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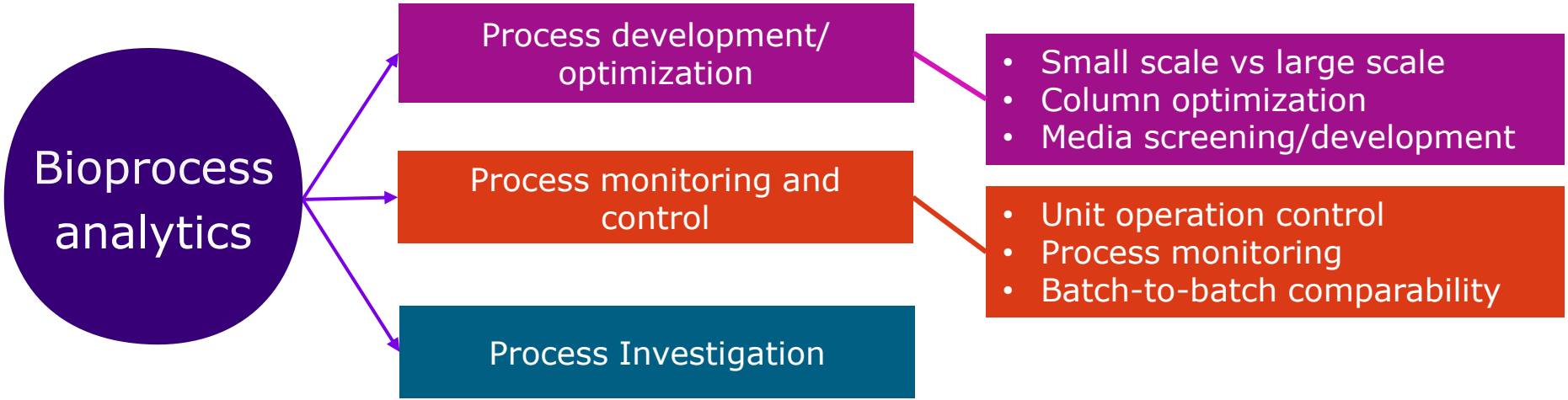
Utilizing high-throughput capillary electrophoresis for bioprocess development support

Brian Wei, PhD

A high throughput analysis is needed to support screening large numbers of samples



- Supports the analytical needs of different functions and aspects.
- Sample throughput and fast data turnaround are key enablers for process development and optimization.

High-throughput CE-SDS & Glycan assay using BioPhase 8800 system

PA 800 Plus

Next generation

BioPhase 8800 system

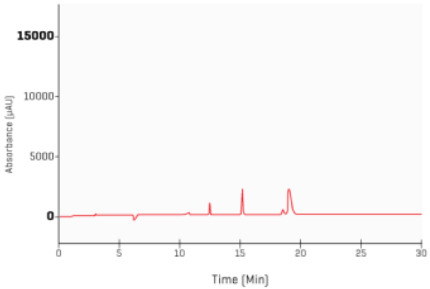
- Next generation of PA 800 Plus system
- Multi-Capillaries (8) system
- 96 well-plate format
- CE-SDS
- Glycan
- CIEF
- RNA 9000

Running 8 capillaries at the same time

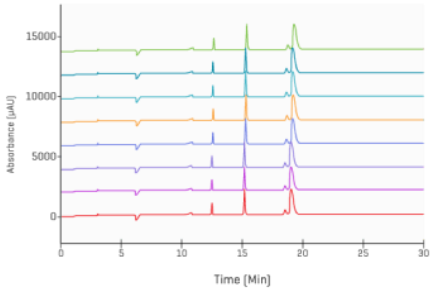
Example for CE-SDS assay (Reduced IgG) with BioPhase 8800

UHPLC	PA 800 Plus	BioPhase 8800
1-2 samples/hr	8-10 samples/hr	64-80 samples/hr

PA 800 Plus



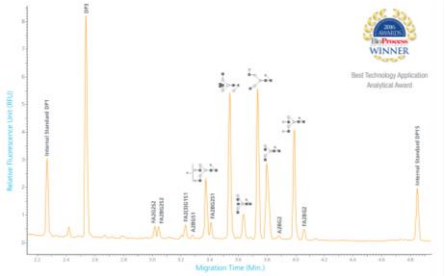
BioPhase 8800



Fast glycan kit can separate glycans in 6 min

	HILIC UHPLC	Sciex Fast Glycan CE
Separation Time	30-120 Min	6 min

Example of Glycan separation



The sample prep/dye labeling can be executed using a liquid handler such as the Biomek system (*Beckman Coulter)

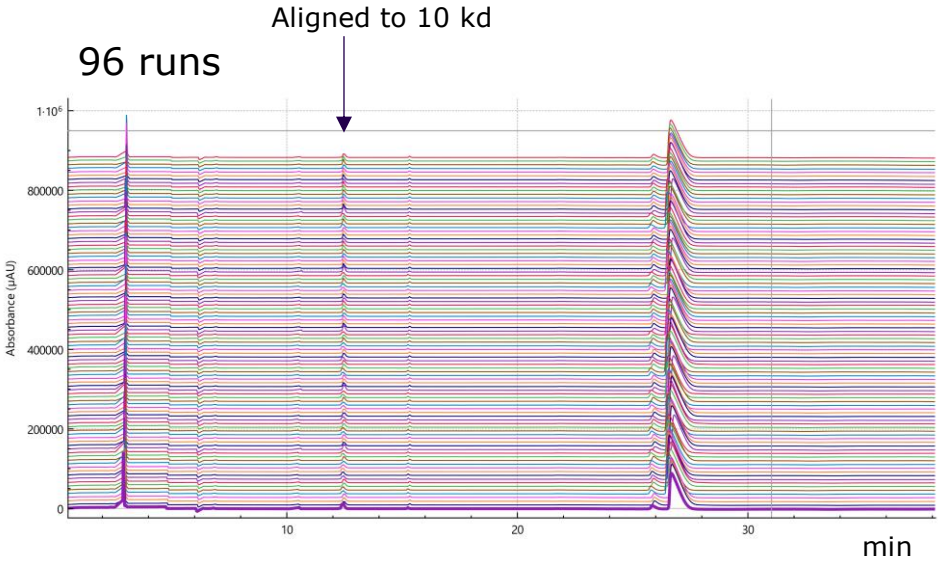
Tech Note: 2023 Fang Wang[1], Sudha Savant[2] and Marcia Santos[1]
[1]SCIEX, USA; [2]Beckman Coulter Life Sciences, USA

Ninety-Six samples can be analyzed in 12 hours

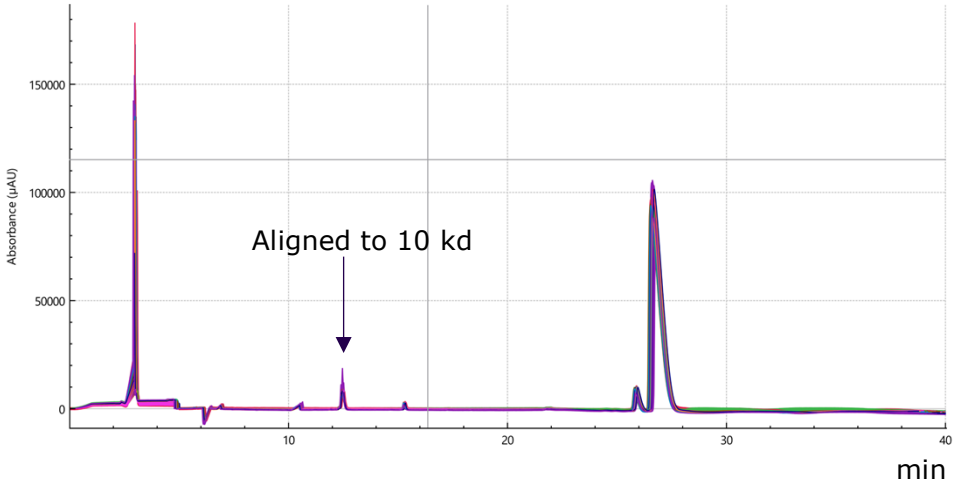
mAb 2

- 16 samples of mAb2 were prepared
  - Each samples was injected 6 times (n=6)
  - 96 data points

