# Rapid and reproducible high-resolution charge variant analysis of adalimumab (mAb) using the IntaBio Imaged CIEF-MS System

Wei Chen, Ben Kojtari, Javier Satulovsky, and Maggie A. Ostrowski
*IntaBio*

## Introduction

Monoclonal antibodies (mAbs) exist as heterogeneous populations that differ in their biophysical and biochemical properties. These differences occur as a result of cellular processes during bioproduction or chemical changes during downstream bioprocessing. The micro-heterogeneity of mAbs requires detailed structural characterization to assess critical quality attributes (CQA) to ensure potency, efficacy and safety.[1,2,3]

Various post translational modifications (PTMs) can result in the formation of charge variants. Examples of these PTMs include deamidation, glycation, succinimide formation, C-terminal lysine formation, N-terminal proline amidation, and sialic acid additions, among others.[4, 5] Imaged capillary isoelectric focusing (iCIEF) has become an essential characterization tool for understanding CQAs of mAbs and its use is required by regulatory agencies for lot release.[6,7]

When new peaks are seen in an iCIEF profile, the effort to characterize these peaks is extensive, both in terms of labor and time. New peak identification generally requires ion exchange chromatography (IEX) method development, IEX fraction collection, and mass spectrometry (MS) characterization.

Unlike iCIEF, which generally uses a platform method, IEX generally requires method development for new molecules. In addition, because IEX and iCIEF separations do not result in the same profiles, the IEX fractions must be reanalyzed by iCIEF to ensure correct correlation of the IEX and iCIEF peaks.[8]

Intabio developed the IntaBio System to accelerate the process of biopharmaceutical drug development. The IntaBio System directly couples iCIEF charge variant analysis with high-resolution MS detection for direct in-line peak identification of intact proteins (Figure 1).[9] Analysis of mAbs using the IntaBio System takes about 15 minutes per sample, much faster than the days or weeks required for traditional workflows such as IEX described above.

Here, we demonstrate the unique capability of IntaBio iCIEF-MS analysis to directly identify charge variants of adalimumab, a therapeutic antibody. The IntaBio System was interfaced with the TripleTOF® 6600+ Quadrupole Time-Of-Flight (QTOF) from SCIEX and data were analyzed using a new IntaBio iCIEF-MS workflow in Protein Metrics Byos® software, developed specifically to support the IntaBio System.

The IntaBio System with the TripleTOF® 6600+ Quadrupole Time-Of-Flight (QTOF)

## Materials and methods

The IntaBio System uses a proprietary microchip-based cartridge that couples the iCIEF separation with an electrospray tip for MS peak identification (Figure 2). Relative quantitation of the charge variant peaks is performed using a 280-nm UV imaging system. IntaBio was coupled to the SCIEX TripleTOF 6600+ using the IntaBio MS adaptor. US-licensed adalimumab was mixed with L-arginine at a final concentration at 8 mM, Pharmalyte pH 3-10 at 0.5%, Pharmalyte pH 8-10.5 at 1.5%, and pI markers 8.4 and 9.99 at 0.0125 mg/mL. Anolyte, catholyte, and mobilizer were comprised of 1% formic acid, 0.75% diethylamine and 50:49:1 acetonitrile/water/formic acid, respectively.

In the first step of the analysis, the IntaBio System fills the 5-cm channel of the chip with the sample solution mix containing the carrier ampholytes, analyte protein, and internal pI markers. The sample solution is then focused by applying a voltage between the anolyte and catholyte. The process of iCIEF allows the proteins to migrate and concentrate to their respective isoelectric points. When the focusing is completed, the system records a final image of the protein's charge variant profile using 280-nm UV absorbance. The electrophoresis circuit is then switched to mobilize the separated peaks through the channel toward the ESI tip. An electrophoretic voltage offset of 5.0 kV was applied to electrophorese the focused isoform bands into the MS compatible mobilizer solution. A separate voltage is applied to the chip tip to create an electrospray plume where each peak is converted into gas phase ions by an electrospray ionization process (ESI) for the analysis by mass spectrometry. SCIEX IntaBio™ software applies the stepped voltage profile and monitors the current.

Data were processed and reports were produced using Protein Metrics Byos® software for detailed analysis of iCIEF peaks and corresponding MS peak assignments. Statistical analysis of replicate injections was performed with an in-house statistical analysis software package.

