

## Intact mAb analysis on the timsTOFHT QTOF mass spectrometer with BioPharma Compass

Monoclonal antibodies (mAbs) play a central role in therapeutic, diagnostic, and research contexts. Due to their inherent heterogeneity, the characterization of intact mAbs is crucial for ensuring their efficacy and safety.

### Abstract

Regulatory agencies emphasize strict control over the development of mAbs. This note discusses the application of the Bruker timsTOF HT system and BioPharma Compass® software, and provides a streamlined, fast, and cost-effective approach to screen for mAb variations and modifications.

Keywords:  
mAb characterization,  
timsTOF,  
Biopharma Compass

### Introduction

The biopharmaceutical industry is continuing to grow at a fast rate, and monoclonal antibodies (mAbs) are a dominant drug modality. In addition to their therapeutic use, they are used as diagnostic and research tools in techniques such as ELISA, western blotting, immunohistochemistry, and flow cytometry [1].

Intact mAbs characterization is essential to detail the inherently heterogeneous nature of these proteins due to the presence of PTMs, incomplete processing, susceptibility to degradation, and disulfide shuffling [2,3]. PTMs including oxidation, deamidation, glycosylation, glycation, proteolysis, and others, may significantly alter the efficacy and safety of the protein drug [2,3]. Thus, regulatory agencies require processes related to mAbs development, production, and distribution to be controlled and reproducible [4].

Mass spectrometry (MS) based approaches are one of the most powerful analytical tools for monitoring and defining critical quality attributes of mAbs.

This application note describes the use of the Bruker timsTOF HT system for analyzing intact mAbs with the QTOF mode. Multiple attributes of the mAb were directly measured in a single easy-to-use Multi-Target Screening workflow within Bruker's BioPharma Compass software. Intact mass measurements provide a straightforward, fast, and cost-efficient way to screen for variations from reference sequences or modification profiles.

## Material and Methods

The NIST monoclonal antibody (NISTmAb) reference material RM 8671 (National Institute of Standards and Technology, Gaithersburg, MD) was diluted to a final concentration of 0.5 mg/mL. 1  $\mu$ L of the sample was analyzed by LC-MS (Figure 1). The HPLC separation was performed on a Bruker Elute UHPLC with a BEH 300 C4 2.1x100 mm (Waters, Milford, MA) at 300  $\mu$ L/min, held at 60°C using a 15-minute gradient.

Mobile phase A (aqueous phase): LC-MS grade water with 0.1% Formic Acid

Mobile phase B (organic phase): LC-MS grade acetonitrile with 0.1% Formic Acid

HPLC gradient			
Time [min]	Flow [mL/min]	% A	% B
0.00	0.30	95.0	5.0
1.00	0.30	95.0	5.0
10.00	0.30	10.0	90.0
12.00	0.30	10.0	90.0
12.50	0.30	95.0	5.0
15.00	0.30	95.0	5.0

ESI source parameters		
Nebulizer	Dry gas	Dry temp
1.8 bar	10 L/min	220°C
Scan range	Scan mode	Spectra rate
600-5000 <i>m/z</i>	MS	1 Hz
Tune		
Deflection 1 delta	70 V	
Funnel 1 RF	350 Vpp	
isCID	100 eV	
Funnel 2 RF	600 Vpp	
Multipole RF	400 Vpp	
Quadrupole ion energy	5 eV	
Quadrupole low mass	800 <i>m/z</i>	
Collision energy	10 eV	
Collision RF	2000 Vpp	
Transfer time	150 $\mu$ s	
Pre-pulse storage	15 $\mu$ s	
Options - Vacuum		
TIMS in	2.34 mbar	

## Data processing

The raw LC-MS data were processed in BioPharma Compass using the Multi-Target Screening workflow. Protein screening using intact mAb analysis is a fast way to gain an overview of the major glycoforms and other PTMs. A dynamic range of >100 is typically achieved using this approach, thus peaks down to at least 1% of the base peak can be detected.

For each sample, the target chemical formula was provided. In addition, the expected glycoforms were specified in a Modification Profile, providing their formula and relative intensity.