96 runs



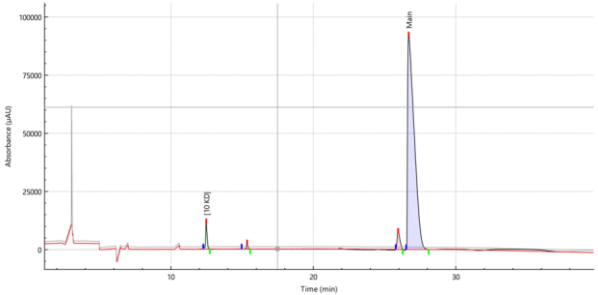
Overlay of 96 runs



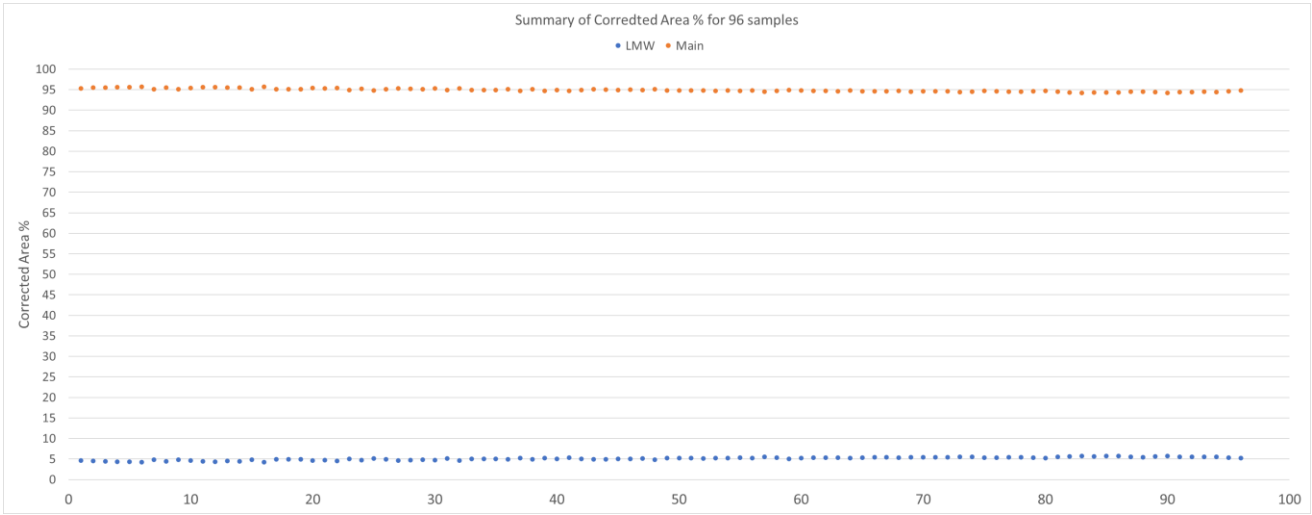
Ninety-Six mAb2 injections have good data repeatability

16 samples prepared and each samples injected 6 times, the CV was calculated for total 96 injections with inter- and intra- capillaries

Example of peak integration

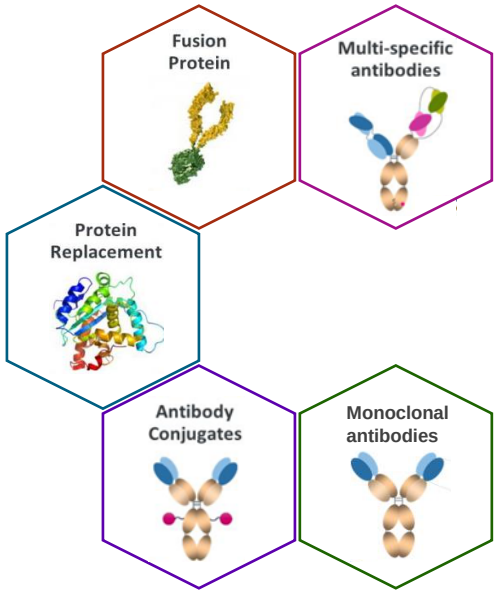


	Corrected area %	
	LMW	Main
Mean	5.11%	94.89%
CV	7.44%	0.40%



N-glycan analysis of protein-based modalities is critical to ensure consistent product quality

Glycosylation may relate to half-life, cell uptake and overall drug potency



- Safety and tolerability
- Biological activity and efficacy
- Immunogenicity
- Batch consistency and quality control
- Regulatory compliance
- Optimization of production processes

Legacy HILIC HPLC assay is currently used for glycan analysis with a lengthy separation time

Timetable

Time	A	B	Flow
Start. Cond. min	95.0 %	5.0 %	1.000 mL/min
2.00 min	95.0 %	5.0 %	--- mL/min
115.00 min	5.0 %	95.0 %	--- mL/min
140.00 min	5.0 %	95.0 %	--- mL/min
141.00 min	95.0 %	5.0 %	--- mL/min
150.00 min	95.0 %	5.0 %	--- mL/min

