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Application News

An Easy and Fast Approach for Monoclonal Antibody N-linked Glycan Analysis from Sample Preparation to Data Analysis

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User Benefits

- A simple and straightforward sample preparation workflow for N-glycan analysis
- Support the automated sample preparation with GlycoAutoPrep™
- Less time-consuming for sample preparation

■ Introduction

Therapeutic monoclonal antibodies (mAbs) and their derivatives are emerging as the fastest-growing category of biologic drugs with a wide range of indications. N-linked glycans play a critical role in mediating many biological processes and affect therapeutics' bioactivity, stability, and immunogenicity. In order to maintain consistent glycosylation profiles during manufacturing, glycan characterization method such as fluorescence-tagging HPLC is required. Traditional methods of N-glycan analysis are very time-consuming (several days typically), and involve many steps, starting with glycan release, followed by purification, labeling with a fluorescence tag (e.g., 2-aminobenzamide, 2-AB), and finally cleanup of labeled glycans prior to LC analysis. In this study, we demonstrate how a simplified and rapid workflow using S-Bio EZGlyco™ mAb-N kit can dramatically reduce sample preparation time for N-glycan characterization to just a few hours. Shimadzu Nexera UHPLC system with highly sensitive fluorescence detector (RF-20A) is used to analyze the labelled glycans.

■ Experimental

mAb Sample:

A bevacizumab biosimilar was used in this study. It was diluted with Milli-Q water to 1 mg/mL prior to sample preparation using S-Bio EZGlyco™ mAb-N kit.

Fast Sample Preparation by S-Bio kit (< 3h):

The kit can work directly with cell culture supernatant, bypassing additional IgG purification step. It provides a speedy protocol with proprietary reagents and cartridges for N-glycan release, 2-AB labeling, and cleanup. The workflow can be readily automated by a robotic system like GlycoAutoPrep™ that can process 24 samples at one go. After final cleanup step, the labeled N-glycans were dissolved in a final volume of 100 µL of 50% acetonitrile solution.

LC-Fluorescence Detection:

Sample analyses were conducted by a Shimadzu Nexera UHPLC system equipped with a highly sensitive fluorescence detector, RF-20A. Table 1 detailed analytical conditions employed in this study.

Data Processing:

Data analysis workflows refer to our previous Application News AD-0191.

Table 1 Analytical conditions

LC conditions	
LC system:	Shimadzu Nexera UHPLC
Column:	HALO® Glycan (150 mm x 2.1 mmI.D., 2.7 µm)
Column Temp.:	40 °C
Flow rate:	0.4 mL/min
Mobile phase A:	50 mmol/L ammonium formate
Mobile phase B:	Acetonitrile
Gradient program:	B. Conc. 78% (0 min)→68% (56 min)→20% (57-62 min)→78% (63 min)
Injection volume:	5 µL
Fluorescence conditions	
Detector:	Fluorescence detector RF-20A
Excitation Wavelength:	330 nm
Emission Wavelength:	420 nm
Gain:	1

■ Results and Discussion

LC-fluorescence analysis of released and 2-AB labeled N-glycans is one of the most commonly used approaches to determining mAb glycosylation. In the previously published Application News of AD-0191, an optimized separation of N-glycan profiles from bevacizumab biosimilar including Man3, G0F-2GN, G0-GN, G0F-GN, G0, Man5, G0F, G1Fa, and G1Fb was achieved (Figure 1). In this study, N-glycan profiles were well separated, especially the isomers of G1Fa and G1Fb (Figure 2). Therefore, accurate quantitation of target glycans and any changes of the mAb glycosylation profiles were able to be done. In the chromatogram, nine N-glycan peaks were detected. Most importantly, the relative abundance of these nine N-glycans in this study is comparable with that of AD-0191 (Figure 3). Additionally, the repeatability of retention time was evaluated. Variations in peak area and retention time were less than 2% for all peaks (Table 2).