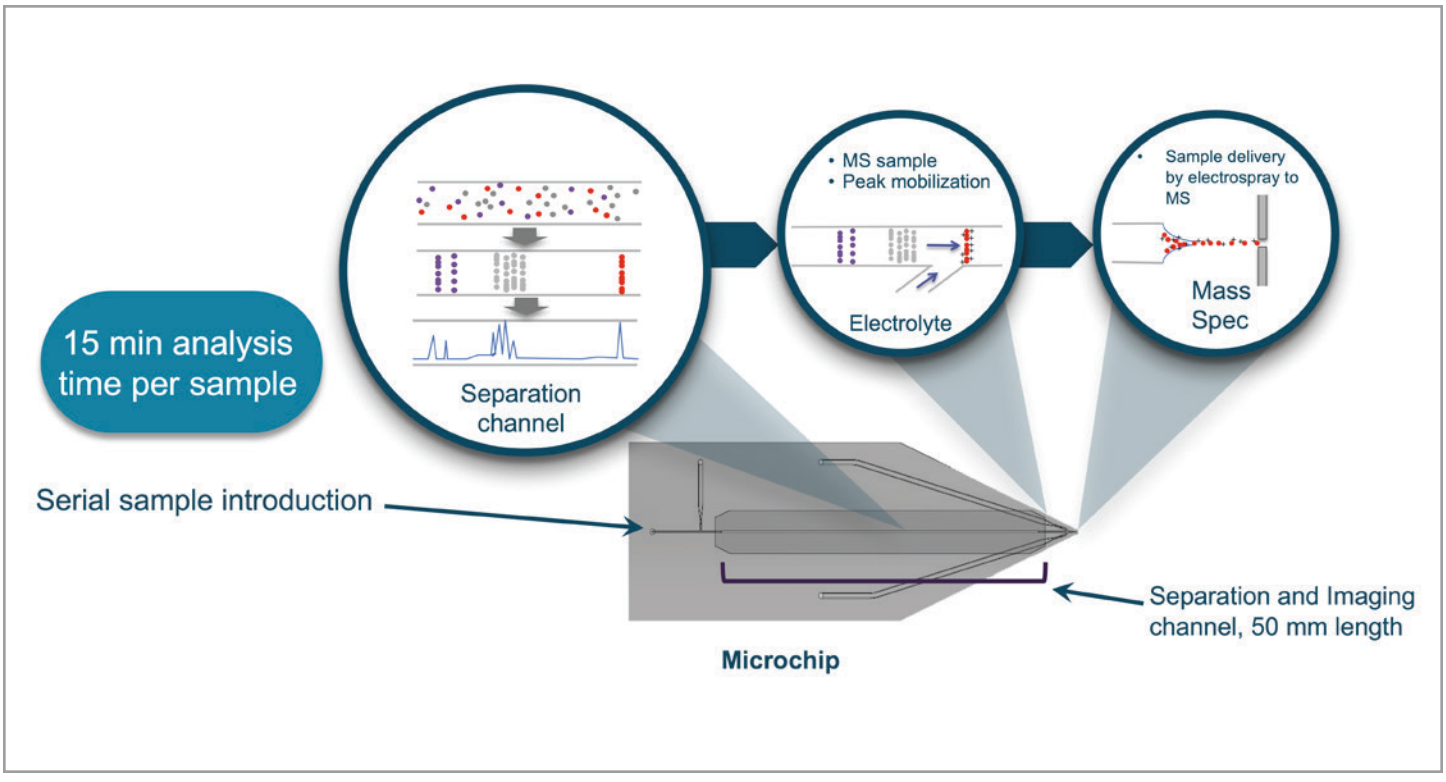


**Figure 1.** Using a proprietary microfluidic chip, Blaze integrates iCIEF with an electrospray ionization tip for direct peak ID by mass spectrometry.

## Results

When focusing is complete, the SCIEX IntaBio software captures a final UV absorbance image of adalimumab charge variant profile (Figure 2). After mobilization, MS peak identification is performed by MS. The SCIEX IntaBio software captures the real-time focusing, shown in the upper panel of Figure 2, as well as the voltages specified by the method and the corresponding current, as shown in the lower panel of Figure 2.

A novel data analysis workflow was developed in collaboration with Protein Metrics Inc. to facilitate the combined analysis of iCIEF data and the corresponding MS results. The new Byos workflow is called Byos-for-IntaBio.

The Byos-for-IntaBio workflow enables the combined analysis of the two different file types; a 3-dimensional electropherogram and the MS output file. These data are captured at different times during the integrated assay (separation vs mobilization) and in different domains (pI vs time) and the new Byos-for-IntaBio workflow enables combined visualization and analysis of the two data sets.

Once the iCIEF data is aligned with the base peak electropherogram (BPE) from the MS data, the iCIEF peaks are automatically defined in the pI domain. The Byos-for-IntaBio workflow then uses the peak boundaries to interrogate the corresponding MS data to perform the deconvolution and peak ID.

Figure 3A shows alignment and overlay of the iCIEF data (black) onto the PBE (red). If the view of the data in the pI domain is preferred, the software can invert the data and display it in the pI domain as seen in Figure 3B.

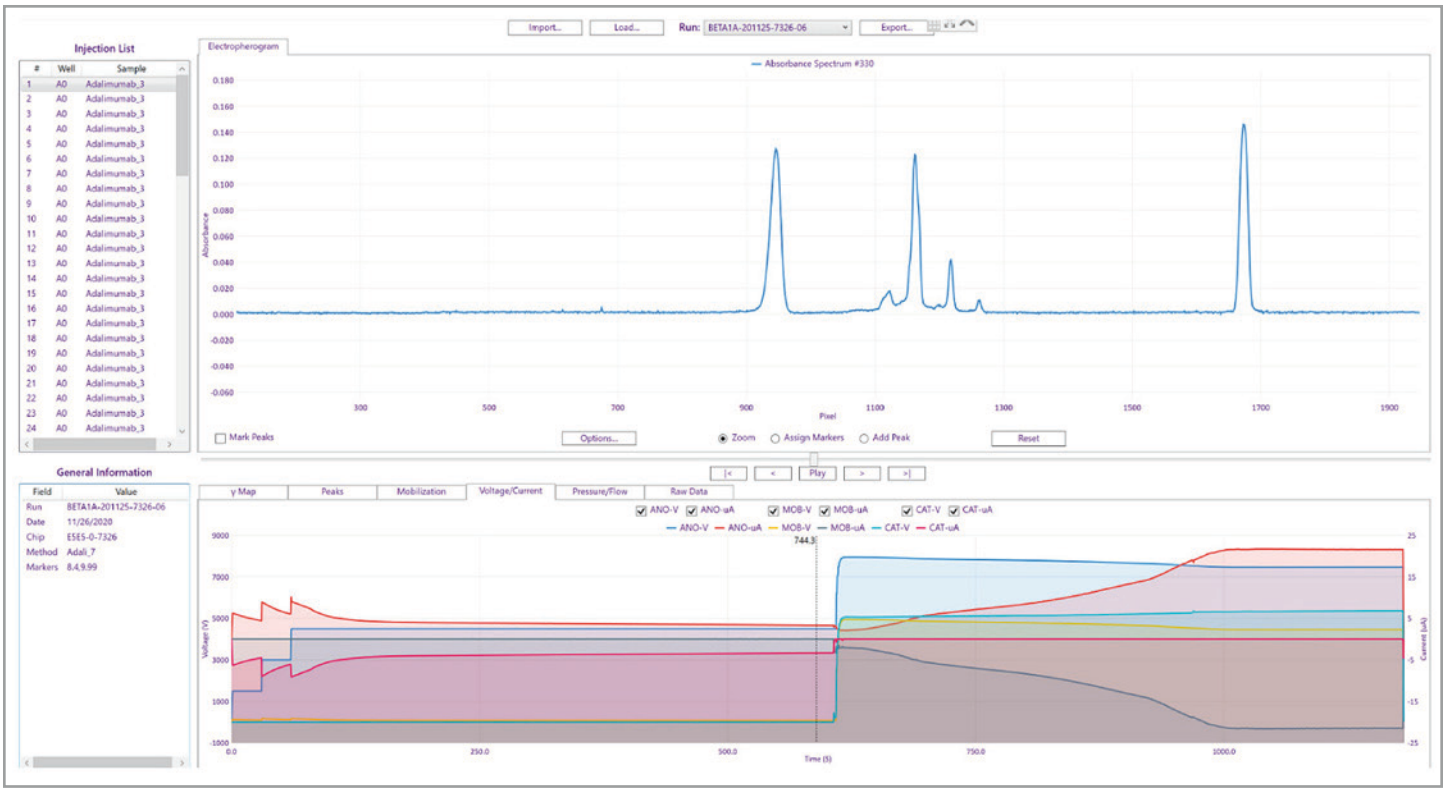


**Figure 2.** A) Adalimumab iCIEF profile from Trailblazer during focusing. B) Trace of the voltage profile and corresponding current. Dashed vertical line indicates the time of the electrophoretic trace above.

