Enhanced CE-SDS Analysis with Maurice's CE-SDS PLUS System

Introduction

CE-SDS is a standard methodology for characterization of biotherapeutic proteins and confirmation of product purity. Maurice and Maurice S. - members of the iCE family that run the CE-SDS application - deliver this analysis in an easy-to-use format that significantly simplifies your workflow and reduce hands-on time and the chance of user error.

Maurice's CE-SDS application has always delivered the speed, automation, reproducibility, and high-resolution data you require[1]. Maurice's CE-SDS PLUS system preserves the same features, and in addition enables enhanced sample stability and data consistency throughout a batch. That translates to highly consistent absolute peak areas and less protein fragmentation. Data for both systems is equivalent with regard to separation linearity, peak resolution, % peak areas, and dynamic range. Added bonus? You'll now also get more injections per cartridge and smoothed baselines for improved signal-to-noise (S/N).

Meet the new CE-SDS PLUS system

The workflow to set up a CE-SDS PLUS batch on Maurice is exactly the same as setting up a CE-SDS batch, so you'll get all the same hands-on time savings when setting up your experiment. Maximum number of injections per batch is still 48 and you'll still get baseline resolution of glycosylated and non-glycosylated reduced IgG heavy chain with 50 µL of prepared sample in less than 25 minutes.

Maurice's CE-SDS PLUS system gives you more consistent data due to improved consumables, reagents, and software. As demonstrated in this study, you'll see stable absolute peak areas with linearity of R^2>0.99, along with RSDs of <10% when measuring absolute peak area in consecutive, triplicate injections. You'll also get more data with the new CE-SDS PLUS cartridge, with 100 guaranteed and 500 maximum injections per cartridge (**Figure 1**).

Maurice systems running Compass for iCE v2.1 and above are capable of running the CE-SDS PLUS system. Compass

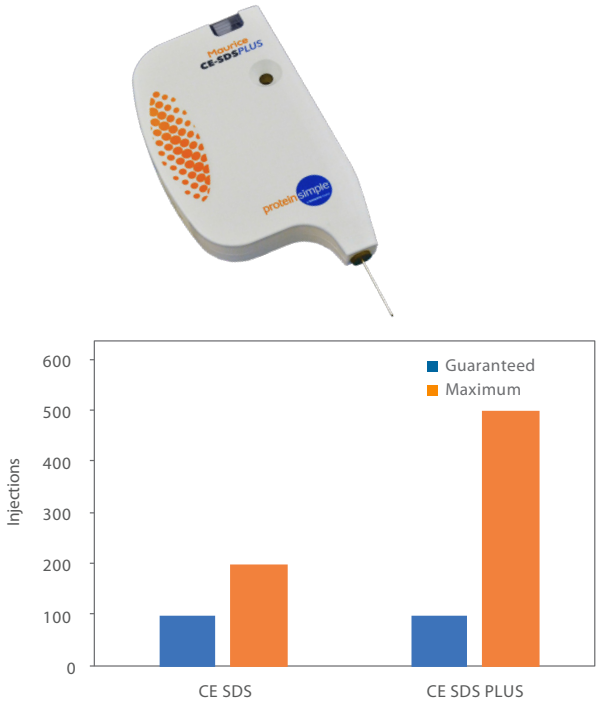

FIGURE 1. The CE-SDS PLUS Cartridge supports more injections.

for iCE v2.1 also gives you the option to apply baseline smoothing to your data so it'll be easier to monitor small peaks in your IgG profile.

To run a CE-SDS PLUS method, just swap out a few CE-SDS consumables with CE-SDS PLUS-specific consumables and select a CE-SDS PLUS method when setting up your batch (**Table 1**). Got a molecule you're already analyzing in QC? You'll still be able to run your validated CE-SDS protocol – just use the original CE-SDS cartridge, reagents, and CE-SDS method when setting up your batch.

