Analysis of N-Linked Glycans from Antibody-Drug Conjugate (ADC) Using the Agilent AssayMAP Automated Sample Preparation and Agilent 1290 Infinity LC System

Application Note

Biotherapeutics

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Abstract

N-linked glycans are attributed with crucial biological properties that they confer to antibody-drug conjugates (ADCs) for their biological functions. This study demonstrates a workflow with an Agilent AssayMAP Bravo automated liquid handling platform and an Agilent 1290 Infinity LC system for the analysis of N-linked glycans from ADC samples. The AssayMAP Bravo was used for automated sample preparation involving the release of N-glycans from the ADC, and derivatization using fluorescent dye. The 2-AB labeled N-glycans were then chromatographically separated using an Agilent AdvanceBio Glycan Mapping column and a 1290 Infinity LC system. The glycoforms were identified using a standard mix, and the percentages of each glycan are reported.

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Introduction

Antibody-drug conjugates (ADCs) are engineered from monoclonal antibodies (mAbs), and contain three components: an mAb, a linker, and a cytotoxic drug, as depicted in (Figure 1A). ADCs combine the strategies of the potent cytotoxic agent with the high specificity of an antibody[1]. ADCs have changed the pharmaceutical landscape from small molecules to larger biomolecules, and are viewed as a significant step towards personalized medicine[2]. Although these revolutionary cancer therapeutics were recently introduced, they have undergone a fast transformation due to their promising clinical efficiency, currently accounting for about 35 ADCs in clinical studies[3].

ADCs are often subjected to detailed characterization. Of several characterization studies, glycosylation patterns are well studied and monitored during manufacturing. The different glycans present in the mAbs affect the biomolecule's target binding capacity, stability, charge, and mass[4]. Extensive analytical methods are required to characterize the glycoforms present in the mAbs. These often include time-consuming sample preparations, multiple handling steps, and demands for higher throughput. An automated sample preparation solution would dramatically increase the efficiency of analysis.

The Agilent AssayMAP Bravo Automated Liquid Handling Platform is a precise liquid-handling system that supports a wide range of applications. The Bravo Automated Liquid Handling Platform with AssayMAP technology combines automation with miniature 5-µL pack bed cartridges for sample preparations, enabling high-throughput chromatography and sample preparations in less time[5].

This Application Note presents an automated solution for N-glycan sample preparation using an AssayMAP Bravo Liquid Handling Platform. The glycosylation patterns of the ADCs were then analyzed with an Agilent 1290 Infinity LC system using an Agilent AdvanceBio Glycan Mapping column, providing a fast separation of under six minutes.

Experimental

Materials

The ProZyme's GlykoPrep-plus Rapid N-Glycan Sample Preparation with 2-AB kit (GPPNG-AB) was purchased from ProZyme. An Agilent AdvanceBio Glycan Mapping Column, 2.1 × 150 mm, 1.8 µm (p/n 859700-913), and Agilent 2-AB labeled human IgG N-linked glycans standards (p/n 5190-6996) were purchased from Agilent Technologies. A lysine-conjugated ADC sample was purchased commercially from a local pharmacy. All other chemicals were procured as HPLC grade from Sigma.

Sample preparation

The ADC sample was diluted to 1 mg/mL in water, then loaded into three columns of a 96-well plate (24 replicates). Reagents from the ProZyme GlykoPrep plus kit were used for the sample preparation. After placing the sample plates and reagent plates as specified in the guideline[6], the samples were processed by launching the *N-Glycan Sample Prep: RX digestion & 2-*AB *labeling* module from Agilent V-Works software. The protocol consists of five modules performed in sequential order.

Figure 1B presents a schematic of the AssayMAP Bravo workflow. The antibodies were denatured to allow enzyme access. The denatured samples were then immobilized onto the column, and digestion with the deglycosylation enzyme was completed. The released glycans were then labeled using 2-AB dye, and purified to remove excess dye. The final purified labeled glycans from each well were then transferred to HPLC vials, and analyzed immediately or stored at –80 °C.