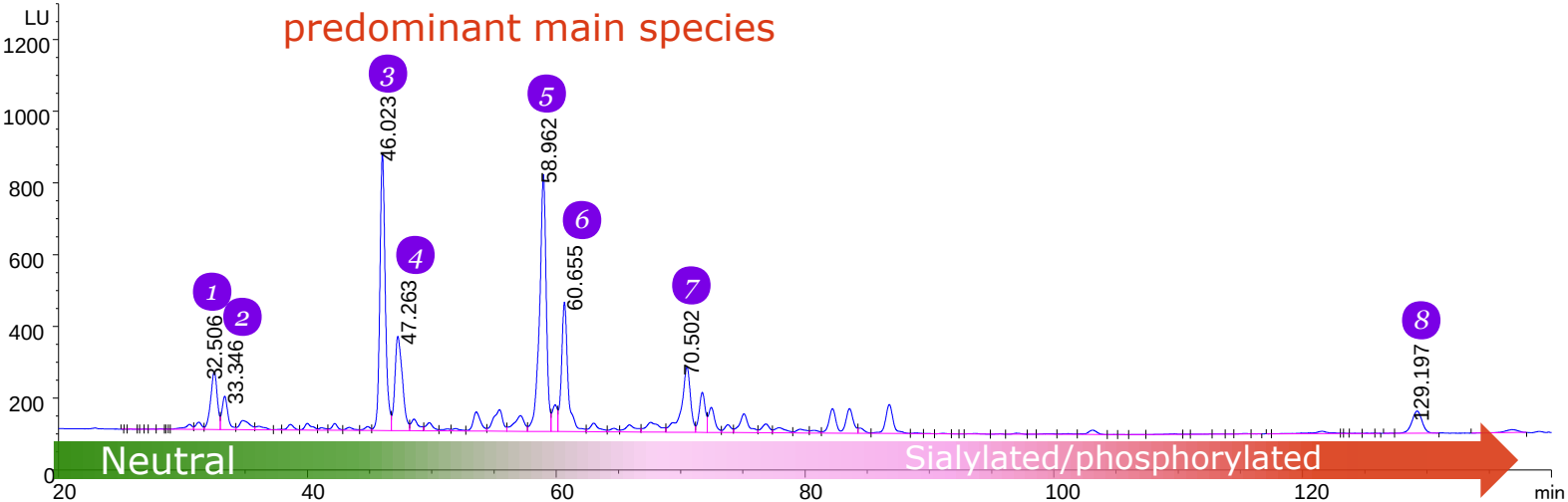
Column: Phenomenex Luna NH2

Mobile Phase A: AcOH in EtOH

Mobile Phase B: AcOH, DEMA, EtOH and EDTA in water

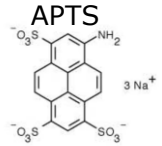
- Note: Each glycan peak is a cluster of glycans with one predominant main species

Peak	Annotation
1	Neutral +F
2	Neutral
3	+1S+F
4	+1S
5	+2S+F
6	+2S
7	+3S+F
8	+2P



Two monoclonal antibodies were selected for a proof-of-concept study

- BioPhase Fast Glycan Labeling And Analysis Kit
- LIF detection



Two monoclonal antibodies-mAb1 and mAb2 as examples

Sample was labeled with APTS dye according to the application guide. Glycan CE separation is enabled by the 3 negative charges provided by the APTS dye.

Experiment 1- mAb1 run with the commercially available APTS labeled glycan standards, G0, Man5, G0F, G1F, G1F' and G2F

- mAb1 was prepared and run on two different days. Day 1 (one capillary (Cap A), four repeats) and day 2 (one capillary (Cap A) three repeats)
- 7 data points were generated using mAb1 to assess intra-capillary precision (repeatability), 4 data points from day 1 and 3 data points from day 2
- Glycan standards were used for the peak assignment

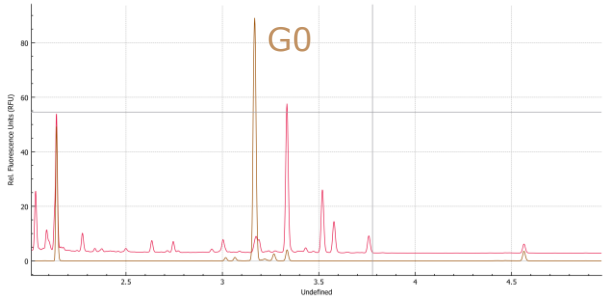
Experiment 2- mAb2 run with the commercially available APTS labeled glycan standards, G0, Man5, G0F, G1F, G1F' and G2F

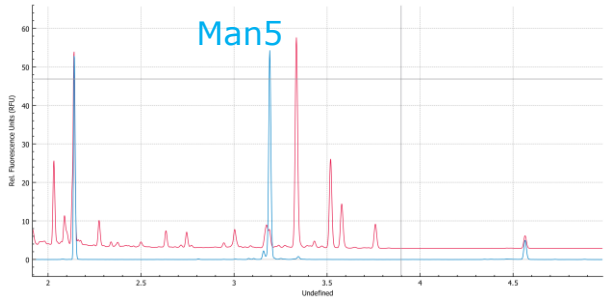
- mAb2 was tested with five repeats in 3 capillaries (Cap B,C,D)
- 15 data points of mAb2 were generated (inter-capillary precision)
- Glycan standards were used for the peak assignment (e-gram data not shown)

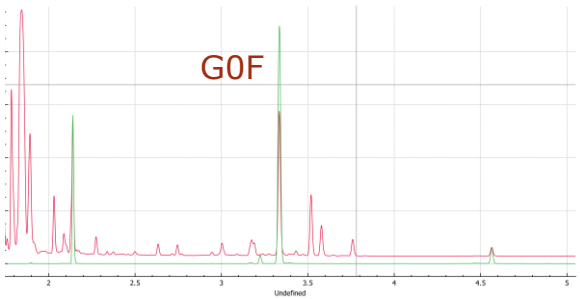
Experiment 3- Total of 72 samples mAb1 (Cap A-D) and mAb2 (Cap E-H) was tested

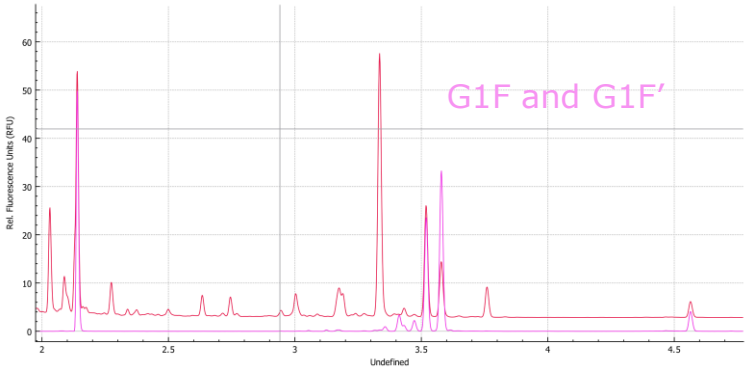
- 36 samples of mAb1 and mAb2 each were analyzed (inter & Intra capillary)

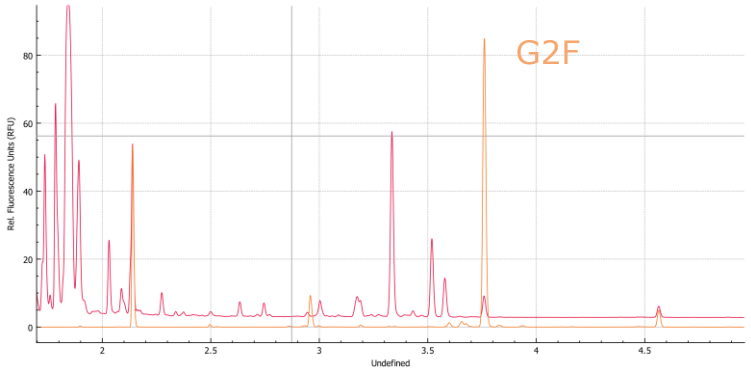
Main peak assignments of mAb1(red trace) were performed by comparing migration times to individual standards



