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Application News

Fully Automated Sample Preparation Module for Glycan Analysis
High Performance Liquid Chromatograph

Profiling Antibody N-Linked Glycans in Culture Supernatant Using Fully Automated Sample Preparation Module for Glycan Analysis

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User Benefits

- By simply placing the dedicated reagent kit and the culture supernatant in the system, it can automatically perform all processes, including antibody purification, glycan release, and fluorescent labeling.
- In addition to relieving operators from repetitive routine tasks, the system can also automatically pre-treat samples overnight.
- Using validated methods to further automate operations, the system enhances laboratory productivity.

■ Introduction

Antibody drugs and other biopharmaceuticals are manufactured using genetic engineering and cell culturing technologies, based on peptides or proteins derived from biological organisms as active ingredients. In recent years, many companies have been researching or developing biopharmaceuticals as therapeutic or diagnostic drugs.

Antibody drugs include N-linked glycans introduced by a post-translational modification. Due to the potential impact on the safety and efficacy of pharmaceuticals, these glycans are considered critical quality attribute of antibody drugs. However, the glycan has structural diversity as an inherent character and the diversity trends can be affected by slight variations in the cell strains and cultivation parameters used in manufacturing process. Therefore, the glycans attached to antibodies are strictly monitored and controlled during manufacturing processes using appropriate analytical methods that align with the intended purpose.

One method used to characterize glycan modifications is glycan profiling, which involves releasing glycans from antibodies using enzymes or other methods, followed by fluorescent labeling and analysis via HPLC, LC/MS, or other techniques. If the given samples are culture supernatants, the process of purifying antibodies has to be included. However, purifying antibodies, releasing glycans, and applying fluorescent labels is a labor-intensive process that can take 2 days or more when performed manually, making the procedure highly person-dependent.

■ Overview of the MUP-3100 System

By simply placing the Auto-EZGlyco mAb-N Kit for SHIMADZU, a dedicated consumable, along with the cell culture supernatant or other samples into the MUP-3100 fully automated sample preparation module for glycan analysis, and clicking the [Run] button in the control software, the module quickly, reliably, and automatically performs all the necessary steps, from antibody purification to labeled glycan preparation. The MUP-3100 is compatible with N-linked glycans in antibodies and can process up to 24 samples within 6 hours.

The MUP-3100 is equipped with the following features to support stable operation.

- ✓ Check the placement of reagents by image processing before automatic operations
- ✓ Check the attachment or detachment of pipette tips using sensors
- ✓ Save a video image if a problem occurs
- ✓ Automatically generate pretreatment reports

For more details about the MUP-3100 system, please refer to the brochure C190-E304.

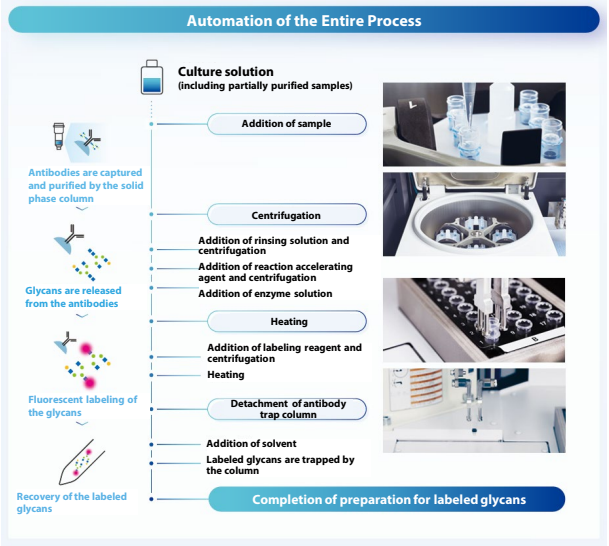

Fig. 1 MUP-3100 Fully Automated Sample Preparation Module for Glycan Analysis and Overview of Automatic Pretreatment Process Flow

■ Analysis of Antibody Glycans in Culture Supernatant

Culture Solution

Supernatant was collected on the third, fourth, fifth, and seventh day of culturing a CHO-MK cell line producing trastuzumab.

Sample Preparation

The culture supernatant was diluted to achieve an antibody concentration within the 20 to 80 µg/1200 µL range. The Auto-EZGlyco mAb-N Kit for SHIMADZU antibody capturing solution was used as the diluting agent.

LC Analysis

The glycan samples obtained through MUP-3100 pretreatment were analyzed under the conditions specified in Table 1. Due to 2-AB (2-aminobenzamide) labeling, the glycans were detected using a fluorescence detector.

