Application Note

Save on Time and Cost with the New Turbo CE-SDS™ Assay

Introduction

Fast CE-SDS analysis with great data quality—once a dream—is now a reality! The new Maurice Turbo CE-SDS cartridge enables rapid high-throughput analysis of protein size and purity without the need for fluorescent labeling, giving you direct detection and high-quality data fast. Get data on reduced samples in 5.5 minutes, and non-reduced samples in 8 minutes. If you're already using Maurice or Maurice S. systems, all you need for faster CE-SDS analysis is the new Turbo CE-SDS cartridge and reagents and the latest Compass for iCE (v3.0.0) and instrument software. The rest is the same easy and simple Maurice workflow you're used to. With the added speed—five times what you're used to—and throughput of Turbo CE-SDS, Maurice systems now have the versatility to get you the data you need across all analytical stages, making for easy and seamless transitions from early discovery all the way to QC, all on a single platform.

This application note demonstrates the reproducibility, separation linearity, dynamic range, and limit of detection (LOD) for the Turbo CE-SDS assay, which were generated using either a monoclonal IgG System Suitability Reference Standard from the United States Pharmacopeia (USP), bovine serum albumin (BSA), Maurice CE-SDS Molecular Weight Markers, or Maurice CE-SDS Internal Standard (IS).

The Maurice Family

Maurice is a dual CE instrument designed for automated whole-column icIEF and CE-SDS analysis. Both methods share an unparalleled ease-of-use, as switching between them is as simple as plugging in the relevant cartridge and reagents for either application. No extensive sample prep and no laborious instrument set up or clean up; simply load your sample vials or a 96-well plate, add your assay reagents, set up your batch on Compass for iCE software, and hit "Start". You'll get rapid, high-quality results on protein purity or charge heterogeneity while staying 21CFR Part 11 compliant.

FIGURE 1. **A.** The Turbo CE-SDS cartridge and **B.** the Maurice instrument.

Materials and Methods

All materials used for experiments in this application note are listed in **TABLE 1**. The Maurice Turbo CE-SDS Size Application Kit (PS-MAK01-TS) and Maurice CE-SDS Molecular Weight (MW) Markers (PN 046-432) were obtained from ProteinSimple, a Bio-Techne brand. The monoclonal IgG System Suitability Reference Standard (PN 1445550, lot F00760) was procured from the USP. β mercaptoethanol (β-ME, PN M-3148), Iodoacetamide (IAM, PN 16125), and BSA (PN A9418) were obtained from Millipore Sigma.

All experiments were conducted using Turbo CE-SDS cartridges on Maurice instruments. The USP mAb Standard 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 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 Maurice CE-SDS MW Markers were prepared according to the product insert. 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 the Compass for iCE software.

Results

FIGURE 2 shows the non-reduced profiles of the USP IgG standard, either non-degraded (**FIGURE 2A**) or degraded (**FIGURE 2B**). The degraded profile clearly shows an increase in fragmentation, as expected, likely due to the breaking of disulfide bonds. **FIGURE 3** shows the reduced profile of the non-degraded USP IgG.

Material	Vendor	Catalog #
Maurice Turbo CE-SDS Size Application Kit	ProteinSimple	PS-MAK01-TS
Maurice Turbo CE-SDS cartridge	ProteinSimple	PS-MC02-TS, PS-MC01-TS
Maurice CE-SDS 1X Sample Buffer	ProteinSimple	046-012
Maurice CE-SDS Separation Matrix	ProteinSimple	046-386
Maurice Turbo CE-SDS Running Buffer-Bottom	ProteinSimple	046-579
Maurice CE-SDS Wash Solution	ProteinSimple	046-569
Maurice CE-SDS Conditioning Solution 1	ProteinSimple	046-014
Maurice CE-SDS Conditioning Solution 2	ProteinSimple	046-015
Maurice CE-SDS 25X Internal Standard	ProteinSimple	046-144
Maurice 96-well plates	ProteinSimple	046-021
Maurice glass reagent vials, 2 mL	ProteinSimple	046-017
Maurice clear screw caps	ProteinSimple	046-138
Maurice CE-SDS orange pressure caps	ProteinSimple	046-572
Maurice CE-SDS Molecular Weight Markers	ProteinSimple	046-432
Monoclonal IgG System Suitability Reference Standard	USP	PN 1445550, lot F00760
β mercaptoethanol (β-ME)	Millipore Sigma	M-3148
Iodoacetamide (IAM)	Millipore Sigma	16125
Bovine serum albumin (BSA)	Millipore Sigma	A9418

TABLE 1. Contents of the Turbo CE-SDS Size Application Kit and other reagents used in this study.