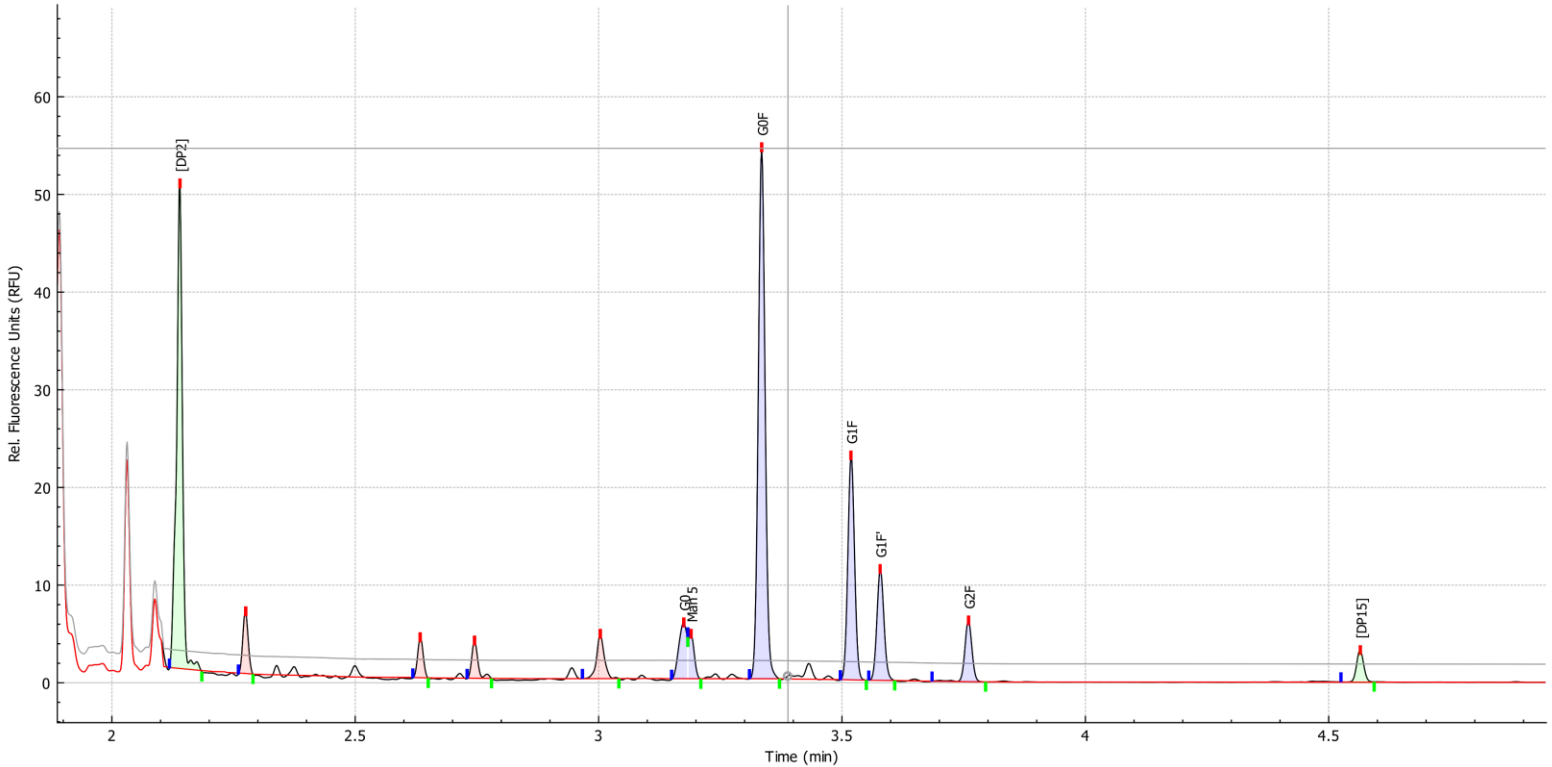








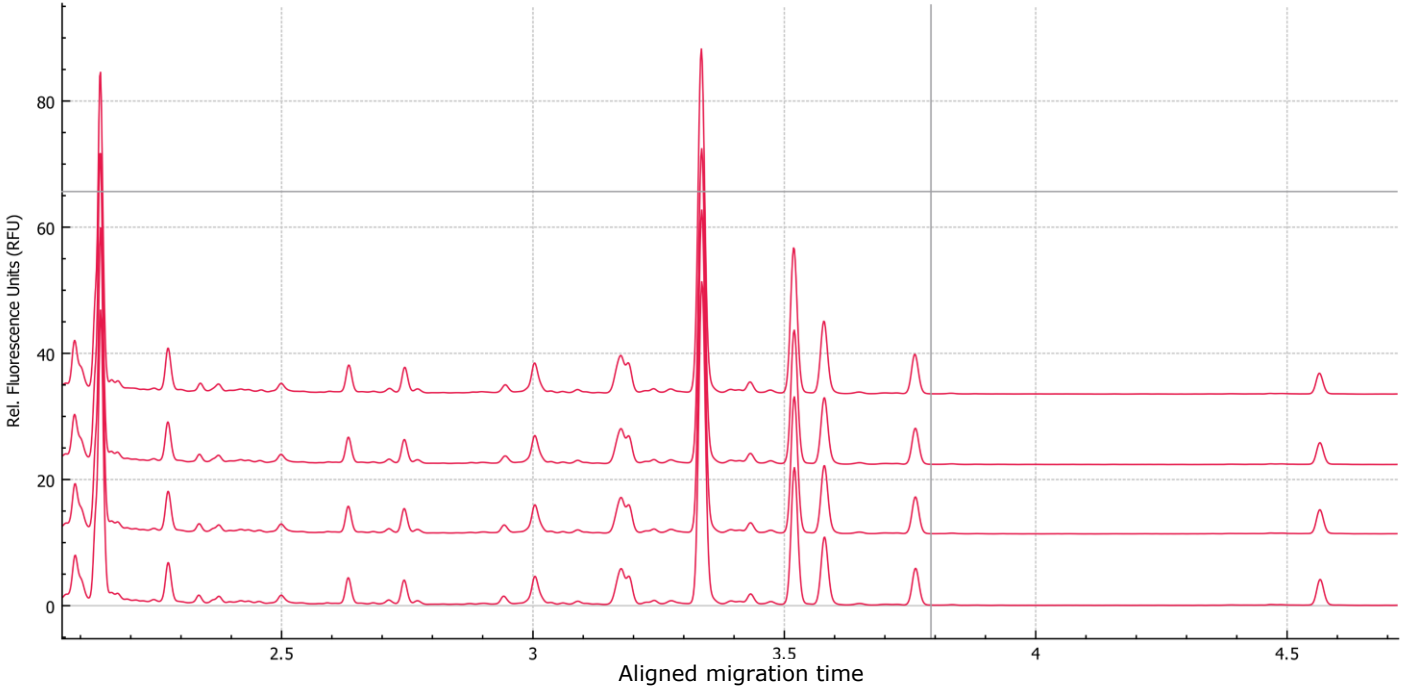
Main glycan profile peaks of mAb1 can be separated using a six-minute method



