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LCMS – Biopharma – Monoclonal Antibody (mAb) Characterization

Untargeted N-glycans Analysis Using Shimadzu LCMS-9050 Q-TOF

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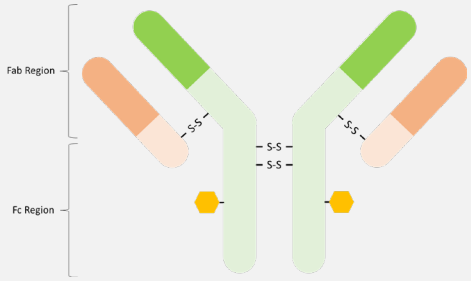
Abstract

The biopharmaceutical industry has experienced significant growth in monoclonal antibody (mAb) therapeutics, which are engineered immunoglobulins designed to mimic natural antibodies for targeting threats like cancer cells. Accurate characterization of mAb physiological properties, particularly their glycosylation profiles and post-translational modifications (PTMs), is essential for assessing safety and efficacy, as well as for regulatory compliance. This study focused on the characterization of N-glycans present on the fragment crystallizable (Fc) region of mAbs, as mandated by regulatory requirements. Samples were prepared using Procainamide-labeled dye in conjunction with the EZGlyco™ mAb-N Kit for LC fluorescent detection, coupled with Shimadzu LCMS-9050 Q-TOF mass spectrometer in Data Dependent Acquisition (DDA) mode. Data processing was conducted using Protein Metrics software for N-glycan identification.



Structure of monoclonal antibody (mAb)

Keywords:
Procainamide, High-Resolution Mass Spectrometry (HRMS), N-glycans, LCMS-9050 Q-TOF, Shim-pack Scepter, LCMS, EZGlyco ™ mAb-N Kit

Highlights

- *Comprehensive analysis of Procainamide labeled N-glycan analysis using EZGlyco ™ mAb-N Kit for Humanized IgG1k monoclonal antibody.*
- *End-to-end workflow for untargeted analysis is successfully established using LCMS-9050 Q-TOF and Protein Metrics software, delivering accurate, high-resolution data with enhanced efficiency.*

Technologies Featured

EZGlyco™ mAb-N Kit With 2-AB

LCMS-9050 Q-TOF

1. INTRODUCTION

In the biopharmaceutical industry, there has been signification growth in monoclonal antibody (mAb) therapeutics, which are engineered immunoglobulins designed to mimic natural antibodies to target threats such as cancer cells within the human body. To ensure the safety and efficacy of mAb therapeutics, it is critical to accurately characterize their physiological properties, including glycosylation profiles, post-translational modifications (PTMs) and molecular size. This characterization plays a pivotal role in assessing the potential severity of adverse drug reactions and meeting the stringent safety and efficacy standards required for therapeutic approval.

Regulatory requirements mandate the complete characterization of N-glycans located on the fragment crystallizable (Fc) region of mAbs. N-glycans are attached to the asparagine (N) within the consensus sequence asparagine-X-serine/threonine (NXS/T), where X represents any amino acid except proline. (Fig. 1). This glycosylation is critical for the structural stability, biological activity, and immunogenicity of mAbs.

To achieve accurate glycan analysis, the sample preparation involves the use of Procainamide-labeled dye in conjunction with EZGlyco™ mAb-N Kit, enabling efficient labeling for LC fluorescent detection. The analysis was conducted using Shimadzu LCMS-9050 Q-TOF in Data Dependent Acquisition (DDA) mode. For data processing and N-glycan identification, Protein Metrics software was employed, ensuring accurate and reliable results.

This integrated workflow highlights the advancements in analytical techniques for mAb characterization, supporting the development of safe and effective biopharmaceuticals drugs.