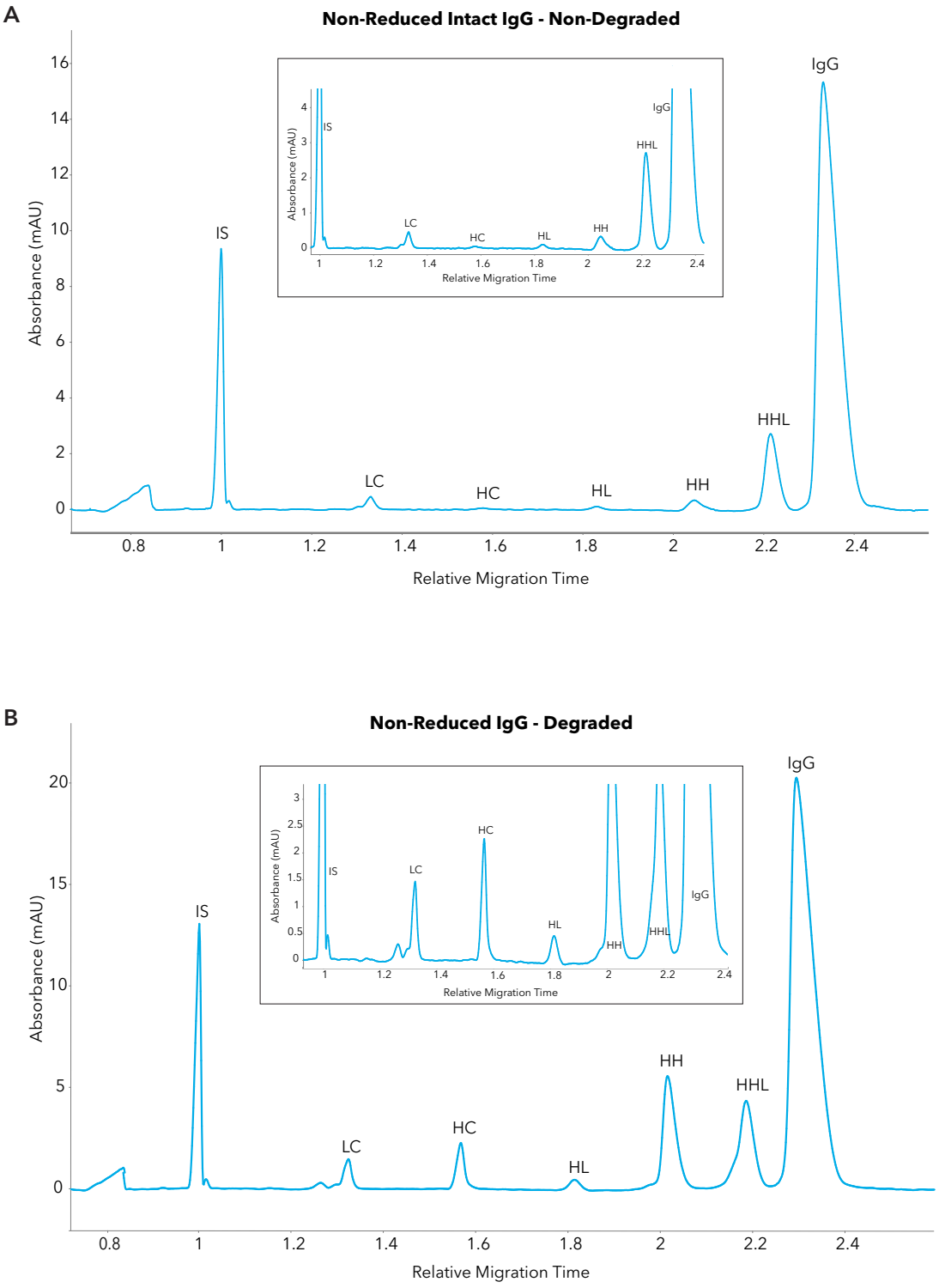

FIGURE 2. Non-reduced CE-SDS analysis of the USP IgG Standard with Turbo CE-SDS. A. Profiles of non-degraded samples and B. degraded samples are shown. The differences between both samples are clearly seen in the insets; an increase in fragmentation is seen in the degraded sample profile, as expected. The peaks identified in both datasets are IgG: intact IgG; HHL: heavy-heavy-light; HH: heavy-heavy; HL: heavy-light; HC: heavy chain; LC: light chain; IS: Internal Standard.

