

Reproducibility study of a high-throughput released N-glycan workflow

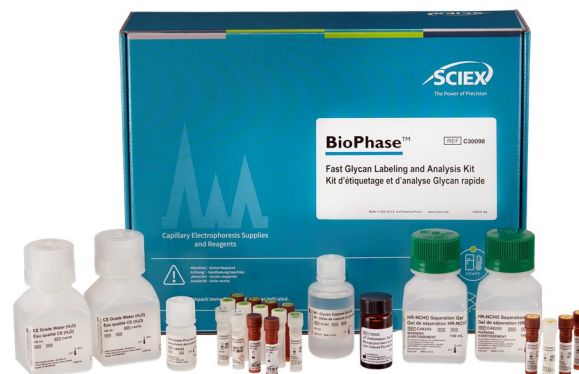
Featuring the BioPhase 8800 system and the BioPhase Fast Glycan Labeling and Analysis kit

Fang Wang and Marcia Santos
SCIEX, USA

Introduction

This technical note demonstrates the robustness and consistency of the high-throughput analysis of APTS-labeled N-linked glycans by capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection. The inter- and intra-capillary reproducibility validation using the BioPhase Fast Glycan Labeling and Analysis kit yielded 576 data points from 2 instruments, 2 cartridges and 3 chemistry kits.

The development of biopharmaceuticals involves many integrated stages. One critical demand in early development is to quickly screen many clones and cell culture expression conditions. The screening process typically monitors the critical quality attributes (CQAs) of therapeutics. As glycosylation plays a significant role in the stability, safety and efficacy of protein therapeutics, glycan expression profiling is one of the CQAs that must be monitored closely. In this technical note, we used the BioPhase Fast Glycan Labeling and Analysis kit to demonstrate a streamlined N-linked glycan workflow that is compatible with the high-throughput capabilities of the BioPhase 8800 system. This workflow enables upstream bioprocessing scientists to analyze a 96-well plate in 1.5 hours or up to 5 96-well plates in 1 working day, which can support fast cell culture condition optimization.



Key features of the released N-linked glycan analysis

- **High-throughput glycan analysis workflow:** Parallel processing of 8 samples in 8.5 min (Figure 1) enables the separation of a 96-well plate in 1 hour and 42 min
- **Highly robust and exceptional performance:** The migration time of the DP2 and DP15 marker peaks from data collected using 2 instruments, 2 cartridges and 3 chemistry kits demonstrated %RSD values of 0.95% and 0.91%, respectively, indicating strong reproducibility
- **Excellent intra-capillary reproducibility:** %RSD for the glucose unit (GU) was less than 0.1% for the 9 glycan species studied