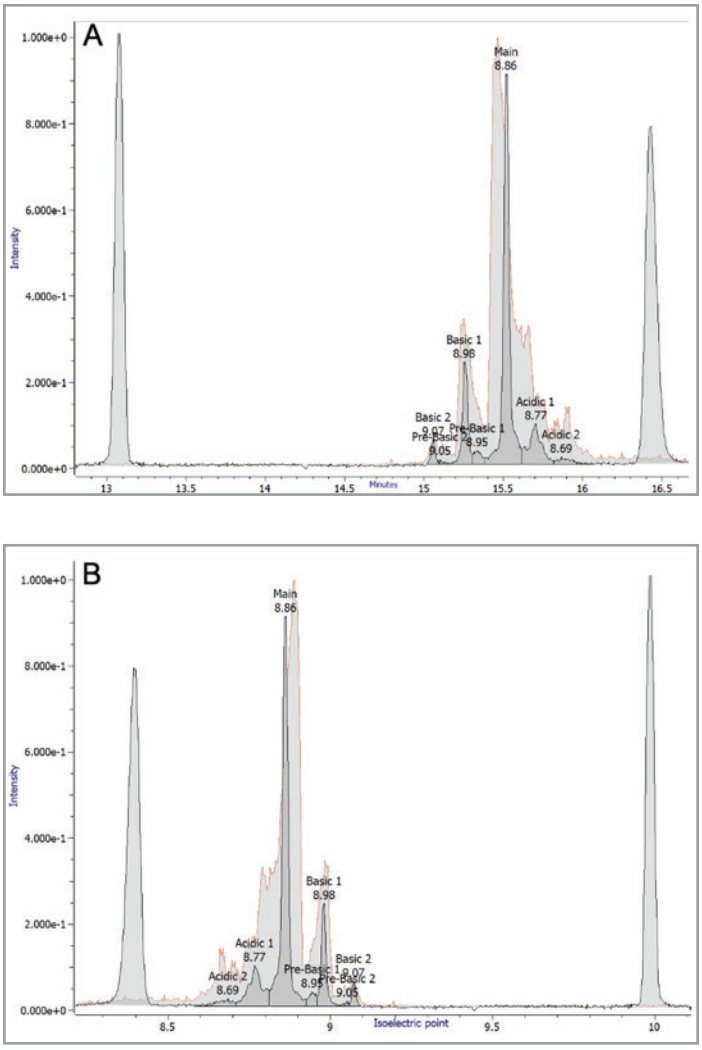


**Figure 3.** A) Overlay of the adalimumab data. iCIEF is shown in black and MS results in red in the time domain. Note that the iCIEF data trace is inverted because the basic variants are introduced into the MS first and are detected before the acidic peaks B) Overlay of the adalimumab data in the pI domain - iCIEF in black and MS in red.

## Critical quality attributes

IntaBio analysis facilitated by the Protein Metrics Byos-for-IntaBio workflow enables straightforward CQA analysis, including the number of charge variants, the pI value for each separated variant, relative peak quantitation, and peak identification by MS.

The IntaBio iCIEF separation of adalimumab resolved the following peaks: Main, Pre-Basic 1, Basic 1, Pre-Basic 2, Basic 2, as well as Acidic 1 and Acidic 2. The pI of the Main peak was calculated to be 8.86. The pI of the peaks and relative peak areas are summarized in Table 1.

| Peak | pI | Relative Area % |
|---|---|---|
| Acidic 2 | 8.69 | 2.1 |
| Acidic 1 | 8.77 | 12.2 |
| Main | 8.86 | 66.5 |
| Pre-Basic 1 | 8.95 | 2.7 |
| Basic 1 | 8.98 | 14.2 |
| Pre-Basic 2 | 9.05 | 0.3 |
| Basic 2 | 9.07 | 2.0 |

**Table 1.** Peak name, pI, and relative percent area for adalimumab iCIEF peaks, analyzed with the Byos for Blaze workflow in Protein Metrics Byos software.

## Main peak analysis by mass spectrometry

### *Glycosylation profile*

The presence of asparagine-linked glycan moieties on therapeutic proteins can affect their potency and immunogenicity. Therefore, it is necessary to detect and identify these glycan structures. Using the IntaBio coupled to the SCIEX TripleTOF® 6600+, both high abundance and low abundance glycoforms of adalimumab were observed (Figure 4). The expected galactose-containing fucosylated oligosaccharide series was observed, including G0F/G0F, G0F/G1F, G1F/G1F (or G0F/G2F), and G1F/G2F. In addition to these, high mannose glycoforms such as man5/man5 and G0F/man5 were detected in the main peak.

These mannose structures are considered important CQAs due to observed augmentation of serum clearance.10 Lower abundance glycoforms including G0F/G0 and G0F/G0F-GlcNAc, were also observed (Figure 4).

The rich and complex deconvoluted mass spectra also revealed very minor abundances of a proteoform containing a single sugar moiety on one heavy chain. While this single glycosylated species would not contribute to a shift in pI of the protein, it is not observed in the other charge variant peaks due to its lower abundance.

## Acidic peak identification by Mass Spectrometry

### *Deamidation*

Deamidation is one of the more challenging PTMs to detect on the intact protein level because the mass shift is only 0.984 Da. Factors such as pH, temperature, formulation buffer components, and protein structure all contribute to the rates

and quantities of deamidation.[11] Deamidation occurs when side chain residues, such as Asn or Asp, undergo an intramolecular cyclization, resulting in a succinimide intermediate. The 5-membered succinimide ring is subsequently hydrolyzed with a water molecule, yielding the iso-Asp product. This succinimide intermediate will be further mentioned below, where its detection in adalimumab basic variants is discussed.

The separation of deamidated and non-deamidated adalimumab is enabled by the charge-based separation of iCIEF with mass information provided by the MS. This approach allows direct comparison of the nondeamidated and the deamidated species.

To properly identify the deamidated species, the G0F/ G0F subpopulation in the Main peak (pI = 8.86) was compared to the same subpopulation in Acidic 1. Here, we see a shift in the average mass in the deconvoluted mass spectra between the main G0F/G0F glycoform and the same glycoform in the Acidic 1 peak of Δm = 1-1.9 Da (Table 2). Since a single deamidation event corresponds to an overall 0.984 Da increase, these results suggest singly or doubly deamidated charge variants. This observed delta mass shift was further confirmed in multiple replicate injections and the mass difference of 1-2 Da was observed between the main peak and acidic variants for multiple glycoforms.

| Glycoform | Main Peak Mass (Da) | Acidic 1 Peak Mass (Da) | Delta (Da) |
|---|---|---|---|
| G0F/G0F | 148082.5 | 148083.5 | 1.0 |
| G0F/G1F | 148245.0 | 148246.5 | 1.5 |
| G1F/G1F | 148406.5 | 148408.4 | 1.9 |

**Table 2.** Identification of deamidation Acidic 1 peak. Column 2 shows the mass for the three most abundant glycoforms in the Main peak. Column 3 shows the measured mass for the Acidic 1 peak, demonstrating a consistent increase in mass of the glycoforms in the Acidic 1 peak, compared to the glycoform measured masses in the Main peak. The mass difference is 1-1.9 Da and suggests singly of doubly deamidated charge variants.

