



Released N-linked glycan analysis employing automated sample preparation on a Biomek i5 liquid handler

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This technical note demonstrates an automated workflow for released N-linked glycan analysis that combines magnetic bead-mediated sample preparation on the Biomek i5 MC automated workstation with high-throughput CE-LIF analysis on the BioPhase 8800 system [Figure 1]. The approach addresses a key bottleneck in biotherapeutics development: the labor-intensive digestion, labeling, cleanup and analysis required for glycan profiling across large sample sets.

This workflow offers the ability to increase throughput while improving consistency and reducing manual effort. By automating key sample preparation steps and pairing them with multi-capillary electrophoresis, the method enables more efficient screening of released N-linked glycans, supports reproducible peak area and glucose unit measurements and reduces user exposure to hazardous reagents.

Reproducibility studies using human serum IgG showed strong intra- and inter-capillary performance for key glycan species, including CPA%, RMT GU and GU measurements. Together, these results support the automated workflow for glycan analysis on the Biomek i5 MC workstation as a solution for high-throughput N-linked glycan profiling in biopharmaceutical development.

For broader automation, while not used in this work, the optional BioPhase Instrument application programming interface [API*] enables external software to monitor instrument status, track sequence progress, and execute supported instrument and sequence operations.

Key features

- **The Biomek i5 MC automated workstation** enables magnetic bead-mediated glycan preparation in a format suited for high-throughput biotherapeutic screening.
- **The BioPhase 8800 system provides multi-capillary CE-LIF analysis** for efficient separation and detection of APTS-labeled N-linked glycans.
- **The combined automated preparation and CE workflow supports reproducible glycan profiling** across multiple capillaries and repeated injections.
- **Inter-capillary reproducibility** was demonstrated with %RSD values below 10% for CPA%, below 0.08% for RMT GU and below 0.07% for GU.
- **Optional BioPhase Instrument API***: Enables software-based monitoring and run control for integration into automated laboratory workflows.



Figure 1. [A] The Biomek i5 MC automated workstation, [B] the BioPhase Fast Glycan Labeling and Analysis kit and [C] the BioPhase 8800 system.

Introduction

This technical note presents a streamlined N-linked glycan analysis that employs an automated sample preparation with the magnetic bead-mediated glycan workflow followed by capillary electrophoresis [CE]. The inter- and intra-capillary reproducibility results demonstrate the robustness of the assay with automated sample preparation.

Thousands of samples, including those containing N-linked glycans, must be screened monthly in the early stages of development. Additionally, this type of profiling has become more important in other stages of development, as the number and complexity of therapeutic modalities have increased. Therefore, the ability to achieve high-throughput N-linked glycan profiling is essential for biotherapeutics development. However, the digestion, labeling and cleanup of these samples are bottlenecks for this type of analysis because these steps are labor-intensive and time-consuming¹. Therefore, automated sample preparation is important to enhance the throughput of these experiments. By adopting the Biomek i5 multichannel [MC] automated workstation and the multi-capillary BioPhase 8800 system with the BioPhase Fast Glycan Labeling and Analysis kit [Figure 1] and an easy-to-use data analysis software², a high-throughput N-linked glycan analysis can be efficiently achieved. Moreover, the automated workflow can reduce user exposure to hazardous chemicals and improve sample preparation reproducibility, resulting in more consistent results.

In this technical note, an automated N-linked glycan preparation was developed and evaluated to demonstrate large-scale, reproducible screening on released N-linked glycans. The inter- and intra-capillary reproducibility results indicated that this workflow can reduce the time needed to screen for thousands of therapeutics.

Materials

Reagents: The BioPhase Fast Glycan Labeling and Analysis kit [P/N: C30098] was from SCIEX [Marlborough, MA]. Acetonitrile was Burdick-Jackson brand obtained from Sigma-Aldrich [St. Louis, MO]. The human serum IgG [hu IgG] sample, 2-picolineborane [pic-BH3] and DMSO were from Sigma-Aldrich [St. Louis, MO]. PNGase F was obtained from New England Biolabs [Boston, MA]. The reagents used to prepare the denaturation,

digestion and labeling solutions were provided in the chemistry kit.

Liquid handler for sample preparation: The Biomek i5 MC workstation from Beckman Coulter® [Indianapolis, IN] was set up with 96-well plate holders, a 96-well plate magnet and stand and 230 μ L and 90 μ L pipette tips and holders. The Biomek i5 MC workstation was also equipped with a 300 μ L 96 pipetting head, heat block [Peltier1], orbital shaker, trash [TR1] and tip washing station [WS1]. Biomek control software, version 5.1 [Beckman Coulter] was used. Figure 2 illustrates the deck setup of the Biomek i5 MC workstation. The labware and other consumables used in this work are presented in Tables 1 and 2. Appendix 1 provides a step-by-step guide for sample preparation.

