

ZipChip

High-Throughput Intact Mass Analysis of mAb Based Therapeutics

INTRODUCTION

Intact mass analysis of biotherapeutic proteins using mass spectrometry is a key characterization technique performed from upstream drug development to downstream product analysis. It is routinely used for sequence confirmation, clone screening, glycoform quantification, and to assess purity and heterogeneity during product quality control screening.

ZipChip's method for intact mAb analysis using denaturing conditions is the simplest and most universal method for intact protein analysis using the platform. The low pH of the BGE effectively mobilizes and ionizes intact proteins resulting in high-quality mass spectra with fast run times. Additionally, it is very tolerant of sample matrix components, such as salts and detergents, so there is generally little to no sample prep necessary.

In this work we demonstrate the high throughput analysis of monoclonal antibodies. Migration times on the order of 0.5 min are obtained and glycoforms of the mAbs are identified from concentrations ranging 1 mg/mL to 1 μ g/mL. Overall, the ZipChip method for intact mass analysis is a simple, high-throughput approach that can facilitate rapid MS characterization of biotherapeutic proteins.

MATERIALS AND METHODS

Materials. Four monoclonal antibodies were used for this study: the NIST mAb Reference Standard, Trastuzumab (Herceptin), Sigma SILu Lite mAb Universal Antibody Standard (Millipore Sigma), and Lokivetmab (Cytoint). The ZipChip Peptides Kit was used for sample preparation and analysis.

Sample Preparation. All antibody stock solutions were diluted to 0.25 mg/mL with Peptide BGE. No further sample processed was necessary.

Analysis. Peptide background electrolyte was used for denatured analysis following the ZipChip protocol

Intact mAb Analysis Using Denaturing Conditions. A 5 nL on-chip injection was performed and an HS chip operated at a field strength of 1000 V/cm was used to maximize throughput. Pressure assist was set to enable at 0.2 minutes.

Data Collection and Processing. All data was collected on an Exactive Plus EMR orbitrap mass spectrometer (Thermo Fisher Scientific). A data acquisition sequence was created in the ZipChip App and a corresponding sequence was created in Xcalibur (Thermo Fisher Scientific). Data collection was triggered via contact closure relay. Data was processed using QualBrower and BioPharmaFinder (Thermo Fisher Scientific).

RESULTS AND DISCUSSION

To prepare the mAbs for denaturing intact analysis the only sample preparation necessary was a simple dilution from formulation strength to the working concentration of 0.25 mg/mL. For all mAbs analyzed under denaturing conditions migration times were less than 1 minute. Each mAb was analyzed 9 times. Figure 1a shows the overlay of all 9 replicate runs of trastuzumab. With these analysis conditions separation of charge variants is not expected. A single peak corresponding to the mAb is detected and is about 6 seconds wide. The average migration time was calculated to be 0.53 min with RSD of 2.1%. Figure 1b plots the migration time as a function of run number for each mAb. Migration times for all 4 mAbs were consistently at approximately 0.5-0.6 minutes. The average migration times for NIST, trastuzumab, Sigma mAb, and Cytoint were 0.56 (\pm 2.5%), 0.53 (\pm 2.1%), 0.56 (\pm 4.6%), and 0.54 (\pm 2.4%) respectively.

Figure 2 shows a raw mass spectrum for trastuzumab. The spectrum was generated by averaging the scans across the width of the peak. Glycoforms of the mAb are clearly visible within a charge state as shown in the inset. Several intact glycoforms were identified after deconvolution using BioPharmaFinder. Figure 2 shows

