Lightning capillary electrophoresis sodium dodecyl sulfate (CE-SDS) workflow for high-throughput analysis of biotherapeutics

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Introduction

There are hundreds to thousands of drug candidates to screen during early drug development, posing a considerable bottleneck in the biopharma industry. High-throughput analytical platforms and fast analysis methods are attractive approaches to help solve the high sample volume issue. CE-SDS is the gold-standard method widely used for drug purity, integrity and stability analysis. However, this need is not met with the current single-capillary system. In this technical note, we propose the lightning CE-SDS workflow, which, together with a multi-capillary system, this workflow speeds up separation to 1.5 x faster than SCIEX gold standard CE-SDS workflow (Figure 1). This method can analyze 192 reduced samples in 14 hours (4.3 min/sample) with high data quality. The relative standard deviation (RSD) % of relative migration time (RMT) and corrected peak area (CPA) % across 192 injections are below 1% and 3%, respectively. Through a systematic evaluation of multiple variables in only 6days, we proved the high accuracy and separation efficiency with no carryover.

During early drug development, screening a large number of clones for lead clone selection is a critical step in cell line development. This process can be time-consuming and a labor-intensive process without high-throughput methodology.[1]

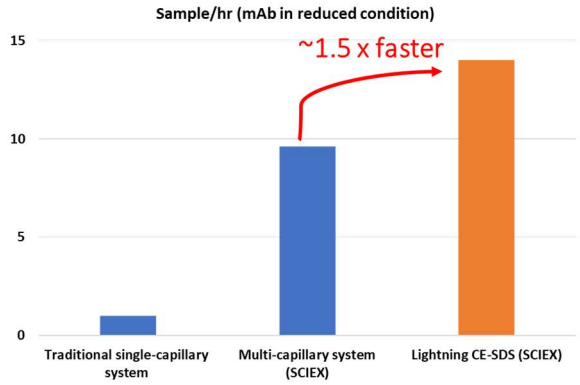

Figure 1. Throughput capability achieved by lightning CE-SDS compared to validated gold-standard workflows.

Meanwhile, developability assessment studies, as a screening strategy to identify process development issues associated with product stability, purity and integrity, require robust and fast analytical approaches to redirect resources to more promising products.[2] To this point, CE-SDS is widely used in biotherapeutics analyses for lot release, stability testing, formulation-buffer screening, process development, cell line development and product characterization. CE-SDS is an automation-friendly application that when combined with a robust multi-capillary electrophoresis system that offers high-throughput, becomes a powerful analytical tool for product characterization during process and cell line development.[3] In this technical note, we increased the throughput on the CE-SDS workflow, enabling the analysis of 192 injections (2 full 96-well plates worth of samples) of IgG standard under reduced (R) and non-reduced (NR) conditions in 14 and 18 hours, respectively, with remarkable reproducibility. This study demonstrates that lightning CE-SDS is accurate and precise as the original workflow with no carryover or loss of separation efficiency.

Key features

- The lightning CE-SDS workflow is up to 1.5 x faster than SCIEX original CE-SDS workflow enabling the analysis of 192 injections of IgG standard in reduced and non-reduced condition within 14 and 18 hours, respectively. That equals to 4.3 min and 5.5 min per injection.
- Exceptional repeatability over 192 injections was achieved with < 1% RSD% and < 3% RSD of relative MT and CPA%, respectively, for all major peaks of IgG standard
- Highly robust workflow with excellent intermediate precision, accuracy and no carryover or loss of separation efficiency

Materials and methods

Chemicals: IgG control standard (PN: 391734) and CE-SDS Protein Analysis Kit (PN: C30085) and the IgG control standard (PN 391734) were from SCIEX, (Framingham, MA). The NIST mAb (RM 8671) reference material 8671 was from NIST (Gaithersburg, MD). The iodoacetamide (PN: I6125-5G) and the 2-mercaptoethanol (PN: M3148-25ML) were from Sigma Aldrich (St. Louis, MO). Chromeo P503 dye (PN: 15106) was from Active Motif, Inc (Carlsbad, CA).

***Materials and instruments*:** BioPhase 8800 system (PN: 5083590F) equipped with UV absorbance detection at 220 nm and LIF detector with 488 nm excitation and 600 nm emission. BioPhase BFS capillary cartridge - 8 x 30 cm (PN: 5080121) and Sample and Reagent Plates (PN: 5080311) were from SCIEX (Framingham, MA). Multi-Therm shaker incubator (Part # H5000-H) was from Benchmark Scientific (Sayreville, NJ). 600 nm/80 nm bandpass filter FWHM 12.5 mm (PN 65736) was from Edmond Optics Worldwide (Barrington, NJ).