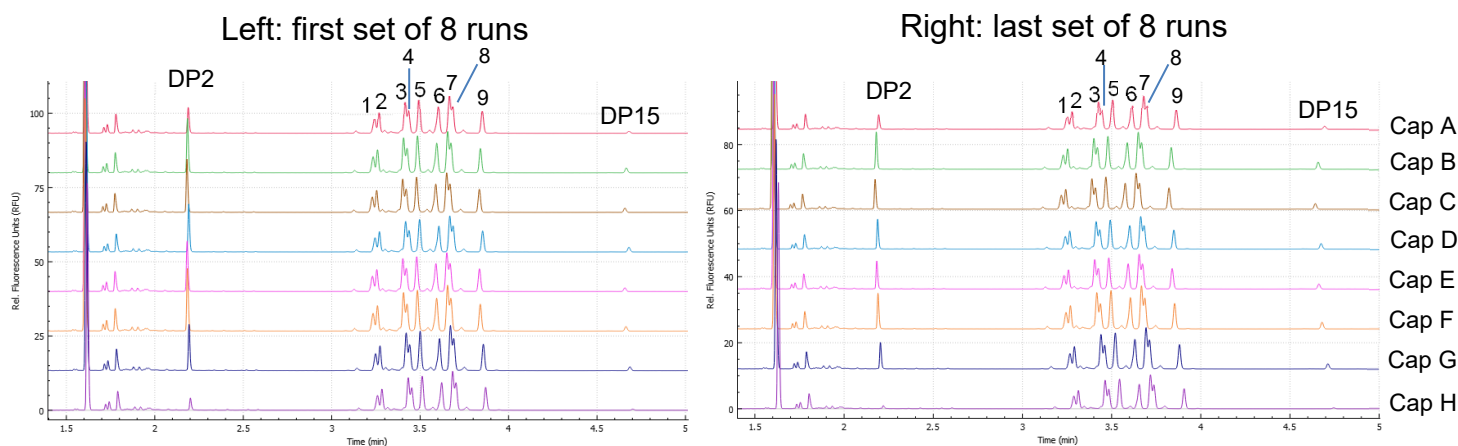


Figure 1. Representative overlays of the electropherograms from 8 runs of glycan standards on capillaries A–H. The left panel shows electropherograms acquired from the first set of 8 runs. The right panel shows those acquired from the last set of 8 runs. Peaks observed include: 1) A2, 2) M5, 3) FA2, 4) A2(6)G1, 5) A2(3)G1, 6) FA2(6)G1, 7) FA2(3)G1, 8) A2G2 and 9) FA2G2.

Methods

Materials: The BioPhase Fast Glycan Labeling and Analysis kit (P/N: C30098) was from SCIEX (Framingham, MA). The 9-glycan panel was a mixture of A2, FA2, A2(3)G1, A2(6)G1, FA2(3)G1, FA2(6)G1, A2G2, FA2G2 and M5 and was a gift from SCIEX research and development team.

Instruments: The BioPhase 8800 system (P/N: 5083590F), BioPhase BFS capillary cartridge – 8 x 30 cm (P/N: 5080121) and BioPhase sample and reagent plates (4,4,8) (P/N: 5080311) were from SCIEX. The BioPhase 8800 system was equipped with a LIF detector and 520 nm emission filter (P/N: 144940).

Software: The BioPhase 8800 software, version 1.2 was used to create the instrument method and sequence and for data acquisition and processing.

Methods and sequence: Figure 2 shows the conditioning (Figure 2A), separation (Figure 2B) and shutdown (Figure 2C) methods for glycan analysis. A typical fast glycan analysis sequence starts with the conditioning method, progresses to the separation method for the analysis of standards and unknown samples and then ends with the shutdown method.

Instrument set up: Reagents and sample plates were loaded according to the reagent plate map for the corresponding sequence. Multiple sequences were performed using 2 different instruments, 2 different cartridges and 3 different reagent lots.

Results and discussion

Inter-capillary migration time and GU value reproducibility

In the upstream bioprocessing stage, thousands of samples must be screened monthly. To meet this need, a robust, high-throughput analytical characterization tool that can deliver reliable instrumentation and efficient, user-friendly and flexible data analysis software for large-scale sample analysis is needed. The BioPhase 8800 system provides exceptional performance, including the robust temperature control that leads to highly reproducible migration times and accurate glycan peak assignments. Additionally, the BioPhase 8800 software streamlines data processing and the fast glycan analysis module provides accurate N-linked glycan peak identifications based on the GU database.

Representative electropherograms from the first and last set of 8 runs of the 9-glycan panel mixture (capillaries A – H) demonstrate excellent separation consistency (Figure 1). Marker peaks DP2 and DP15 were used as anchor peaks for glycan assignments based on 576 data points collected from 2

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 2. Instrument method settings. A) Conditioning method, B) separation method and C) shutdown method.

instruments, 2 lots of cartridges and 3 chemistry kits. The migration time of these peaks showed high reproducibility. The consistency of migration time for DP2 and DP15 demonstrated good instrument stability and assay reliability between separations and equivalence among the 8 capillaries within a single experiment (Figure 3).

