Streamlined protein characterization workflows for capillary electrophoresis using automated sample preparation

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This technical note describes a streamlined process for protein characterization utilizing the Biomek i5 multichannel (MC) workstation for downstream analysis using the BioPhase 8800 system.

Biotherapeutics have revolutionized modern medicine and created a multi-billion-dollar business, with hundreds more currently in the development pipeline. Biotherapeutic molecules range from traditional monoclonal antibodies (mAb) and more complex mAb-derived modalities to cell and gene therapy products. In the process development environment of a biotherapeutic lifecycle, thousands of samples must be prepared and analyzed consistently and reliably by chromatographic or electrophoretic means to determine and quantify the purity, charge heterogeneity, and glycan composition.

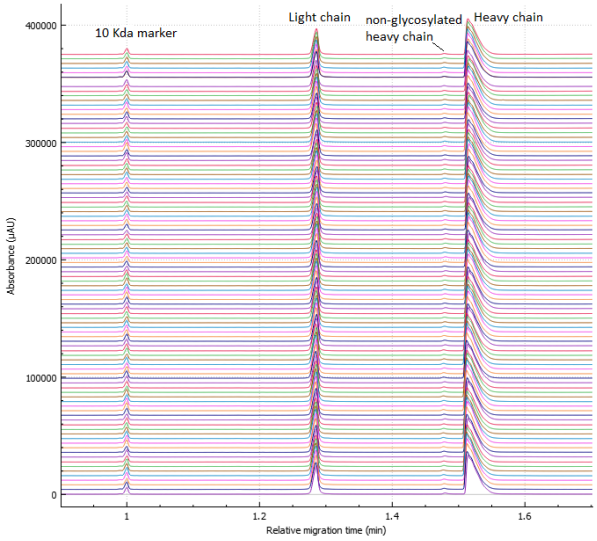
Key features of the automated CE workflows

High-throughput automated sample preparation: Hands-free sample preparation of an entire 96-well plate in less than 3 hours for capillary electrophoresis sodium dodecyl sulfate (CE-SDS) – Figure 1 and 2.3 hours for capillary isoelectric focusing (cIEF) and glycan analysis

Robust and reproducible analytical performance: Exceptional intra- and inter-capillary performance and reproducibility

Enhanced usability and sample preparation flexibility: The Biomek i5 MC workstation from Beckman Coulter® features flexible configurations that increase user confidence and walk-away time

Streamlined workflow: An integrated assay coupling automated sample preparation, multichannel CE data acquisition and intuitive data analysis software for rapid reporting and decision-making



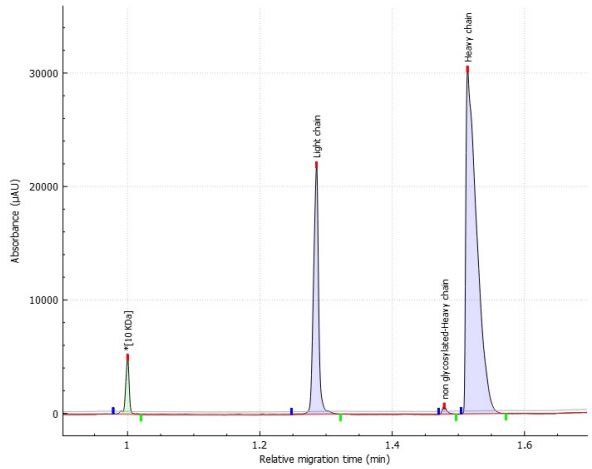


Figure 1:(Top) CE-SDS electropherograms of 96 reduced USP mAb IgG SS RF samples. The different colors represent the different capillaries, with the first run displayed at the top. (Bottom) An electropherogram from a single run shows the 4 peaks (Capillary A).