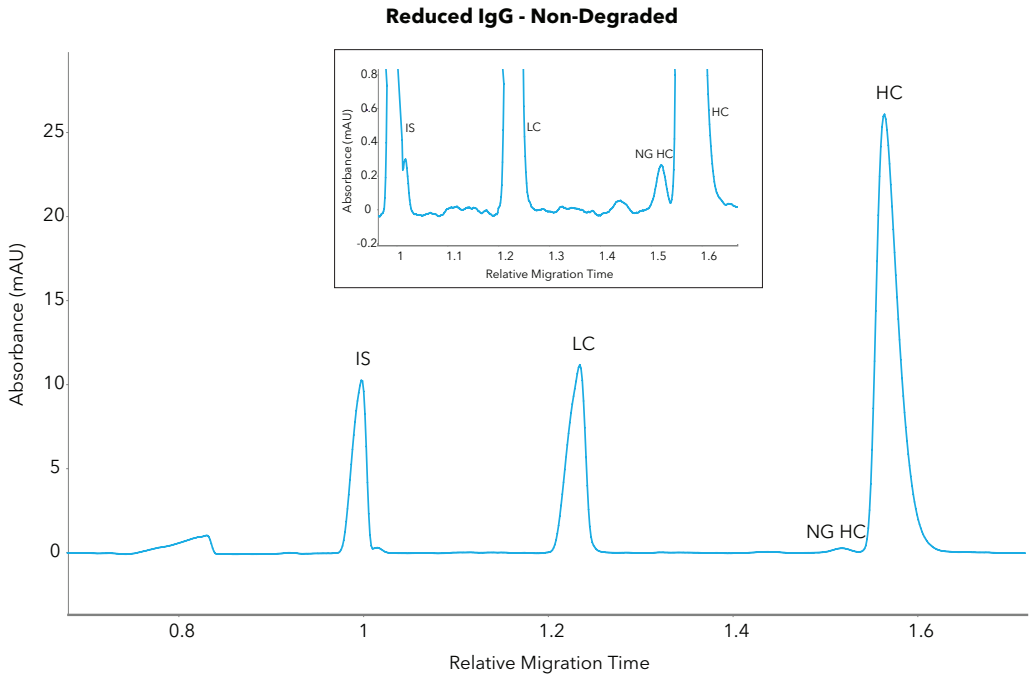

FIGURE 3. Reduced CE-SDS analysis of the USP IgG Standard with Turbo CE-SDS. Peaks identified are heavy chain (HC), non-glycosylated heavy chain (NG HC), light chain (LC), and internal standard (IS).

Separation Linearity

Separation linearity of Turbo CE-SDS was evaluated using the MW Markers. **FIGURE 4** shows the results, where an excellent R^2 value of 0.9927 was obtained for the separation linearity in the 10 270 kDa molecular weight range.