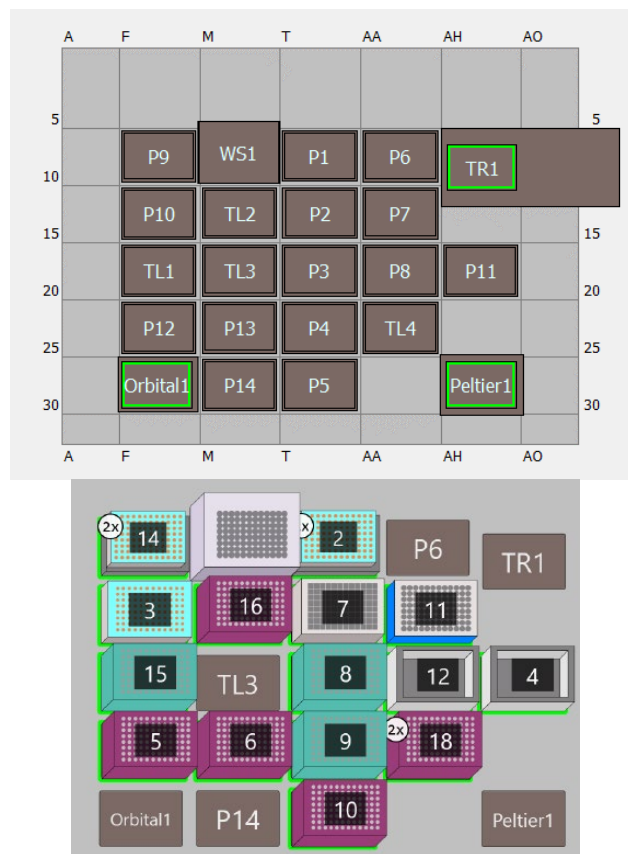


Figure 2. (A) Deck layout and (B) labware organization of the Biomek i5 MC workstation.

Capillary electrophoresis: The BioPhase 8800 system [P/N: 5083590F] was used. The separations were accomplished using the BioPhase BFS capillary cartridge - 8 x 30 cm [P/N: 5080121] and BioPhase sample, reagent and outlet plates [P/N: 5080311] that were acquired from SCIEX [Marlborough, MA]. N-linked glycans labeled with 9-aminopyrene-1,4,6-trisulfonic

acid [APTS] were detected using laser-induced fluorescence (LIF) with excitation and emission wavelengths of 488 and 520 nm, respectively.

Methods and sequence: Figure 3 shows the conditioning [Figure 3A], separation [Figure 3B] and shutdown [Figure 3C] methods used for glycan analysis. A typical fast glycan analysis sequence starts with the conditioning method, then the separation method is performed to analyze standards and unknown samples and finally the sequence terminates with the shutdown method.

Data processing: The BioPhase software, version 1.2, was used to create methods and sequences and to perform data acquisition and processing.

Table 1. Accessories required for the automated sample preparation using the Biomek i5 MC workstation.

Description	Vendor	Part number	Quantity
MicroAMP Splash Free 96-Well Base	Applied Biosystems	4312063	3
Alpaqua Magnum FLX Magnet	Alpaqua	A000400	3

Table 2. List of pipette tips and plates used in this sample preparation workflow.

Description	Vendor	Part number	Quantity
230 µL pipette tips, non-sterile	Beckman Coulter	B85903	3
90 µL pipette tips, non-sterile	Beckman Coulter	B85881	5
Microplate 96 deep well	Chrom Tech, Inc.	96-6009	1
Thermowell 96 well polypropylene PCR microplates	Corning	6551	2
Reservoir	Agilent	201244-100	2
BioPhase 8800 sample plate	SCIEX	5080313	1

A) Method Duration: 5.0 min. Number of Actions: 2

Settings	Capillary Cartridge: 25.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA, Enabled	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 100 Peak Width: 1 sec Data Rate: 10 Hz
Rinse	Duration: 2.5 min. 80.0 psi	Inlet: Water Rinse Outlet: Waste
Rinse	Duration: 2.5 min. 80.0 psi	Inlet: HR-NCHO gel Rinse Outlet: Waste

