CHARACTERIZATION OF MAURICE™ CE-SDS *PLUS* FOR USP <129> SUITABILITY

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INTRODUCTION

Since the first therapeutic monoclonal antibody (mAb) was commercialized in the mid-80's, close to 100 therapeutic mAb products (accounting for around a quarter of all biotech drugs) have hit the market; making it a $125 billion industry that targets critical pathological health conditions – including but not limited to products for antitumor, antiviral, and antiplatelet therapies. From early-stage process development to batch lot release testing, the efficacy, safety, identity, stability, and purity of therapeutic mAb products throughout their shelf life are of crucial importance. Capillary electrophoresis sodium dodecyl sulfate (CE-SDS) has become the gold standard technique for the quality-control of therapeutic mAbs and proteins due to its ease of implementation, robustness, and reproducibility, replacing the more traditional and labor-intensive technique such as SDS-PAGE gel. Successful CE-SDS method development, under both reducing and non-reducing conditions, aims to reduce assay-associated impurities, fragmentations, and aggregations.

Here, we have used the monoclonal IgG System Suitability Reference Standard developed by U.S. Pharmacopeia (USP) to assess the rigor and robustness of an optimized Maurice™ CE-SDS *PLUS* method compared to the recommended USP protocol provided in monograph <129>. The optimization leveraged Design of Experiments (DOE) to optimize key components in sample preparation, denaturing conditions, and sample injection. The results show that the optimized methods: (1) cause less fragmentation compared to the USP <129> method, (2) are not susceptible to sample injection variations that might differ between instruments, and (3) provide comparable data to the USP <129> monograph for mAbs.

MATERIALS & METHODS

Maurice™

CE-SDS *PLUS* Cartridge

REAGENT/KIT	VENDOR	PART #
Maurice™ CE-SDS *PLUS* Application Kit	ProteinSimple	PS-MAK03-S
Monoclonal IgG System Suitability	U.S. Pharmacopeia	1445550
β-mercaptoethanol (β-ME)	Sigma-Aldrich	M-3148
Iodoacetamide (IAM)	Sigma-Aldrich	I6125

MAURICE™ CE-SDS METHOD

Lyophilized monoclonal IgG system suitability was reconstituted with CE-SDS *PLUS* buffer to a concentration of 1mg/mL in a final volume of 50µL, mixed with 2µL of reconstituted 25X Internal Standard (included in the kit), and either 2.5µL of 14.2M β-ME (reduced IgG samples) or 2.5µL of 20mM IAM (non-reduced IgG samples) before heat denaturation (10min at 70°C for reduced IgG samples and 5min at 65°C for non-reduced IgG samples) in a thermocycler. Samples were kept on ice for 5min before transferring to a 96-well plate, then centrifuged for 10min at 1000×g, and inserted in Maurice™. Batch reagents (included in the kit) were loaded into Maurice™ based on the application guide. Samples were injected for 20sec at 4600V and separated for 25min (reduced IgG) or 35min (non-reduced IgG) at 5750V using the CE-SDS *PLUS* cartridge.

DESIGN OF EXPERIMENT AND DATA ANALYSIS

Suitable statistical methods, Box-Behnken and D-optimal designs, were applied using the JMP® software for design of experiment. Sample concentration, sodium dodecyl sulfate (SDS) concentration, β-ME and IAM concentration, denaturation condition for reduced and non-reduced methods, separation time, and voltage were optimized to ensure complete reaction, while minimizing method-induced fragmentation. Assay robustness was tested through 48-injection batches and the methods were compared to the recommended U.S. Pharmacopeia protocol <129>. All data were analyzed with Compass for iCE software and JMP®.

RESULTS

Maurice™ CE-SDS *PLUS* Method Optimization

We started the method optimization by first comparing commonly used CE-SDS sample buffers. We used SCIEX™ sample buffer, CE-SDS buffer, and CE-SDS *PLUS* buffer for this purpose. CE-SDS *PLUS* buffer outperformed the other two options in both reduced and non-reduced experiments with higher injection efficiency (**Figure 1**). We further evaluated the robustness of methods by probing sample (Sample concentration: 0.6-1.4mg/mL and Buffer SDS concentration: 0.5-1.5X of CE-SDS *PLUS* buffer) and instrument (Separation time: 10-30 min and Injection voltage: 4500-5500V) variables. While sample concentration was linearly correlated with fragmentation as expected, we saw strong assay performance through other parameters; showing method suitability across instruments and SDS concentrations (**Figure 2**).