Overlay of four electropherograms for mAb1 showing good repeatability

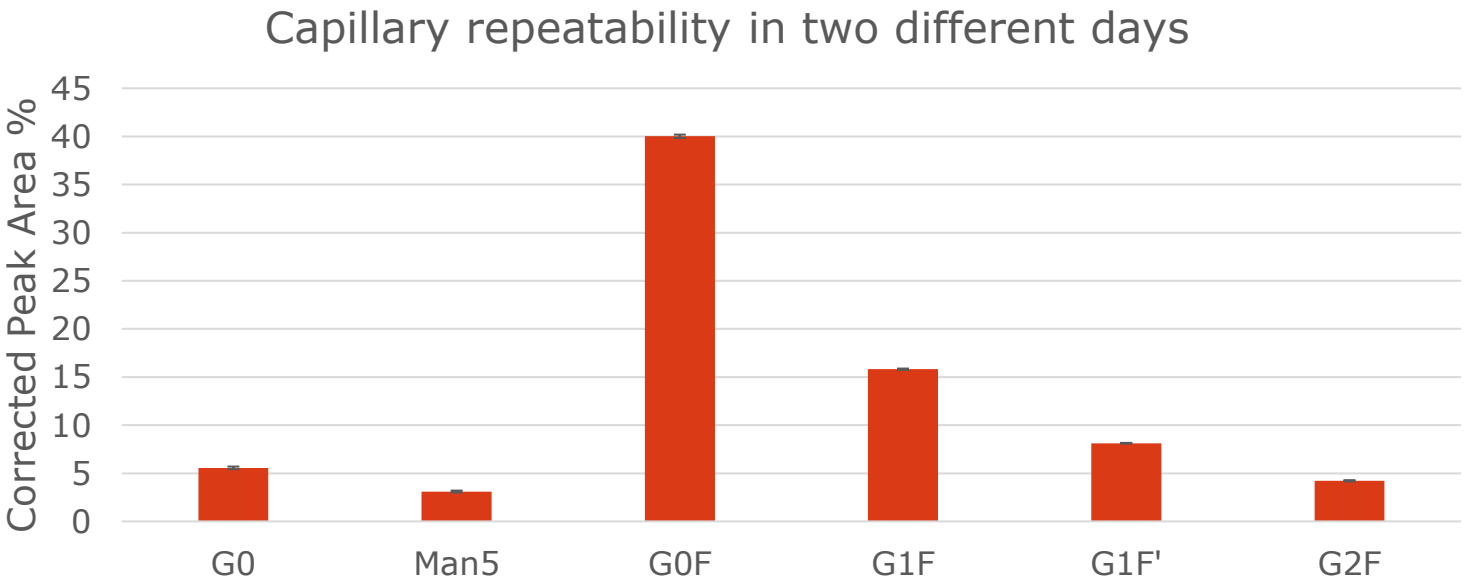
mAb1

Overlay with 4 runs



Intra-capillary precision (repeatability) (Capillary A) shows an RSD for the corrected peak area% of less than 4%

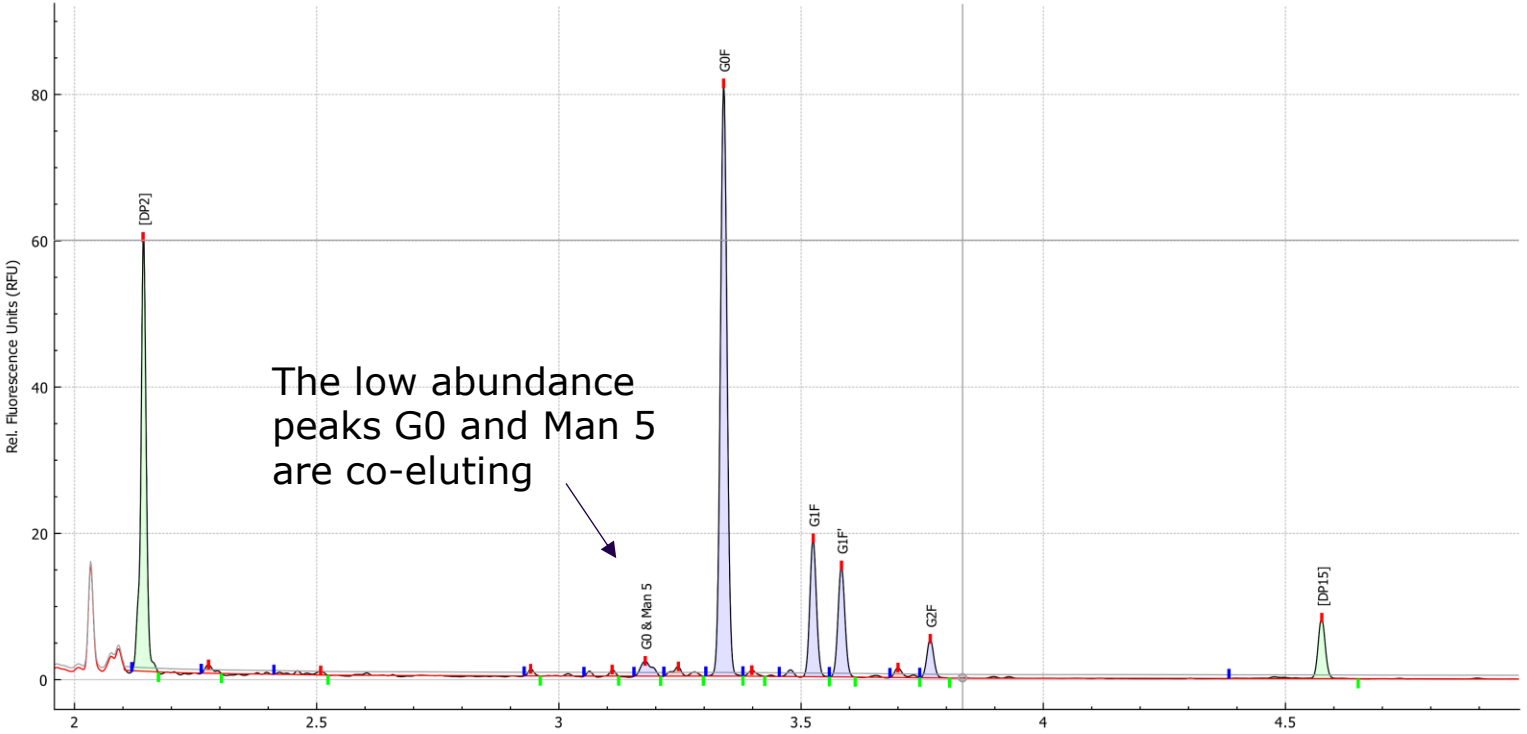
mAb1



	G0	Man5	G0F	G1F	G1F'	G2F
RSD (%)	2.48	3.27	0.44	0.46	0.47	1.64

Main glycan peak of mAb2 also can be separated using a six-minute method

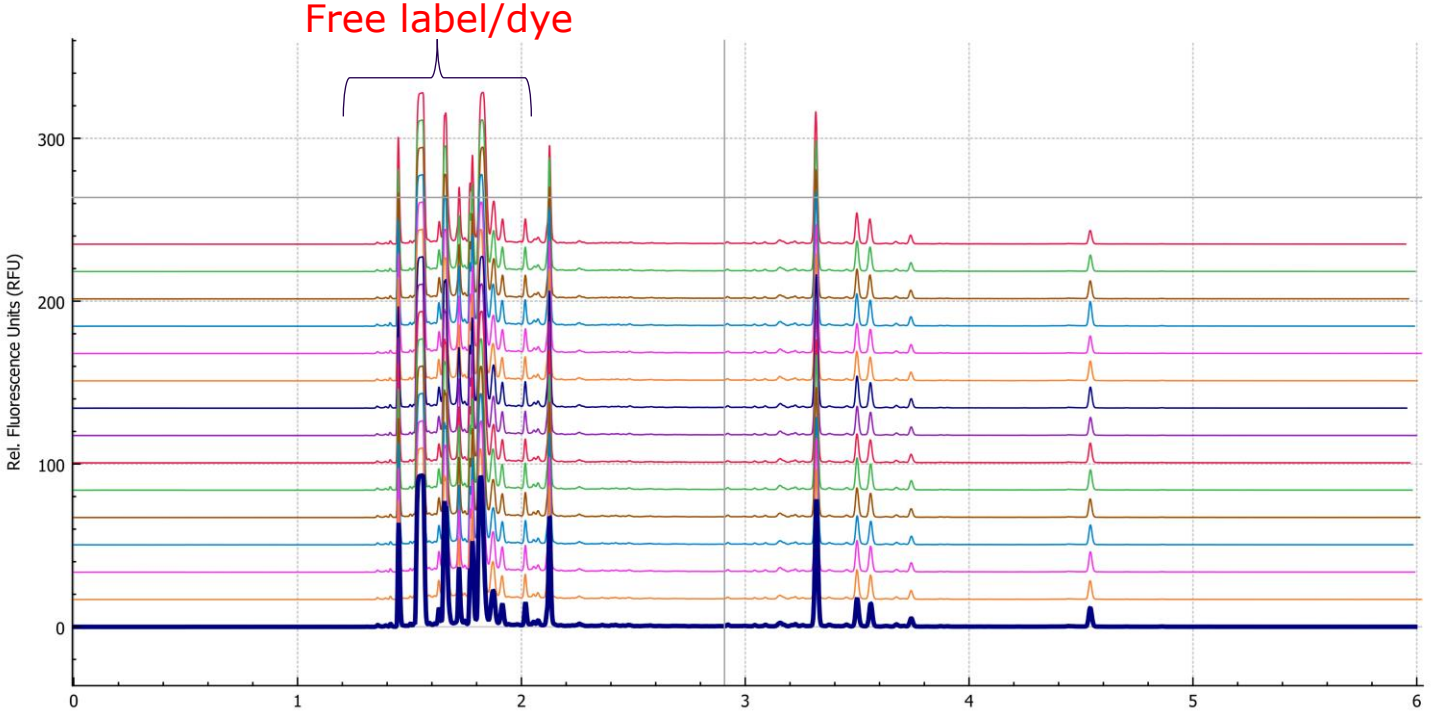
Peaks are assigned by comparison to different glycan standards, data not shown