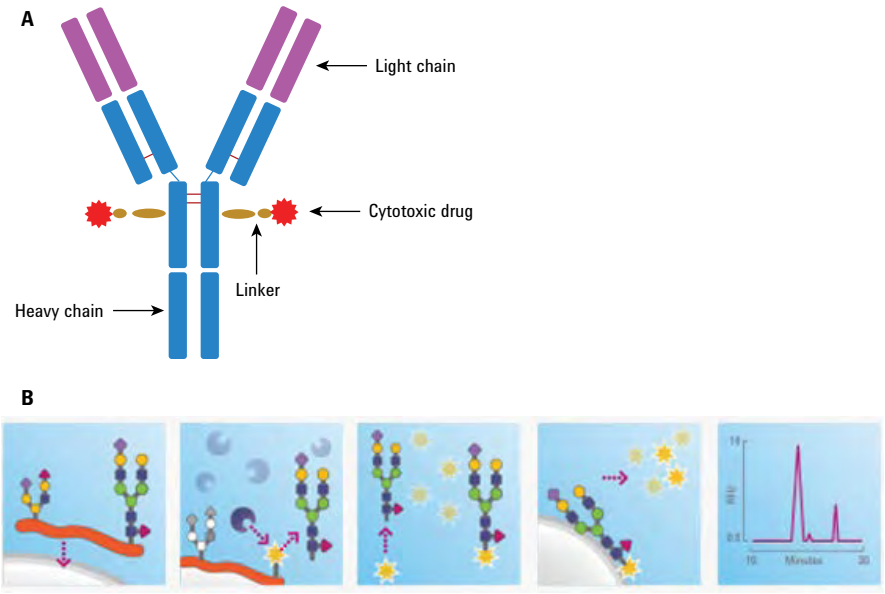

Figure 1. A) Schematic showing an antibody-drug conjugate. B) Diagram showing the denaturation, immobilization, glycan cleavage, 2-AB labeling, and purification performed in the workflow.

Instruments

Agilent 1290 Infinity LC System including:

- Agilent 1290 Infinity Binary Pump G4220A
- Agilent 1290 Infinity Autosampler G4226A
- Agilent 1290 Infinity TCC G1316C
- Agilent 1260 Infinity Fluorescence Detector G1321 B

An LC method described earlier was adopted for this study[7]; each sample was analyzed in quadruplet injections followed by blank injections.

Software

- Agilent VWorks Automation Control 11.4.0.1233
- N-Glycan Sample Prep: RX digestion and 2-AB labeling protocols 1.0
- Agilent AssayMAP Launch Pad 3.0
- Agilent ChemStation C.01.06

Results and Discussion

AssayMAP sample preparation

The AssayMAP Bravo software suite consist of five modules, as shown in Figure 2. The software suite consists of a deck layout, labware table, and application settings tab for each module to be performed (Figure 3). The user is prompted to move the appropriate consumables and reagents listed in the labware table to the specified deck positions. After setting up all labware, the protocol is executed, and AssayMAP Bravo completes the protocol. A confirmation message is displayed to proceed to the next module.

The final Cleanup Protocol elutes the labeled and purified glycans in the aqueous buffer into a clean and fresh 96-well plate. The samples were then analyzed using the downstream UHPLC analysis in replicates of four each to demonstrate a similar high-throughput application.

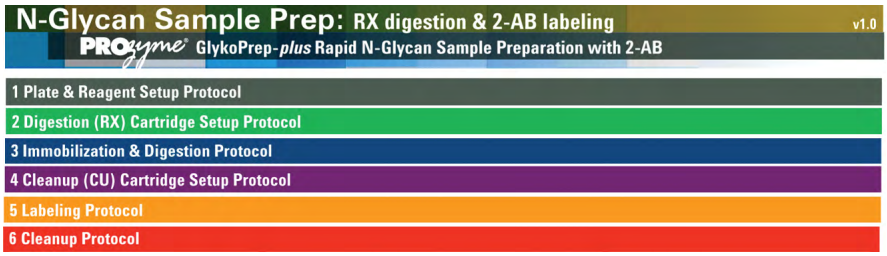

Figure 2. Modules of an Agilent AssayMAP N-Glycan sample preparation, in Agilent VWorks.

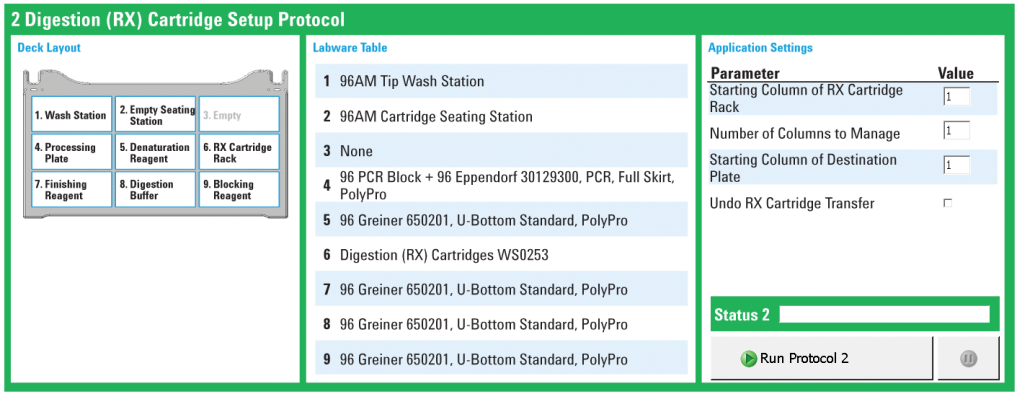

Figure 3. Deck layout, labware table, and application settings within Module 2 of the Agilent VWorks sample preparation shown in Figure 2.