CONSUMABLE	PART NUMBER
Maurice CE-SDS PLUS Application Kit	PS-MAK03-S
Maurice CE-SDS PLUS Cartridge (2/pack)	PS-MC02-SP
Maurice CE-SDS PLUS Sample Buffer	046-567

TABLE 1. CE-SDS PLUS specific consumables. To run the CE-SDS PLUS system, use CE-SDS PLUS consumables with a CE-SDS PLUS method on a Maurice running Compass for iCE v2.1 and above.

Enhanced system, same great data

SEPARATION LINEARITY

To demonstrate data equivalency, we first ran ProteinSimple MW Markers using both the CE-SDS and CE-SDS PLUS system on Maurice. No significant difference was detected as data points and linear regression lines in the Log MW vs 1/Mobility (RMT, Relative Migration Time) plot all overlapped (**Figure 2**). The plot also confirmed separation linearity in the 10 – 270 kDa molecular weight range with a R^2 of 0.992 and 0.993 using the CE-SDS and CE-SDS PLUS systems, respectively.

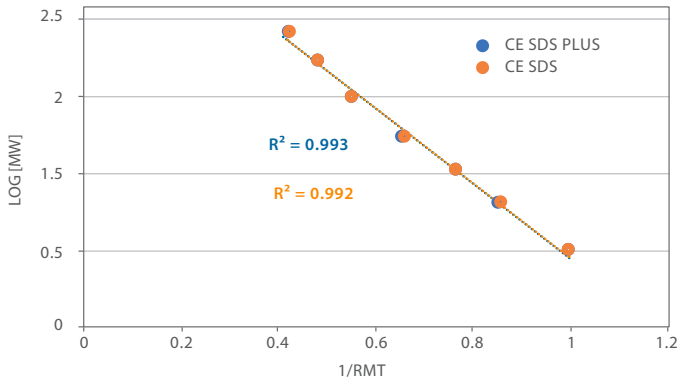

FIGURE 2. CE-SDS (orange) and CE-SDS PLUS (blue) separation linearity based on the mobility of the MW Markers. Separation for both were linear with an R^2 of 0.992 for the CE-SDS system and 0.993 for the CE-SDS PLUS system.

DYNAMIC RANGE

We next checked the linear dynamic range equivalency using both the 10 kDa Internal Standard and BSA. Samples were serially diluted 5-fold from 100 to 0.16 µg/mL using either the original CE-SDS 1X Sample Buffer or CE-SDS PLUS 1X Sample Buffer and run using a CE-SDS or CE-SDS PLUS method, respectively, on Maurice. The dynamic range for both molecules was at least 2 logs on both systems. Data was linear with an R^2 for the Internal Standard of 0.998 for the CE-SDS system and 0.994 for the CE-SDS PLUS system and an R^2 for BSA of 0.999 for both systems (**Figure 3**).

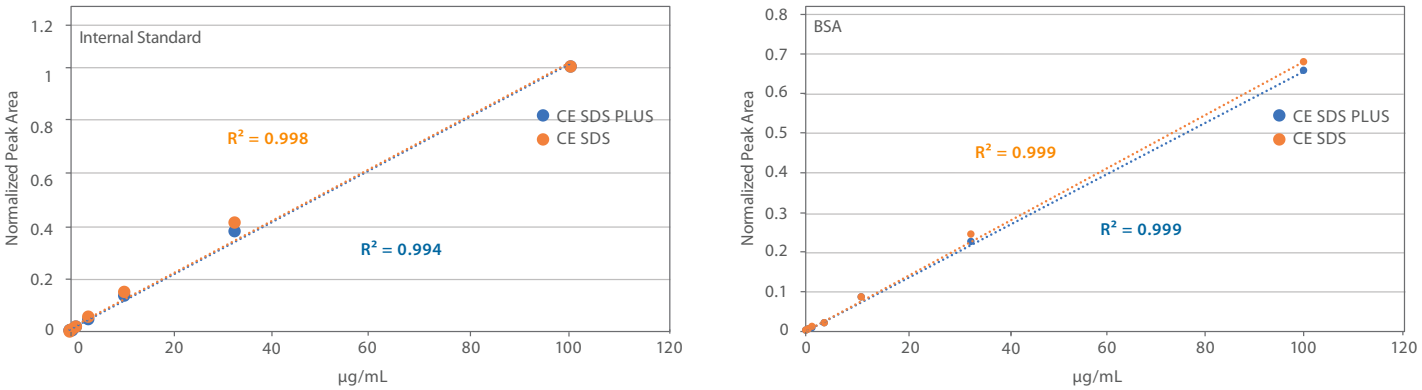

FIGURE 3. Maurice's CE-SDS system (orange) and CE-SDS PLUS system (blue) both have a dynamic range of at least 2 logs with an R^2 of at least 0.994 or greater with either the ProteinSimple Internal Standard (left) or BSA (right).

