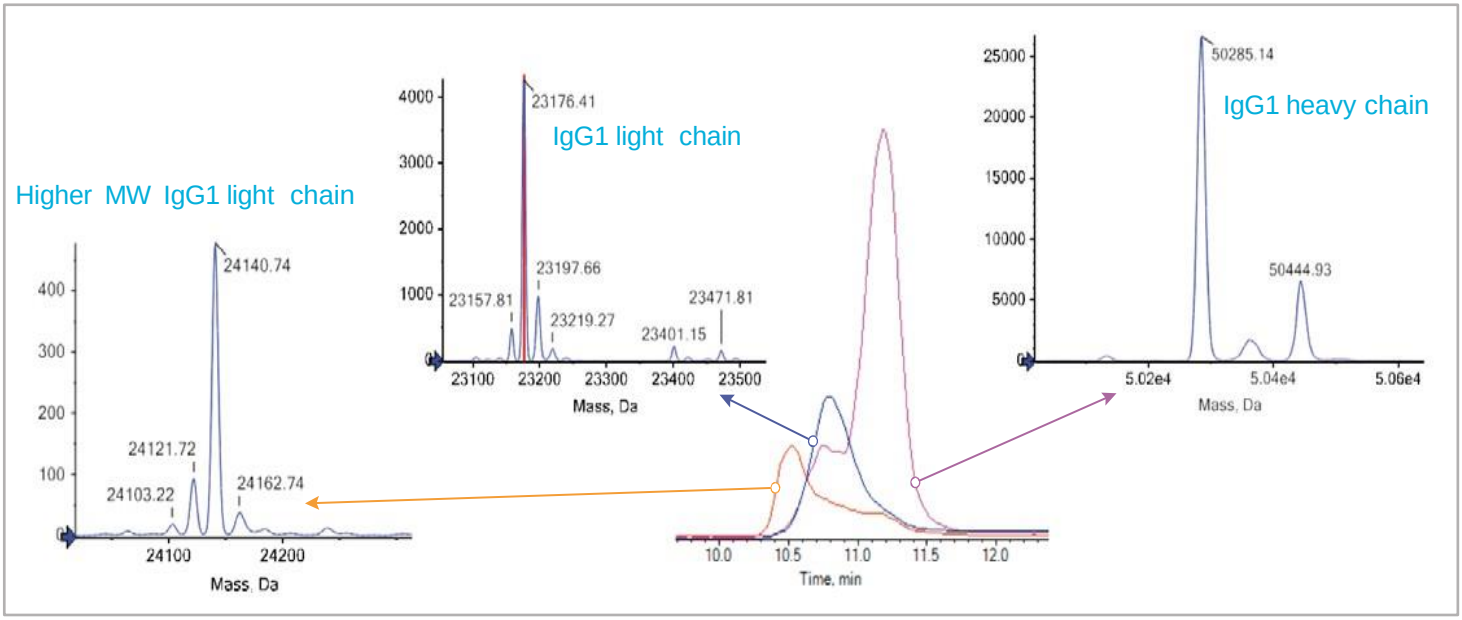

Figure 4: Reduced IgG1 analysis with MS detection. Extraction and deconvolution of reduced IgG1 species MS spectra from the CESI separation. The extracted ion electropherograms (XIEs) from the most abundant charge states (~3 – 6) from the same charge state envelopes were summed.