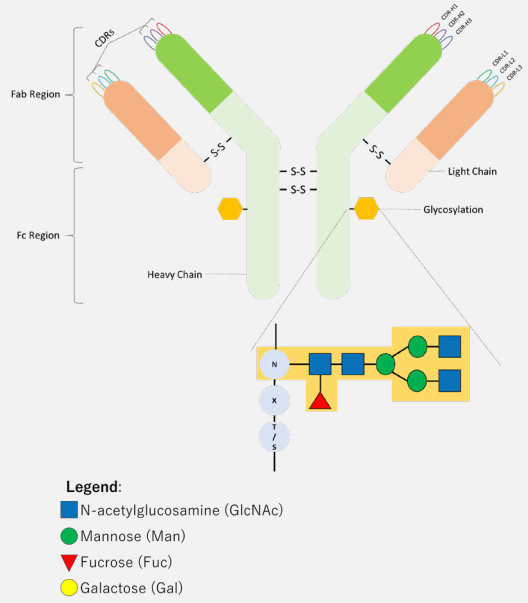


Fig. 1 Structure of monoclonal antibody with N-glycans

2. EXPERIMENT

2.1 Reagents and Chemicals

- **NISTmAbs Humanized IgG1k monoclonal antibody reference material 8671** was obtained from Merck Pte Ltd.
- **Procainamide hydrochloride** was obtained from Sigma-Aldrich, Cat No. PHR1252.
- **Procainamide labeled IgG N-glycans library** was obtained from Ludger.
- **TORAST-H Bio Vial (370-04350-00)** was obtained from Shimadzu.
- **EZGlyco™ mAb-N Kit (APASBC-BSX4410Z)** was obtained from S-BIO Business Division, Sumitomo Bakelite Co., Ltd., Japan.
- **Acetonitrile (LCMS grade)** was obtained from commercial suppliers.
- **Ammonium formate (>99%) of LCMS grade** was used as an additive in the mobile phase prepared from Milli-Q water.

2.2 Sample Preparation

Procainamide solution was prepared by dissolving 146 mg of Procainamide in a solution of DMSO, acetic acid and MilliQ water in the ratio of 40: 120: 80 µL respectively. The procainamide solution was then vortexed and transferred to the tube containing reducing reagent 7 available in the EZGlyco™ mAb-N Kit for step [10]. Sample was extracted as per the protocol provided by the kit, excluding step [10]. Procainamide solution prepared for step [10] was used in step [10] instead of 2-AB solution. Step [11] to [16] was performed as per the protocol. Samples were transferred into a Shimadzu TORAST-H Bio Vial and inject into Shimadzu LCMS-9050 Q-TOF. The analytical conditions are detailed in Table 1.

IgG N-glycans library obtained from Ludger was reconstituted in 50 µL of water and injected into the Shimadzu LCMS-9050 Q-TOF.

Table 1. Analytical conditions on LCMS-9050 Q-TOF

LC Conditions	
Column	Shim-pack GIST-HP Amide 1.9 µm, 150 × 2.1 mm (P/N: 227-30947-05)
Flow Rate	0.4 mL/min
Mobile Phase	A: 50 mM Ammonium Formate in Water
	B: 100% Acetonitrile
Elution mode	Gradient elution, 50 mins
Gradient Program	%B conc.: 0-1 min., 75%; 1-35 min., 75% to 60%; 35-38.5 min., 60% to 75%; 38.5-50 min., 75% to 75%
Oven Temp.	60°C
Injection Vol.	5 µL
Fluorescence Conditions	
Fluorescence detector	Shimadzu RF-20A
Excitation	308 nm
Emission	359 nm
Interface Conditions (LCMS-9050)	
Interface Temp.	300°C
DL Temp.	250°C
Heat Block Temp.	400°C
Nebulizing Gas	3 L/min
Heating Gas Flow	15 L/min
Drying Gas Flow	15 L/min
Data acquisition (Q-TOF)	
MS Mode	DDA positive
DDA event #	2-8
TOF m/z range	200-2500 m/z
DDA m/z range	50-2000 m/z
CE	Auto
CE spread	0
Event Time (s)	0.100
Pulser Inj. Time	193