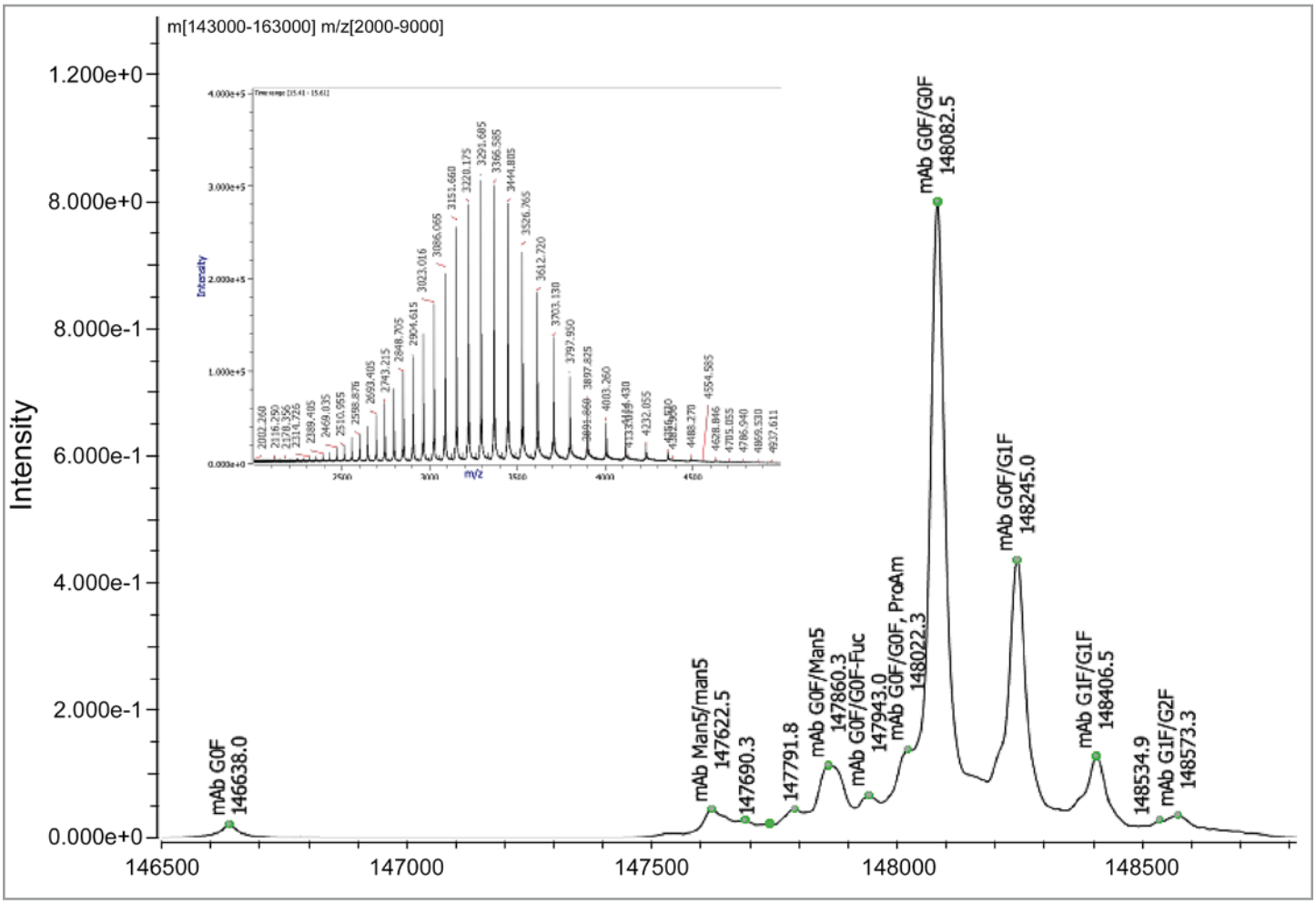


**Figure 4.** Deconvoluted mass spectrum of the Main peak demonstrating detection of high abundance and low abundance glycoforms, as well as a single glycosylation on the protein. Inset shows the raw mass spectrum for the main peak. The measured mass of the G0F/G0F proteoform was 14882.5 Da; the theoretical mass was 148080.1 Da.

### *Glycation*

Glycation is another common PTM, where sugars become covalently (non-enzymatically) bound to a free amine on an amino acid side chain (e.g. Lys), resulting in an acidic shift in the protein pI. This hexose addition is observed as a +162 Da shift in the deconvoluted MS spectrum. Glycation can be particularly challenging to track in glycosylated therapeutic antibodies, since the hexose addition mass shift can be confounded with the addition of isobaric sugars that are covalently bound to the more commonly observed and abundant N-linked biantennary glycans. To determine whether glycation is present in the acidic peaks, changes in the ratios of the most abundant protein glycoforms can be compared between the main peak and the acidic 1 peak.

In the analysis of adalimumab, this approach was employed to evaluate putative glycation. By comparing the relative abundances of the three main glycoforms (G0F/G0F, G0F/G1F, and G1F/G1F) via spectral peak height, a glycation event was detected. In this example, an increase in the ratio of G0F/G1F and G1F/G1F relative to G0F/G0F was observed in Acidic 1 vs the Main peak (Figure 5). As expected, the ratios of the three main glycoforms of the Main peak to Basic 1 were nearly identical. Coupling these observations with the acidic shift in pI, it is therefore likely that this change in relative abundance of adalimumab glycoforms can be attributed to the glycated protein. The change in relative abundance for the Main, Acidic 1 and Basic 1 peaks are shown in the inset of Figure 5. Comprehensive iCIEF-MS integrated analysis can provide meaningful detection capabilities of this CQA.