Sample preparation for CE-SDS analysis using UV detection: The IgG control standard was prepared by adding 16 µL of 10 kDa Internal Standard and 40 µL of 250 mM iodoacetamide (IAM) for non-reduced sample or β-mercaptoethanol (β-ME) for the reduced sample to 760 µL of the IgG control standard solution. The sample mixture was vortexed, centrifugedand then heat denatured at 70°C for 10 min. The sample was then cooled to room temperature and 100 µL aliquots were transferred to the 8 wells of the sample plate for CE-SDS analysis. The NIST reference standard was prepared by adding 995 µL SDS-MW sample buffer, followed by adding of 25 µL of 10kD, 60 µL of 250 mM IAM for non-reduced sample or β-ME for reduced sample to 120 µL of 10mg/mL NIST. The sample mixture was vortexed, centrifuged and then heat denatured at 70°C for 10 min. The final concentration of NIST reference standard was 1 mg/mL.

Sample preparation for CE-SDS analysis using LIF detection: 12 µL of 10 mg/mL NIST mAb was added to 1128 µL SDS-MW sample buffer, followed by either 60 µL of 250 mM IAM for non-reduced sample or β-ME for reduced sample. The sample mixture was vortexed, centrifuged and then heat denatured at 70°C for 10 min. After cooling down to room temperature, 4 µL Chromeo P503 dye (1 mg/mL) was added to the sample. The sample mixture was vortexed, centrifuged and then heated at 70°C for 10 min for fluorescent labeling. The final concentration of NIST mAb for CE-SDS-LIF analysis is 0.1 mg/mL.

50 µL of treated NIST mAb was transferred to the sample plate for CE-SDS analysis (UV and LIF). Figure 2 shows the sample plate layout used in the systematic study.

CE methods: Figures 3, 4 and 5 show the cartridge conditioning, original sample separation and the lightning CE-SDS separation methods used in this work.

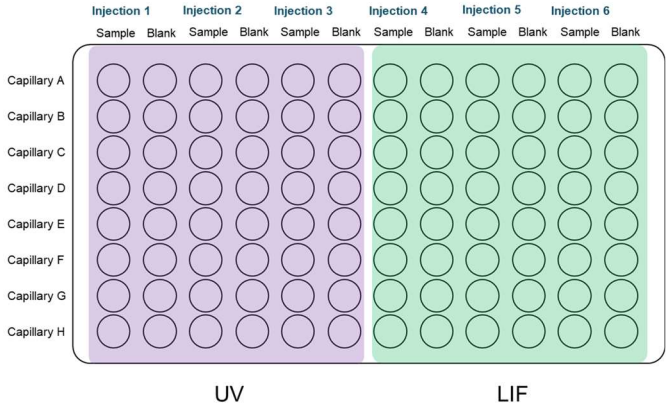

Figure 2. Layout of the sample plate for 1 analysis run of the systematic study.

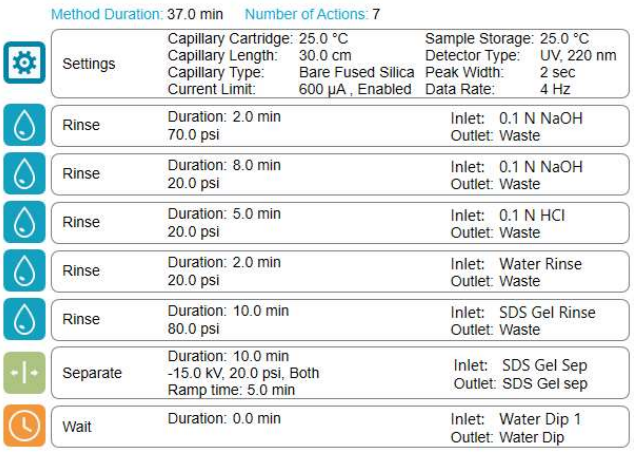

Figure 3. Screenshot of cartridge conditioning method.