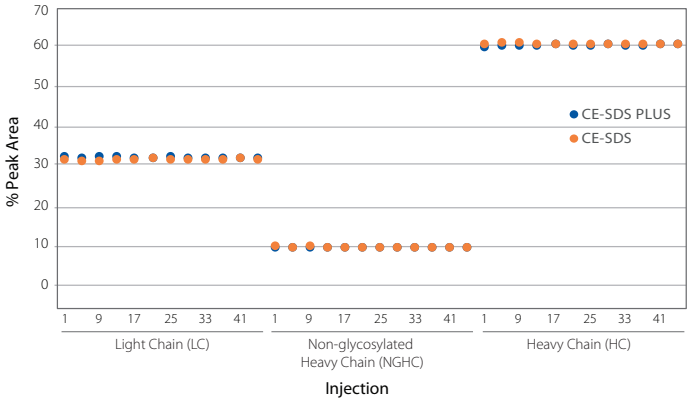

FIGURE 4. % Peak area of reduced light chain (LC), non-glycosylated heavy chain (NGHC), and heavy chain (HC) across 48 injections run using the CE-SDS system (orange) and CE-SDS PLUS system (blue) confirms system equivalency. RSDs were all <1% for all peaks. Data from 12 injections shown.

QUANTIATION PRECISION

To compare the quantitation precision between the CE-SDS and CE-SDS PLUS system, we next ran 1.0 mg/mL of reduced IgG standard across 48 injections (**Figure 4**). Percent peak areas for the reduced light chain (LC), non-glycosylated heavy chain (NGHC), and heavy chain (HC) as well as the HC/LC ratio were analyzed using all 48 injections (**Table 2**). Data points all overlapped, proving equivalent quantitation between both systems. Average peak percentages were within 0.5% or less for the LC, NGHC, and HC. Data quality was consistent over the course of the batch with RSDs for all peaks ≤1%, confirming you'll get the same answer at injection 1 and injection 48 with either system. The average HC/LC ratio was also equivalent with a ratio of 1.9 for both systems.

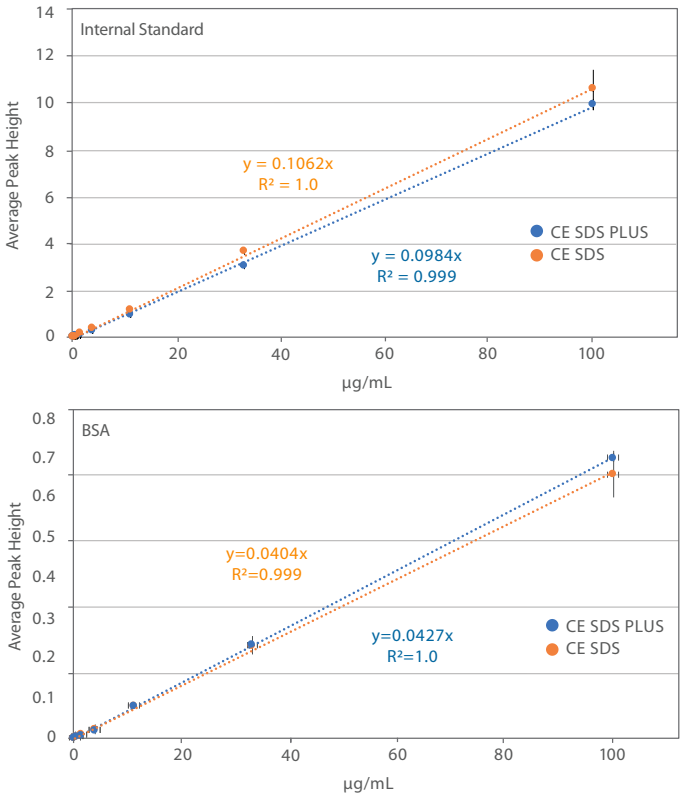

FIGURE 5. A serial dilution of the 10 kDa Internal Standard (top) and BSA (bottom) was used to demonstrate similar LODs between the CE-SDS (orange) and CE-SDS PLUS system (blue).