Introduction

Analyzing thousands of samples in the early development can quickly become a bottleneck for sample testing throughput. Streamlined workflows with automated sample preparation and high-throughput data acquisition and analysis can maximize sample processing time, minimize human intervention, and eventually address the throughput limitation in the analytical characterization of intermediate samples.

This work demonstrates the full automation of 3 key applications in the characterization of biopharmaceuticals using capillary electrophoresis: CE-SDS[1], cIEF[2] and glycan[3] from sample preparation to data analysis.

Methods

The automated CE-SDS preparation method (workflow shown in Figure 2) started with 95 µL of the USP system suitability standard reference standard (USP mAb IgG SS RS) in a 96-well plate. To each sample, 2 µL of 10 kDa internal standard and 5 µL of 2-Mercaptoethanol (β-ME) were added. The plate was then sealed with a film and placed back on the deck. The plate was then heated to 70°C for 10 minutes before 90 µL aliquots of these samples were transferred to the sample inlet tray. The sample inlet tray was centrifuged at 177x g for 20 minutes and then placed into the BioPhase 8800 system for electrophoretic separation. For the reagent inlet plate, all reagents were pipetted column-wise, as described in the application guide.[4]

For the reagent outlet, a single tip was used for each reagent. For the sample outlet tray, a tip was used for each well to be filled, which depended on the number of samples to be analyzed. The reagent inlet and sample outlet trays were prepared with the Biomek i5 MC workstation and then placed directly into the BioPhase 8800 system. The capillary conditioning was performed according to the application guide[4] before the samples were separated.

The automated cIEF sample preparation (workflow in Figure 3) started with 8 µL per well of 5 mg/mL USP mAb IgG SS RS in a deep well plate format. The cIEF master mix was prepared manually in advance, according to the cIEF for the BioPhase 8800 system application guide.[5] The final cIEF master mix contained 4M urea cIEF gel, cathodic stabilizer, anodic stabilizer, Pharmalyte 3-10 and pI 4.1, 5.5 and 10 markers. A 248 µL aliquot of master mix was added to each well and tip-mixed 5 times with the sample. Once mixed, 100 µL aliquots of the prepared samples were transferred to the sample inlet tray. All solutions for the inlet reagent plate were pipetted column-wise, as described in the application guide.[5] For the reagent outlet plate, a single tip was used for each reagent.

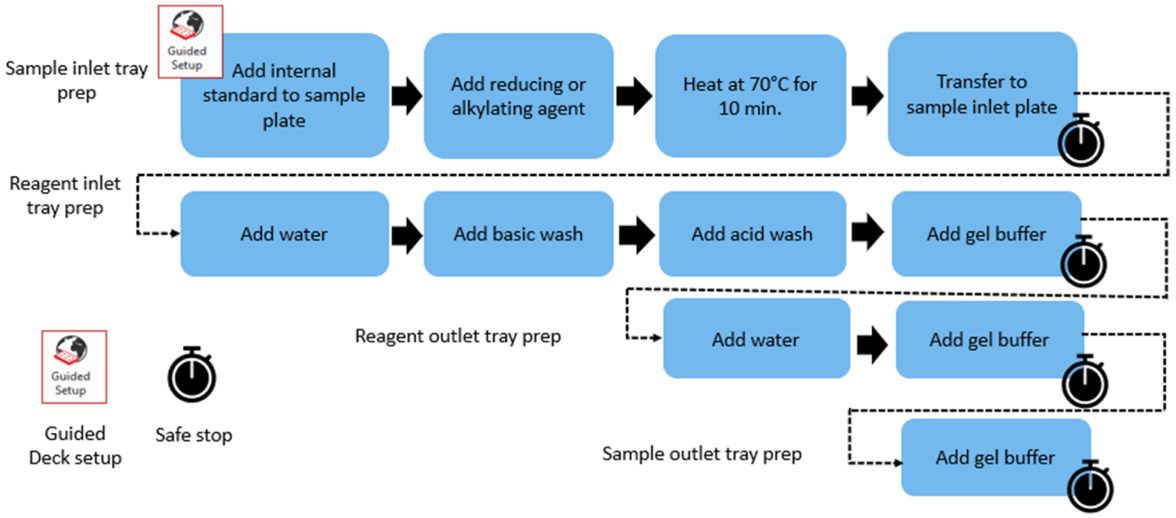


Figure 2. The sample and reagent preparation workflow for automated CE-SDS analysis using the Biomek i5 MC workstation.

