Sizing-up IgG with Maurice's CE-SDS Application

Introduction

If you're in the biopharmaceutical industry, you're probably using monoclonal antibodies (mAbs) routinely as therapeutic products. So it's always a good thing when you can find better assessment tools like CE-SDS for product characterization and purity. Maurice, the newest member of the iCE family, takes CE-SDS to the next level by giving you way more throughput with a lot less hassle.

Maurice combines the best of two worlds: a high-resolution CE-SDS application and the gold-standard iCE data you get when analyzing molecule charge heterogeneity. Maurice's CE-SDS application gives you everything you'd expect with capillary gel electrophoresis, like automation and reproducible, high-resolution data, but simplifies things to cut your hands-on time and minimize user error. A simple workflow lets you monitor the degree of heavy chain glycosylation and degree of fragmentation in reduced and non-reduced IgG molecules quickly and easily. And Compass for iCE software lets you to set up batch and method parameters, monitor your run in real-time, and analyzes data for you in a snap.

Meet Maurice and Maurice S.

Maurice and Maurice S. use the CE-SDS Cartridge to analyze your IgG purity and heterogeneity. The cartridge lets you run 48 samples per batch and up to 100 injections in total (Figure 1). Plus set up couldn't be easier! Just prepare your samples, batch reagents, CE-SDS Cartridge and place everything in Maurice. Then set up your batch parameters in Compass for iCE and hit Start (Figure 2). Everything from cartridge prep to starting your run takes less than 10 minutes.

FIGURE 1. CE-SDS Cartridge.

You'll get baseline resolution of glycosylated and non-glycosylated reduced heavy chain in less than 35 minutes, and assay performance can't be beat! We're talking <2% RSDs, a 0.3 µg/mL LOD based on an internal standard, a 2-log dynamic range, and application linearity greater than 0.995.

At the end of the batch, just run a simple, automated clean-up that takes less than 6 minutes and you're ready for the next run.

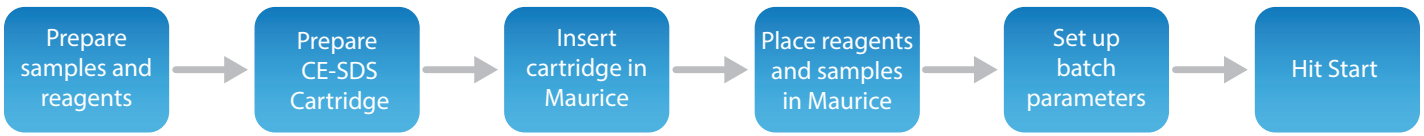

FIGURE 2. Maurice workflow. It takes less than 10 minutes to start a run once your samples and reagents are prepared.

CE-SDS application basics

BATCH REAGENTS

All the batch reagents you'll need for a run are available in our CE-SDS Application Kit (Table 1). Simply add the amount shown in Table 1 into 2 mL glass reagent vials, cap them with either a pressure or clear screw cap as noted, then put them in Maurice. Reagents with pressure caps are pressure loaded into the capillary in the CE-SDS Cartridge, and reagents with screw caps are vacuum loaded or used for capillary end washing.

REAGENT	NUMBER OF VIALS	VOLUME	CAP
Conditioning Solution 1	1	1.5 mL	Orange pressure cap
Conditioning Solution 2	1	1.5 mL	Orange pressure cap
Deionized (DI) water	1	1.5 mL	Orange pressure cap
Wash Solution	2	1.5 mL	Clear screw cap
Wash Solution	1	1.0 mL	Orange pressure cap
Separation Matrix	1	1.0 mL	Orange pressure cap
Running Buffer	1	1.0 mL	Clear screw cap
Empty (air)	1	N/A	Orange pressure cap

TABLE 1. Reagents you'll need for each CE-SDS batch.

SAMPLE PREP

CE-SDS 1X Sample Buffer (ProteinSimple), β-mercapto-ethanol (βME, Sigma PN M6250-250ML), and iodoacetamide (IAM, Sigma PN I1149-5G) were used to prepare reduced and non-reduced IgG samples. IgG samples were diluted in SDS-containing 1X Sample Buffer to a final concentration of 1 mg/mL and a final volume of 50 µL. Samples were reduced with 2.5 µL of 14.2 M βME, and non-reduced samples were alkylated with 2.5 µL of 250 µM IAM. All samples contained 2 µL of the 10 kDa 25X CE-SDS Internal Standard (ProteinSimple) that was previously reconstituted in 240 µL of 1X Sample Buffer. The prepped samples were heated in a thermocycler for 10 minutes at 70 °C. Each 50 µL sample was then transferred to the wells of a 96-well plate and spun at 1000 xg for 10 minutes before placing in Maurice. Samples can also be transferred to sample vials with integrated inserts (ProteinSimple) if you're only running a few samples.