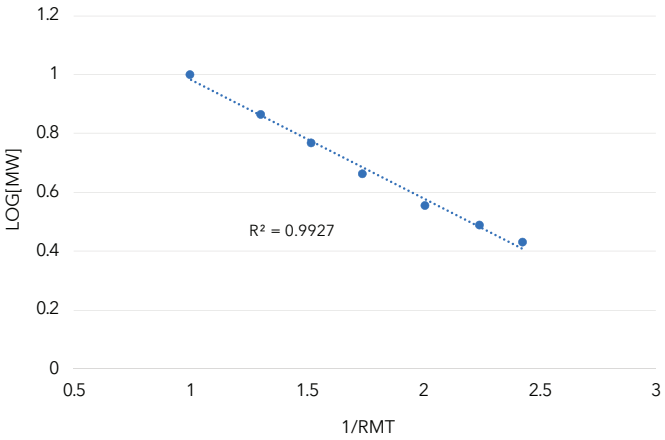

FIGURE 4. Separation linearity of Turbo CE-SDS. Using MW markers, the cartridge demonstrated linear separation with an R^2 value of 0.9927.

Reproducibility

To evaluate the reproducibility of Turbo CE-SDS, the profiles of 96 sample injections were analyzed for both non-reduced and reduced USP IgG samples. Excellent reproducibility within a batch was obtained for both types of samples, as shown in **FIGURES 5 and 6**. The figures show stacked plots of ten injections, including the first injection, eight subsequent injections at regular intervals throughout a batch, and the 96th injection. In addition, three cartridges were used to assess cartridge reproducibility for reduced and non-reduced USP IgG samples. The percent peak area and corresponding CV values are reported in **TABLE 2**, while the relative migration time (RMT) and CV values are reported in **TABLE 3**. The data indicates excellent reproducibility between cartridges, with average peak percent area CV values ≤0.4% and ≤0.8% for non-reduced and reduced samples, respectively, and RMT CV values within 0.7% and 0.2%.