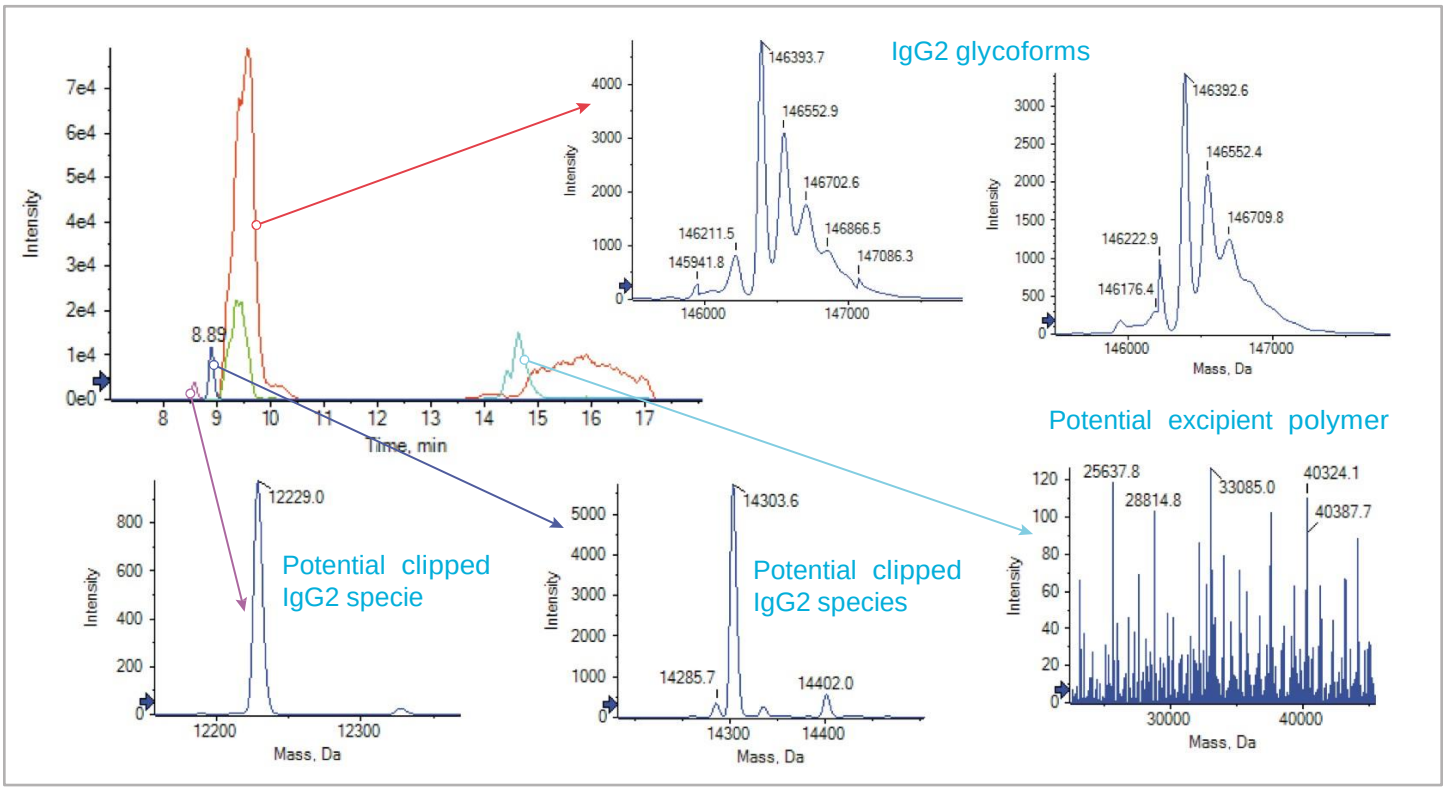

Figure 5: Charge heterogeneity separation of IgG2 with MS detection. Extraction and deconvolution of non-reduced IgG2 species MS spectra from the CESI separation. The extracted ion electropherograms (XIEs) from the most abundant charge states (~3 – 6) from the same charge state envelopes were summed.