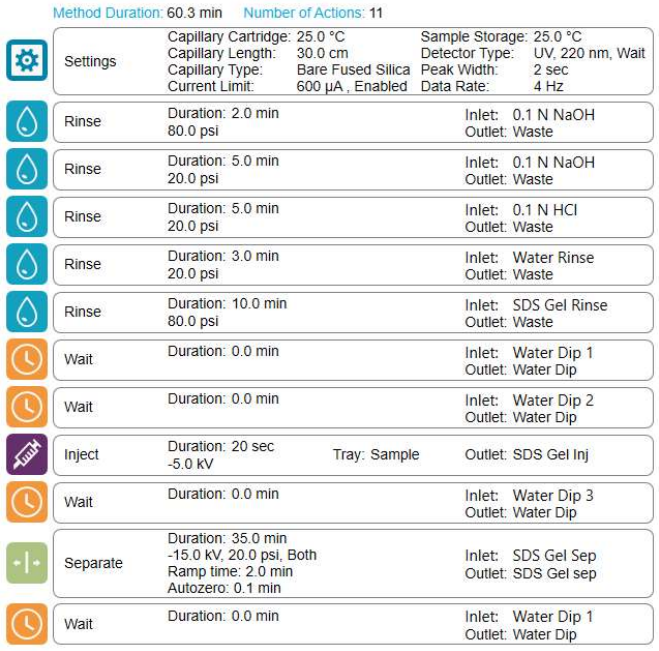

Figure 4. Screenshot of CE-SDS original separation method (for non-reduced antibody analysis condition). Separation time is set to 25 min for reduced antibody analysis.

Figure 5. Screenshot of lightning CE-SDS separation method (for non-reduced antibody analysis condition). Separation time is set to 25 min for reduced antibody analysis.

The rinse cycles in the lightning CE-SDS method has been reduced to only 9 min while maintaining all the rinsing reagents. That reduced the total separation time by 64% compared to the original workflow.

Data analysis: The BioPhase analysis software package version 1.0 was used to create methods and sequences followed by data acquisition (not data analysis) and data processing.

Results and discussions

The high-throughput capabilities of the lightning CE-SDS workflow: To increase throughput by reducing the cycle time of CE-SDS analysis while maintaining separation efficiency and workflow robustness is critical to retaining high data quality. The rinsing time in the SCIEX original workflow takes up to 50% of the total cycle time. One strategy to reduce the cycle time is by reducing the rinsing time. However, for a thorough capillary surface cleaning and conditioning, we only reduced the duration of the rinse steps while keeping all necessary reagents used in the current method. The combination of high pressure (Figure 5) allows for effective capillary surface treatment while reducing the overall rinsing time to only 9 minutes, or 64% compared to the original workflow. As a result, the separation efficiency and migration time requirements remain the same as the original CE-SDS workflow. Table 1 summarizes the throughput results for the lightning and the original CE-SDS workflows. The lightning CE-SDS workflow took only 6.9 and 8.8 hours to complete one 96-well plate for reduced and non-reduced IgG control standards, translating into an average of 14 reduced and 11 non-reduced samples/hr.

Table 1. Throughput result of lightning and original CE-SDS workflow.

Condition	Workflow	Cycle time	Min/Sample*	Hrs/Plate	Samples/hr
Reduced	*Lightning*	*34 min*	*4.3*	*6.9*	*14*
	Original	*50 min*	*6.25*	*10*	*9*
Nonreduced	*Lightning*	*44 min*	*5.5*	*8.8*	*11*
	Original	*60 min*	*7.5*	*12*	*8*

*min/sample acquired by cycle time divided by 8

To determine the reproducibility of the lightning CE-SDS workflow, we performed 24 consecutive injections of IgG control standard from 1 column of sample plate, that is 8 samples injected 24 times for a total of 192 injections, under reduced and non-reduced conditions. Figure 6 highlights the separation consistency of the lightning CE-SDS workflow. The separation profiles between the first and 24th injection were comparable, indicating that the reduced rinsing conditions did not compromise the separation efficiency.