## Results and discussion

Accelerating the transition of a drug candidate from initial identification to pre-clinical development, clinical trials, and eventual regulatory approval and commercialization is paramount for the success of biological drugs. The attributes of an antibody—such as homogeneity, stability, and specificity—are intrinsically linked to its structure and play a critical role in this process. Optimizing these attributes at the discovery stage is significantly more cost-effective than making adjustments during later development stages. Given that antibody drugs are derived from living cells, they inherently lack the homogeneity seen in chemically synthesized drugs. This variability introduces product-related impurities, which are variants of the

**Table 1**  
timsTOF HT parameters – MS (tims off mode)

main drug product that differ in activity, efficacy, and stability. Regulatory requirements mandate that these impurities be maintained at acceptable levels to ensure the drug's safety and effectiveness [5].

The Multi-Target Screening workflow in BioPharma Compass offers a comprehensive solution for drug discovery efforts. This tool provides researchers with all the necessary information to evaluate the key attributes of antibodies through user-friendly, interactive views. By leveraging this workflow, scientists can effectively screen and optimize antibody candidates early in the discovery process, facilitating a smoother and more cost-efficient progression to subsequent development stages.

The Results table (Figure 1) provides a summary of the sample analyses, presenting critical information such as the MAM reporting icon, the base peak of the spectrum, and the mass difference to the base peak. This consolidated view enables users to quickly assess the overall quality and integrity of their samples. For more in-depth analysis, the Expected view provides detailed results of individual analyses. Each compound in the Modification Profile, as defined in the wizard, is evaluated for its presence or absence in the sample, with results displayed using a red/green color code. This immediate visual feedback helps users easily determine whether the expected compounds are present, facilitating quick decision-making. Additionally, the relative intensities of the sample versus the reference are compared, allowing users to assess how closely the measured profile matches the reference profile. This comparison is crucial for identifying deviations that affect the sample's quality or efficacy. The Unidentified view lists all peaks in the spectrum that do not correspond to the Modification Profile and exceed the intensity threshold set in the Workflow wizard. This feature helps users identify and investigate unexpected components in their samples, providing valuable insights into potential impurities or anomalies.