B) Method Duration: 8.6 min. Number of Actions: 9

Settings	Capillary Cartridge: 25.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA, Enabled	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 100 Peak Width: 1 sec Data Rate: 10 Hz
Rinse	Duration: 2.5 min. 80.0 psi	Inlet: HR-NCHO gel Rinse Outlet: Waste
Wait	Duration: 0.1 min.	Inlet: Water Dip Outlet: Water Dip
Inject	Duration: 5 sec. 1.0 psi	Plate: Reagent Inlet: Water Injection Outlet: Waste
Inject	Duration: 5 sec. -1.0 kV	Plate: Sample Outlet: HR-NCHO gel Separation
Wait	Duration: 0.0 min.	Inlet: Water Dip Outlet: Water Dip
Inject	Duration: 2 sec. -1.0 kV	Plate: Sample Location: Column 1 Outlet: HR-NCHO gel Bracketing Standard...
Inject	Duration: 5 sec. 1.0 psi	Plate: Reagent Inlet: HR-NCHO gel Rinse Outlet: Waste
Separate	Duration: 6.0 min. -30.0 kV, 5.0 psi, Both Ramp Time: 0.1 min. Autozero: 0.5 min. Advance after: 12 actions	Inlet: HR-NCHO gel Separation Outlet: HR-NCHO gel Separation
Wait	Duration: 0.0 min.	Inlet: Water Dip Outlet: Water Dip

C) Method Duration: 16.0 min. Number of Actions: 3

Settings	Capillary Cartridge: 25.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA, Enabled	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 100 Peak Width: 1 sec Data Rate: 10 Hz
Rinse	Duration: 6.0 min. 80.0 psi	Inlet: Water Rinse Outlet: Waste
LIF Laser	OFF	
Wait	Duration: 0.0 min.	Inlet: Water Dip Outlet: Water Dip

Figure 3. BioPhase 8800 system method settings for fast glycan analysis. Conditioning method [A], separation method [B] and shutdown method [C].

Discussion

N-linked glycan sample preparation on the Biomek i5 MC liquid handler involves three main steps: magnetic bead-mediated digestion, labeling and cleanup/release. Detailed instructions are provided in the Fast Glycan application guide.³ Briefly, the sample is mixed with magnetic beads, then denatured and digested with PNGase F. The beads are washed with acetonitrile to capture glycans while the deglycosylated protein is removed. The captured glycans are then labeled by reductive amination with APTS and picoline borane. Excess APTS is removed through three acetonitrile washes. Finally, the labeled glycans are released from the beads with distilled deionized (DDI) water and analysed on the BioPhase 8800 system. Total preparation time for a 96-well plate was approximately 2 hours and 37 minutes.

Robustness of the automated sample preparation process and CE-LIF analysis

The robustness of the automated sample preparation was assessed by evaluating the intra- and inter-capillary consistency of corrected peak area percentage [CPA%], relative migration time of glucose units, which is the same as the relative migration time of the glycan species [RMT GU] and

glucose units [GU] for FA2G2S1/M5, FA2, M7[D1]/FA2[6]G1, FA2G2 and FA2[3]G1/FA2B[6]G1 species.

The samples used in this study were human serum IgG and negative control. In the negative control sample, the IgG was replaced with water. The negative control was used to determine any potential artifact introduced by the denaturation, digestion, glycan capture, cleanup and release processes.

The labeled N-linked glycans were placed in rows A, C, E and G of the sample plate. The negative control was placed in rows B, D, F and H.

Inter-capillary reproducibility

Figure 4 demonstrates consistent inter-capillary CPA% measurements across the five evaluated glycan species, supporting the reproducibility of the automated sample preparation and CE-LIF analysis workflow. Most glycan species showed low variability across capillaries, with CPA% %RSD values ranging from 1.78% to 3.79% for M5, FA2, FA2[6]G1, and FA2G2. FA2[3]G1 showed the highest variability at 9.92%, but it remained below 10%, indicating acceptable inter-capillary reproducibility. Overall, the bar graph supports the robustness and consistency of the automated N-linked glycan analysis workflow.

Accuracy in glycan peak assignment is critical and relies on the calculated GU values. Figure 5 showcases the inter-capillary reproducibility of the GU values calculated using the BioPhase software across all 4 capillaries.

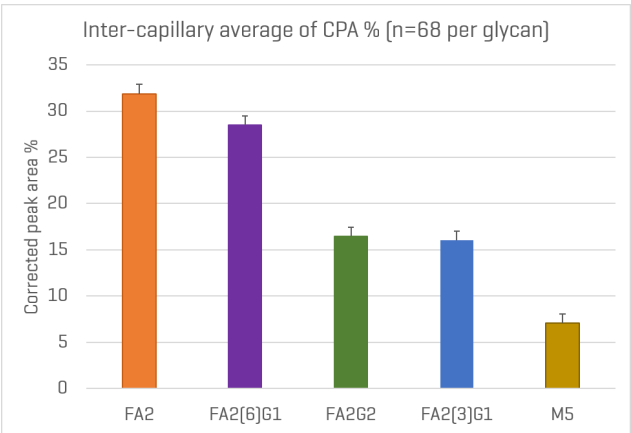


Figure 4. Inter-capillary average CPA% for each glycan species.