Nexera™

Table 1 Analysis Conditions

System:	Nexera XR
Column:	Shim-pack™ GIST-HP Amide [Metal free] (100 mm × 2.1 mmI.D.,1.9 µm)*1
Mobile Phases:	A) 100 mM Ammonium formate (pH 4.4)
	B) Acetonitrile
Time Program	B Conc. 77.5 % (0 min) → 68.5 % (50 min) → 50 % (50.01-55 min) → 77.5 % (55.01-62 min)
Column Temp.:	40 °C
Flowrate:	0.5 mL/min
Injection Volume:	1 µL
Detection:	Fluorescence (Ex. 330 nm, Em. 420 nm) (using RF-20Axs, semi-microcell)
Vial:	TORAST PP Vial screw*2

*1 P/N: 227-30951-02
*2 P/N: 370-04050-01

Chromatograms obtained by analyzing supernatant samples from the third day of culturing are shown in Fig. 2. The glycan structures corresponding to the detected peaks were identified by comparing their retention times with those of standard samples or through LC/MS analysis.

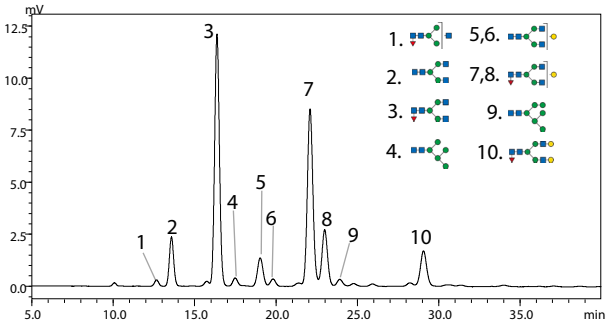

Fig. 2 Chromatogram of N-Linked Glycans in Antibodies Contained in the Supernatant from Culturing Trastuzumab-Producing Cells

● Mannose ▲ Fucose ○ Galactose ■ N-Acetylglucosamine

Pretreatment Reproducibility

Six Lot 1 samples collected on the third day of culturing were pretreated using the MUP-3100 system and measured by HPLC to evaluate pretreatment reproducibility based on the area of each peak measured. The results demonstrated excellent pretreatment reproducibility, with 5 % or less RSD for major glycans and 8 % or less RSD for minor glycans.

Table 2 Reproducibility of Pretreatment for Culture Supernatant Samples (%RSD for n = 6 Measurements of Glycan Peak Areas)

Peak No.	Average Area	Area Value %RSD	Peak No.	Average Area	Area Value %RSD
1	5193	7.11	6	8062	4.20
2	43765	4.27	7	201774	4.21
3	236938	4.16	8	65241	4.11
4	7414	7.48	9	7947	7.79
5	32348	4.97	10	44387	4.58

■ Changes in Glycan Profiles Based on Culturing Duration

Fig. 3 shows chromatograms obtained from culturing days three to seven. Fig. 4 shows the change in the area of each glycan peak for each day of culturing, indicated as a ratio of the total area of 10 types of glycan peaks. From the third to fifth day of culturing, the peak ratio of the glycan No. 3, a non-galactosylated complex-type glycan, increased, while the peak ratio of galactosylated glycans and the glycan No.2, a non-fucosylated glycan, decreased. Furthermore, the results confirmed that the peak ratios for high mannose-type glycans No. 4 and No. 9 increased during the fifth to seventh days of culturing.

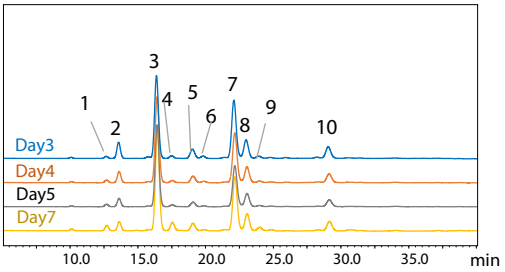

Fig. 3 Chromatograms for Respective Number of Days of Culturing

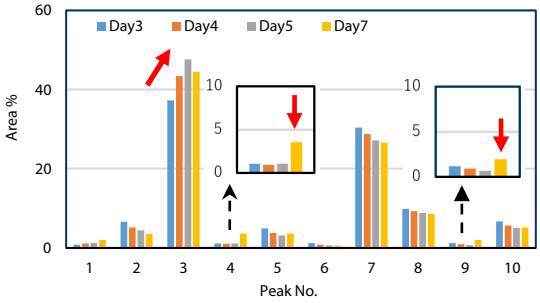

Fig. 4 Comparison of Glycan Profiles for Each Culturing Day

■ Conclusion

The MUP-3100 fully automated sample preparation module for glycan analysis was used to pretreat 24 supernatant samples from antibody-producing cell cultures within 6 hours. The resulting N-linked glycan samples were then analyzed using the Nexera HPLC system to confirm changes in glycan profiles over the elapsed days of culturing. The MUP-3100 offers a more efficient workflow compared to manual pretreatment methods and supports the development of manufacturing process for antibody drugs.

Acknowledgments

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