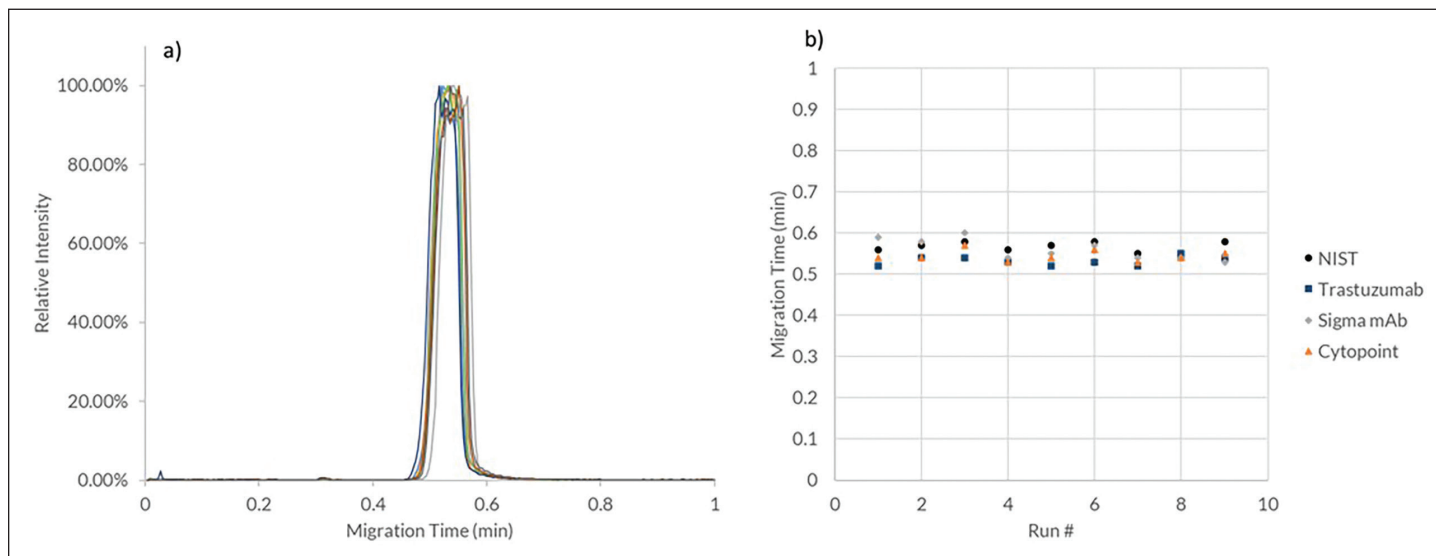


Figure 1: a) Overlaid electropherograms of nine replicates of trastuzumab. Under denaturing conditions, a single peak corresponding to the mAb is detected. Migration time RSD for the replicate runs is 2.1%. b) Plot of migration time versus run number for all 4 mAbs analyzed. Migration times for each mAb were consistently between 0.5 and 0.6 min.