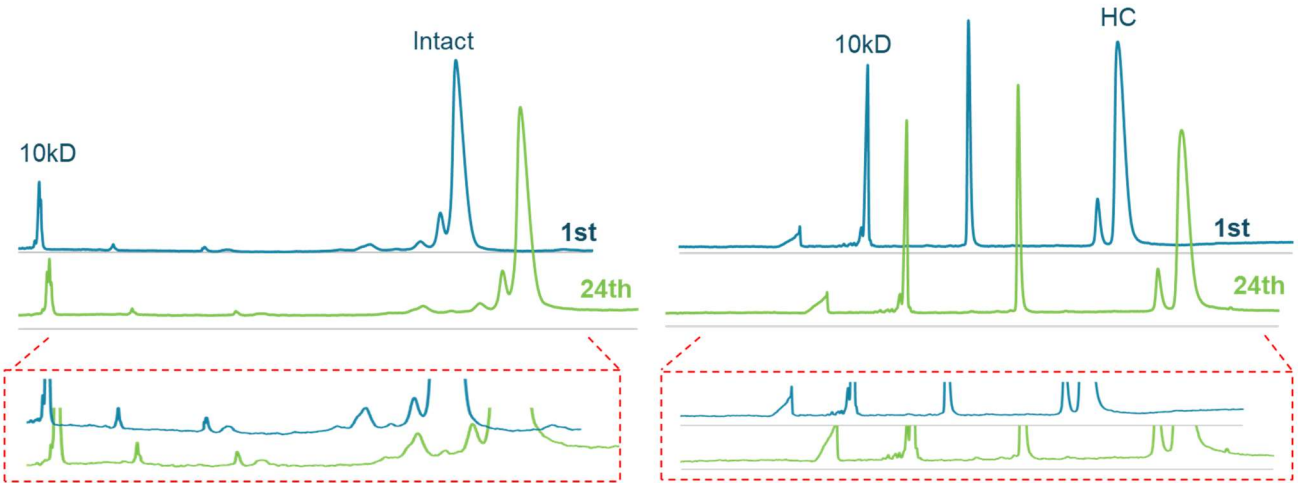

Figure 6. The separation profile comparison between 1st and 24th injection of IgG control standard under non-reduced (left panel) and reduced (right panel) conditions. The figures inside the red blocks showcase the consistency of the profiles at the baseline level for each separation.

To quantify the consistency of the lightning CE-SDS workflow, we calculated the RSD% (N=192) of relative migration time (RMT) and corrected peak area (CPA) % of the major peaks

such as, light chain (LC), heavy chain (HC), non-glycosylated heavy chain (NG-H) of the reduced IgG and HC:HC:LC (HHL) and intact IgG peak of non-reduced IgG. As shown in Figure 7, the RSD% for RMT was below 0.5% for all major peaks. The

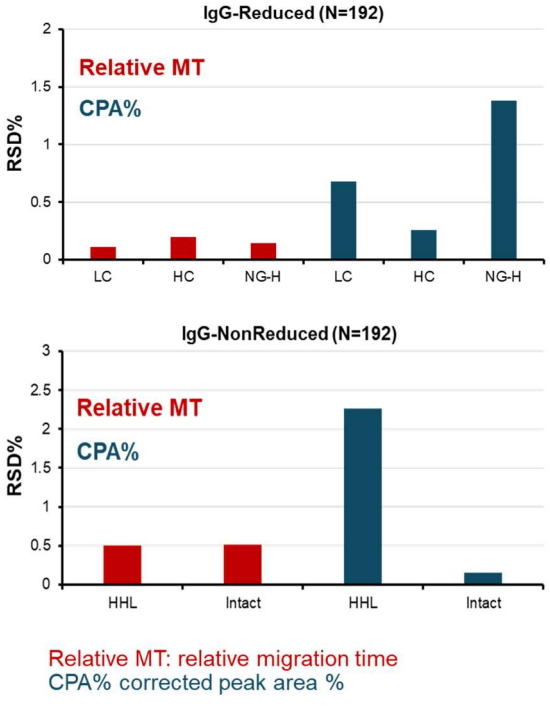

Figure 7. RSD% of lightning CE-SDS workflow for the major peaks of reduced and non-reduce IgG control standard.

RSD% for CPA% was < 1.5% for reduced IgG control peaks and < 2.5% for non-reduced IgG control peaks. The low RSD% of both figures of merit indicates the high reproducibility of the workflow. Most notably, the minor species such as, NG-H and HHL achieved RSD% of less than 3% over 192 replicates for CPA%.