An optional IgG Standard (ProteinSimple) is available as a positive control sample. The IgG Standard comes lyophilized and was reconstituted in 50 µL of 1X Sample Buffer. 2 µL of 25X Internal Standard was added to each sample, and reduced or non-reduced IgG Standard samples were prepared as described prior before heating at 70 °C for 10 minutes.

Optional Molecular Weight (MW) Markers (ProteinSimple) are also available if you're interested in determining the molecular weight of the IgG heavy chain and light chain, or of any IgG fragments detected. MW Markers come lyophilized and are prepared by reconstituting with 50 µL of 1X Sample Buffer and then adding 2 µL of 25X Internal Standard and 2.5 µL of βME before heating at 70 °C for 10 minutes.

CARTRIDGE PREP AND MAINTENANCE

CE-SDS Cartridge preparation is easy. All you have to do is load a single-use Top Running Buffer vial in the cartridge, insert the cartridge in Maurice and you're ready to go.

Once the run's done, if you're going to start another experiment within 2 hours all you have to do is insert a new Top Running Buffer vial in the cartridge before you hit **Start**. If you're done for the day, just run the 6-minute automated cartridge clean-up procedure and then store the cartridge in its original packaging. And did we mention all waste is contained in the Top Running Buffer vial for safe handling of hazardous samples?

SEPARATION

The CE-SDS Cartridge contains a 50 µm ID capillary with 15 cm of separation length made of bare fused silica. Maurice conditions the capillary at the beginning of the run with Conditioning Buffer 1 (base), Conditioning Buffer 2 (acid), DI water and the Separation Matrix. That makes sure the capillary surface is uniformly charged, so you get optimal resolution and assay reproducibility. Capillaries are then conditioned every 12 injections to maintain surface integrity.

Samples are electrokinetically injected into the cartridge capillary by applying voltage for 20 seconds at 4600 V before samples are electrophoresed at 5750 V. Separation is monitored in real time in seconds as peaks pass a single-point detector. The peak relative migration time (RMT) compared to the 10 kDa Internal Standard is reported by

Compass for iCE after the separation is finished. For those who prefer not to add the 10 kDa Internal Standard to their sample, Compass for iCE will also let you determine peak RMTs in relation to any peak you designate. Reduced IgG samples, non-reduced IgG samples, and the MW Markers were separated for 25, 35, and 30 minutes respectively, to optimize sample resolution. At the end of the run, all data was analyzed using Compass for iCE.

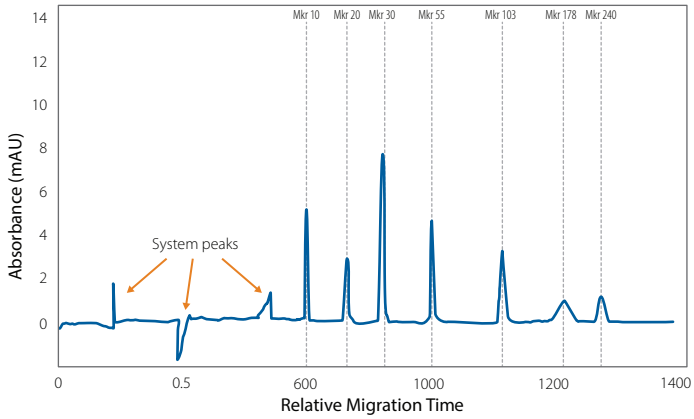

FIGURE 3. Electropherogram of the MW Markers which includes recombinant proteins at 10, 20, 33, 55, 103, 178, and 240 kDa. Samples were reduced with βME and heat denatured at 70 °C for 10 minutes before separating on Maurice for 30 minutes.

The separation linearity you need

Proteins are separated in the capillary using an entangled polymer separation matrix. The MW Markers consist of seven recombinant proteins, including the 10 kDa Internal Standard, which gave us a range from 10 to 240 kDa to demonstrate the CE-SDS Separation Matrix resolution (Figure 3). All proteins were baseline resolved in only 30 minutes, and plotting the Log MW vs. 1/Mobility (RMT) indicated the separation was extremely linear across the molecular weight range with a R^2 of 0.9924 (Figure 4).