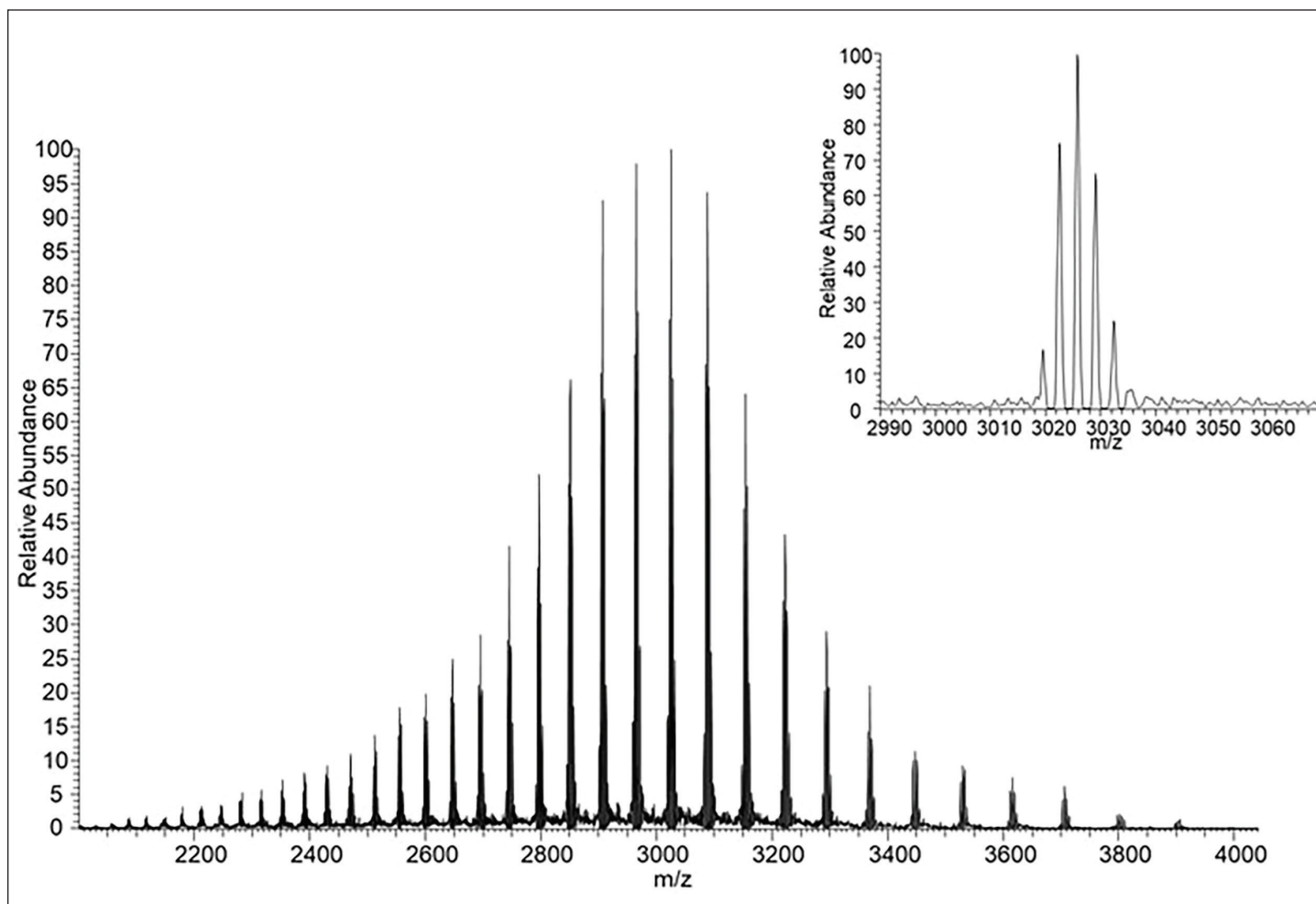


Figure 2: Raw mass spectrum for trastuzumab. The protein envelope spans approximate 2000-4000 m/z. Glycoforms are resolved within the charge states (inset).

the deconvoluted spectrum for trastuzumab. The six most abundant glycoforms were identified based on the sequence of the mAb and the known masses of the glycoforms. Table 1 lists the 6 most abundant identified glycoforms and the mass error observed. Mass errors for all identified species were less than 20 ppm.

A dilution series of the NIST mAb was prepared to assess the sensitivity of the method. The limits of detection were estimated based on the lowest concentration sample where the mass spectrum was able to be processed to return accurate masses for the 3 most abundant glycoforms. Based on this criterion the estimated LODs for a typical IgG biotherapeutic mAb are 1 µg/mL. Figure 4 compares the analysis of a 0.5 mg/mL NIST mAb sample and a 1 µg/mL NIST mAb sample. As seen in Figure 4b, four of the main glycoforms were identified within 20 ppm despite the low concentration of antibody. More complex sample matrices containing a higher level of non-volatile salts or higher complexity proteins, like ADCs, may impact the LOD.

To demonstrate the matrix tolerability of the ZipChip intact mass assay NIST mAb was analyzed in a growth media matrix to mimic a harvest sample of drug product from a bioreactor. The sample was analyzed after a 4x dilution with BGE which resulted in a mAb concentration of 0.25 mg/mL. Figure 5 shows the electropherogram and processed mass spectrum of the

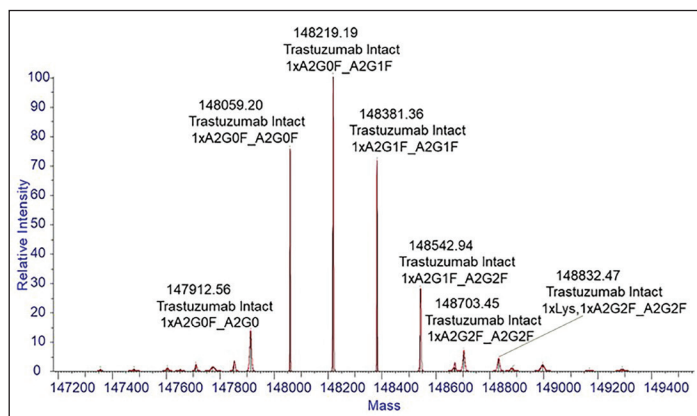


Figure 3: Processed mass spectrum of trastuzumab with the most abundant glycoforms labeled.

| Average Mass | Matched Delta Mass (ppm) | Matched Sequence |
|--------------|--------------------------|------------------|
| 147912.6 | 14.6 | A2G0F_A2G0 |
| 148059.2 | 18 | A2G0F_A2G0F |
| 148219.2 | 3.4 | A2G0F_A2G1F |
| 148381.4 | 3.6 | A2G1F_A2G1F |
| 148542.9 | 0.1 | A2G1F_A2G2F |
| 148703.5 | 11 | A2G2F_A2G2F |

Table 1: Identified glycoforms of trastuzumab from intact analysis via ZipChip.