Validation of the Lightning CE-SDS workflow by multiple factorial design using NIST reference standard mAb:

Because of the multi-capillary environment of the BioPhase 8800 system, to better understand the study design and the data output this platform can generate in 1 sequence, the terms used in this work are defined as follows. Data point refers to one separation from 1 well using 1 capillary. Each run refers to 8 data points. The sample plate layout (Figure 2) used in this work comprises 3 columns (or 8 samples) for UV detection and 3 columns of samples for LIF detection. Between each column of samples, a column of sample buffer is used as blanks to assess carryover. One sequence is defined as a single separation of 1 sample (NIST antibody) plate with the layout on Figure 2, generating 48 datapoints. Table 2 illustrates the experimental design to evaluate the robustness of the lighting CE-SDS workflow. The study required each of the 3 analysts to prepare 1 sample plate daily as described in Figure 2. Each analyst ran different instruments using 3 different cartridges (triplicate runs per plate/day) with 9 runs, generating 432 data points for reduced and non-reduced samples. This study provided insights into variation potentially caused by instruments, analyst operation, cartridges, capillaries and different injections. These multiple factors were effectively tested in triplicates to highlight their impact in the overall results directly. Most notably, the multi

capillary environment of the BioPhase 8800 system allowed to execute this study in only 6 days.

Table 2. Systematical study plan of NIST under reduced or non-reduced conditions.

# of Runs	Instrument	Person	Cartridge	Day
1	1	Analyst-1	3	1
2	2	Analyst-2	2	
3	3	Analyst-3	1	
4	1	Analyst-2	1	2
5	2	Analyst-3	3	
6	3	Analyst-1	2	
7	1	Analyst-3	2	3
8	2	Analyst-1	1	
9	3	Analyst-2	3	

Additionally, we used the original CE-SDS workflow as a control experiment but a reduced statistical sampling. In summary, 2 sequence analyses were performed using the same sample plate layout as Figure 2 for reduced and non-reduced NIST antibodies. Therefore, our control runs generated 48 data points for reduced or non-reduced conditions.

This comprehensive study systematically assessed factors that may impact MT, resolution and CPA% of major peaks of the NIST mAb.

Each bar graph shown in Figure 8 represents the average value of 24 data points collected with the original workflow or 216 data points (UV or LIF) collected by the lightning CE-SDS workflow with standard deviations as error bars. The average MT, CPA% and resolution values were very close between the original CE-SDS and the lightning CE-SDS workflow for all major peaks observed under reduced and non-reduced conditions. Most notably, the low CV% found for raw MT, CPA% and resolution was overall below 5%, indicating the robustness and reproducibility of the lightning workflow compared to the original for both detection modes. Additionally, the data also suggested that the duration of the separation is the same between the 2 workflows, facilitating method adoption. Similarly, the resolution between NG-H, HC peaks, HHL and the intact IgG peaks indicated the separation efficiency observed in the lightning CE-SDS workflow was not only maintained across the 216 data points but was equivalent to the original CE-SDS workflow.

Assessment of carryover: To check for any carryover issues due to the shortening of the rinsing steps especially when using LIF was also evaluated. We incorporated 6 blank injections in the plate layout where the separation of a blank sample always followed each sample separation. A closer look at the data from blank injections in Figure 9 revealed no carryover detected in the lightning CE-SDS workflow for both UV and LIF detection schemes.