Overlay of fifteen electropherograms for mAb2 showing good repeatability

mAb2

Overlay with 15 runs, Cap B,C,D were used for five injections per capillary



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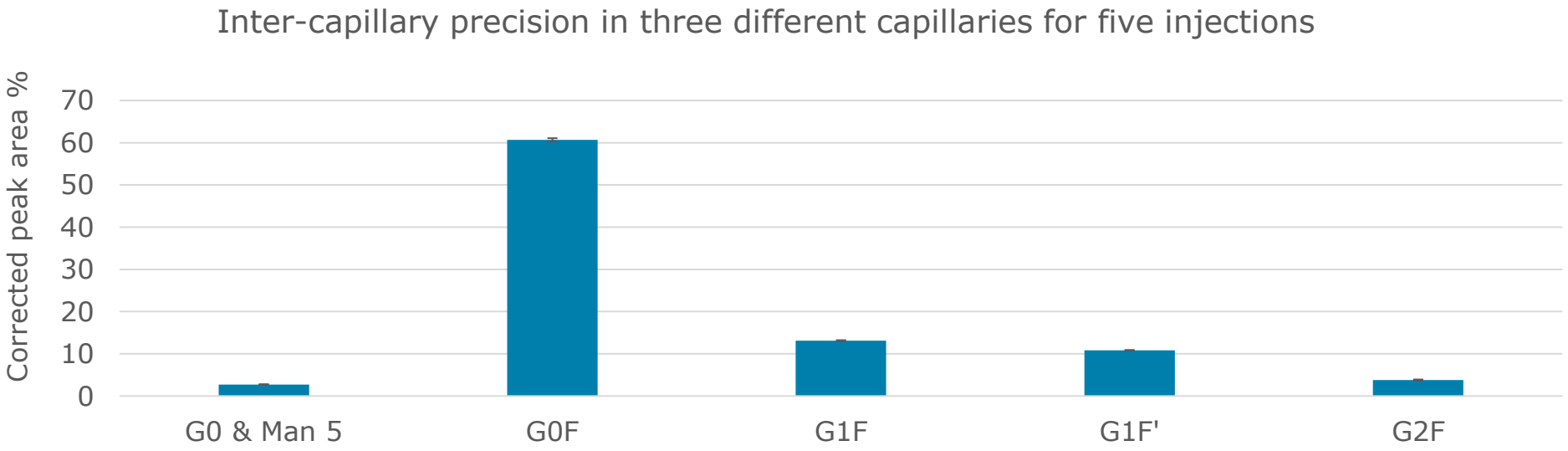
Inter-capillary precision shows an RSD for the corrected peak area% of less than 4%

Capillary B, C, D were run and repeated 5 times (15 data point)

mAb2



	G0 & Man 5	G0F	G1F	G1F'	G2F
RSD (%)	0.78	0.70	0.87	0.95	3.32

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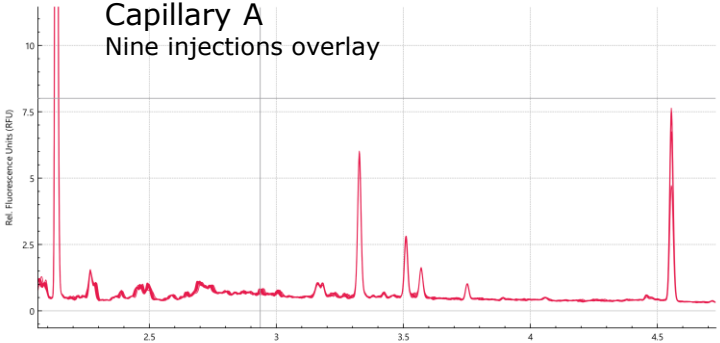
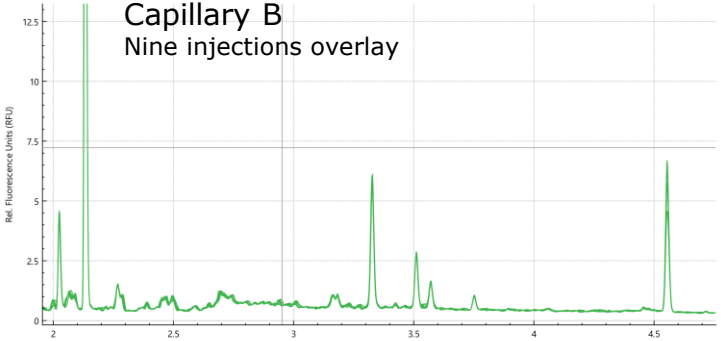
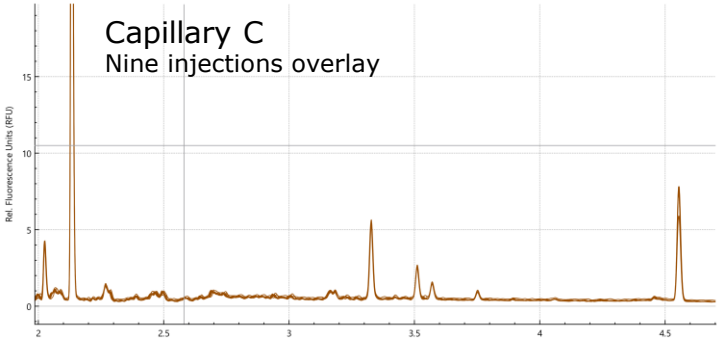
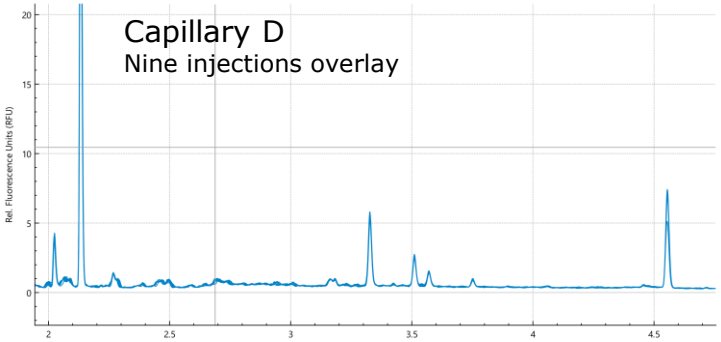
Overlay of thirty-six electropherograms for mAb1 showing good inter-capillary precision in different capillaries (A-D)