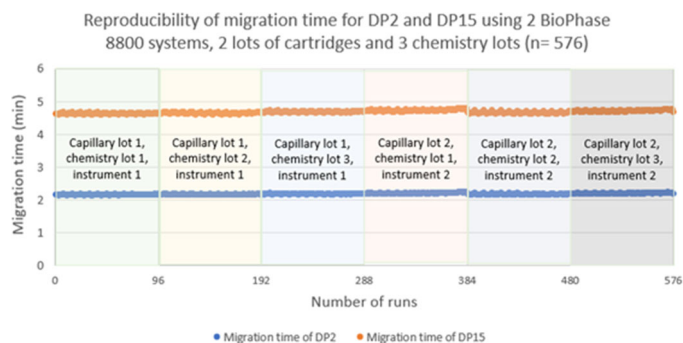


Figure 3. Reproducibility of migration time for marker peaks DP2 and DP15.

The average migration times for DP2 and DP15 were 2.19 min and 4.69 min, respectively. The calculated reproducibility values of DP2 and DP15 were 0.95% and 0.91% RSD, respectively. The migration time of each 9-glycan standard was converted into

relative migration time and later converted to the GU value for glycan identification.²

The calculated GU values were evaluated to confirm peak identification accuracy. Figure 4 shows the inter-capillary reproducibility of the GU values for each of the 9 glycans in the sample. The results demonstrate that the BioPhase 8800 software offers accurate and reliable glycan identification.

Table 1 shows the inter- and intra-capillary average GU values identified for each of the 9 glycan species. Table 2 shows the %RSD of the data shown in Figure 5 and these results demonstrate the robustness of the fast N-linked glycan analysis. On average, the %RSD for GU peak assignment (n=576 separations) was <0.1% for all 9 species for intra- and inter-capillary conditions.

Inter- and intra-capillary corrected peak area percentage (CPA%) composition

N-linked glycan distribution can affect drug efficacy, therefore the relative abundance of these glycans must be monitored in addition to their identification during data analysis. Table 3 indicates that the average CPA% for all 9 glycans is consistent between intra- and inter-capillary conditions. Table 4 showcases the average %RSD values calculated from the CPA% values, indicating highly reproducible results for all 9 glycans. The smallest %RSD observed was 1.44% for FA2(3)G1, whereas the largest %RSD was <12% for FA2G2. These statistical results corroborate the observations made in Table 3.

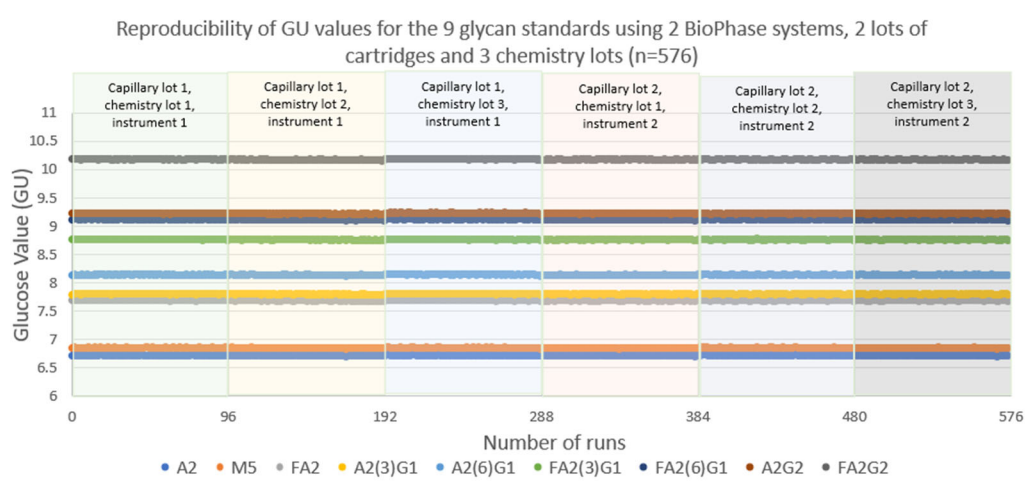


Figure 4. Reproducibility of GU values. Inter-capillary glycan values were calculated for the 9-glycan panel analyzed with 2 capillary cartridge lots, 3 chemistry lots and 2 BioPhase 8800 systems.