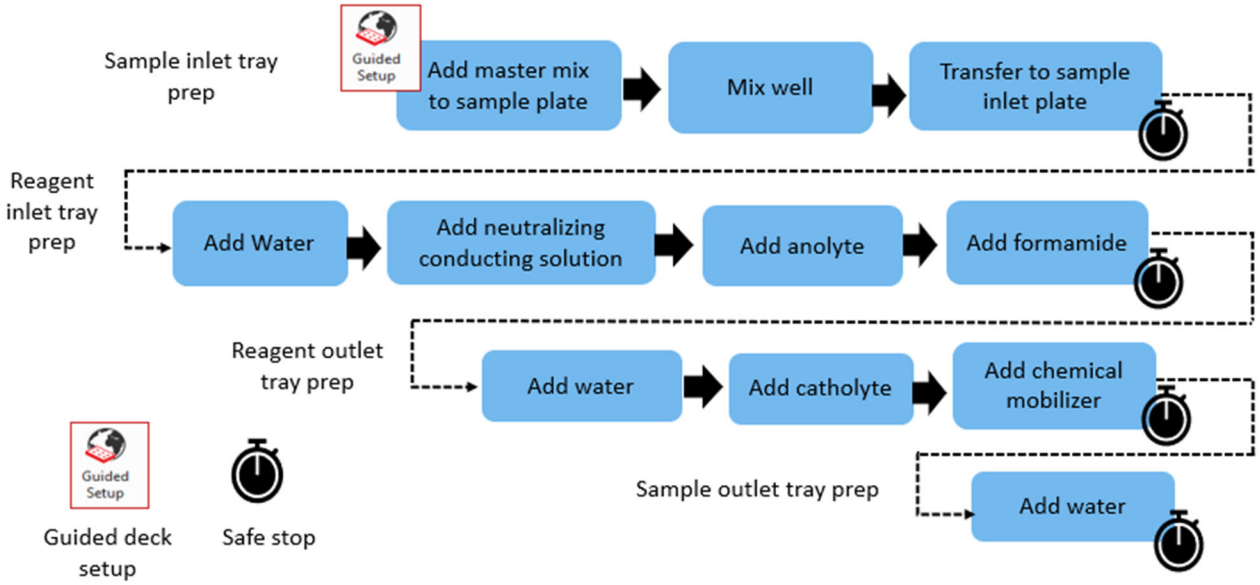


Figure 3. The sample and reagent preparation workflow for automated cIEF analysis using the Biomek i5 MC workstation.

After the Biomek i5 MC workstation completed the method, all 4 trays were manually placed directly into the BioPhase 8800 system.

The automated N-linked glycan sample preparation method (workflow in Figure 4) can be run from start to finish with full walk-away capability. This workflow features a magnetic bead-meditated denaturation, digestion, labeling and clean up. The labeling is performed via reductive amination using 1-aminopyrene-3,6,8-trisulfonic acid (APTS), a highly charged fluorescent tag that allows for high sensitivity and fast separation of N-linked glycans. The denaturing, digestion and labeling solutions were prepared according to the application guide.[6]

Performance of automated sample and reagent preparation of the CE-SDS workflow

CE-SDS analysis: Inter-capillary reproducibility

Figure 1 demonstrates the inter-capillary reproducibility for the analysis of the USP mAb IgG SS RS.