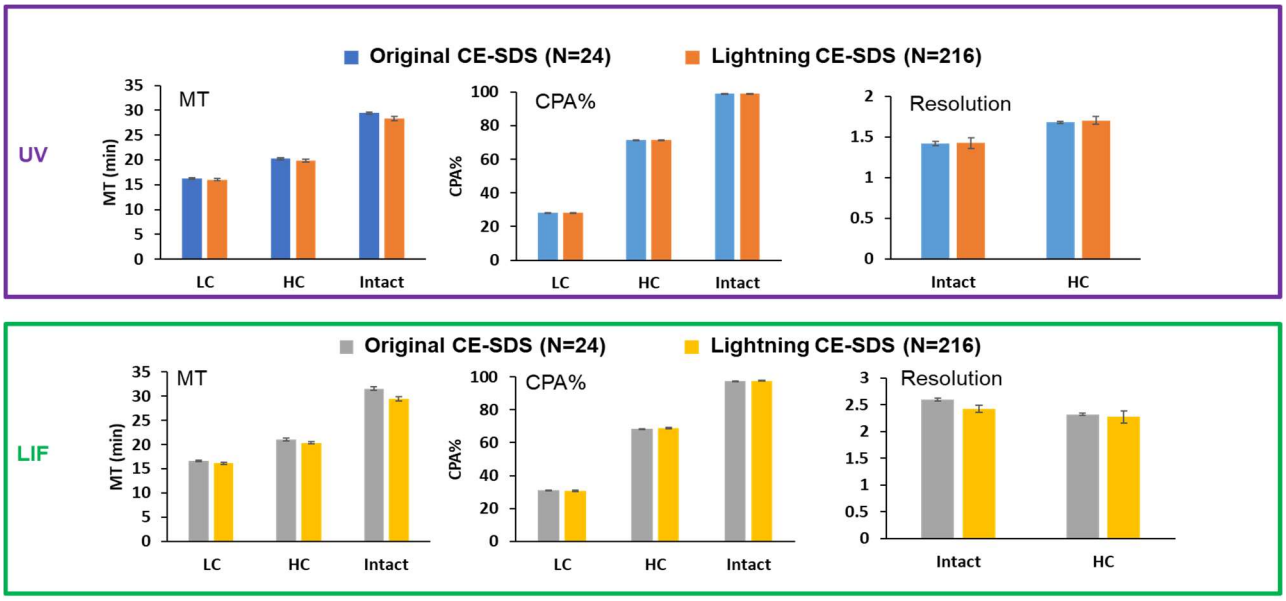

Figure 8. Attributes comparison (MT, CPA% and resolution) between original and lightning CE-SDS workflow (for both UV and LIF). The numbers in parentheses are the number of replicates. The error bars indicate the standard deviation.

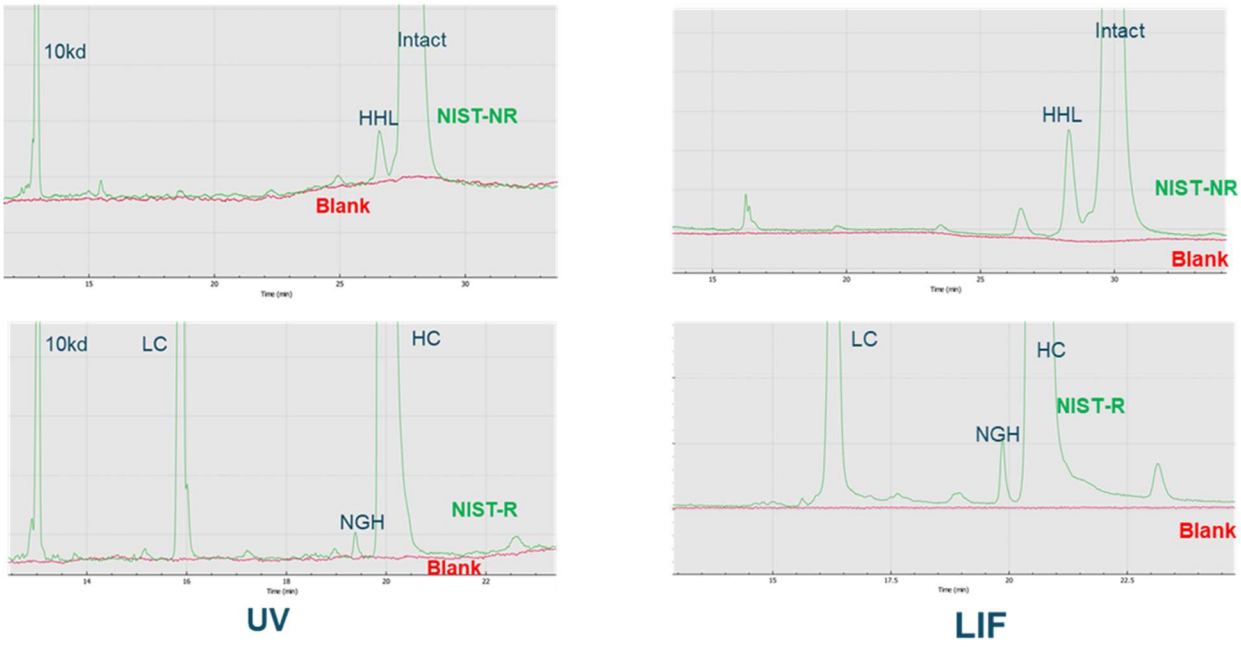

Figure 9. Electropherograms of non-reduced and reduced NIST mAb between sample and blank. The green trace was from sample at 1 mg/mL (UV) and 0.1 mg/mL (LIF). The red trace was from blank (SDS-MW sample buffer). Upper panels show results of non-reduced NIST mAb. Lower panels show results of reduced NIST mAb.