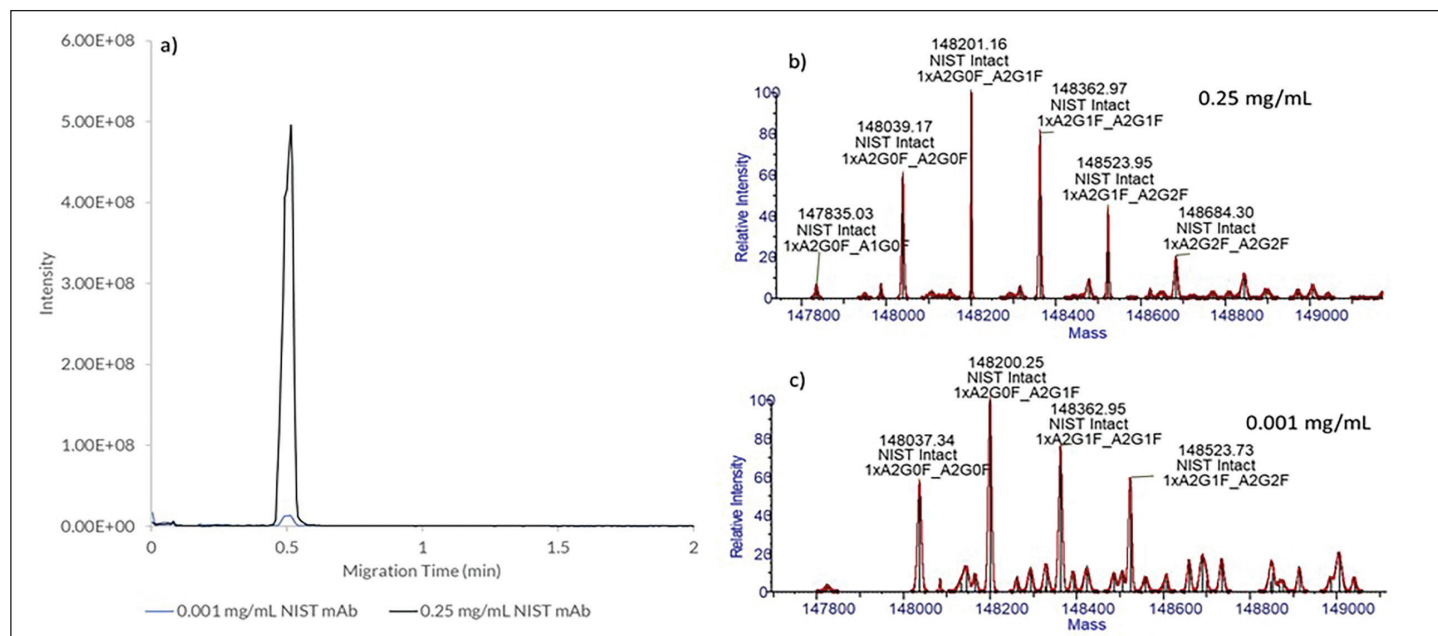


Figure 4: High and low concentration of NIST mAb. a) Electropherograms for a 0.25 mg/mL and 0.001 mg/mL sample of NIST mAb. b) Processed mass spectra for the NIST mAb samples. At the 0.001 mg/mL 4 glycoforms of the NIST were identified with a mass error of less than 20 ppm.

mock harvest sample. Despite the high salt content of the sample matrix, a peak corresponding to the mAb is easily detected. As seen in 5b, the five most abundant glycoforms were identified with less than 20 ppm mass error.