Row	Result	Position	Base Peak Mr [Da]	$\Delta$ Base Peak Mr [Da]	Sample Name	Formula or Mr for Molecule1																																																																																																																																								
1		2	148201.2	-1.0	Intact NIST mAb	145439.8																																																																																																																																								
<div style="display: flex; border-bottom: 1px solid black;"> <span style="margin-right: 10px;">Expected</span> <span style="margin-right: 10px;">Unidentified</span> <span>All</span> </div> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Formula</th> <th>Annotation</th> <th>Mr Ref</th> <th>Mr Sample</th> <th><math>\Delta</math> Mr [ppm]</th> <th><math>\Delta</math> Mr [Da]</th> <th>Int. [a.u.]</th> <th>Rel. Int. Ref [%]</th> <th>Rel. Int. Sa...</th> <th>Rt Sample [...]</th> <th>Confirmed</th> </tr> </thead> <tbody> <tr> <td>Da</td> <td>G2F/- 2pQ-2K</td> <td>146919.0</td> <td></td> <td></td> <td></td> <td>0.000E+00</td> <td>unexpected</td> <td>0.0</td> <td></td> <td>Yes</td> </tr> <tr> <td>Da</td> <td>G3F/- 2pQ-2K</td> <td>147081.2</td> <td></td> <td></td> <td></td> <td>0.000E+00</td> <td>unexpected</td> <td>0.0</td> <td></td> <td>Yes</td> </tr> <tr> <td>Da</td> <td>native</td> <td>145439.8</td> <td></td> <td></td> <td></td> <td>0.000E+00</td> <td>unexpected</td> <td>0.0</td> <td></td> <td>Yes</td> </tr> <tr> <td></td> <td></td> <td></td> <td>147998.6</td> <td></td> <td></td> <td>1.017E+03</td> <td></td> <td>13.0</td> <td>5.17</td> <td>No</td> </tr> <tr> <td>Da</td> <td>G0F/G0F-2K 2pQ</td> <td>148040.1</td> <td>148039.0</td> <td>-7.35</td> <td>-1.1</td> <td>4.576E+03</td> <td>12.9</td> <td>15.8</td> <td>5.17</td> <td>Yes</td> </tr> <tr> <td>Da</td> <td>G0F/G0F-K 2pQ</td> <td>148168.2</td> <td>148162.8</td> <td>-36.75</td> <td>-5.4</td> <td>1.519E+03</td> <td>5.3</td> <td>5.2</td> <td>5.17</td> <td>Yes</td> </tr> <tr style="background-color: #e6f2ff;"> <td>Da</td> <td>G0F/G1F-2K 