Four samples of mAb1 were prepared









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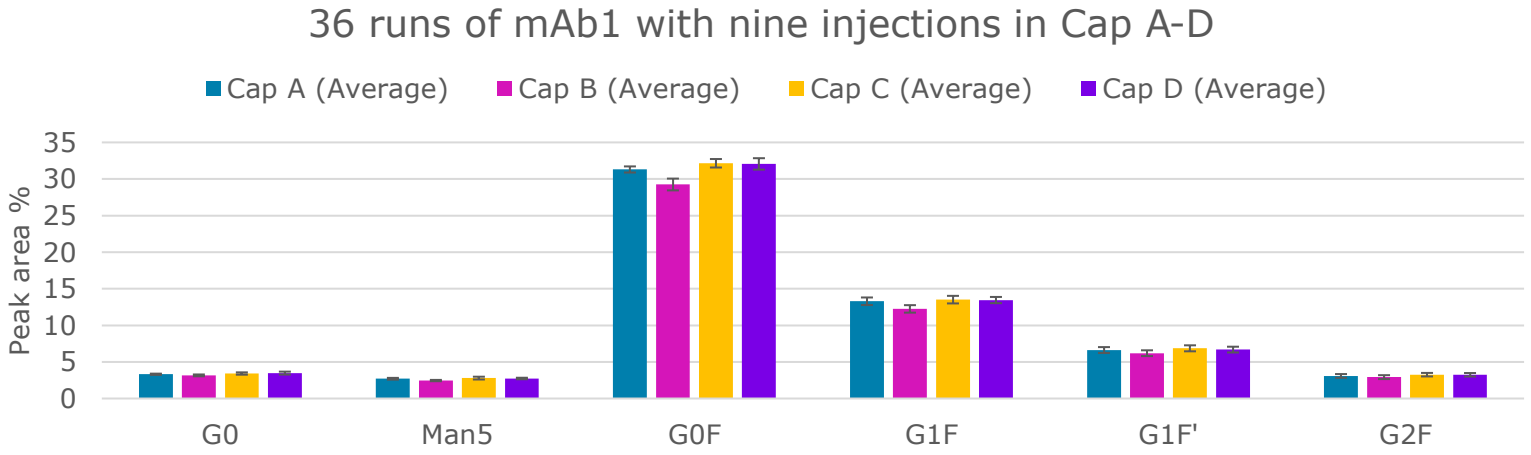
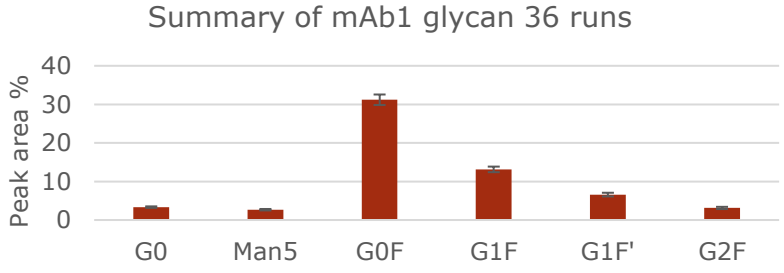
Thirty-six samples of mAb1 have good data repeatability in each injections of different capillaries (A-D)





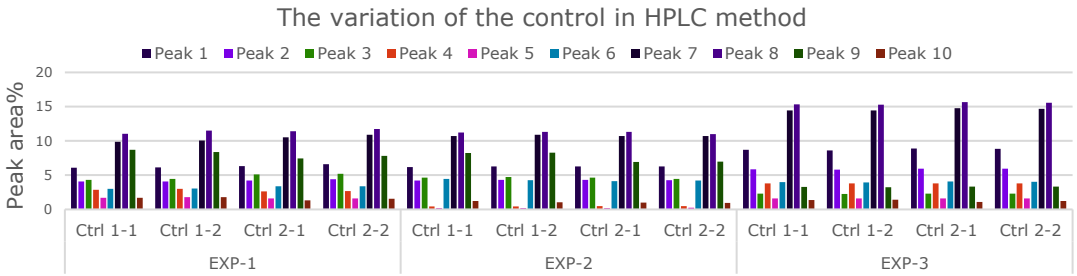
Note: RSD was calculated for 36 injections using 4 different capillaries with 9 repeating injections, 36 data points

	G0	Man5	G0F	G1F	G1F'	G2F
Mean	3.34	2.67	31.19	13.13	6.59	3.13
SD	0.19	0.19	1.36	0.72	0.48	0.29
RSD (%)	5.73	6.99	4.37	5.51	7.22	9.22

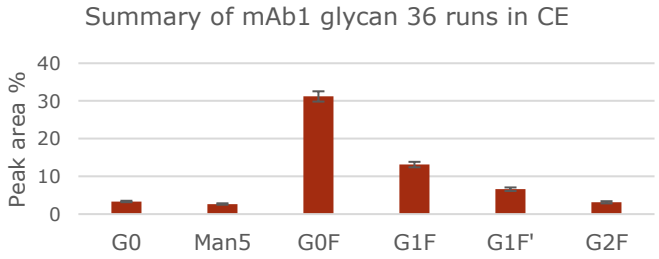
Three examples of historical HPLC control data also showed the similar variation by comparing to CE

RSD (%)<15 usually is the System Suitability pass criteria

- Two controls were prepared each experiment dates
  - Same date preparation showed RSD (%)<12, across two control samples
  - There is higher variation for different date sample preparation
    - This is due to the deglycosylation and labeling and there is not a key difference in the separation method (CE vs HPLC)



	Peak Name	1	2	3	4	5	6	7	8	9	10
EXP1	RSD (%)	3.15	3.08	8.26	5.52	5.27	5.32	3.92	2.11	6.14	11.02
EXP2	RSD (%)	0.62	0.78	2.22	2.81	9.04	2.76	0.74	1.30	8.78	9.47
EXP3	RSD (%)	1.15	1.08	0.72	0.42	0.77	1.31	1.01	1.05	1.08	9.74



