HIGH SPEED AND HIGH THROUGHPUT CE-SDS ANALYSIS WITH THE MAURICE TURBO CE-SDS™ ASSAY

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INTRODUCTION

Analytical tools that deliver fast while providing high quality data are increasingly in demand today, driven by the need for new and improved biologics as well as competition in the market. To meet this demand, we have developed a new assay for the Maurice CE instrument, Turbo CE-SDS, which enables rapid CE-SDS analysis with high sample throughput. Protein size and purity analysis is now five times faster with Turbo CE-SDS, allowing for analysis in 5.5 minutes for reduced samples and 8 minutes for non-reduced samples. Because the data is generated with the Compass for iCE software, which is 21 CFR Part 11 compliant, analysis with Turbo CE-SDS provides data to enable faster informed decisions from a large number of samples, especially in early discovery stages like cell culture and clonal selection. Furthermore, because Maurice is also used in late-stage development processes like QC, method transfer between cartridges and labs is significantly easier and faster, thus precluding the need to buy different phase-appropriate instruments for the same type of analysis. This poster highlights quantitative aspects of the Maurice Turbo CE-SDS assay using the IgG System Suitability Reference Standard from the United States Pharmacopeia (USP), a 10 kDa Internal Standard (IS), and bovine serum albumin (BSA).

METHODS

Maurice (Figure 1A) and Maurice S. instruments are both compatible with the Turbo CE-SDS cartridge (Figure 1B). All experiments presented in this poster were conducted using Turbo CE-SDS cartridges and Maurice instruments. The reagents and consumables necessary to run CE-SDS on Maurice were from The Turbo CE-SDS Application Kit. Additional reagents used were β-mercaptoethanol (β-ME), iodoacetamide (IAM), and bleach (to neutralize β-Me in waste).

A

B

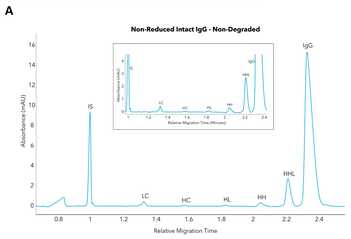
Figure 1. A. The Maurice instrument, and B. the Maurice Turbo CE-SDS cartridge.

The USP mAb Standard (#144555) samples were prepared at a final concentration of 1 mg/mL in 50 µL of Maurice CE-SDS 1X Sample Buffer, with 1X Internal Standard (IS). The samples were treated with either 2.5 µL of 250 mM IAM or 2.5 µL of 14.2 M β-ME to make non-reduced or reduced samples, respectively. The samples were then denatured for 10 minutes at 75°C. For limit of detection (LOD) calculations, the IS and BSA were serially diluted in the Maurice CE-SDS 1X Sample Buffer, resulting in concentrations ranging from 0.4 to 200 µg/mL. Samples were denatured for 5 minutes at 95°C. After denaturing, all samples were diluted in deionized water at a 1:1 ratio to make a 100 µL sample. Each 100 µL sample can be used for up to 10 injections.

The samples and batch reagents were loaded onto Maurice with the Turbo CE-SDS cartridge according to the Turbo CE-SDS Application Kit product insert. The samples were injected for 8 seconds at 3500 V and separated at 4200 V for 5.5 minutes for reduced samples and 8 minutes for non-reduced samples. The data were analyzed using Compass for iCE software.

RESULTS

A



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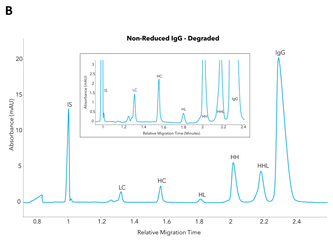

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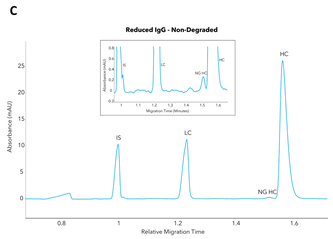