2pQ</td> <td>148202.2</td> <td>148201.2</td> <td>-6.55</td> <td>-1.0</td> <td>7.837E+03</td> <td>21.6</td> <td>27.0</td> <td>5.17</td> <td>Yes</td> </tr> <tr> <td>Da</td> <td>G0F/G1F-K 2pQ</td> <td>148330.4</td> <td>148326.8</td> <td>-24.11</td> <td>-3.6</td> <td>1.511E+03</td> <td>5.4</td> <td>5.2</td> <td>5.17</td> <td>Yes</td> </tr> <tr> <td>Da</td> <td>G1F/G1F-2K 2pQ</td> <td>148364.3</td> <td>148363.5</td> <td>-5.74</td> <td>-0.9</td> <td>6.581E+03</td> <td>18.6</td> <td>22.7</td> <td>5.17</td> <td>Yes</td> </tr> <tr> <td>Da</td> <td>G0F/G1F 2pQ</td> <td>148458.6</td> <td>148461.4</td> <td>19.44</td> <td>2.9</td> <td>3.833E+02</td> <td>1.9</td> <td>1.3</td> <td>5.17</td> <td>Yes</td> </tr> <tr> <td>Da</td> <td>G1F/G1F-K 2pQ</td> <td>148492.5</td> <td>148490.2</td> <td>-15.45</td> <td>-2.3</td> <td>9.247E+02</td> <td>3.5</td> <td>3.2</td> <td>5.17</td> <td>Yes</td> </tr> </tbody> </table>											Formula	Annotation	Mr Ref	Mr Sample	$\Delta$ Mr [ppm]	$\Delta$ Mr [Da]	Int. [a.u.]	Rel. Int. Ref [%]	Rel. Int. Sa...	Rt Sample [...]	Confirmed	Da	G2F/- 2pQ-2K	146919.0				0.000E+00	unexpected	0.0		Yes	Da	G3F/- 2pQ-2K	147081.2				0.000E+00	unexpected	0.0		Yes	Da	native	145439.8				0.000E+00	unexpected	0.0		Yes				147998.6			1.017E+03		13.0	5.17	No	Da	G0F/G0F-2K 2pQ	148040.1	148039.0	-7.35	-1.1	4.576E+03	12.9	15.8	5.17	Yes	Da	G0F/G0F-K 2pQ	148168.2	148162.8	-36.75	-5.4	1.519E+03	5.3	5.2	5.17	Yes	Da	G0F/G1F-2K 2pQ	148202.2	148201.2	-6.55	-1.0	7.837E+03	21.6	27.0	5.17	Yes	Da	G0F/G1F-K 2pQ	148330.4	148326.8	-24.11	-3.6	1.511E+03	5.4	5.2	5.17	Yes	Da	G1F/G1F-2K 2pQ	148364.3	148363.5	-5.74	-0.9	6.581E+03	18.6	22.7	5.17	Yes	Da	G0F/G1F 2pQ	148458.6	148461.4	19.44	2.9	3.833E+02	1.9	1.3	5.17	Yes	Da	G1F/G1F-K 2pQ	148492.5	148490.2	-15.45	-2.3	9.247E+02	3.5	3.2	5.17	Yes
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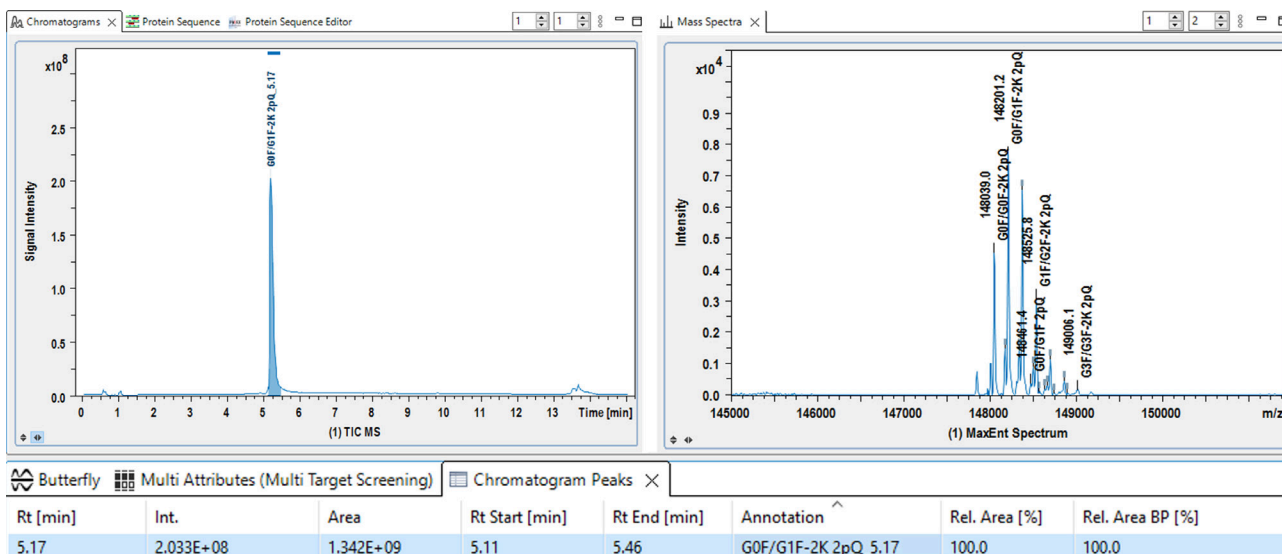
**Figure 1**  
Result Table including All expected compounds as defined in the Modification Profile, and the unidentified compounds.