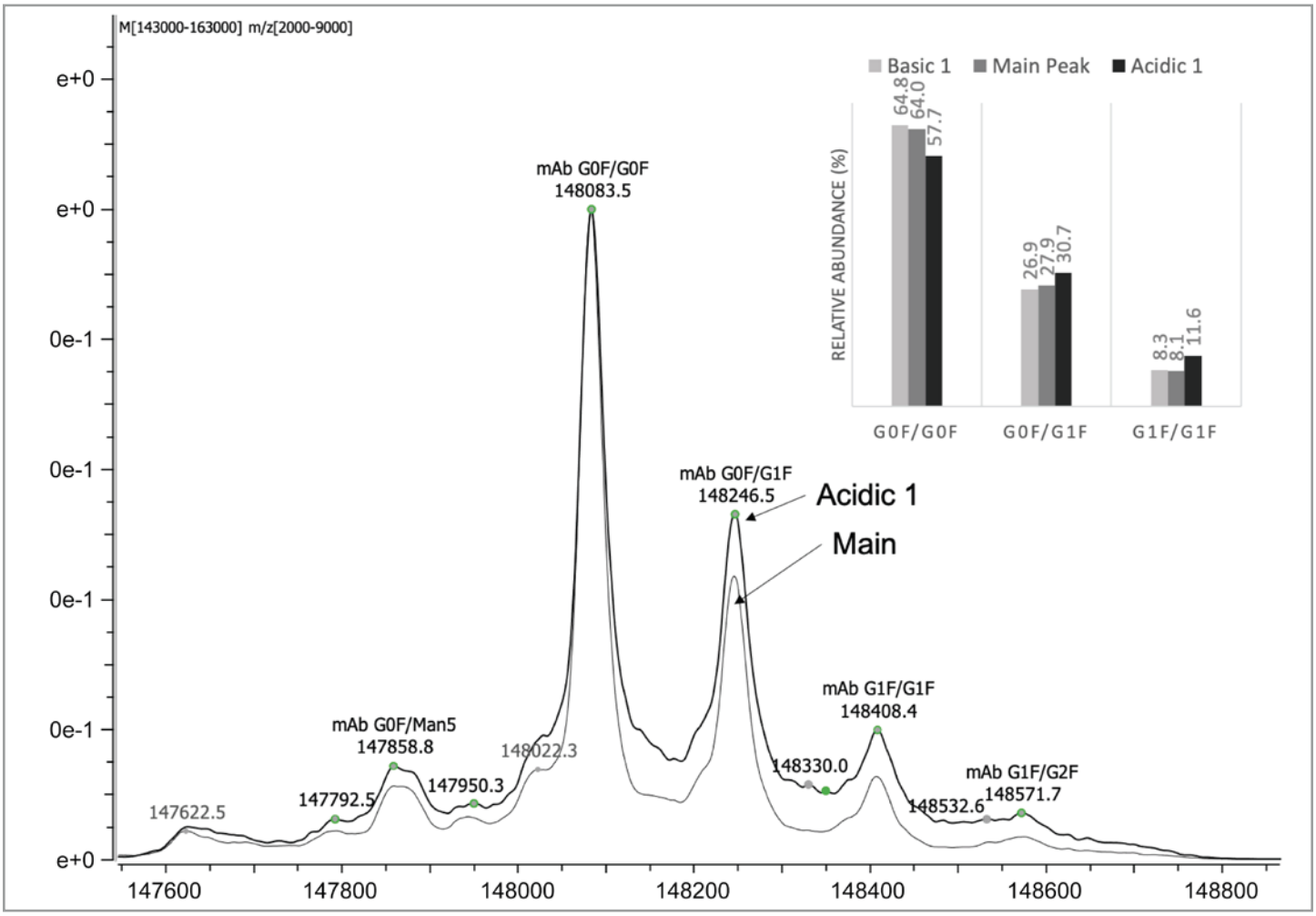


**Figure 5.** Overlay of the deconvoluted mass spectra of the Main and Acidic 1 peaks showing an increase in the abundance of larger proteoforms indicative of glycation. An increase in the apparent ratio of G0F/G1F and G1F/G1F relative to G0F/G0F was observed in Acidic 1 vs the Main peak. This ratio change occurs because the apparent G1F glycoform signal is compromised of two populations: G1F and the G0F proteoform containing glycation. Coupling these observations with the acidic shift in pI, it is therefore likely that this change in relative abundance of adalimumab glycoforms can be attributed to the glycated protein. Inset shows the apparent increase in the higher mass glycoforms for Acidic 1 relative to the Main and Basic 1 peaks.

## Basic peak identification by Mass Spectrometry

Among the more dominant proteoforms of adalimumab are the C-terminal lysine variants, which were nearly baseline resolved (Figure 6A). The two most readily observed basic peaks were associated with one or two unclipped lysine residues at the C-terminus (Figure 6C, E). These peaks are accompanied by a 128-Da shift for each clipping event relative to the deconvoluted mass spectra associated with the main peak (Figure 4).

### *Identification of succinimide formation*

The two abundant, basic lysine charge variant peaks are also accompanied by less abundant basic charge variants found in the valleys between Basic 2 and Basic 1 (Figure 6D), and Basic 1 and the Main peak (Figure 6B). Deconvoluted mass spectra for these regions, contained minor species with a nominal loss of 17 Da, were detected in the valleys. Loss of 17 Da is found in two peaks, pI = 9.05 (Pre-Basic 2) and 8.95 (Pre-Basic 1).

By considering the pI of these two variants in combination with their mass shifts, these two minor species were identified as succinimide formation of the Main and Basic 1 charge variants, consistent with previous reports[2]. Succinimide formation is a commonly observed intermediate of isoaspartic acid conversion from aspartic acid, which yields a loss of acidity within the protein. Although these minor species are difficult to resolve in the iCIEF electropherogram, they can be more confidently identified by using combined MS data with iCIEF and the associated knowledge that these modifications cause basic charge shifts.

### *Proline amidation*

Other minor subcomponents were also identified within the basic peaks. Proline-amidated species were observed and were accompanied by a nominal -57 Da shift in the deconvoluted mass spectra. This small subpopulation can be found in the Main and Basic 1 peaks and (Figure 4, Figure 6C, Table 3).