The inter- and intra-capillary values for reproducibility are quantified in Table 1 (n=96). The observed relative standard deviation (RSD) was approximately 1% for migration time (MT) and for the corrected peak area percentage (CPA %) for the 10 kDa marker, the LC and HC peaks and the HC/LC ratio.

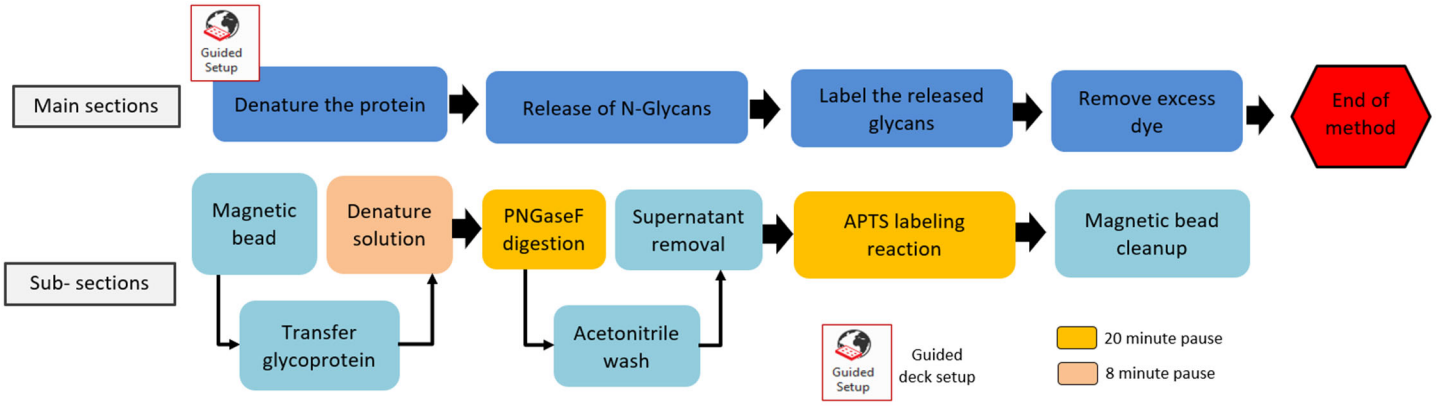


Figure 4. Direct comparison between PA 800 plus and the BioPhase 8800 systems of a mAb sample under non-reduced conditions.

Table 1. Intra- and inter-capillary %RSD for MT, CPA% and HC/LC ratio for the 10 kDa marker and IgG control standard (N=96).

Intra-capillary reproducibility (%RSD)								
		Migration time (min)			Corrected peak area percentage			Ratio
Capillary	10 Kda	LC	ng-HC	HC	LC	ng-HC	HC	HC/LC
A	0.69	0.67	0.66	0.65	0.21	2.15	0.07	0.43
B	0.56	0.52	0.52	0.50	0.23	1.77	0.08	0.34
C	0.55	0.51	0.50	0.48	0.36	2.21	0.13	0.38
D	0.64	0.61	0.60	0.59	0.21	2.71	0.09	0.38
E	0.74	0.73	0.73	0.70	0.34	2.23	0.14	0.34
F	0.76	0.74	0.72	0.71	0.22	2.00	0.08	0.27
G	0.96	0.93	0.93	0.92	0.14	1.77	0.05	0.24
H	1.11	1.10	1.11	1.09	1.12	1.82	1.04	0.44
Inter-capillary	0.83	0.81	0.82	0.80	0.47	2.24	0.38	0.37

Table 2 shows the average values observed, demonstrating the inter- and intra-capillary consistency for MT, CPA% and HC/LC ratio.

Performance of the automated sample and reagent preparation for the cIEF workflow

Figure 5A shows the overlay of 96 runs, demonstrating the reproducibility of both instrument and sample preparation.

Figure 5B shows a typical USP IgG SS RS profile and the integration strategy to report the area percentage composition of the main, basic and acidic variants.