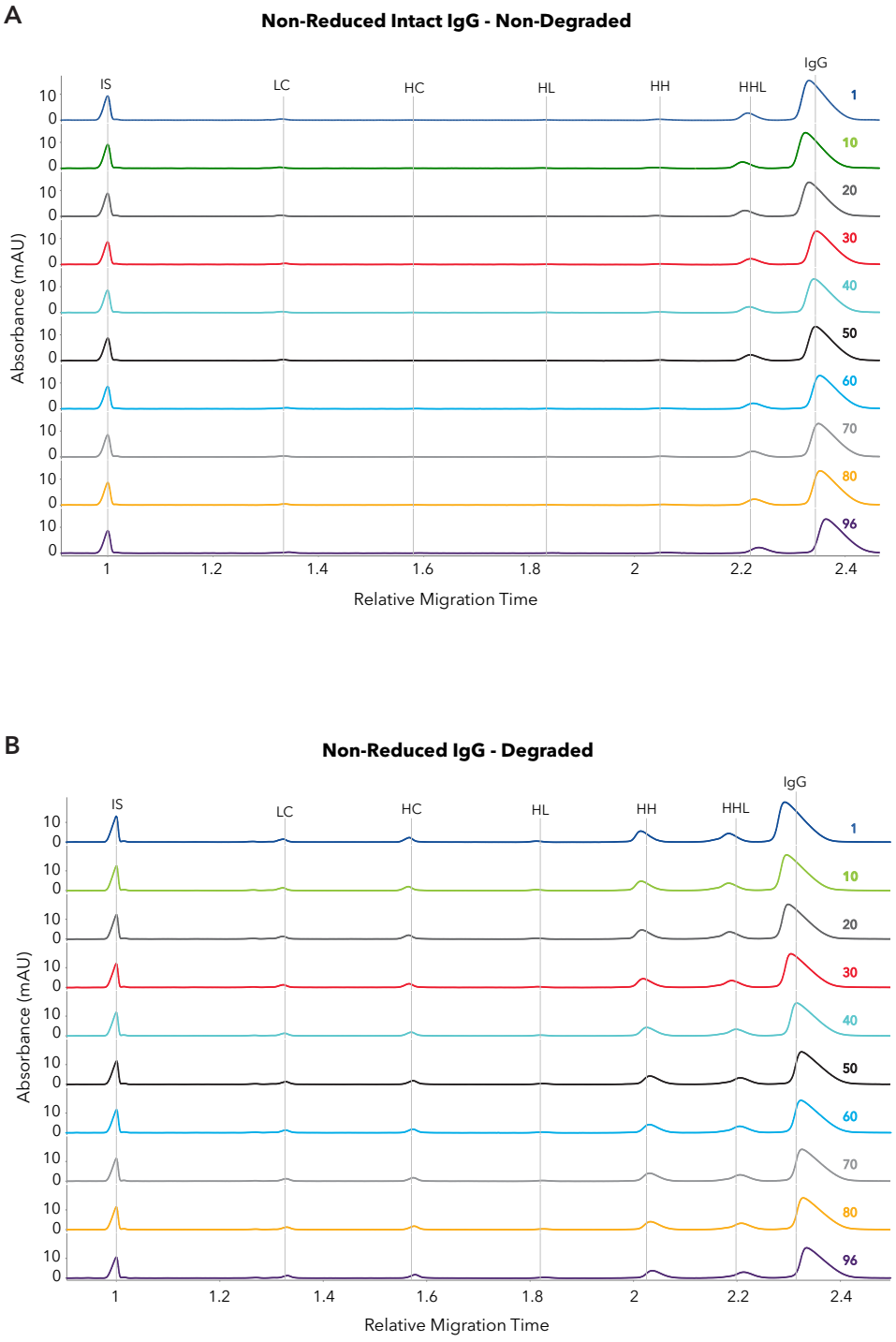

FIGURE 5. Data reproducibility with Turbo CE-SDS for non-reduced samples. A. Stacked plots of ten injections for non-reduced intact samples and B. degraded samples are shown, demonstrating excellent reproducibility of the Turbo CE-SDS assay in a 96-injection batch. The injection number is listed next to each plot and includes first and last injection.

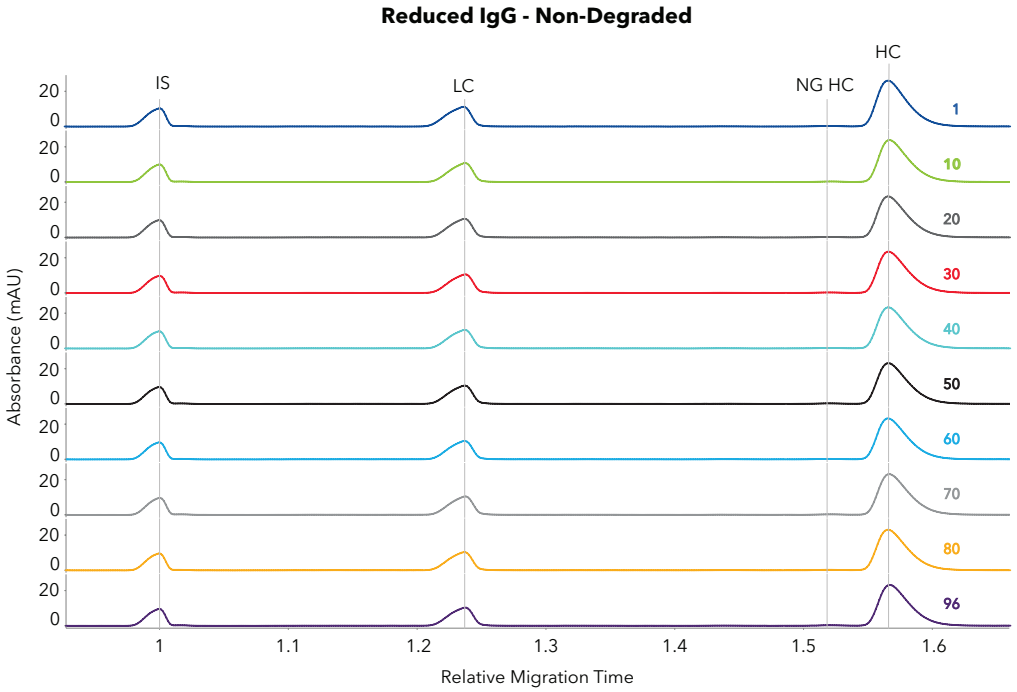

FIGURE 6. Data reproducibility with Turbo CE-SDS for reduced samples. Stacked plots of ten sample injections are shown from a 96-injection batch. The injection number is listed next to each plot and includes the first and last injection. All major peaks show excellent reproducibility.