Table 1. Inter- and intra-capillary average GU values for the 9-glycan panel.

	Intra-capillary average of GU for the 9-glycan panel (n=72)								
	A2	M5	FA2	A2(3)G1	A2(6)G1	FA2(3)G1	FA2(6)G1	A2G2	FA2G2
Capillary A	6.70	6.83	7.67	7.78	8.12	8.74	9.10	9.20	10.16
Capillary B	6.70	6.84	7.67	7.79	8.13	8.75	9.10	9.21	10.16
Capillary C	6.70	6.84	7.67	7.78	8.12	8.75	9.10	9.21	10.16
Capillary D	6.70	6.84	7.67	7.79	8.13	8.75	9.10	9.21	10.16
Capillary E	6.70	6.84	7.67	7.79	8.12	8.75	9.10	9.21	10.16
Capillary F	6.70	6.84	7.67	7.79	8.12	8.75	9.10	9.21	10.16
Capillary G	6.70	6.84	7.67	7.79	8.12	8.75	9.10	9.21	10.16
Capillary H	6.70	6.83	7.67	7.78	8.12	8.74	9.10	9.20	10.16
Inter-capillary average of GU (n=576)	6.70	6.84	7.67	7.78	8.12	8.75	9.10	9.21	10.16

Together, these results demonstrate remarkable consistency between capillaries for each of the 9 glycan species in the panel. These results further showcase the intra-capillary robustness of the BioPhase 8800 system and other components, including the instrument, cartridges and chemistry kits.

Table 2. Inter- and intra-capillary %RSD average for the GU values for the 9-glycan panel.

	Intra-capillary %RSD of GU for the 9-glycan panel (n=72)								
	A2	M5	FA2	A2(3)G1	A2(6)G1	FA2(3)G1	FA2(6)G1	A2G2	FA2G2
<i>Capillary A</i>	0.07	0.06	0.06	0.07	0.06	0.06	0.05	0.06	0.05
<i>Capillary B</i>	0.06	0.06	0.06	0.07	0.07	0.05	0.07	0.07	0.06
<i>Capillary C</i>	0.06	0.06	0.06	0.06	0.06	0.05	0.06	0.06	0.05
<i>Capillary D</i>	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05
<i>Capillary E</i>	0.07	0.07	0.07	0.07	0.07	0.06	0.05	0.07	0.05
<i>Capillary F</i>	0.06	0.07	0.06	0.07	0.06	0.06	0.05	0.06	0.05
<i>Capillary G</i>	0.07	0.08	0.08	0.08	0.08	0.06	0.07	0.07	0.08
<i>Capillary H</i>	0.08	0.08	0.07	0.09	0.09	0.07	0.07	0.08	0.06
<i>Inter-capillary %RSD of GU (n=576)</i>	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.07	0.06

Table 3. Inter- and intra-capillary average CPA% for the 9-glycan panel.