LIMIT OF DETECTION (LOD)

Finally, a serial dilution of the 10 kDa Internal Standard was performed to compare the LOD of the CE-SDS and CE-SDS PLUS systems and to evaluate the impact of smoothing the baseline in Compass for iCE v2.1. The Internal Standard and BSA were both diluted from 100 to 0.16 mg/µL in either CE-SDS 1X Sample Buffer or CE-SDS PLUS 1X Sample Buffer (**Figure 5**) and normalized peak heights were used

INJECTION	CE-SDS SYSTEM				CE-SDS PLUS SYSTEM			
	%LC	%NGHC	%HC	HC/LC	%LC	%NGHC	%HC	HC/LC
Average	30.7%	9.8%	59.5%	1.9	31.2%	9.6%	59.2%	1.9
Std Dev.	0.16	0.09	0.13	0.01	0.11	0.05	0.12	0.01
%RSD	0.53%	0.98%	0.23%	0.73%	0.34%	0.51%	0.20%	0.53%

TABLE 2. Quantitation summary statistics for the % peak areas and HC/LC ratio of reduced IgG using the CE-SDS and CE-SDS PLUS system. Data confirms the equivalent relative quantitation and precision of both systems.

to calculate LODs by dividing three times the standard deviation of the noise by the dilution curve slope. LODs with baseline smoothing turned on were similar between both systems with a LOD for the internal standard of 0.29 +/- 0.02 µg/mL and 0.27 +/- 0.03 µg/mL and LOD for BSA of 0.89 +/- 0.2 µg/mL and 0.88 +/- 0.07 µg/mL for the CE-SDS and CE-SDS PLUS systems, respectively (**Table 3**).

	INTERNAL STANDARD	BSA
CE-SDS	0.29 +/- 0.02 µg/mL	0.89 +/- 0.2 µg/mL
CE-SDS PLUS	0.27 +/- 0.03 µg/mL	0.88 +/- 0.07 µg/mL

TABLE 3. Calculated LODs for the 10 kDa Internal Standard and BSA are similar between the CE-SDS and CE-SDS PLUS system.