Figure 5C shows the detection times for the main peak, pI markers 4.1, 5.5 and 10. In Figure 5D, each bar represents the average peak area percentage for the basic, main or acidic isoforms, calculated from 96 data points collected on the BioPhase 8800 system.

Table 2: Intra- and inter-capillary average MT, CPA% and HC/LC ratio values for the 10 kDa marker and IgG control standard (N=96).

Intra-capillary average								
		Migration time (min)			Corrected peak area percentage			Ratio
Capillary	10 Kda	LC	ng-HC	HC	LC	ng-HC	HC	HC/LC
A	13.18	16.95	19.5	19.96	28.19	0.75	71.06	2.52
B	13.13	16.89	19.43	19.89	28.14	0.75	71.10	2.53
C	13.13	16.88	19.42	19.88	28.17	0.74	71.08	2.52
D	13.12	16.87	19.4	19.85	28.15	0.74	71.09	2.53
E	13.17	16.94	19.48	19.95	28.2	0.74	71.05	2.52
F	13.16	16.90	19.46	19.92	28.22	0.75	71.02	2.52
G	13.19	16.96	19.51	19.78	28.24	0.75	71.00	2.51
H	13.28	17.08	19.65	20.12	28.09	0.74	70.86	2.52
Inter-capillary	13.17	16.93	19.48	19.92	28.18	0.75	71.03	2.52

Error bars indicate the standard deviation. The RSD values for peak area percentage across all data points were 1.5%, 0.67% and 1.5% for basic, main and acidic isoforms, respectively, indicating robustness across sample preparations.

Excellent intermediate precision was demonstrated for the calculated pI value.

Table 3 shows the reproducibility of cIEF analysis for USP mAb

Table 3 Reproducibility of cIEF analysis for USP mAb IgG SS RS isoforms on a BioPhase 8800 system.

	Intra-capillary average					
Capillary	**Average (n=12)**	**%RSD**	**Average (n=12)**	**%RSD**	**Average (n=12)**	**%RSD**
A	17.72	1.20	60.92	0.35	21.28	0.99
B	17.52	0.84	60.94	0.24	21.51	0.87
C	17.64	1.36	60.76	0.70	21.57	1.52
D	17.70	1.34	60.90	0.96	21.37	2.00
E	17.80	1.63	60.58	0.64	21.59	1.12
F	17.84	1.46	60.65	0.73	21.48	2.01
G	17.81	1.19	60.83	0.76	21.31	1.61
H	17.89	1.59	60.60	0.72	21.46	1.54
Inter-capillary average (n=8)	17.74		60.77		21.45	
% RSD	0.68		0.24		0.53	