Figure 2. Representative electropherograms generated using Turbo CE-SDS. A. Non-reduced analysis of the USP mAb Standard sample resulted in the expected peaks (IgG: intact peak; HHL: heavy-heavy-light; HH: heavy-heavy; HL: heavy-light; HC: heavy chain; LC: light chain; IS: Internal Standard). **B.** Non-reduced analysis of the degraded USP mAb Standard sample clearly shows an increase in fragmentation, likely due to the breakage of disulfide bonds. **C.** Reduced analysis of the USP IgG sample resulted in expected peaks, which are heavy chain (HC), non-glycosylated heavy chain (NG HC), light chain (LC), and internal standard (IS). The inset shows well-resolved peaks generated by the Compass for iCE software.

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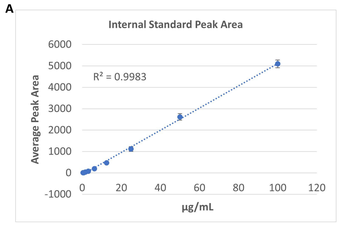

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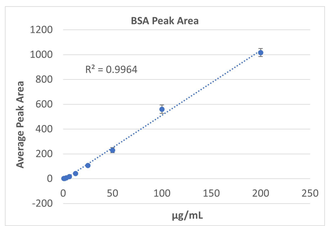
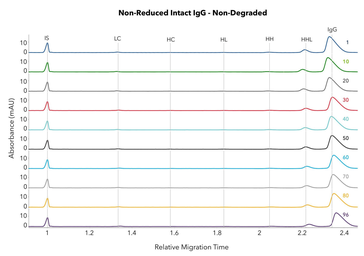


Figure 3. Dynamic range of Turbo CE-SDS. A. Plot of dynamic range for the Internal Standard generated using sample concentrations ranging from 100-0.4 µg/mL. The resulting R^2 value was 0.998 . **B.** Plot of dynamic range for BSA generated using sample concentrations ranging from 200 to 0.8 µg/mL. The resulting R^2 value was 0.996. Both samples were serially diluted 2-fold using the Maurice CE-SDS 1X Sample Buffer. The dynamic ranges for both samples were at least 2 logs based on peak area and the data were linear.

RESULTS

A



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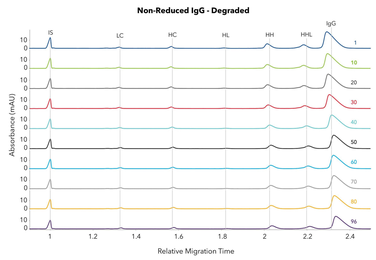

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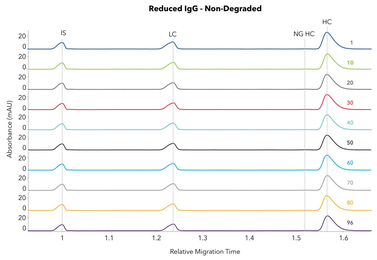

Figure 4. Reproducibility of Turbo CE-SDS. Batches of 96 injections were run for each type of the USP mAb Standard sample (non-degraded and degraded non-reduced, and reduced), and stacked plots are shown for ten injections for each batch, including the first injection, eight injections chosen at regular intervals throughout the batch, and the last (96th) injection. **A.** Reproducibility of non-reduced CE-SDS for intact samples in a 96-injection batch **B.** Reproducibility of non-reduced CE-SDS for degraded samples in a 96-injection batch, where all samples clearly show an increase in fragmentation. **C.** Reproducibility of reduced CE-SDS in a 96-injection batch. All three datasets demonstrate excellent reproducibility of the Turbo CE-SDS assay.