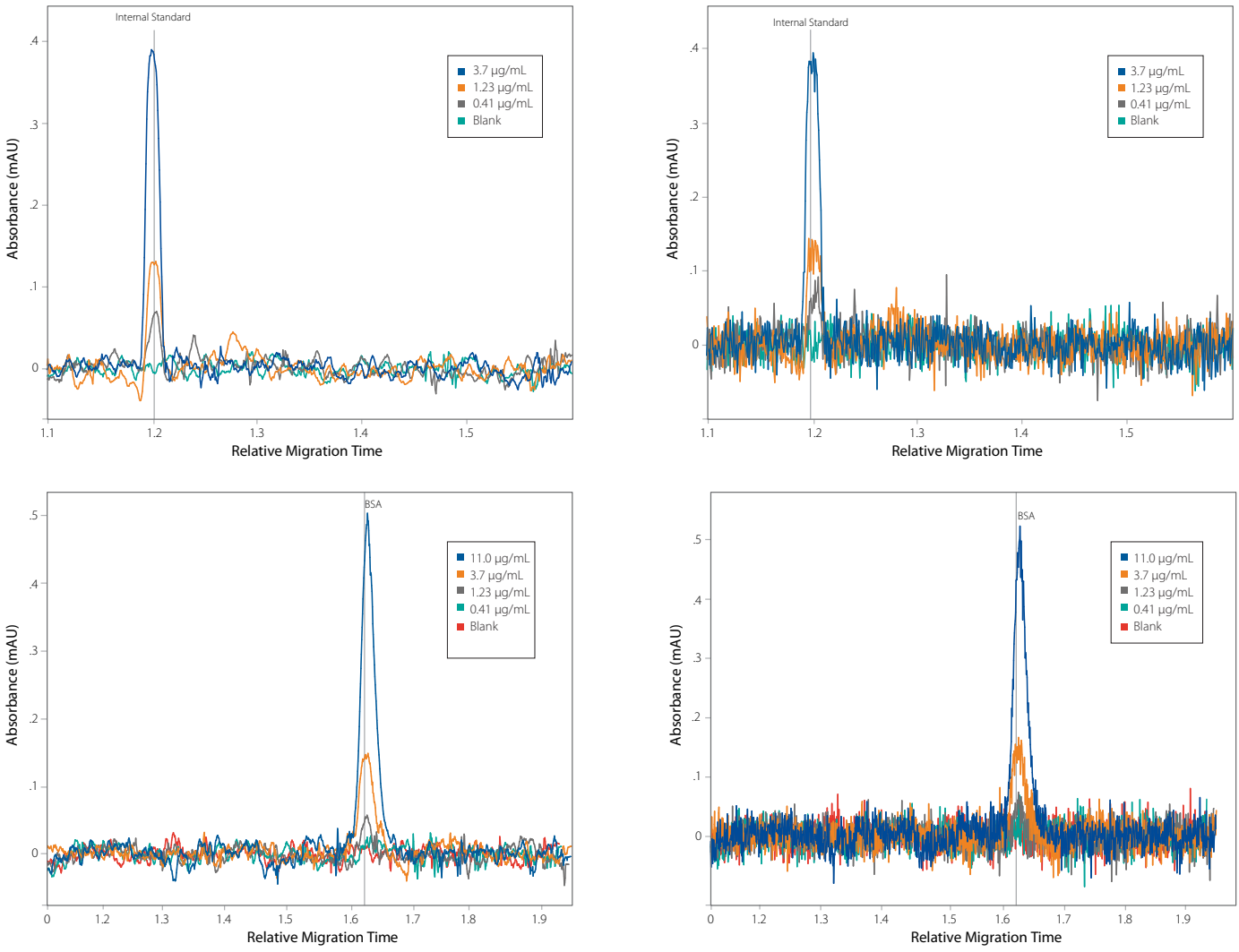

FIGURE 6. Data with baseline smoothing on (left) and off (right) for the Internal Standard (top) and BSA (bottom) exhibited a significant decrease in noise with smoothing, making it easier to detect small peaks.

	INTERNAL STANDARD		BSA	
	SMOOTHING	NO SMOOTHING	SMOOTHING	NO SMOOTHING
Noise	0.028	0.066	0.036	0.060
LOD	0.27 +/- 0.03 µg/mL	0.67 +/- 0.01 µg/mL	0.88 +/- 0.07 µg/mL	1.41 +/- 0.08 µg/mL

TABLE 4. Turning baseline smoothing on results in approximately 2X less baseline noise that translates to lower calculated LODs.

LIMIT OF DETECTION WITH AND WITHOUT BASELINE SMOOTHING

When we compared the titration data with and without baseline smoothing in Compass for iCE v2.1, an observable difference was noted between the same set of data with baseline smoothing on and off (**Figure 6**). The baseline noise, calculated as 3X the standard deviation of data points using the region in a blank sample where the molecule typically migrates, was approximately 2X lower after baseline smoothing, improving the S/N values especially for low end sample concentrations. This decrease in noise also resulted in lower LOD calculations (**Table 4**).