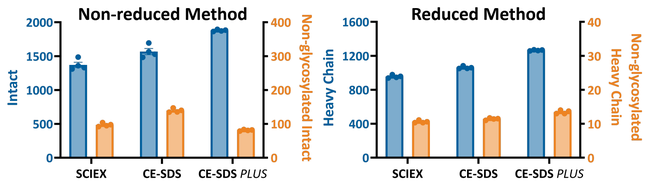

Figure 1. Evaluating different sample buffers. Compared to the SCIEX™ and CE-SDS buffer, CE-SDS *PLUS* buffer helps injecting the species of interest better in each method; resulting in the highest area for intact and heavy chain peak.

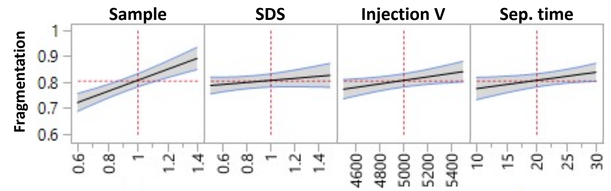

Figure 2. Evaluating sample and instrument conditions. Sample concentration is linearly correlated with fragmentation. However, our method is not susceptible to changes of %SDS in CE-SDS *PLUS* buffer, as well as instrument parameters.

Reduced Method

A successful reduced CE-SDS method is achieved when denaturation is completed with minimal induced fragmentation and background peaks. Higher injection voltage and elevated temperatures improve resolution. We sought to optimize the reducing agent (here β-ME) concentration, denaturation time, and denaturation temperature. We probed 0.3-1.1M of β-ME and denatured the samples at 65-75°C for 5-15min. Samples treated at a temperature lower than 70°C showed extra peaks associated with incompletely reduced IgG, that ran later than heavy chain peak (**Figure 3**). Our results showed that increasing the amount of reducing agent and denaturation time did not change the profile; therefore, we selected the following optimized CE-SDS *PLUS* method conditions:

Reducing agent	Denaturation Temp.	Denaturation Time
0.71M β-ME	70°C	10 min

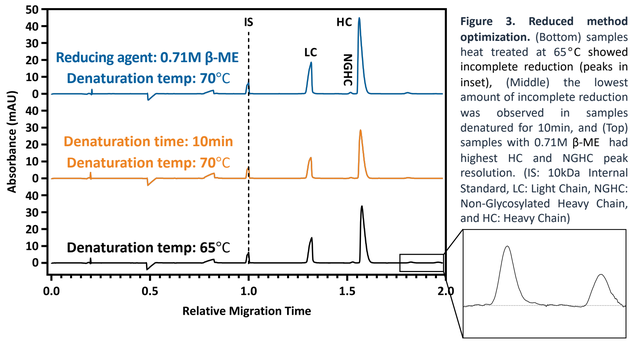

Figure 3. Reduced method optimization. (Bottom) samples heat treated at 65°C showed incomplete reduction (peaks in inset), (Middle) the lowest amount of incomplete reduction was observed in samples denatured for 10min, and (Top) samples with 0.71M β-ME had highest HC and NGHC peak resolution. (IS: 10kDa Internal Standard, LC: Light Chain, NGHC: Non-Glycosylated Heavy Chain, and HC: Heavy Chain)

Non-reduced Method

A non-reduced CE-SDS method is used for evaluating sample purity, measuring intact product, fragments, covalently bound aggregates, and non-product related impurities. mAbs are incubated at high temperatures for denaturation (accelerates SDS binding) and alkylation; however, it can induce mAb fragmentation. We sought to optimize alkylating agent (here IAM) concentration, as well as denaturation time and temperature, by minimizing the peak area of fragments. Our results showed that the most significant factor was IAM concentration. Moreover, denaturing samples at lower temperatures and for shorter time led to a ~15% reduction in the peak area of fragments (**Figure 4**); therefore, we selected the following optimized CE-SDS *PLUS* method conditions:

Alkylating agent	Denaturation Temp.	Denaturation Time
20mM IAM	65°C	5 min

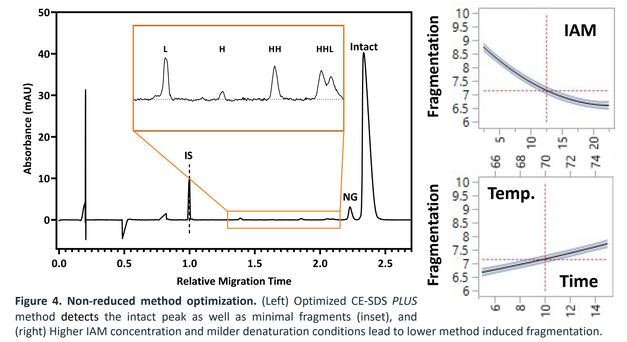

Figure 4. Non-reduced method optimization. (Left) Optimized CE-SDS *PLUS* method detects the intact peak as well as minimal fragments (inset), and (right) Higher IAM concentration and milder denaturation conditions lead to lower method induced fragmentation.

Method Robustness

To study the robustness and reproducibility of our optimized assays, we ran 48-injection batch experiments. We evaluated robustness by looking at the relative standard deviation (%RSD) of peak area of heavy chain (%RSD: %2.5) and intact peaks (%RSD: %2.7) in reduced and non-reduced methods, respectively (**Figure 5**).

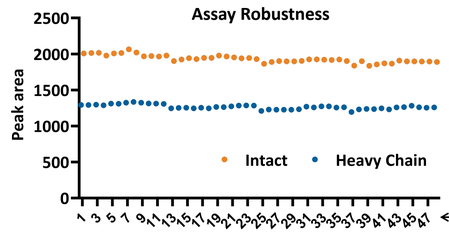

Figure 5. Assay robustness assessment. Performing 48-injection batch experiments, using both non-reduced and reduced methods showed strong assay robustness with low degree of injection-to-injection deviation during long hours of experiment (48 hours). The slight drop after every 12 injection is due to the cartridge clean-up step in our method.

Comparison to USP <129> Protocol

Finally, we compared the optimized CE-SDS *PLUS* methods with the analytical procedure for recombinant therapeutic mAb described in U.S. Pharmacopeia <129> protocol. We ran three replicates for each method (total of 12 samples) in the same run where each sample was injected four times. Results showed that the optimized CE-SDS *PLUS* methods match the sensitivity and reproducibility of USP <129> standard protocol. There were no significant differences between the amount of incomplete reduction in reduce methods (%CE-SDS *PLUS*: 0.98±0.09 and %USP <129>: 1.00±0.04). However, having lower method-induced fragmentation, the non-reduced CE-SDS *PLUS* method showed significant improvements (*P*-value < 0.0001) over the USP non-reduced method (**Figure 6**).

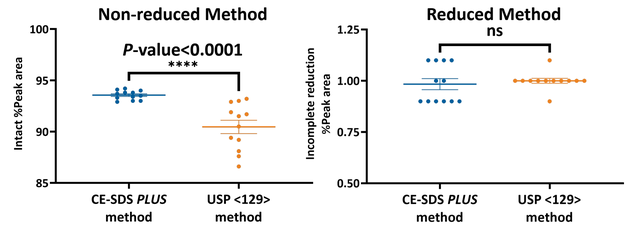

Figure 6. Comparing CE-SDS *PLUS* method to USP <129> standard protocol. Statistical analysis (Mann-Whitney test) shows that non-reduced CE-SDS *PLUS* method induces significantly less fragmentation compared to USP <129> method; while both reduced methods perform equally acceptable in minimizing incomplete reduction (ns: not significant).

CONCLUSIONS

- Maurice™ CE-SDS *PLUS* methods developed here show robust performance over 48-hour-long CE-SDS experiments.
- The optimized reduced method matches the sensitivity of USP <129> protocol, while the non-reduced method significantly outperforms it.

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