A

AVERAGE PERCENT PEAK AREA				
Non-Reduced		Reduced		
Cartridge	Intact IgG	Cartridge	LC	HC
1	89.86	1	29.92	69.51
2	86.49	2	29.80	69.71
3	85.95	3	29.44	70.04
PERCENT PEAK AREA CV (%)				
Non-Reduced		Reduced		
Cartridge	Intact IgG	Cartridge	LC	HC
1	0.29	1	0.40	0.20
2	0.40	2	0.80	0.30
3	0.29	3	0.60	0.20

B

AVERAGE RMT										
Non-Reduced							Reduced			
Cartridge	IgG	LC	HC	HL	HH	HHL	Cartridge	LC	HC	NG HC
1	2.31	1.33	1.57	1.82	2.03	2.20	1	1.24	1.57	1.52
2	2.32	1.33	1.57	1.82	2.03	2.20	2	1.25	1.60	1.55
3	2.34	1.33	1.58	1.83	2.05	2.22	3	1.24	1.57	1.53
RMT CV (%)										
Non-Reduced							Reduced			
Cartridge	IgG	LC	HC	HL	HH	HHL	Cartridge	LC	HC	NG HC
1	0.36	0.22	0.27	0.36	0.21	0.28	1	0.03	0.05	0.05
2	0.38	0.21	0.29	0.28	0.28	0.34	2	0.05	0.15	0.14
3	0.44	0.34	0.36	0.69	0.28	0.37	3	0.06	0.17	0.12

Table 1. Percent peak area and relative migration time (RMT) performance of Turbo CE-SDS for non-reduced and reduced USP IgG samples using three cartridges.
A. Averages for the major peaks detected in reduced and non-reduced samples were very similar between cartridges, with CV values of ≤0.4% and ≤0.8% for non-reduced and reduced samples, respectively. **B.** The average RMT for all peaks detected were highly similar between cartridges, with reported CV values within 0.7% and 0.2% for non-reduced and reduced samples, respectively. Overall, the intra-cartridge reproducibility of Turbo CE-SDS was remarkable.

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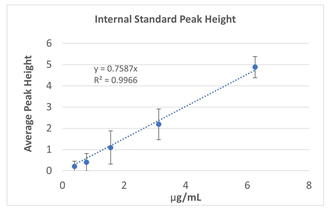

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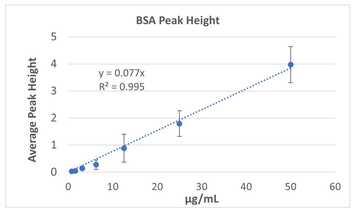

Sample	Internal Standard	BSA
LOD (µg/mL)	0.20	0.90

Table 2. LOD values for IS and BSA.

Figure 5. Dilution series for IS and BSA to determine limit of detection (LOD) for Turbo CE-SDS. Both samples were serially diluted 2-fold, as described in Figure 3. **A.** The average peak height for serial dilutions of IS were plotted and a linear regression was performed. The LOD was calculated by dividing three times the standard deviation of the noise by the slope of the linear regression, resulting in 0.20 µg/mL. **B.** The LOD for BSA was calculated as described above, resulting in a value of 0.90 µg/mL.

CONCLUSION

- The Maurice Turbo CE-SDS assay allows 5X faster CE-SDS analysis while providing high resolution and high throughput without requiring any protein labeling.
- The Turbo CE-SDS assay generates distinct non-reduced and reduced profiles of IgG samples, and fragmentation is clearly visible in degraded samples.
- The dynamic range of Turbo CE-SDS assay is of at least 2 logs, as seen from analysis of the Internal Standard and BSA, with R^2 values of 0.998 and 0.996, respectively.
- The LOD on Turbo CE-SDS for IS and BSA were 0.20 and 0.90 µg/mL, respectively.
- The Turbo CE-SDS assay is highly reproducible. The intra-cartridge average peak percent area CV values were ≤0.4% and ≤0.8% for non-reduced and reduced CE-SDS analysis, respectively. The RMT CV values were <0.7% and <0.2%.

To learn more about how Turbo CE-SDS can enhance your workflow across multiple stages, visit us at www.bio-techne.com/turbo-ce-sds