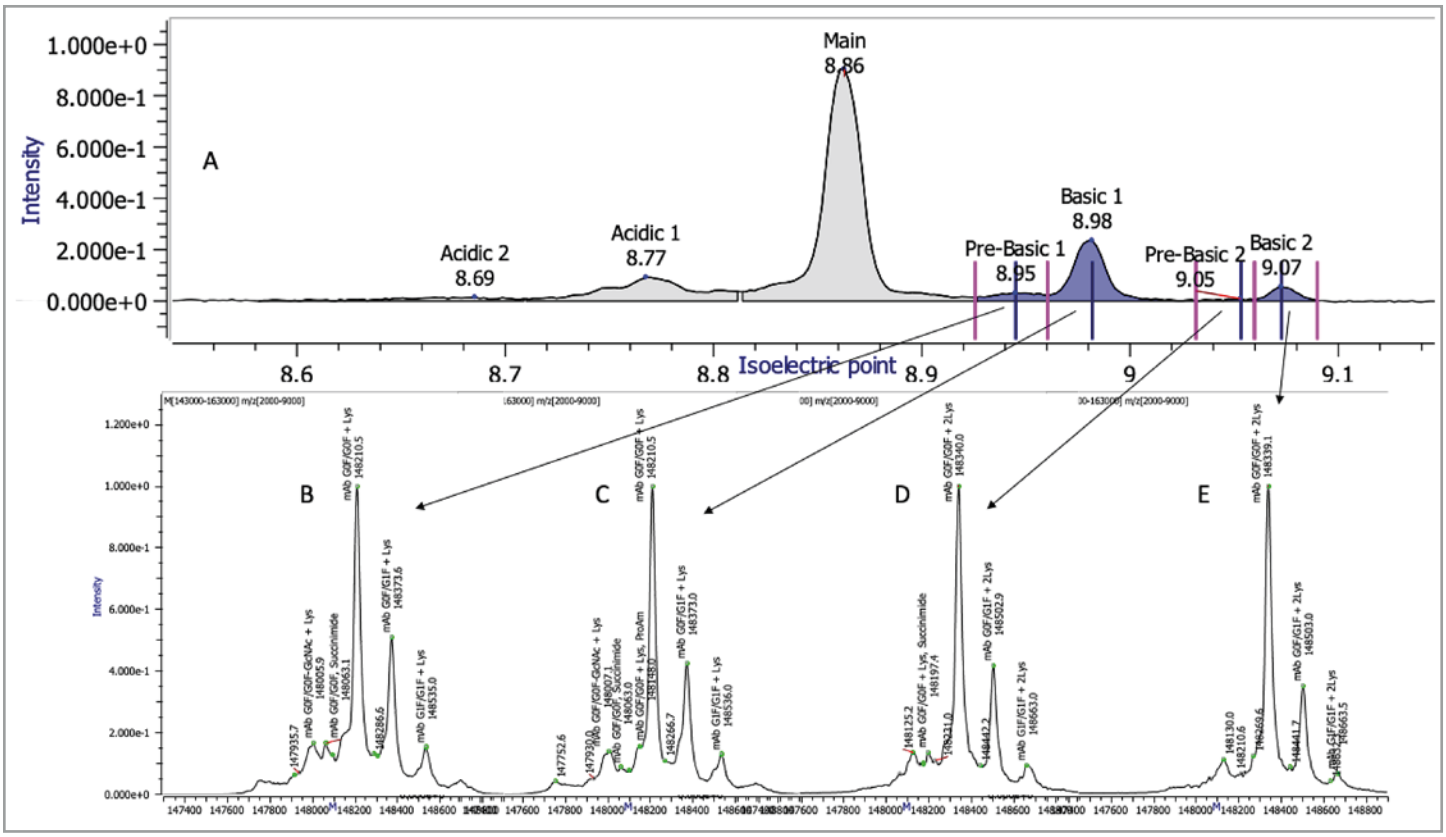


**Figure 6.** iCIEF separation (A) and corresponding deconvoluted mass spectrum for the basic variants including the Pre-Basic 1, Basic 1, Pre-Basic 2, and Basic 2 peaks. Basic 1 and Basic 2 peaks are nearly baseline resolved with one lysine or two lysine additions to the glycoforms, respectively, as seen in the deconvoluted MS results (C, E). Pre-Basic 1 contains the G0F/G0F + succinimide proteoform (B) and Pre-Basic 2 contains the G0F/G0F+lysine+succinimide proteoform (B).

## Summary reporting of results

To facilitate data review and archiving of the charge variant analysis, common reports are pre-loaded and can also be highly customized in the Byos-for-IntaBio workflow. Features include an in-depth report formatting and intuitive design, removing the ambiguity for future analyses of a therapeutic protein. The CQA analysis can be summarized by combining results obtained from the iCIEF separation and the MS confirmation data into a combined report, displaying relative quantitation and absolute intensity of charge variants via UV absorbance and identification of specific subpopulations found via the intact mass analysis (Table 3). Additional reporting formats, heatmaps, charts, and tabulations can be tailored for each user's preferences, for research and development and regulatory filings. Protein Metrics enables analysis and reporting of IntaBio iCIEF data and MS data using the Byos-for-IntaBio workflow.

PROTEIN METRICS

| | | | | | | Sample name ← | B2E-20201125_7326_Adali_06_inj8 (%) |
|---|---|---|---|---|---|---|---|
| Apex pI ↑ | Peak Comment | Intensity ↓ | Name ↑ | Mass ↑ | Delta mass from calc. ↑ | | |
| 8.69 | Acidic 2 | 11640.8 | mAb , G0F/G0F | 148083 | 2.8 | | 49.1 |
| | | 5632.0 | mAb , G0F/G1F | 148244 | 1.3 | | 23.7 |
| | | 2345.6 | mAb , G1F/G1F | 148408 | 2.8 | | 9.9 |
| | | 1585.3 | mAb , G0F/Man5 | 147862 | 9.5 | | 6.7 |
| | | 1440.1 | mAb , G0F/G0F-GlcNAc | 147879 | 2.0 | | 6.1 |
| | | 1083.5 | mAb , G1F/G2F | 148570 | 3.3 | | 4.6 |
| 8.77 | Acidic 1 | 23688.3 | mAb , G0F/G0F | 148084 | 2.9 | | 51.4 |
| | | 12588.4 | mAb , G0F/G1F | 148246 | 3.6 | | 27.3 |
| | | 4727.3 | mAb , G1F/G1F | 148408 | 3.4 | | 10.3 |
| | | 3413.8 | mAb , G0F/Man5 | 147859 | 6.3 | | 7.4 |
| | | 1699.9 | mAb , G1F/G2F | 148572 | 4.6 | | 3.7 |
| 8.86 | Main | 80422.2 | mAb , G0F/G0F | 148082 | 1.8 | | 51.0 |
| | | 35098.2 | mAb , G0F/G1F | 148245 | 2.1 | | 22.3 |
| | | 11135.0 | mAb , G0F/G0F, ProAm | 148022 | -1.4 | | 7.1 |
| | | 10295.8 | mAb , G1F/G1F | 148406 | 1.5 | | 6.5 |
| | | 9086.9 | mAb , G0F/Man5 | 147860 | 7.8 | | 5.8 |
| | | 5320.7 | mAb , G0F/G0F-Fuc | 147943 | 8.5 | | 3.4 |
| | | 3548.9 | mAb , Man5/man5 | 147623 | -1.7 | | 2.2 |
| | | 2831.0 | mAb , G1F/G2F | 148573 | 6.1 | | 1.8 |
| 8.95 | Pre-Basic 1 | 8719.3 | mAb , G0F/G0F + Lys | 148211 | 1.6 | | 47.1 |
| | | 4457.5 | mAb , G0F/G1F + Lys | 148374 | 2.5 | | 24.1 |
| | | 1454.6 | mAb , G0F/G0F-GlcNAc + Lys | 148006 | 0.3 | | 7.9 |
| | | 1450.6 | mAb , G0F/G0F, Succinimide | 148063 | -0.6 | | 7.8 |
| | | 1341.8 | mAb , G1F/G1F + Lys | 148535 | 1.8 | | 7.2 |
| | | 1104.6 | mAb , G0F/G1F + Lys , ProAm | 148305 | -9.5 | | 6.0 |
| 8.98 | Basic 1 | 18685.6 | mAb , G0F/G0F + Lys | 148211 | 1.6 | | 51.4 |
| | | 7971.5 | mAb , G0F/G1F + Lys | 148373 | 1.9 | | 21.9 |
| | | 2930.0 | mAb , G0F/G0F + Lys, ProAm | 148148 | -3.9 | | 8.1 |
| | | 2619.5 | mAb , G0F/G0F-GlcNAc + Lys | 148007 | 1.5 | | 7.2 |
| | | 2448.8 | mAb , G1F/G1F + Lys | 148536 | 2.8 | | 6.7 |
| | | 1665.4 | mAb , G0F/G0F, Succinimide | 148063 | -0.6 | | 4.6 |
| 9.05 | Pre-Basic 2 | 3174.3 | mAb , G0F/G0F + 2Lys | 148340 | 3.0 | | 60.6 |
| | | 1326.6 | mAb , G0F/G1F + 2Lys | 148503 | 3.9 | | 25.3 |
| | | 436.2 | mAb , G0F/G0F + Lys, Succinimide | 148197 | 5.6 | | 8.3 |
| | | 303.0 | mAb , G1F/G1F + 2Lys | 148663 | 1.7 | | 5.8 |
| 9.07 | Basic 2 | 3458.8 | mAb , G0F/G0F + 2Lys | 148339 | 2.1 | | 70.5 |
| | | 1220.4 | mAb , G0F/G1F + 2Lys | 148503 | 4.0 | | 24.9 |
| | | 229.3 | mAb , G1F/G1F + 2Lys | 148664 | 2.3 | | 4.7 |