Running the MW Markers in your batch is optional. But, if you include them, as an added bonus Compass for iCE will automatically assign a molecular weight to the different IgG peaks for you.

Baseline-resolve those immunoglobulins

Current default methods for the Maurice CE-SDS application are optimized for resolution and quantitation of reduced and non-reduced IgGs (Figure 5). The reduced IgG method gives you fast, consistent baseline resolution of the non-glycosylated heavy chain and heavy chain in only 25 minutes. The non-reduced IgG method is

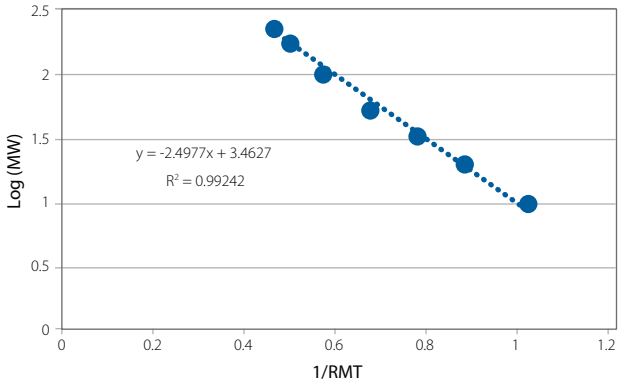

FIGURE 4. CE-SDS Separation Matrix linearity based on the mobility of the MW Markers. The separation was linear with an R^2 of 0.9924.

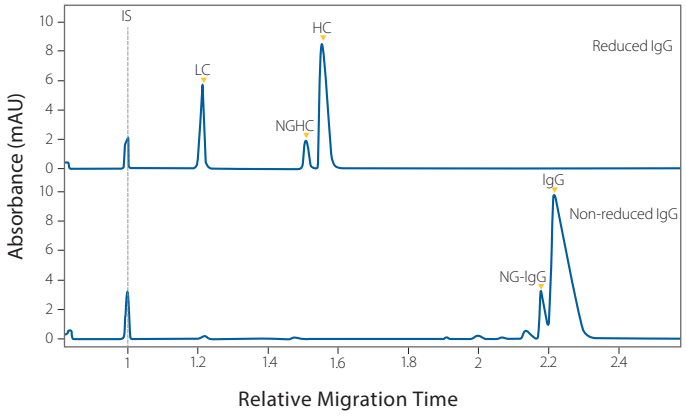

FIGURE 5. Separation of the reduced (top) and non-reduced (bottom) Maurice IgG Standard with the 10 kDa Internal Standard (IS). The IgG standard was reconstituted to 1 µg/mL with 1X Sample Buffer containing SDS. Samples were reduced with βME and non-reduced samples alkylated with IAM before heat denaturing at 70 °C for 10 minutes. Reduced and non-reduced samples were separated for 25 and 35 minutes, respectively.

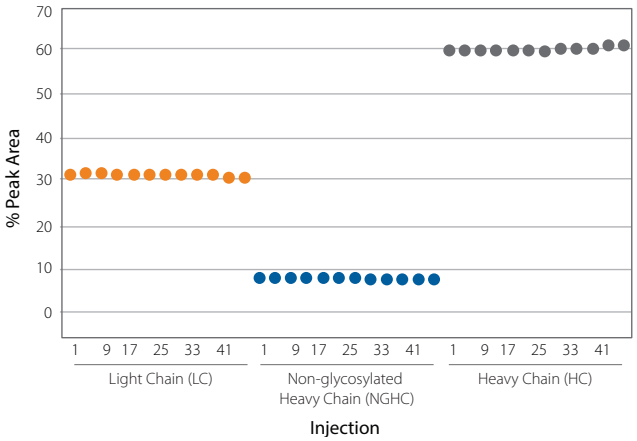

FIGURE 6. %Peak area of reduced light chain (orange), non-glycosylated heavy chain (blue) and heavy chain (grey) across 48 injections with <2.5% RSDs for all peaks. Data from 12 injections shown.