3. RESULTS AND DISCUSSION

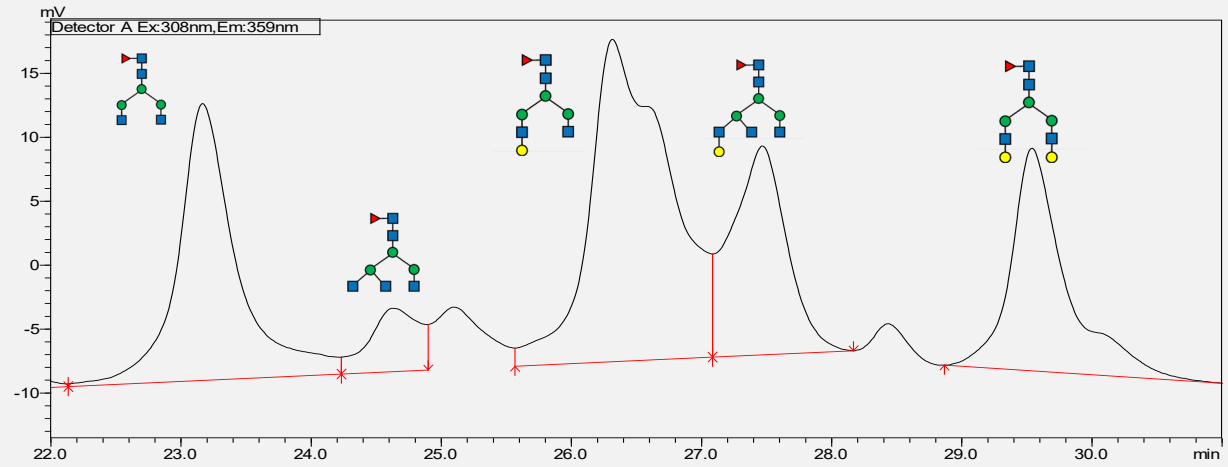


Fig. 2 IgG N-glycans Library Fluorescence Chromatogram

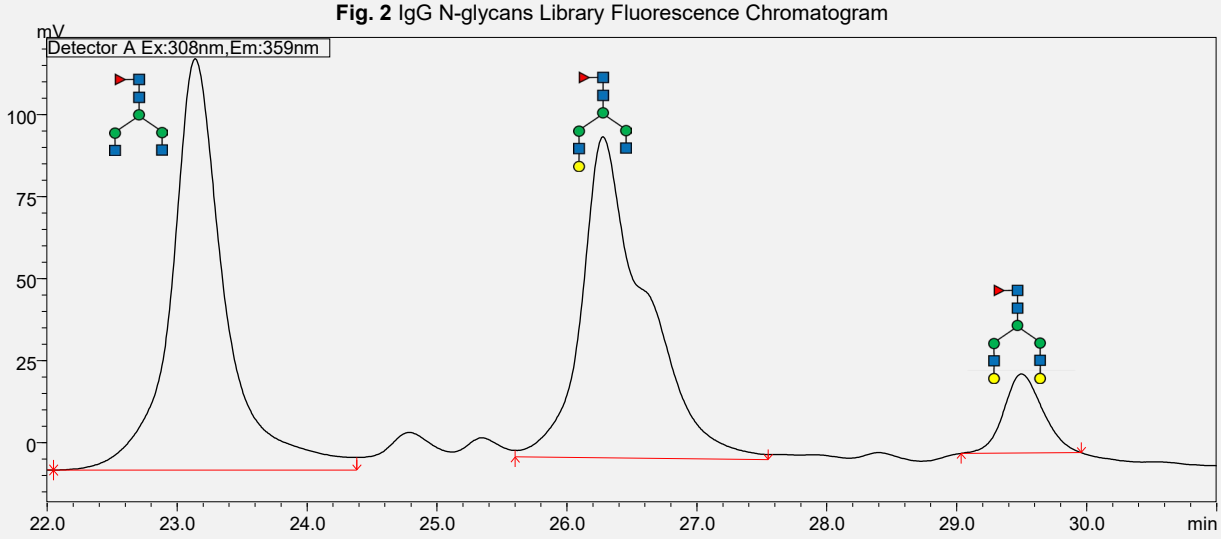


Fig. 3 NISTmAbs Fluorescence Chromatogram

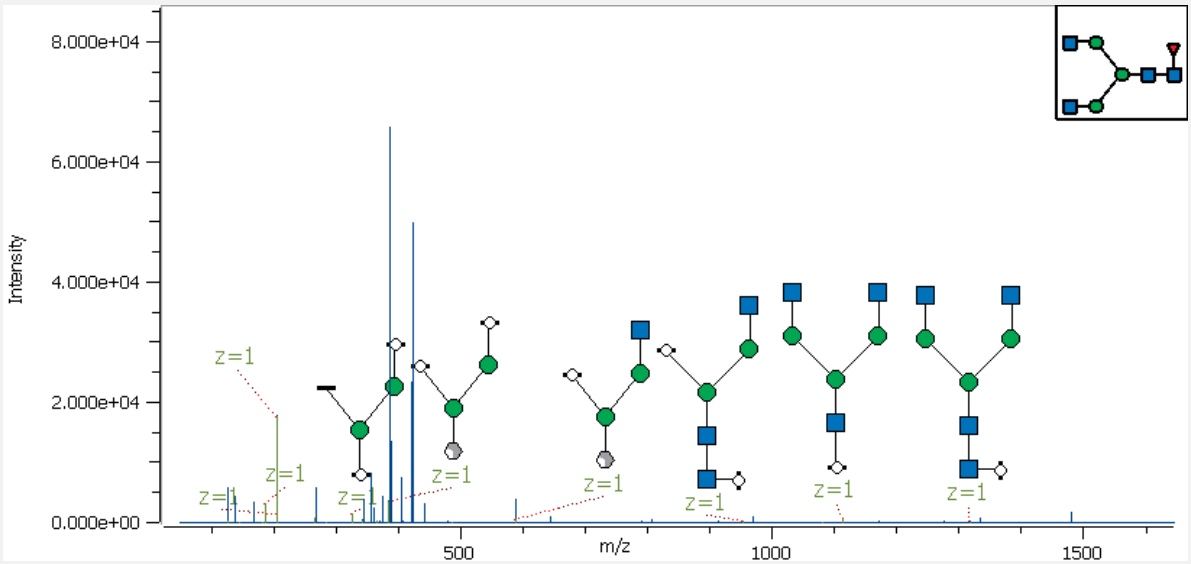


Fig. 4 IgG N-glycans Library G0F or FA2 MS/MS Spectra

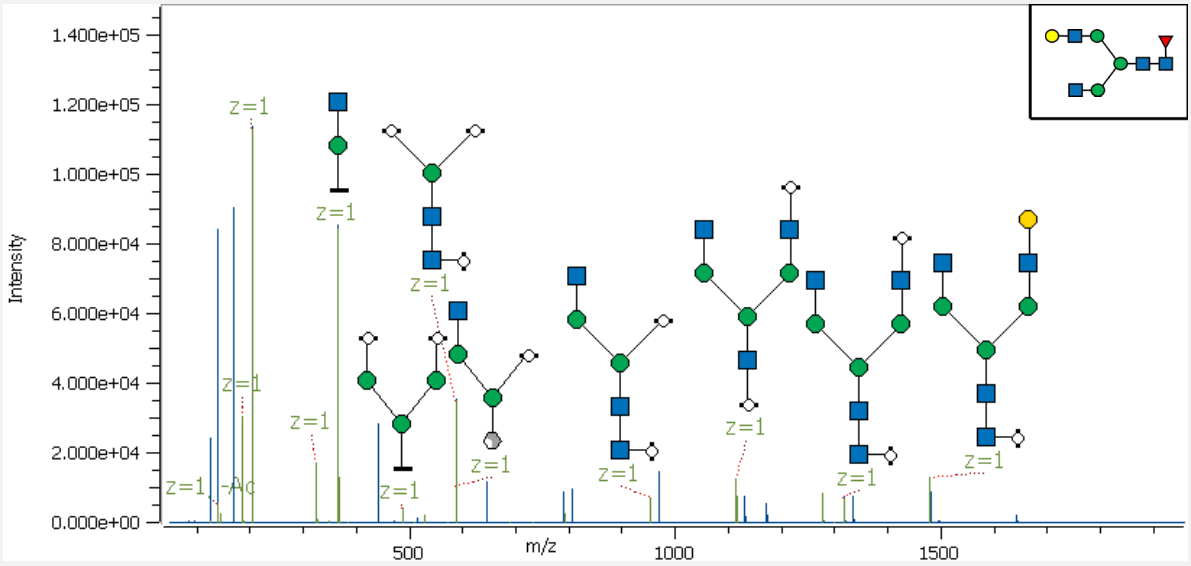
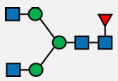
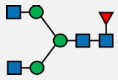
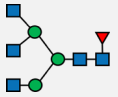
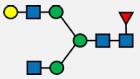
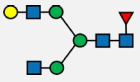
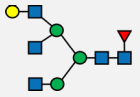
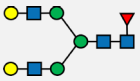
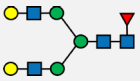