CE-SDS data, plus…

ABSOLUTE PEAK AREA PRECISION

Maurice's CE-SDS PLUS application delivers precise quantitation so you'll get consistent peak areas as well as % peak areas throughout a batch. To evaluate this, we ran reduced ProteinSimple IgG Standard, non-reduced ProteinSimple IgG Standard, and non-reduced Trastuzumab across 48 injections on Maurice using the CE-SDS PLUS system (**Figure 7**). To also factor in sample preparation variability in our analysis, we prepared each sample in quadruplicate and alternated injections between four sample wells in a 96-well plate. Data from all three batches were extremely precise, with absolute peak area % RSDs for triplicate capillaries run consecutively

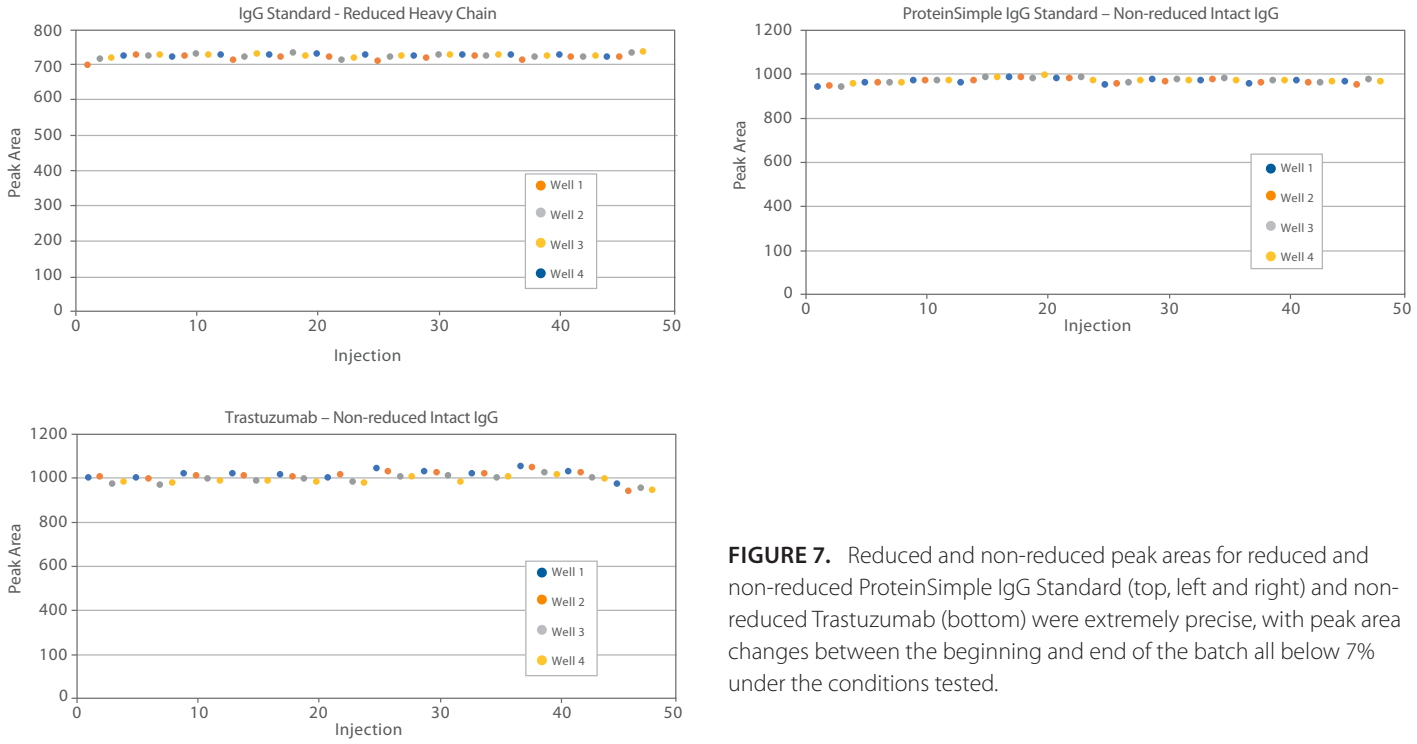

FIGURE 7. Reduced and non-reduced peak areas for reduced and non-reduced ProteinSimple IgG Standard (top, left and right) and non-reduced Trastuzumab (bottom) were extremely precise, with peak area changes between the beginning and end of the batch all below 7% under the conditions tested.