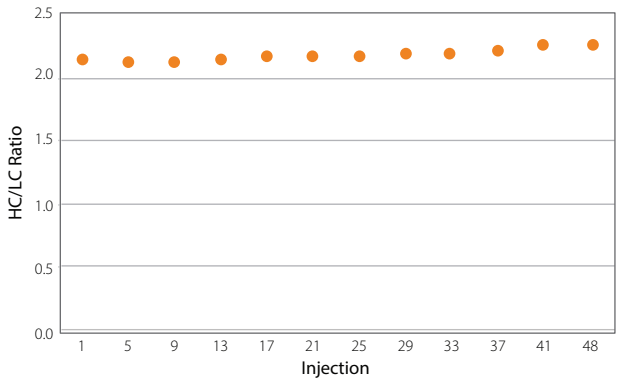

FIGURE 7. Heavy chain to light chain ratio of the reduced IgG across 48 injections with <2.0% RSDs for all peaks. Data from 12 injections shown.

INJECTION	% LC	% NGHC	% HC	HC/LC
1	31.7	8.2	60.1	2.2
5	31.9	8.2	60.0	2.1
9	31.9	8.1	60.0	2.1
13	31.7	8.2	60.1	2.2
17	31.7	8.2	60.2	2.2
21	31.7	8.1	60.2	2.2
25	31.5	8.3	60.1	2.2
29	31.5	8.0	60.5	2.2
33	31.4	7.8	60.8	2.2
37	31.3	7.9	60.7	2.2
41	30.8	7.8	61.5	2.3
48	30.7	8.0	61.3	2.3
Average	31.5	8.0	60.5	2.2
Std. Dev.	0.35	0.19	0.49	0.04
%RSD	1.1	2.4	0.8	1.8

TABLE 2. Quantitation and summary statistics for the % peak area and HC/LC ratio of reduced IgG shows the precision of the CE-SDS application.

optimized for IgG fragment detection and only requires a 35 minute separation for resolution of all IgG fragments and glycosylated/non-glycosylated intact IgG.

Top-notch precision

A reduced IgG Standard was run across 48 injections, and 12 of the injections were analyzed with Compass for iCE to demonstrate application precision. The average percent light chain (LC), non-glycosylated heavy chain (NGHC), and glycosylated heavy chain (HC) was 31.5%, 8.0%, and 60.5% respectively with RSDs less than 2.5% for all peaks (Figure 6, Table 2). The HC/LC ratio was 2.2 with an RSD of 1.8% (Figure 7, Table 2). Data quality stayed consistent over the course of the entire batch, so you'll get the same great data at injection 1 and injection 48.

Non-reduced IgG Standard was also run across 48 injections, and 12 injections were used for quantitation. The average percent of low molecular weight (LMW) fragments running in front of the NGHC was 5.1%, while the amount of NGHC and IgG monomer present was 9.9% and 85.0% respectively, indicating the sample contained 95% intact IgG (Figure 8, Table 3). RSDs were all below 3% (Table 3). And like the reduced IgG sample, data quality stayed consistent across the entire batch.

Different concentrations of the reduced IgG Standard were also run to confirm peak areas stay consistent over a range of sample concentrations. Samples were titrated 1:2 in 1X Sample Buffer from 1.0 mg/mL down to 0.062 mg/mL, then injected eight times in a single batch. The average percent peak area was calculated for the light chain, non-glycosylated heavy chain, and glycosylated heavy chain

INJECTION	% LMW FRAGMENT	% NGHC	% IgG MONOMER
1	5.0	9.7	85.3
5	5.0	9.9	85.1
9	5.1	10.1	84.8
13	5.2	9.9	84.9
17	5.0	9.6	85.4
21	5.0	10.1	85.0
25	5.3	9.6	85.1
29	5.2	9.8	85.0
33	5.3	9.9	84.8
37	5.0	10.1	85.0
41	5.1	10.1	84.8
48	48	10.0	85.3
Average	5.1	9.9	85.0
Std. Dev.	0.2	0.2	0.2
%RSD	3.0	1.7	0.2

TABLE 3. Quantitation and summary statistics for non-reduced IgG shows the precision of the CE-SDS application.