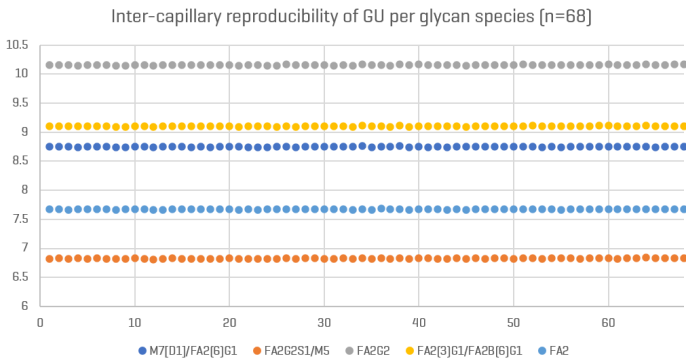


Figure 5. Inter-capillary GU value for each glycan species.

Table 3 shows the inter-capillary reproducibility in terms of %RSD for CPA%, and RMT GU and GU to substantiate the findings presented in Figures 4 and 5.

Table 3. Inter-capillary reproducibility of CPA% and GU by glycan species.

Glycan species	%RSD of CPA%	%RSD of RMT GU	%RSD of GU
FA2[3]G1	9.92	0.06	0.05
M5	1.82	0.08	0.07
FA2	1.78	0.06	0.05
FA2[6]G1	3.41	0.05	0.05
FA2G2	3.79	0.05	0.05

Intra-capillary reproducibility

The graphs in Figure 6 demonstrate strong intra-capillary consistency across the glycan species evaluated. For CPA%, the grouped bar profiles indicate comparable quantitative responses across capillaries, supporting consistent peak area measurement and reliable quantitation within the cartridge.

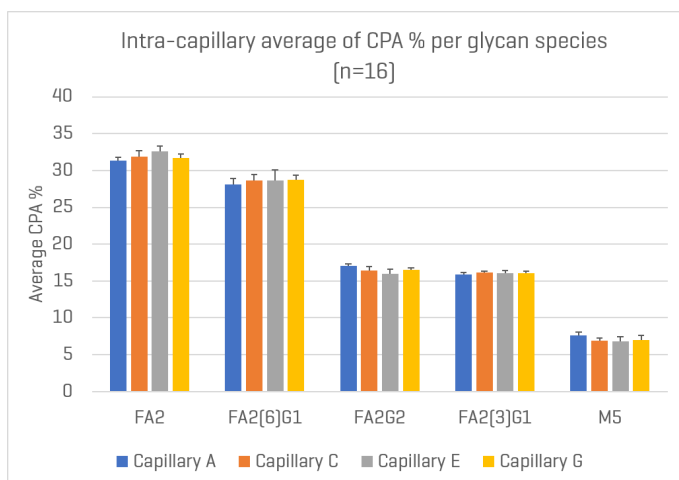


Figure 6. Intra-capillary average of CPA%. Each bar represents data collected from a given capillary. Bars are grouped by glycan species.

Figure 7 demonstrates the consistency within a capillary and also a comparison between various capillaries of GU values. These results also indicate the reliability and precision of the glycan identification based on GU values.

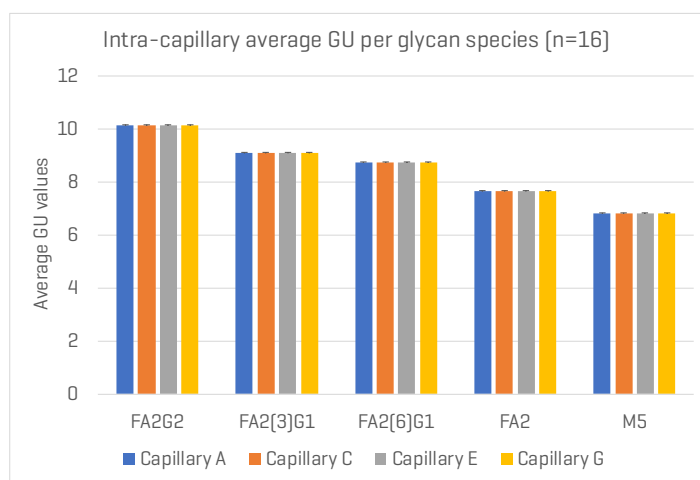


Figure 7. Intra-capillary average of GU. Each bar represents data collected from a given capillary. Bars are grouped by glycan species.