Average Percent Peak Area				
Non-Reduced		**Reduced**		
Cartridge	**Intact IgG**	**Cartridge**	**LC**	**HC**
1	89.96	1	29.92	69.51
2	89.49	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

TABLE 2. Percent peak area performance for Turbo CE-SDS cartridges for reduced and non-reduced USP IgG. Three cartridges were evaluated for average percent peak area and CVs. Averages for the major peaks detected in reduced and non-reduced samples were very similar between cartridges, and the CV values across all cartridges were ≤0.4% and ≤0.8% for non-reduced and reduced samples, respectively.

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 3. RMT performance for Turbo CE-SDS cartridges for reduced and non-reduced UPS IgG. Three cartridges were evaluated for average RMT and CVs. Averages for all peaks detected were very similar between cartridges, and the CV values across all cartridges were within 0.7% and 0.2% for non-reduced and reduced samples, respectively.

Dynamic Range

Turbo CE-SDS dynamic range was evaluated using IS and BSA. Both samples were serially diluted 2-fold using the Maurice CE-SDS 1X Sample Buffer. Data shown for IS ranged from 100 to 0.4 µg/mL, and BSA from 200 to 0.8 µg/mL.

The samples were run using the Turbo CE-SDS assay, and the dynamic ranges for both samples were at least 2 logs based on peak area. The data were linear, with R^2 values of 0.998 and 0.996 for IS and BSA, respectively, as shown in **FIGURES 7A** and **7B**.

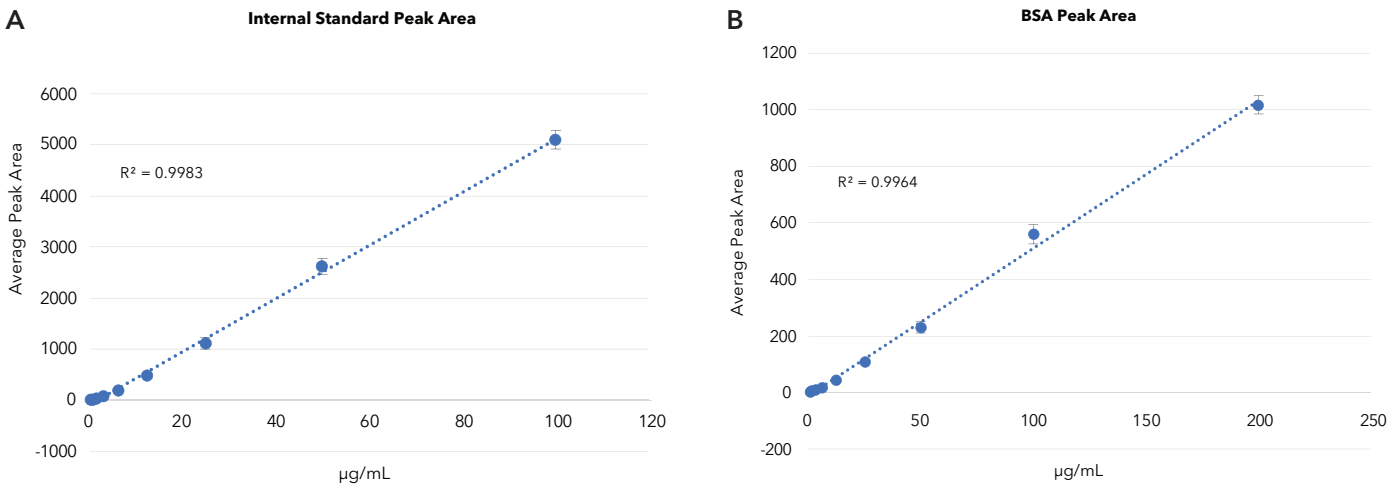

FIGURE 7. Dynamic range of Turbo CE-SDS. The assay demonstrated a dynamic range of at least 2 logs and an R^2 value of ≥0.99 for the **A**. Internal Standard and for **B**. BSA.

Limit of Detection

To ascertain the LOD, 2-fold serial dilutions of IS and BSA were made using the CE-SDS 1X Sample Buffer as described earlier. Average peak heights were used to calculate the LOD, which was done by dividing three times the standard deviation of the noise by the slope of the linear regression for peak height. The LOD for IS was found to be 0.20 µg/mL and 0.90 µg/mL for BSA.