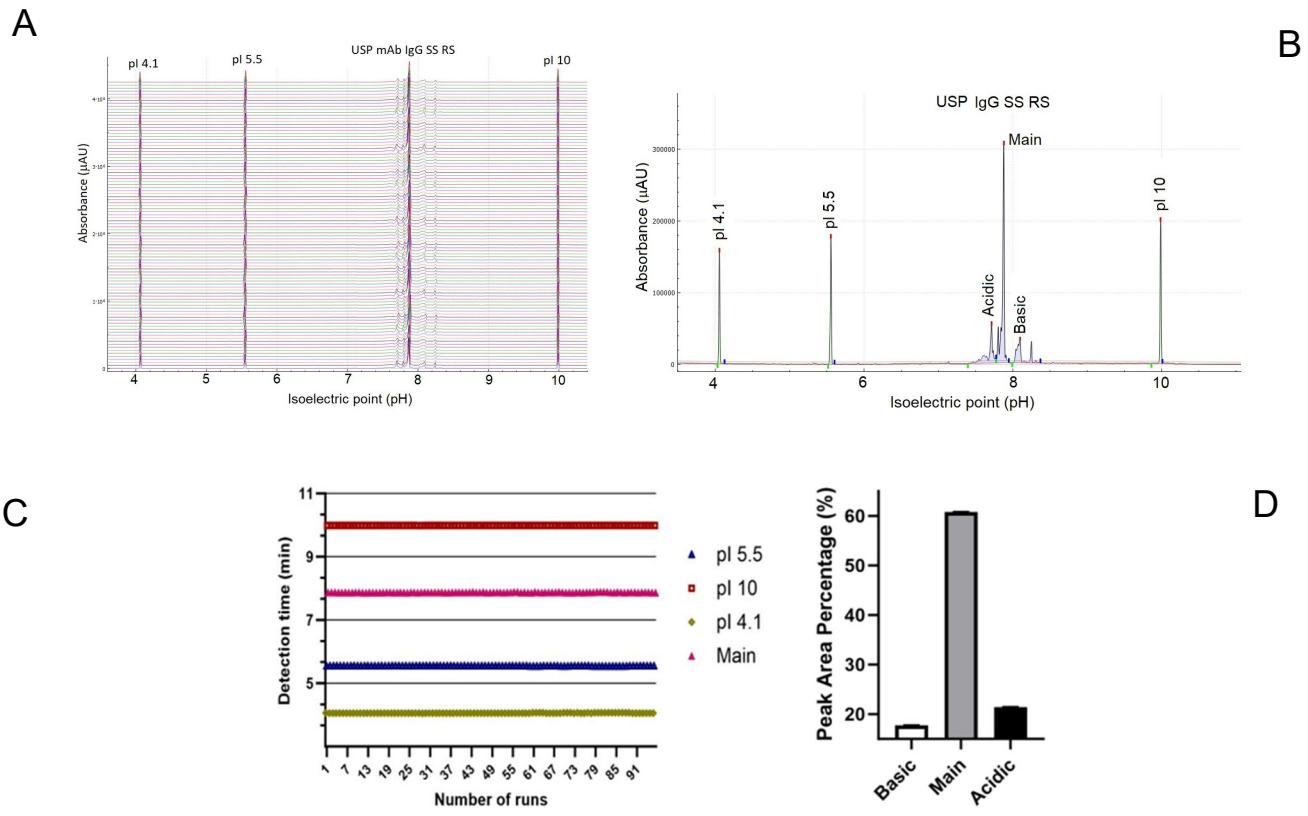


Figure 5. (A) Electropherograms of 96 samples of USP mAb IgG SS RF. Peaks 1, 2 and 7 show the pI 4.1, 5.5 and 10 markers, respectively. Peaks 3, 4 and 5 represent the acidic, main and basic groups, respectively. The color of each trace (A-H) indicates the capillary used for analysis. The first run is shown at the top. (B) A representative electropherogram for USP mAb IgG SS RF with absorbance and pI value indicated on the y- and x-axes, respectively. Each peak is annotated with peak number and name. (C) Plot of detection times observed for the 96 runs for pI 4.1 (olive), pI 5.5 (blue), main group (pink) and pI 10 (maroon). (D) Graph shows assay robustness with a plot of peak area % values for different isoforms of USP mAb IgG SS RS.

IgG SS RS isoforms on a BioPhase 8800 system. The average values and %RSD values were calculated for the peak area percentage from 12 data points per capillary for the basic, main or acidic isoforms. The inter-capillary values were calculated across 8 capillaries.

Performance of the automated sample and reagent preparation for the fast glycan workflow

The performance of the automated sample and reagent preparation workflow for the fast glycan analysis using a human serum IgG sample is demonstrated in Figure 8.

The reproducibility of the automated, fast glycan sample preparation was assessed by evaluating the inter- and intra-capillary %RSD of CPA% (Table 4), relative migration time (RMT GU) and glucose units (GU) for FA2G2S1/M5, FA2, M7[D1]/FA2(6)G1, FA2G2 and FA2(3)G1/FA2B(6)G1 species.

Table 4 demonstrates the excellent reproducibility of automation in complex sample preparation schemes.