Figure 8 further illustrates the advantage of automated sample preparation. This figure shows a representative overlay of 17 runs obtained on capillary A to showcase the good consistency in sample preparation.

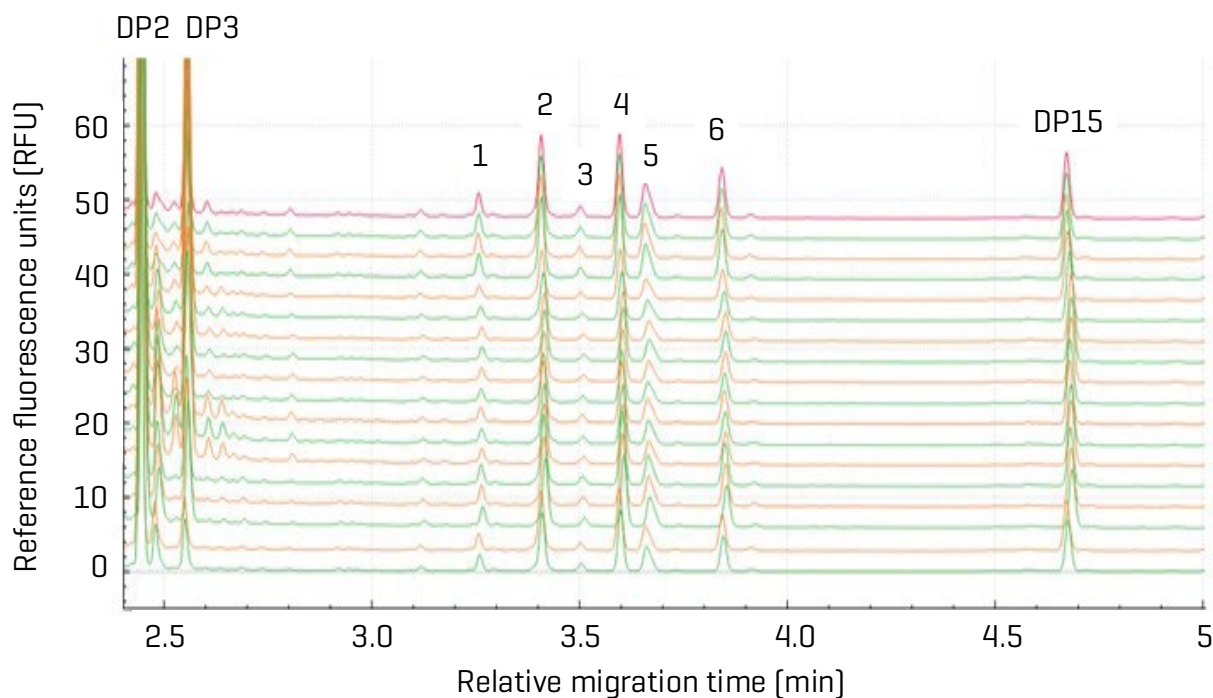


Figure 8. Overlay of 17 injections of released N-linked glycans. Sample preparation and separation conditions are described in the experimental section. Peaks correspond to 1) M5, 2) FA2, 3) FA2B, 4) FA2[6]G1, 5) FA2[3]G1 and 6) FA2G2.

Conclusions

- The automated sample preparation yielded good intra-capillary reproducibility data for CPA%, RMT GU and GU.
- Inter-capillary reproducibility values for CPA%, RMT GU and GU were less than 10%, 0.08% and 0.07%, respectively.
- Automated sample preparation reduces labor-intensive bench work and provides a higher throughput in N-linked glycan analysis with consistent results.
- The BioPhase 8800 was operated using standard BioPhase software for instrument control and data acquisition. For greater automation, even though the API* was not evaluated in this work, SCIEX offers the BioPhase Instrument API*, which allows external software to monitor instrument status, trigger and track a sequence progress, and integrate the system into broader lab automation workflows.

3. Fast glycan labeling and analysis kit for the BioPhase 8800 system application guide. SCIEX application guide, RUO-IDV-05-4648-A.

* available separately and under the API EULA T&Cs

References

1. Characterization of 2-AB labeled released N-linked glycans using the X500B QTOF System. SCIEX technical note, RUO-MKT-02-9202-A.
2. High throughput released N-glycan identification using CE and a customizable glycan library. SCIEX technical note, MKT-26425-A.

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