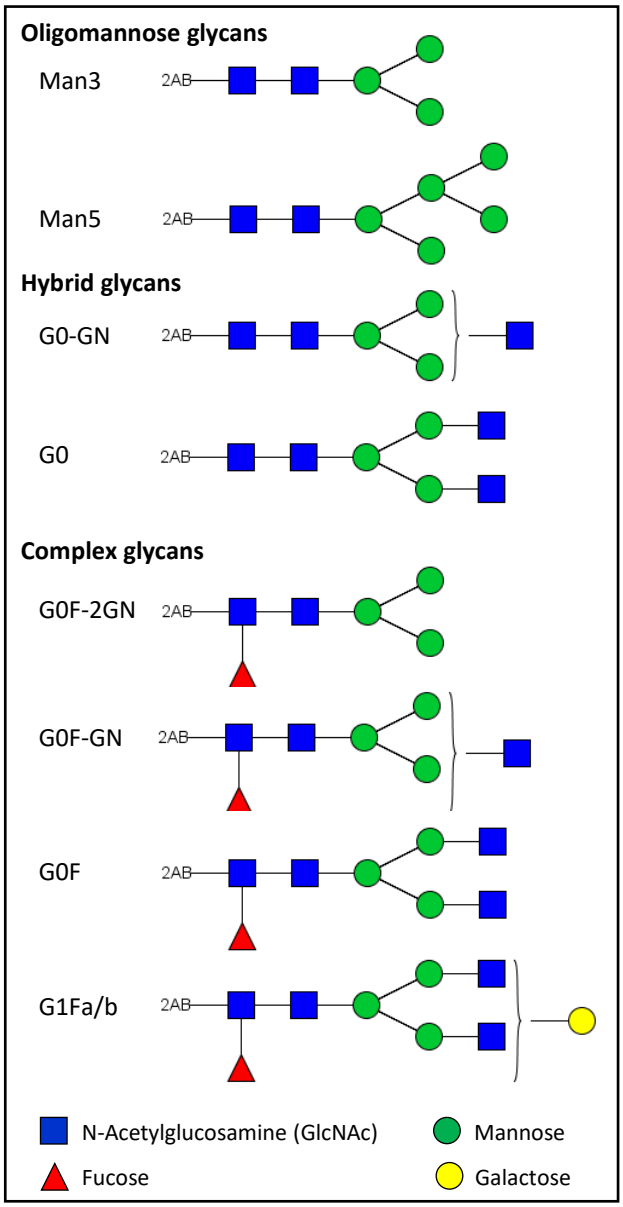

Figure 1. N-glycans from bevacizumab biosimilar.
GN = GlcNAc

■ Conclusion

This study demonstrated an easy and fast solution for mAb N-linked glycan analysis. It took less than 3 hours for sample preparation. Meanwhile, this new workflow considerably improved the peak resolutions. Therefore, more accurate quantitation of the target glycan species can be done.

Table 2. Repeatability of relative abundance and retention time of N-glycans (n = 3)

Glycans	Relative Abundance (%)	Area(%RSD)	RT (min)	RT(%RSD)
Man3	0.07	0.32	8.173	0.19
G0F-2GN	0.31	0.73	10.567	0.14
G0-GN	0.36	0.26	12.100	0.14
G0F-GN	1.91	0.22	15.131	0.14
G0	1.20	0.27	16.972	0.14
Man5	2.02	0.19	18.547	0.15
G0F	87.38	0.03	20.374	0.17
G1Fa	5.10	0.41	26.679	0.15
G1Fb	1.65	0.41	27.323	0.15

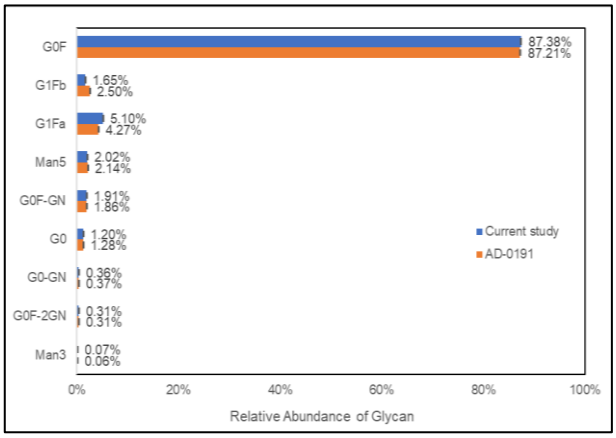

Figure 3. Relative abundance of 9 N-glycans from bevacizumab biosimilar.

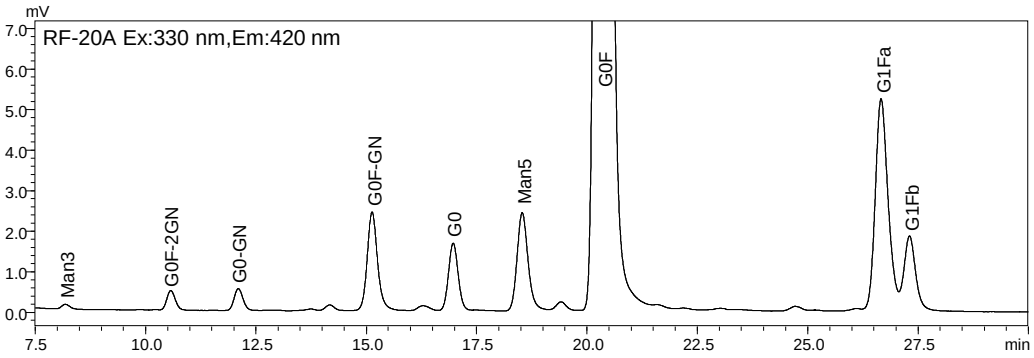

Figure 2. Chromatogram of labeled N-glycans from bevacizumab biosimilar.

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