Table 4 Inter- and intra-capillary reproducibility for CPA%, RMT GU and GU values.

Inter-capillary reproducibility			
Glycan species	**CPA%**	**RMT GU**	**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	.041	0.05	0.05
FA2G2	3.79	0.05	0.05
Intra-capillary reproducibility			
Glycan species	**CPA%**	**RMT GU**	**GU**
FA2(3)G1	1.10	0.04	0.04
M5	1.73	0.05	0.05
FA2	2.26	0.04	0.03
FA2(6)G1	0.94	0.07	0.06
FA2G2	1.10	0.04	0.04

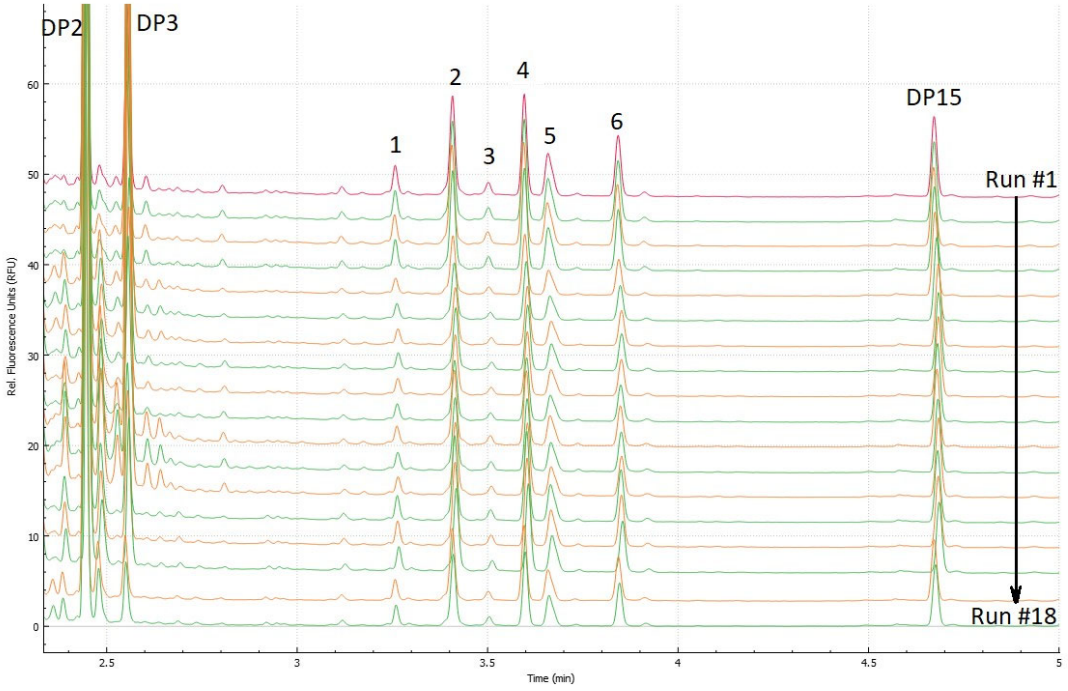


Figure 6. Overlay of 18 capillary electrophoretic separations of released N-linked glycans labeled with APTS from a serum IgG sample. Peak legend: 1) M5, 2) FA2, 3) FA2B, 4) FA2(6)G1, 5) FA2(3)G1 and 6) FA2G2.

Conclusions

- Automated sample and reagent inlet and outlet plate preparation on the Biomek i5 MC workstation eliminated labor-intensive bench work and provided high-throughput with highly consistent results when combined with the BioPhase 8800 system
- Robust, automated sample preparation yielded excellent intra-capillary reproducibility data for migration time, relative migration time and corrected peak area percentage compared to manual preparation
- The streamlined workflow coupling automated sample preparation, high-throughput data acquisition and intuitive data analysis facilitated a rapid question-to-answer process in a fast biopharma environment

References

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