Sample Position: 2   Sample ID: Intact NIST mAb				
Method Attribute	Narrow	Wide	Sample Result	Unit
Mass Accuracy [Da]	< 5.00	≥ 8.00	2.36	Da
Base peak as expected	≥ 100.0	< 80.0	100.0	Rel. int. [%] vs. base peak
Target Profile Matching	≥ 0.900	< 0.800	0.990	Similarity [cosine score]
Intensity Coverage	≥ 88.0	< 75.0	88.2	Rel. int. [%] of all target signals

**Figure 2**  
Multi Attributes view, displaying the Narrow and Wide acceptance criteria for 4 quality attributes and the values determined as Sample Result.

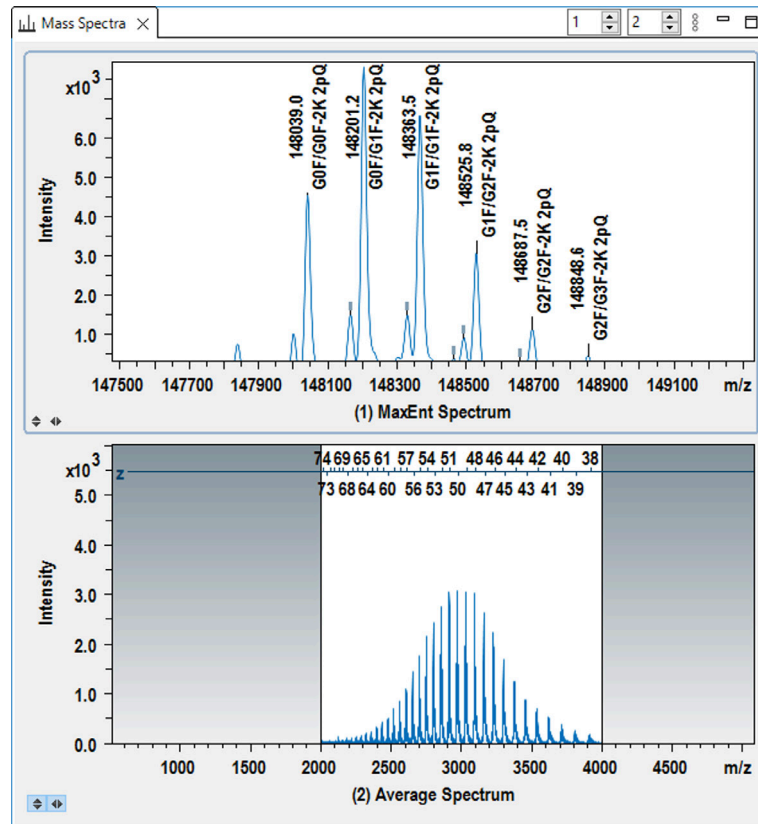
The Multi-Attribute view is designed to provide users with clear, actionable insights into the quality attributes of their samples. Utilizing a traffic light reporting framework, this view visually represents the acceptance criteria and the respective results for each sample, making it easy to interpret complex data at a glance. For each sample, multiple quality attributes are assessed (predefined in the workflow method), and their results are displayed using color-coded indicators. As seen in Figure 2, all reporting fields for this sample are green, signifying that all acceptance criteria have been met according to narrow definitions. A critical aspect of this evaluation is the Mass Accuracy [Da], which is determined by the median of the absolute mass errors of the compounds in the Modification Profile. This precise measurement ensures the mass accuracy is within acceptable limits, further validating the sample's integrity.

The Chromatograms view shows the Total Ion Chromatogram (TIC), where the selected peak is highlighted in dark blue, making it easy to identify and focus on specific peaks of interest. The corresponding Mass Spectra view displays the MaxEnt deconvoluted and annotated spectrum, providing detailed information about the molecular composition of the selected peak. The Chromatogram Peaks view complements this by listing all peaks present in the chromatogram, allowing users to quickly scan and identify key components. In addition to the TIC, the software displays the Base Peak Chromatogram (BPC) and UV Chromatogram. The BPC provides a clear representation of the most intense ions, helping users identify the most significant components in their sample. The UV Chromatogram is particularly beneficial for quantitation purposes, especially when multiple peaks are observed in the chromatogram. The UV trace aids in distinguishing and quantifying these peaks, ensuring accurate measurement and analysis of the sample's components.



**Figure 3**  
Chromatogram and deconvoluted mass spectrum of the intact NISTmAb. The target peak profile is well represented in the spectrum.

Users can inspect the spectra in the Mass Spectra view. The Average Spectrum (Figure 4) is obtained from a selected chromatographic peak or by clicking on single or multiple entries in the Expected table. The  $m/z$  range for the MaxEnt (Maximum Entropy) deconvolution is marked as a white spectra area, with charge states of the peaks shown on top. The top panel displays the charge-deconvoluted neutral mass spectrum. The exceptional data quality and minimal processing by the maximum entropy deconvolution algorithm preserve the fine features in the raw data, ensuring accurate and detailed spectra.



**Figure 4**  
Average and Deconvoluted Spectrum of intact NIST mAb

## Conclusions

- A comprehensive analysis of monoclonal antibodies (mAbs) provided by this assay is crucial for pharmaceutical companies to ensure the development, production, and regulatory approval of safe, effective, and high-quality antibody-based therapies.
- An LC-MS workflow for routine characterization of antibodies has been developed on the timsTOF HT with an Elute UHPLC. Using the provided LC-MS experimental conditions and the Multi-Target ESI workflow, high-quality intact mAb data can be obtained to evaluate key attributes including post-translational modifications and stability-indicating chemical changes to the molecule, such as glycosylation, charge isoforms, phosphorylation, oxidation, and deamidation in a single assay.
- The interactive interface of BioPharma Compass makes it time-efficient to review analysis results in both tabular and graphical forms and to generate meaningful reports, thus reducing analysis turnaround time and ensuring critical quality attributes are met from the earliest stages of development.

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