**Table 3.** Automated report summarizing the key results of the Blaze iCIEF-MS of adalimumab analysis for each iCIEF-separated peak including pI, quantitation by UV with mass identification and assignment by MS. Multiple other predefined and customizable reports are also available.

## Multiple replicates analyzed with the IntaBio System

Replicate injections of the adalimumab sample were analyzed.

Figure 7 shows 25 sequential injections.

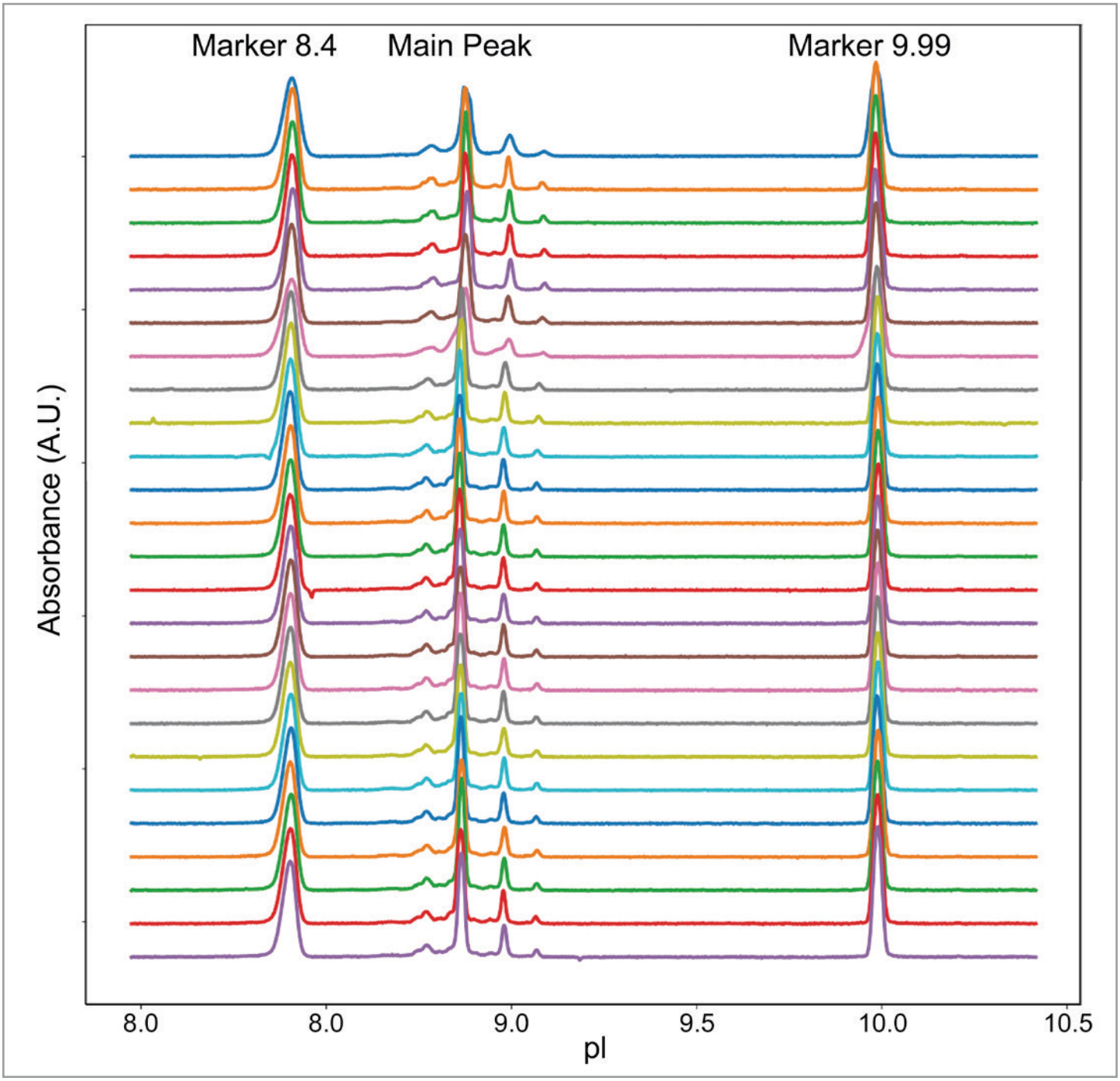


**Figure 7.** 25 sequential Blaze injections of adalimumab demonstrate the reproducibility of the Blaze System. The Main peak is labeled, along with the peaks resulting from the pI markers.