Fig. 5 NISTmAbs G1F or FA2G1 MS/MS Spectra

The detection of various N-glycans was carried out using an RF detector to acquire fluorescence data (Fig. 2 and 3) at an excitation wavelength of 308 nm and an emission wavelength of 359 nm, followed by confirmation through DDA MS/MS. The Protein Metrics software was used for N-glycans candidate assignments and labeling of MS/MS data with their corresponding N-glycans structures and charge states (Fig. 4 and 5). In total, five N-glycans were identified in the IgG N-glycans library sample, while three N-glycans were identified in the NISTmAbs sample.

The observed mass showed an error of less than 10 ppm when compared to the theoretical mass (Table 2). The most prevalent N-glycans in the IgG N-glycans library sample was G1F or FA2G1, with a relative abundance of 32.69%, which was consistent with the Certificate of Analysis (COA) data. Likewise, the N-glycans with the highest abundance in the NISTmAbs sample was also G1F or FA2G1, exhibiting a relative abundance of 45.78%. Both the IgG N-glycans library and the NISTmAbs samples contained G0F or FA2, G1F or FA2G1G2F, and FA2G2 N-glycans. Additionally, FA2B and FA2BG1 N-glycans were identified in the IgG N-glycans library sample. All identified N-glycans were determined to belong to the fucosylated group (Table 2).

Table 2 List of N-glycans identified

Group	Apex time	Glycan Name	Glycan Cartoon	Observed Mass	Theorical Mass	ppm	IgG N-glycans Library (%)	NISTmAbs (%)
Fucosylated	22.63	G0F or FA2		1681.72	1681.72	-1.12		43.51
	23.17	G0F or FA2		1681.72	1681.72	0.69	23.23	
	24.63	FA2B		1884.79	1884.80	-3.85	6.09	
	25.77	G1F or FA2G1		1843.77	1843.77	-0.91		45.78
	26.32	G1F or FA2G1		1843.77	1843.77	-0.89	32.69	
	27.50	FA2BG1		2046.84	2046.85	-7.60	18.01	
	29.13	G2F or FA2G2		2005.83	2005.82	1.76		10.70
	29.59	G2F or FA2G2		2005.82	2005.82	-3.28	19.98	

4. CONCLUSION

Regulatory authorities mandate the characterization of glycosylation profiles on monoclonal antibodies (mAbs) to ensure the safety and efficacy of these drugs. The analysis of procainamide-labeled N-glycans using the EZGlyco™ mAb-N Kit, Shim-pack GIST-HP Amide column, LCMS-9050 Q-TOF mass spectrometer, and Protein Metrics software provides a robust and comprehensive platform for the detailed characterization of N-glycans on mAbs.

A comparative analysis of N-glycan profiles from NISTmAbs and IgG N-glycan library samples demonstrated that similar glycans were identified, with G1F (FA2G1) showing the highest relative abundance.

The application of high-resolution mass spectrometry is essential for untargeted analyses, providing the accuracy needed to identify N-glycans and ensuring the development of biopharmaceuticals drugs that are both safe and highly effective.

5. REFERENCES

Zhao, J., Peng, W., Dong, X., & Mechref, Y. (2021). Analysis of NIST Monoclonal Antibody Reference Material Glycosylation Using the LC-MS/MS-Based Glycoproteomic Approach. *Journal of Proteome Research*, *20*(1), 818–830. https://doi.org/10.1021/acs.jproteome.0c00659

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