Glycan analysis of ADC molecules

The 2-AB labeled and purified glycans from the AssayMAP sample preparation were analyzed using the Agilent 1290 Infinity LC system by injecting 2 µL of samples in quadruplets, followed by a blank injection. The chromatograms of the samples were then compared with the 2-AB labeled IgG glycan standard, and the peaks were annotated and presented in Figure 4.

Table 1 shows the reproducibility of the sample preparation assessed by looking at the retention times (RTs), peak areas, and peak heights of four major glycan species. The CVs for the RTs were less than 0.6 %, and for all other parameters, the CV% was less than 9.3 %. The chromatogram overlay of the five replicates shown in Figure 5 shows the reproducibility of the AssayMAP platform in processing the samples.

Table 2 shows the identified glycans and their overall distribution. The table shows that G0F was the most prevailing glycan species present in the ADC, constituting approximately 43.8 % of total glycans. G1F, G1F′, and G2F were the other major glycans, in decreasing order.

Table 2. Glycans identified from the ADC sample, with their percent distribution.

Glycans	Area percentage
G0	4.9
G0F	43.8
Man5	1.3
G1F	26.8
G1F′	13.0
G2F	6.5
G1FS1	0.6

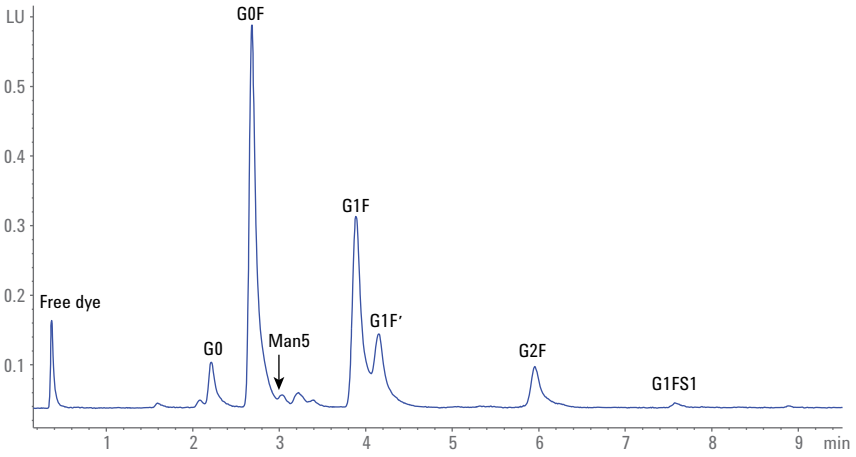

Figure 4. Chromatogram showing N-glycans from a lysine-conjugated ADC.

Table 1. Reproducibility data on retention time, peak area, and peak height (n = 96).

ADC - Reproducibility CV%			
Glycans	RT (min)	Peak area	Peak height
G0F	0.5	7.4	8.1
G1F	0.6	7.6	8.8
G1F′	0.6	8.6	9.3
G2F	0.6	8.1	7.8

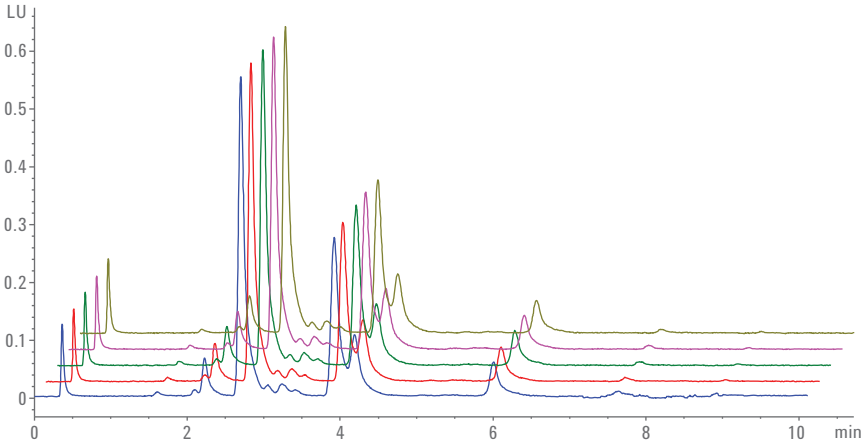

Figure 5. Chromatogram overlay of five replicates of ADC showing good reproducibility (x-axis offset of 1 %, and y-axis offset of 5 %).

Conclusions

- An Agilent AssayMAP Bravo system is a reliable automation system for high-throughput glycan sample preparation from antibody drug conjugates.
- The Agilent VWorks software suite simplifies sample preparation with ready-to-go protocols, resulting in downstream-compatible samples with minimal hands-on operation.
- An Agilent 1290 Infinity LC system with an Agilent Glycan Mapping column offers excellent reproducibility in glycan analysis, with shorter analysis time.
- Using the combination of the AssayMAP Bravo and a 1290 Infinity LC system, the glycan profile of a lysine-conjugated ADC sample was deduced.

References

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Published in the USA, July 1, 2016
5991-7024EN

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