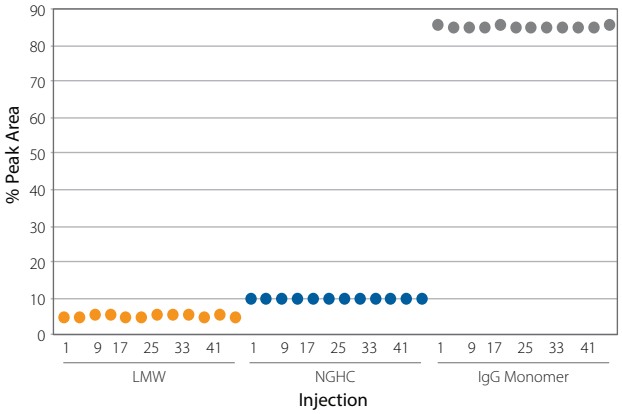

FIGURE 8. %Peak area of non-reduced IgG. LMW fragments (orange), non-glycosylated heavy chain (blue) and heavy chain (grey) across 48 injections with <3% RSDs for all peaks. Data from 12 injections shown.

IgG CONCENTRATION	AVERAGE %LC	AVERAGE %NGHC	AVERAGE %HC
0.062 mg/mL	29.3%	7.2%	62.5%
0.125 mg/mL	29.8%	7.1%	62.9%
0.25 mg/mL	30.1%	6.7%	62.9%
0.5 mg/mL	30.7%	7.1%	62.3%
1 mg/mL	30.5%	7.1%	62.2%

TABLE 4. Quantitation summary for the %peak area measured across five different concentrations of reduced IgG Standard.

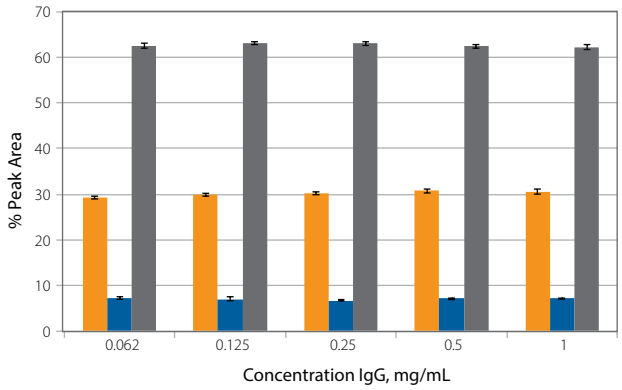

FIGURE 9. The %peak area of reduced light chain (LC), non-glycosylated heavy chain (NGHC), and heavy chain (HC) across different concentrations demonstrates consistent quantitation for the CE-SDS application.

INJECTION	LC	NGHC	HC
1	1.216	1.510	1.554
5	1.216	1.508	1.553
9	1.217	1.509	1.555
13	1.216	1.508	1.554
17	1.216	1.509	1.555
21	1.216	1.509	1.555
25	1.217	1.510	1.557
29	1.216	1.510	1.557
33	1.216	1.508	1.555
37	1.217	1.509	1.556
41	1.216	1.506	1.554
48	1.216	1.505	1.553
Average	1.216	1.508	1.555
Std. Dev.	0.001	0.002	0.001
%RSD	0.037	0.104	0.086

TABLE 5. Summary statistics for the reduced IgG RMT across 48 injections demonstrates consistent peak migration. Data from 12 injections shown.

(Figure 9). %Peak areas were all within 1% of each other across the different concentrations (Table 4).

The migration time of the LC, NGHC, and HC relative to a 10 kDa Internal Standard was also extremely consistent. Relative migration time RSDs of 12 injections taken over the course of a 48-injection batch were all ≤0.1 %, showing sample migration was the same at the beginning and at the end of the run (Figure 10, Table 5).

Robustness you can't beat

To test system robustness, five different analysts ran the reduced IgG Standard on five different Maurice systems with five different cartridges. Only the reagent and IgG Standard lots were kept consistent. Each analyst prepped their own samples before running them on their Maurice and independently decided which injections to analyze. The results across instruments and cartridges were incredibly precise, with relative migration time RSDs for the LC, NGHC, and HC all less than 0.2% (Table 6). And %peak

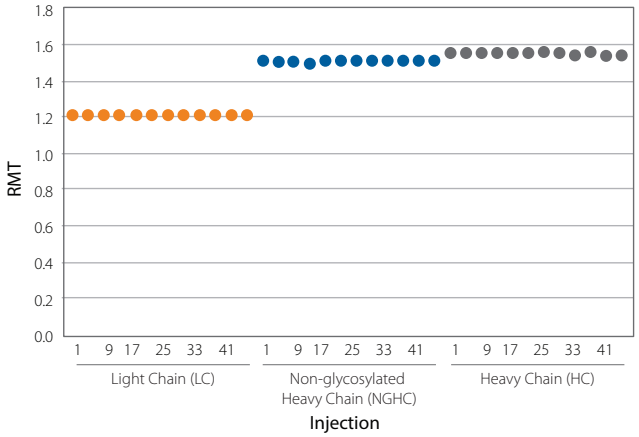

FIGURE 10. Relative migration of reduced IgG light chain (orange), non-glycosylated heavy chain (blue) and heavy chain (grey) across 48 injections with <0.1% RSDs for all peaks. Data for 12 injections shown.