Finally, we systematically evaluated the impact of multiple factors such as, instrument, analyst, cartridge and capillaries on the attributes of resolution and CPA% of the lightning CE-SDS workflow. We isolated the results of each attribute so we could easily underscore how a factor such as, an instrument to instrument has any impact on the average of CPA% and resolution between NG-H and HC. Figure 10 illustrates the average CPA% and resolution between NG-H and HC peaks when data is organized into instrument (green bars), analysts (blue bars), capillary cartridge (yellow bars), day (gray bars), injection (dark blue bars) and capillaries (orange bars). The error bars indicate the standard deviation of the replicates for each factor. Overall, this study revealed that the lightning CE-SDS

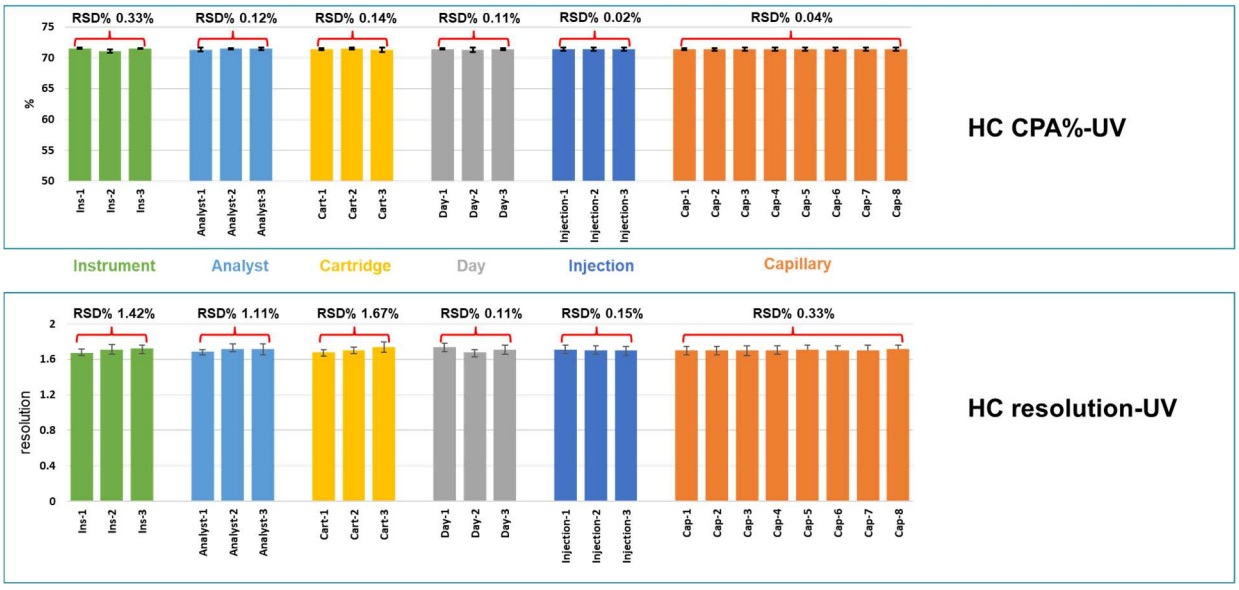

Figure 10. Attributes comparison under different conditions.

generated very reproducible data. For example, when considering instrument to instrument, we observed %RSD as low as 0.4% and below 2% for CPA% and resolution between NH-H and HC peaks, respectively. We also performed a similar analysis on other peaks under reduced and non-reduced conditions (data not shown) and reached the same conclusion.

When considering the 3 analysts as source of variation, our data showed minimum impact on all attributes considered in this study, indicating the robustness of the lightning CE-SDS workflow generating similar results as the SCIEX original CE-SDS workflow.

Conclusions

- The lightning CE-SDS increased the analysis speed up to 1.5 x compared to the original workflow, where 192 injections can be analyzed within 18 hours without the need to change reagent plates
- The reproducibility of the lightning CE-SDS workflow is remarkable with RSD% of < 1% and < 3% for calibrated MT and CPA%, respectively, for all major peaks of IgG standard
- The multi-factorial design of this experiment demonstrated that the lightning CE-SDS workflow is as accurate and precise as the original validated CE-SDS workflow with no carryover and loss of separation efficiency

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