CONCLUSIONS

The ZipChip method for intact mass analysis using denaturing conditions is a simple, high-throughput approach that can facilitate rapid MS characterization of biotherapeutic proteins. Using an HS chip and Peptide BGE run times for intact mass analysis can be on the order of 1 minute or less. Even though run times are rapid a high degree of mass accuracy can be

achieved in the process mass spectra. Glycoforms were identified with less than 20 ppm mass error for the mAbs discussed here. The limits of detection for this method were estimated to be 1µg/mL. The matrix tolerance of the ZipChip intact mass method was demonstrated by analyzing a mAb in growth media. Despite the increase in matrix complexity glycoforms were still identified with <20 ppm mass error. While monoclonal antibodies are the focus of this work, the method is applicable to many different types of proteins of varying size and complexity and can be used as a routine intact protein analysis technique.

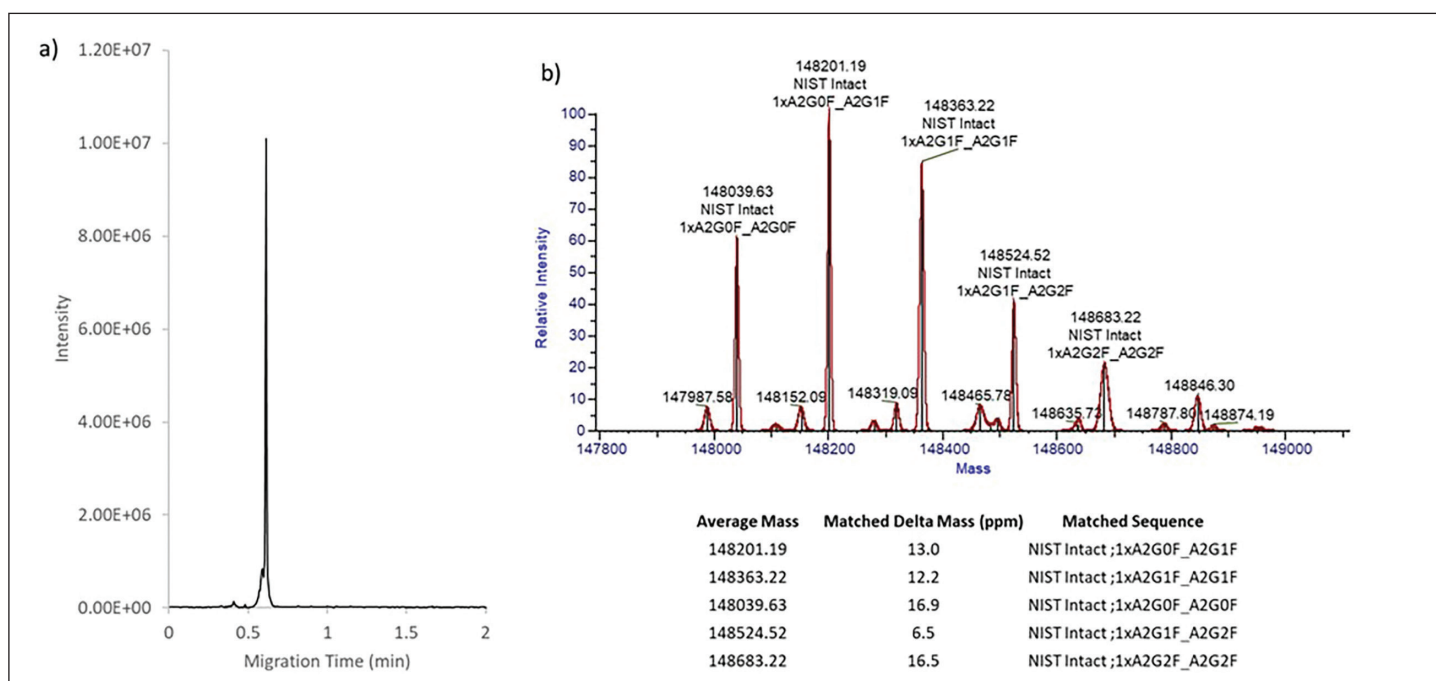


Figure 5: Mock bioreactor harvest sample. a) Base peak MS 2500-4000 m/z of the analysis of NIST mAb in growth media. After dilution the mAb concentration was 0.25 mg/mL. b) Processed mass spectrum for NIST mAb. Despite the complex sample matrix, five glycoforms were identified with a mass error of less than 20 ppm.



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