Summary statistics of 25 sequential, replicate injections of adalimumab are shown in Table 4. The Main peak pI and the Main, Acidic and Basic regions' relative percent area were quantified for the 25 replicates. The pI of the main peak is calculated to be 8.86 and with a coefficient of variation (CV) of 0.11% demonstrating extremely high repeatability. The mean area for the Main, Acidic and Basic regions, was 64.1%, 17.0% and 18.9% respectively.

| Injection | Main pI | Acidic area (%) | Main peak area (%) | Basic area (%) |
|---|---|---|---|---|
| 1 | 8.87 | 15.4 | 62.6 | 22.0 |
| 2 | 8.87 | 15.3 | 63.5 | 21.2 |
| 3 | 8.87 | 17.8 | 65.7 | 16.5 |
| 4 | 8.87 | 17.2 | 66.6 | 16.3 |
| 5 | 8.88 | 16.9 | 66.5 | 16.6 |
| 6 | 8.87 | 17.2 | 65.4 | 17.4 |
| 7 | 8.87 | 16.7 | 62.5 | 20.8 |
| 8 | 8.86 | 17.3 | 64.5 | 18.2 |
| 9 | 8.86 | 17.0 | 62.1 | 20.9 |
| 10 | 8.86 | 18.6 | 61.2 | 20.2 |
| 11 | 8.86 | 18.1 | 64.8 | 17.0 |
| 12 | 8.86 | 17.4 | 65.8 | 16.8 |
| 13 | 8.86 | 17.8 | 65.2 | 17.0 |
| 14 | 8.86 | 17.7 | 65.1 | 17.2 |
| 15 | 8.86 | 17.1 | 63.0 | 20.0 |
| 16 | 8.86 | 16.7 | 65.6 | 17.7 |
| 17 | 8.86 | 17.7 | 64.7 | 17.6 |
| 18 | 8.86 | 16.1 | 63.5 | 20.4 |
| 19 | 8.86 | 17.4 | 65.0 | 17.6 |
| 20 | 8.86 | 16.4 | 62.7 | 21.0 |
| 21 | 8.86 | 16.8 | 65.7 | 17.5 |
| 22 | 8.86 | 16.2 | 63.4 | 20.4 |
| 23 | 8.86 | 16.2 | 63.7 | 20.1 |
| 24 | 8.86 | 16.9 | 62.4 | 20.7 |
| 25 | 8.86 | 17.8 | 62.0 | 20.2 |
| *Mean* | *8.86* | *17.0* | *64.1* | *18.9* |
| *Std dev* | *0.01* | *0.8* | *1.5* | *1.8* |
| *CV %* | *0.11* | *4.6* | *2.4* | *9.7* |

**Table 4.** Summary statistics of 25 sequential, replicate injections of adalimumab. The pI of the main peak is calculated to be 8.86 and with a coefficient of variation (CV) of 0.11% demonstrating extremely high repeatability. The mean area for the Main, Acidic and Basic regions, was 64.1%, 17.0% and 18.9% respectively.

## Conclusion

Here we demonstrate IntaBio iCIEF separation and peak identification for each iCIEF-separated peak by MS, highlighting the detection of charge variants including, C-terminal lysine variants, proline amidation, glycation, deamidation, succinimide intermediates, as well as high abundance and low-abundance neutral glycoforms for the analysis of the adalimumab antibody. The new IntaBio data analysis workflow in Byos software enables users to move rapidly from data through results to decisions. The Byos-for-IntaBio workflow automates data analysis and includes predefined report templates, and customizable reports for streamlined data analysis, interpretation and reporting.

The IntaBio system coupled to the SCIEX TripleTOF 6600+ provides an integrated characterization technique that enables iCIEF-MS analysis of mAbs, all in about 15 minutes. Rapid monitoring of CQAs will help to accelerate the development of therapeutic mAbs by accelerating the analysis and providing more detailed structural mAb characterization earlier in the drug development process.

## References

1. Higel F, Seidl A, Sörgel F, Friess W. N-glycosylation heterogeneity and the influence on structure, function and pharmacokinetics of monoclonal antibodies and Fc fusion proteins. *Eur. J. Pharm. Biopharm.*2016; 100.
2. Füssl F, Trappe A, Cook K, Scheffler K, Fitzgerald O, Bones J. Comprehensive characterisation of the heterogeneity of adalimumab via charge variant analysis hyphenated on-line to native high resolution Orbitrap mass spectrometry. *MAbs* 2019; 11.
3. Wang W, Singh S, Zeng DL, King K, Nema S. Antibody structure, instability, and formulation. *J. Pharm. Sci.*2007; 96.
4. Vlasak J, Ionescu R. Heterogeneity of Monoclonal Antibodies Revealed by Charge-Sensitive Methods. *Curr Pharm Biotechnol* 2008; 9.
5. Du Y, Walsh A, Ehrick R, Xu W, May K, Liu H. Chromatographic analysis of the acidic and basic species of recombinant monoclonal antibodies. *MAbs* 2012; 4:578–85.
6. He XZ, Que AH, Mo JJ. Analysis of charge heterogeneities in mAbs using imaged CE. *Electrophoresis* 2009; 30.
7. Cohen S, Genovese MC, Choy E, Perez-Ruiz F, Matsumoto A, Pavelka K, Pablos JL, Rizzo W, Hrycaj P, Zhang N, et al. Efficacy and safety of the biosimilar ABP 501 compared with adalimumab in patients with moderate to severe rheumatoid arthritis: A randomised, double-blind, phase III equivalence study. *Ann Rheum Dis* 2017; 76.
8. Lyubarskaya Y, Houde D, Woodard J, Murphy D, Mhatre R. Analysis of recombinant monoclonal antibody isoforms by electrospray ionization mass spectrometry as a strategy for streamlining characterization of recombinant monoclonal antibody charge heterogeneity. *Anal Biochem* 2006; 348.
9 Mack S, Arnold D, Bogdan G, Bousse L, Danan L, Dolnik V, Ducusin MA, Gwerder E, Herring C, Jensen M, et al. A novel microchipbased imaged CIEF-MS system for comprehensive characterization and identification of biopharmaceutical charge variants. *Electrophoresis* 2019; 40.
10. Goetze AM, Liu YD, Arroll T, Chu L, Flynn GC. Rates and impact of human antibody glycation in vivo. *Glycobiology* 2012; 22.
11. Liu S, Madren S, Feng P, Sosic Z. Characterization of the acidic species of a monoclonal antibody using free flow electrophoresis fractionation and mass spectrometry. *J Pharm Biomed Anal* 2020; 185:113217.