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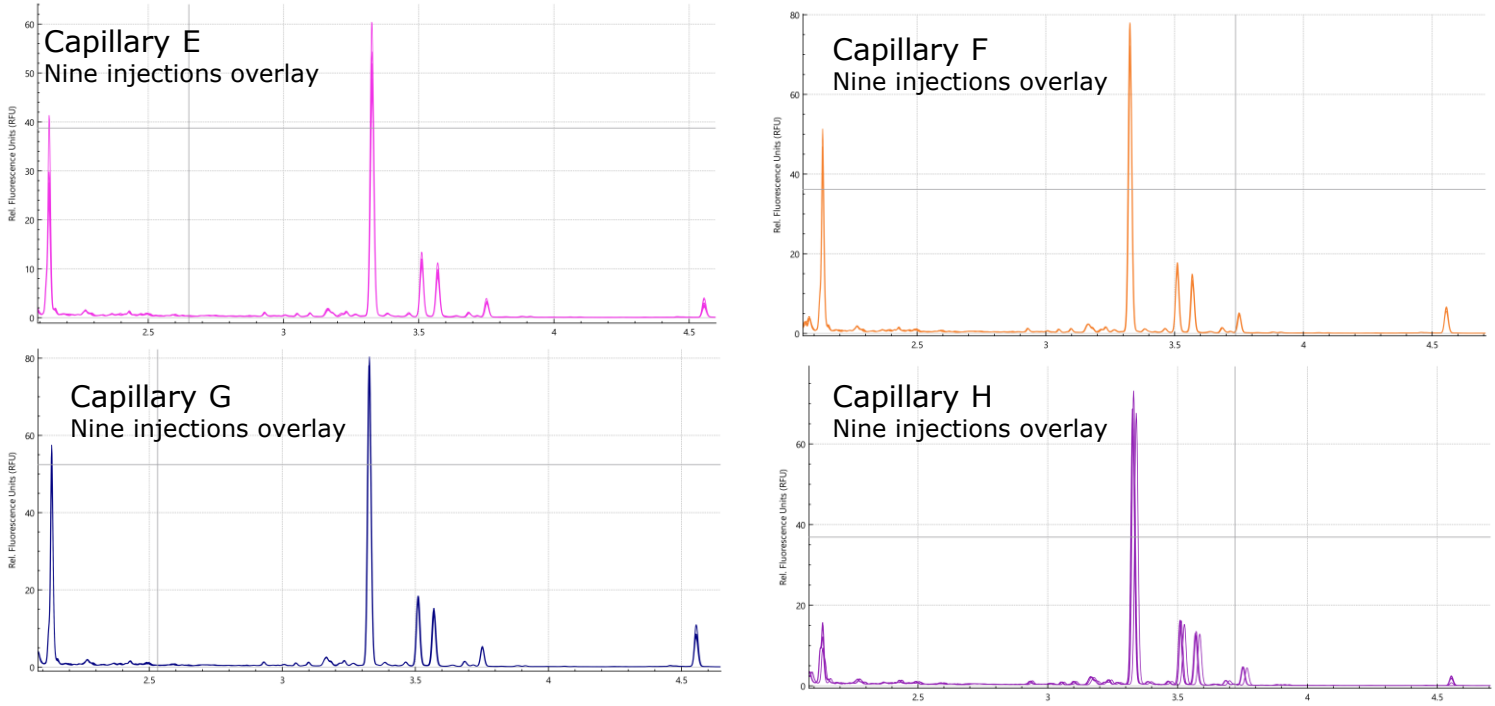
Overlay of thirty-six electropherograms for mAb2 showing good inter-capillary precision in different capillaries (E-H)

Four samples of mAb2 were prepared



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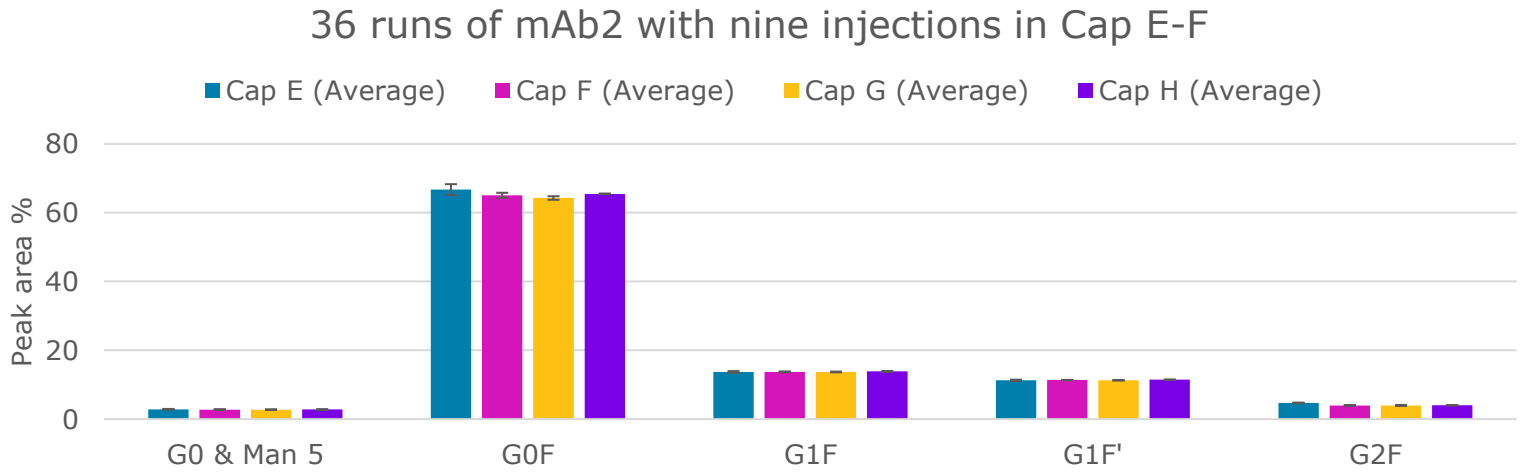
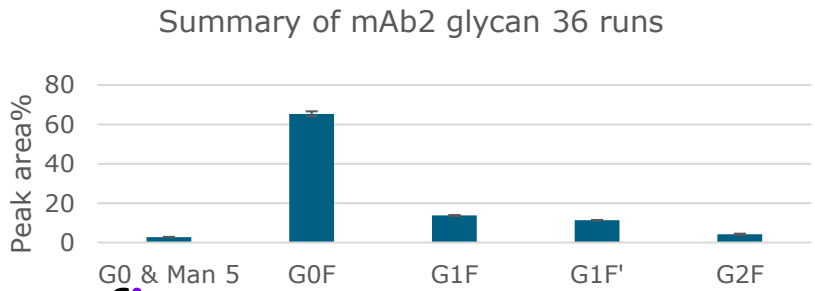
Thirty-six samples of mAb2 have good data repeatability in each injections of different capillaries (E-H)





Note: RSD was calculated for 36 injections using 4 different capillaries with 9 repeating injections, 36 data points

	G0 & Man 5	G0F	G1F	G1F'	G2F
Mean	2.79	65.36	13.75	11.34	4.17
SD	0.09	1.29	0.18	0.13	0.32
RSD (%)	3.21	1.97	1.28	1.12	7.71

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Summary

Pros

- A high-throughput multi-capillary electrophoresis glycan analysis method was developed to monitor glycan profiles of biologics.
  - Repeatable & reproducible
  - Higher throughput, >40x faster
  - Suitable high throughput method for monitoring the glycan profile of biologics to support process development activities

Cons

- The separation mode is not compatible with mass spectrometry making it more difficult to ID less common glycan species. However, the BioPhase 8800 system provides enough glycan information with the commercial standards for our high throughput bioprocess glycan trending purpose

Conclusion

- BioPhase 8800 system offers a high throughput glycan analysis solution for our intended use. However, further resolution improvement (i.e. better separation gel or additives) for G0 and Man5 will be desired for simplifying peak ID and analysis

Acknowledgements

- Global Bioanalytics, Mammalian Bioanalytics, and Bioprocess Analytics
  - Laurent Duhau
  - Marc Verhagen
  - Brad Whittaker
  - **Liam Wilson**
  - **Joe Thompson**

- **Greg Manley**
- **Fang Wang**
- Marcia Santos
- Sahana Mollah

Thank you

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