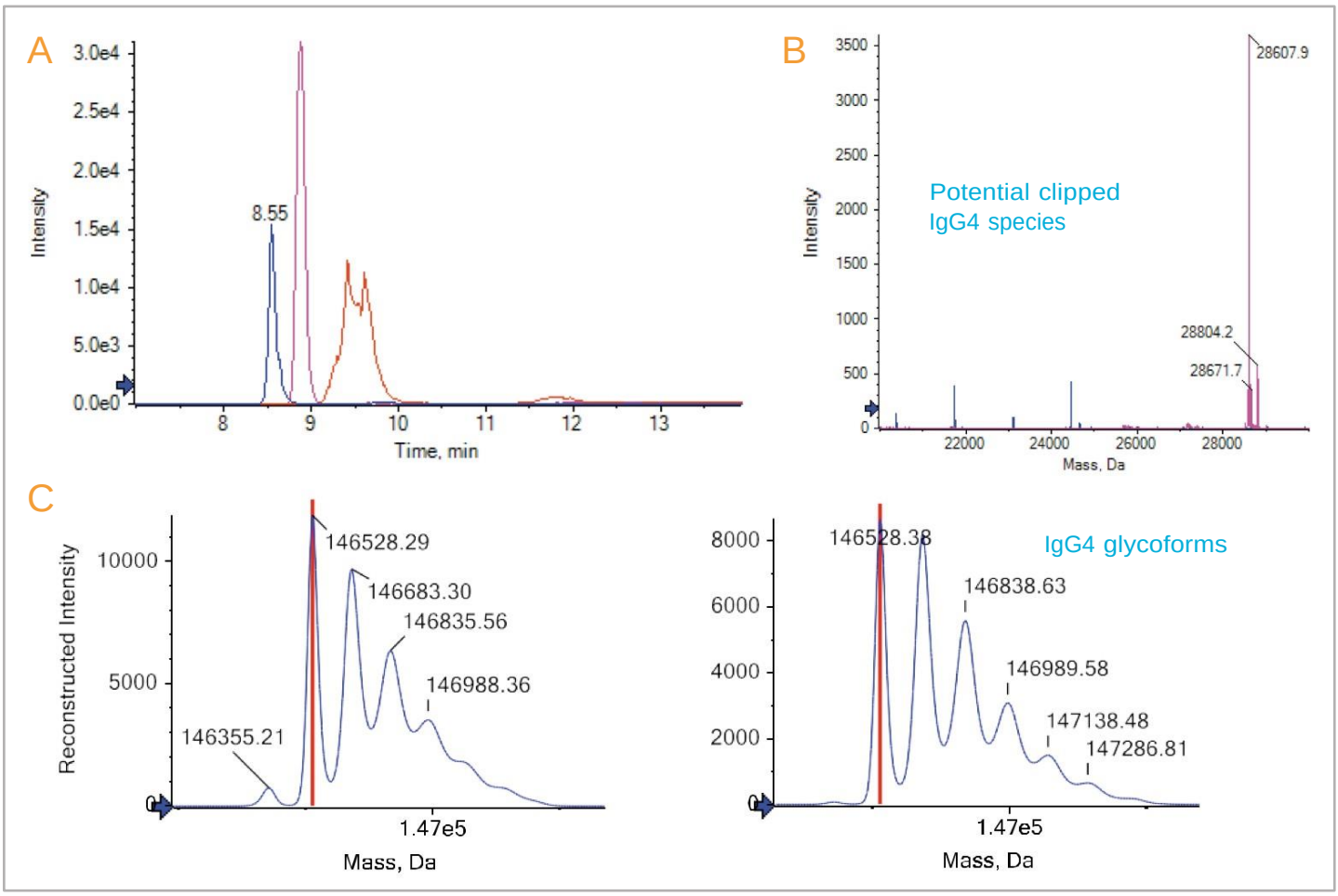

Figure 6: (A) Charge heterogeneity separation of IgG4 with MS detection. The extracted ion electropherograms (XIEs) from the most abundant charge states (~3 – 6) from the same charge state envelopes were summed. (B) Extraction and deconvolution of potential clipped IgG4 species MS spectra from the CESI separation. Deconvoluted MS spectra colors correspond to the XIE peaks of the same color. (C) Extraction and deconvolution of non- reduced IgG4 species MS spectra from the CESI separation. The left and right deconvoluted spectra correspond to spectral extraction of the left and right side of the orange, split XIE peak.

Conclusion

The combined CESI-MS and stand-alone CE functionality of the CESI 8000 Plus system provides powerful methods to characterize intact and reduced IgG forms. Both analyses deliver charge heterogeneity, purity, and molecular weight information. When coupled with the TripleTOF® 6600 system, the CESI- based analysis using a neutral-coated capillary generates high resolution separations with high mass accuracy molecular weight information for characterization of mAb charge variants, glycoforms, and impurities. Additionally, the high sensitivity CESI- MS analyses are achieved from small sample amounts (~10 ng), particularly useful in the mass-limited development phase.

References

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