	REDUCED PROTEINSIMPLE IgG STANDARD HC	NON-REDUCED INTACT PROTEINSIMPLE IgG	NON-REDUCED INTACT TRASTUZUMAB
Average Peak Area (48 Injections)	722.5	962.8	980.0
Std Dev. (48 Injections)	6.9	11.6	23.7
%RSD (48 injections)	1.0%	1.2%	2.4%
Well 1 - % change (Injection 1 and 45)	3.5%	2.6%	-3.1%
Well 2 - % change (Injection 2 and 46)	2.6%	0.5%	-6.6%
Well 3 - % change (Injection 3 and 47)	2.5%	3.3%	-1.9%
Well 4 - % change (Injection 4 and 48)	1.6%	1.1%	-3.9%

TABLE 5. Absolute peak area precision and summary statistics for the peak area of reduced ProteinSimple IgG HC, non-reduced intact ProteinSimple IgG and non-reduced intact Trastuzumab using the CE-SDS PLUS system demonstrates system robustness throughout the batch.

all below 2.4% and % RSDs over 48 injections all <3%. Additionally, the absolute peak area for each well from the first to last injection was also extremely consistent, with percent changes all <7% (**Table 5**).

ABSOLUTE PEAK AREA LINEARITY

We also looked at the absolute peak area linearity using the CE-SDS PLUS system. A 5-point dilution curve from 1.5 to 0.5 mg/mL of the non-reduced IgG Standard was performed in CE-SDS PLUS 1X Sample Buffer and run on Maurice (**Figure 8**). Absolute peak area results were highly linear, with an R^2 of 0.995.

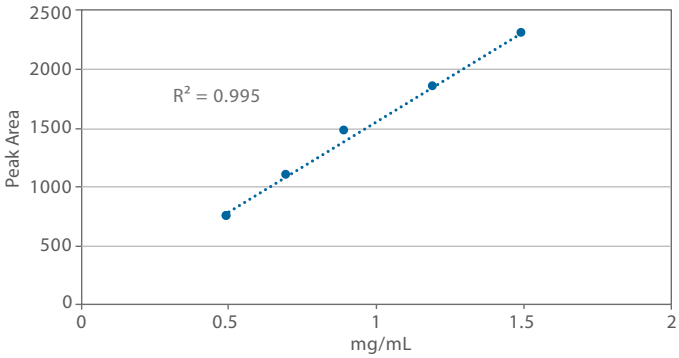

FIGURE 8. Linearity of the non-reduced IgG standard demonstrates CE-SDS PLUS linearity with an R^2 of 0.995.