	Intra-capillary average of CPA% for the 9-glycan panel (n=72)								
	A2	M5	FA2	A2(3)G1	A2(6)G1	FA2(3)G1	FA2(6)G1	A2G2	FA2G2
<i>Capillary A</i>	7.44	10.86	13.35	9.23	14.25	13.11	13.06	11.71	6.99
<i>Capillary B</i>	7.49	10.88	13.32	9.25	14.31	13.13	12.92	11.78	6.93
<i>Capillary C</i>	7.42	10.87	13.35	9.22	14.26	13.14	12.96	11.80	6.97
<i>Capillary D</i>	7.55	11.00	13.34	9.31	14.33	13.07	12.81	11.74	6.84
<i>Capillary E</i>	7.33	10.77	13.42	9.11	14.18	13.11	13.23	11.71	7.13
<i>Capillary F</i>	7.22	10.65	13.43	9.06	14.10	13.14	13.45	11.68	7.26
<i>Capillary G</i>	7.19	10.56	13.44	9.03	14.07	13.19	13.49	11.65	7.38
<i>Capillary H</i>	7.29	10.67	13.43	9.09	14.14	13.11	13.41	11.61	7.25
<i>Inter-capillary average of CPA% (n=576)</i>	7.37	10.78	13.39	9.16	14.20	13.13	13.17	11.71	7.09

Table 4. Inter- and intra-capillary %RSD average for the CPA% for the 9 glycan panel.

	Intra-capillary average of CPA% reproducibility (RSD %) for the 9-glycan panel (n=72)								
	A2	M5	FA2	A2(3)G1	A2(6)G1	FA2(3)G1	FA2(6)G1	A2G2	FA2G2
<i>Capillary A</i>	8.72	6.13	1.90	5.65	3.14	1.46	9.00	2.36	12.27
<i>Capillary B</i>	8.69	5.94	2.11	5.81	3.24	1.28	9.46	2.66	12.36
<i>Capillary C</i>	7.91	5.60	1.93	5.51	3.02	1.11	8.70	2.44	11.69
<i>Capillary D</i>	9.96	6.97	2.24	6.55	3.76	1.55	10.91	2.70	14.52
<i>Capillary E</i>	8.41	6.34	1.97	5.27	3.00	1.52	9.01	2.55	11.54
<i>Capillary F</i>	7.22	5.63	1.71	4.46	2.64	1.26	6.96	2.14	10.09
<i>Capillary G</i>	7.57	4.90	1.88	5.00	2.78	1.59	7.61	2.96	9.86
<i>Capillary H</i>	9.69	6.43	1.77	5.97	3.44	1.75	9.49	2.60	12.53
<i>Inter-capillary RSD% of CPA% (n=576)</i>	8.52	5.99	1.94	5.53	3.13	1.44	8.89	2.55	11.86

Conclusions

- The BioPhase 8800 software performed reliable glycan peak identifications with average GU %RSD values between 0.05 and 0.08% for the 9 glycan species analyzed
- The assay using BioPhase Fast Glycan Labeling and Analysis kit demonstrated remarkable robustness and inter-capillary consistency
- The BioPhase 8800 software provided a consistent relative glycan abundance with an average %RSD value for CPA% <12% for all 9 glycan species
- The temperature control feature of the BioPhase 8800 system enabled highly consistent migration times for the DP2 and DP15 markers used for glycan identification

References

1. BioPhase Fast Glycan Labeling and Analysis kit for the BioPhase 8800 system – application guide, RUO-IDV-05-4648-A
2. High throughput released N-glycan identification using CE and a customizable glycan library. SCIEX technical note, [MKT-26425-A](#)

The SCIEX clinical diagnostic portfolio is For In Vitro Diagnostic Use. Rx Only. Product(s) not available in all countries. For information on availability, please contact your local sales representative or refer to <https://sciex.com/diagnostics>. All other products are For Research Use Only. Not for use in Diagnostic Procedures.

Trademarks and/or registered trademarks mentioned herein, including associated logos, are the property of AB Sciex Pte. Ltd. or their respective owners in the United States and/or certain other countries (see www.sciex.com/trademarks).

© 2023 DH Tech. Dev. Pte. Ltd. MKT- 26648-A.



Headquarters
 500 Old Connecticut Path | Framingham, MA 01701 USA
 Phone 508-383-7700
sciex.com

International Sales
 For our office locations please call the division
 headquarters or refer to our website at
sciex.com/offices