The dilution series plot of peak heights for IS and BSA are shown in **FIGURES 8A** and **8B** respectively, and the LOD results are shown in **TABLE 4**.

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

TABLE 4. LOD values for IS and BSA.

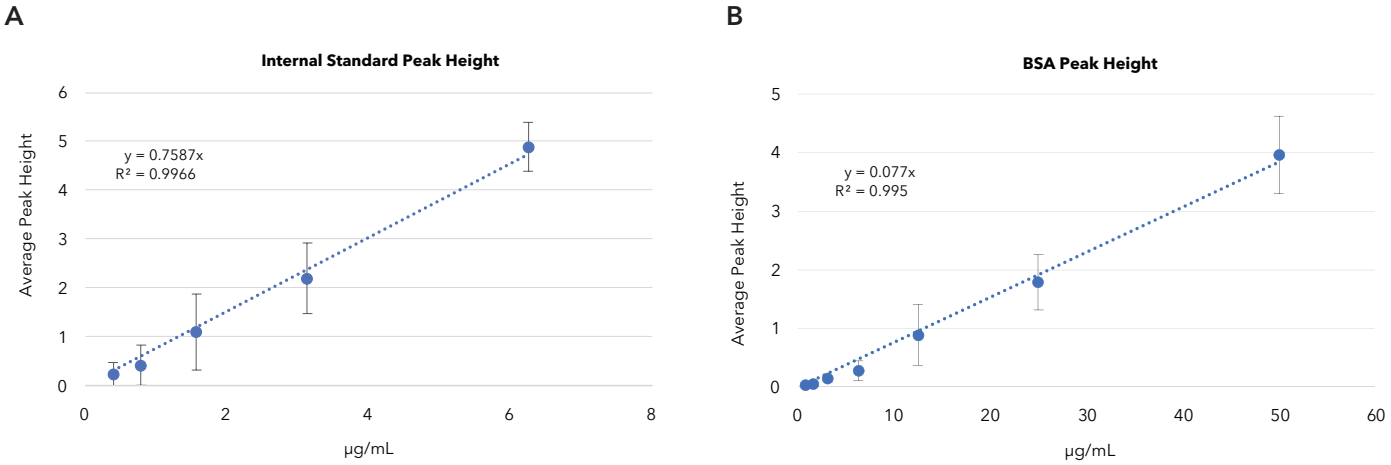

FIGURE 8. Dilution series for BSA and IS to determine limit of detection for Turbo CE-SDS. The average peak height for serial dilutions of **A**. Internal Standard and **B**. BSA were plotted and a linear regression was performed. The LOD values were determined for the Internal Standard and BSA by dividing three times the standard deviation of the noise by the slope of the linear regression, resulting in 0.20 and 0.90 µg/mL for the samples, respectively.

Conclusion

This application note showed the performance and data quality of the new Maurice Turbo CE-SDS™ assay. The assay demonstrated excellent separation linearity, with an R^2 value of 0.9927. Furthermore, the data from 96 samples, for both non-reduced and reduced USP IgG samples, showed high reproducibility with percent peak area CVs of ≤0.4% and ≤0.8%, respectively. Using an Internal Standard (IS) and BSA, the dynamic range of the cartridge was at least 2 logs for both proteins, and the LOD values were 0.20 µg/mL and 0.90 µg/mL respectively.

There is no need to rely on multiple instruments for the same type of analysis which demands a larger investment of time and money for learning, training, method development and transfer, and purchasing systems that are phase appropriate.

The Turbo CE-SDS cartridge addresses these challenges by expanding the Maurice system's capabilities. You can now perform protein size and purity analysis in upstream processes that require greater speed and throughput. You can easily transfer your methods to stages like analytical development and QC, where the Maurice system is extensively used. Turbo CE-SDS eliminates the need for multiple analytical instruments to address sample throughput and data quality challenges. Maurice gives you everything you need to make decisions faster, enabling you to progress seamlessly across your biotherapeutic development so you can reach patients sooner. Learn more about Turbo CE-SDS here.

Contact Us

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