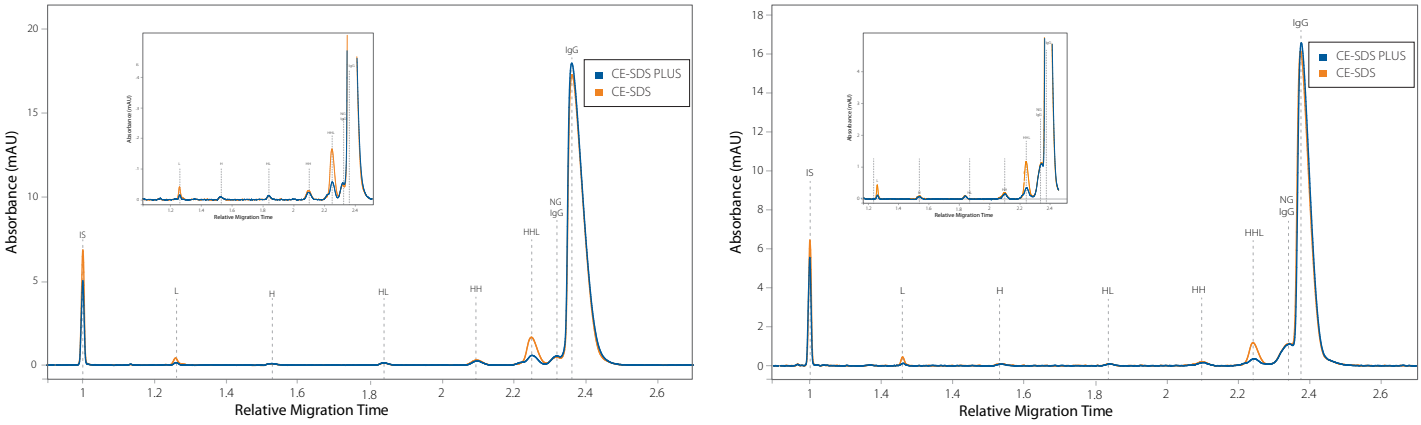

FIGURE 9. Less fragmentation was observed in non-reduced Trastuzumab (left) and Rituximab (right) prepared in CE-SDS PLUS Sample Buffer (blue) compared to samples prepared in CE-SDS Sample Buffer (orange). All samples were run on Maurice using a CE-SDS PLUS method.

LESS FRAGMENTATION WITH CE-SDS PLUS SAMPLE BUFFER

Finally, we assessed the CE-SDS PLUS 1X Sample Buffer for enhanced sample stability under non-reducing conditions as measured by reduced molecule fragmentation when analyzing IgGs on Maurice. Samples containing 1.0 mg/mL of non-reduced Trastuzumab or 1.0 mg/mL non-reduced Rituximab were prepared in either CE-SDS 1X Sample Buffer or CE-SDS PLUS 1X Sample Buffer and run on Maurice using a CE-SDS PLUS method. As expected, less fragmentation was observed with the molecules prepared in CE-SDS PLUS 1X Sample Buffer (Figure 9). There was only 6.8% fragmentation observed when Trastuzumab was prepared with the CE-SDS PLUS 1X Sample Buffer compared to 11.6% with sample prepared with CE-SDS 1X Sample Buffer. Rituximab exhibited similar behavior, with 9.6% and 14.4% fragmentation observed with the CE-SDS PLUS 1X Sample Buffer and CE-SDS 1X Sample Buffer, respectively. Thus, the CE-SDS PLUS 1X Sample Buffer can be used as a low fragmentation buffer without adversely affecting sample injection efficiency.

Conclusion

Maurice's CE-SDS system gives you the capability to monitor the purity and stability of proteins with a vastly simplified workflow. The new Maurice CE-SDS PLUS system further provides precise, absolute quantitation and higher injection numbers thanks to improvements to the cartridge, reagents, and software.

In this study, we first demonstrated system equivalency between the CE-SDS and CE-SDS PLUS system with regards to separation linearity, dynamic range, relative quantitation, and sensitivity. Both systems exhibited linear separation with R^2 of 0.992 for the CE-SDS system and 0.993 for the CE-SDS PLUS system. Both systems also had at least 2 logs of dynamic range and equivalent quantitation with % peak areas all within 0.5% and RSDs all under 1%. LODs were also equivalent with a LOD of 0.29 +/- 0.02 µg/mL and 0.27 +/- 0.03 µg/mL for the 10 kDa Internal Standard and 0.80 +/- 0.20 µg/mL and 0.88 +/- 0.07 µg/mL for BSA for the CE-SDS and CE-SDS PLUS system, respectively. Baseline smoothing also decreased data noise by approximately 2X, thereby lowering LODs approximately 2X as well.

Enhancements made to the CE-SDS PLUS system deliver robust absolute quantitation. Absolute peak area was linear with an R^2 of 0.995 and consistent with %RSDs <3% across 48 injections for three different molecules tested. Absolute peak area was also consistent between the beginning and end of a run, with % change between the first and last injections of each sample all <7%. Additionally, enhanced sample stability using the CE-SDS PLUS 1X Sample Buffer resulted in a decrease in non-reduced IgG fragmentation.

The CE-SDS PLUS system preserves the same great data you've come to expect, with further advances that enable researchers to obtain superior data consistency, significantly increased injection number per cartridge, and more accurate analysis of proteins susceptible to fragmentation.

Reference

1. Sizing-up IgG with Maurice's CE-SDS Application, *ProteinSimple Application Note*.

proteinsimple a bio-techne brand

Toll-free: (888) 607-9692
Tel: (408) 510-5500
info@proteinsimple.com
proteinsimple.com