area RSDs for the LC, NGHC, and HC were 1.4%, 3.5%, and 0.5% respectively (Table 7). This means you'll always get the same great data without analyst, instrument, injection or cartridge bias. All five analysts generated equivalent data, so you'll easily be able to transfer methods between users and labs too.

Compass for iCE also actively monitors the current in the capillary during separation. If the current drops due to a bubble, the software stops the separation and automatically re-injects the sample for you. This minimizes the number of potential failed injections per run, and saves you from having to re-run samples. Reinjections are an optional feature, so if you don't want to use them just turn them off when setting up your batch.

Spot-on quantitation linearity

The linearity of the CE-SDS application was determined by serially diluting either the 10 kDa Internal Standard or BSA (Figure 11). Samples were diluted 5-fold from 100 to 0.16 µg/mL using 1X Sample Buffer, and linearity was determined using peak area versus concentration. The dynamic range for both samples was at least 2 logs with an R^2 of at least 0.9998.

Solid sensitivity

Serial dilutions of the 10 kDa Internal Standard were also used to determine the LOD and LOQ of the CE-SDS application (Figure 12). The Internal Standard was diluted

ANALYST	INSTRUMENT #	CARTRIDGE LOT #	INJECTION	LC RMT	NGHC RMT	HC RMT
1	KF1	3151231271	2	1.217	1.511	1.559
			4	1.217	1.511	1.559
			6	1.217	1.512	1.56
			7	1.218	1.511	1.559
			8	1.216	1.51	1.558
			9	1.217	1.51	1.558
2	KF2	3151231258	2	1.218	1.514	1.561
			3	1.218	1.515	1.561
			4	1.217	1.515	1.562
			6	1.215	1.507	1.552
			7	1.215	1.506	1.552
			8	1.217	1.512	1.559
3	KF3	3151231259	2	1.217	1.514	1.561
			4	1.216	1.512	1.558
			6	1.216	1.513	1.561
			8	1.216	1.513	1.561
			10	1.217	1.513	1.56
			12	1.216	1.512	1.559
4	KF4	3151231276	2	1.216	1.517	1.565
			4	1.216	1.517	1.565
			6	1.216	1.515	1.564
			8	1.215	1.514	1.562
			10	1.215	1.513	1.562
			12	1.215	1.513	1.561
5	KF5	3151231275	2	1.218	1.515	1.563
			3	1.217	1.514	1.561
			4	1.217	1.512	1.56
			6	1.217	1.513	1.56
			7	1.217	1.511	1.559
			8	1.217	1.511	1.558
			Average	1.217	1.513	1.560
			Std. Dev.	0.000	0.002	0.003
			%RSD	0.078	0.162	0.186

TABLE 6. Summary of the RMT data and statistics across multiple instruments, multiple cartridges, and multiple users demonstrates robust protein migration with Maurice.

ANALYST	INSTRUMENT #	CARTRIDGE L/N	INJECTION	% LC	% NGHC	% HC
1	KF1	3151231271	2	30.9	8.2	60.9
			4	30.9	8.3	60.8
			6	30.9	8.5	60.6
			7	31	8.5	60.5
			8	31.1	8.5	60.4
			9	31.1	8.7	60.2
2	KF2	3151231258	2	30	8.7	61.3
			3	30.9	8	61.1
			4	30.5	8.7	60.8
			6	31.3	8.2	60.5
			7	31.2	8.1	60.6
			8	30.5	9	60.5
3	KF3	3151231259	2	30.5	8.6	60.8
			4	30.7	8.8	60.4
			6	30.7	8.8	60.5
			8	30.7	9	60.3
			10	30.9	9	60.1
			12	31	8.8	60.2
4	KF4	3151231276	2	29.9	8.9	61.1
			4	29.8	8.9	61.3
			6	30.2	8.8	61
			8	30.1	8.9	61
			10	30.4	8.9	60.7
			12	30	9.2	60.7
5	KF5	3151231275	2	29.9	8.8	61.3
			3	30.7	8.5	60.8
			4	30.8	8.5	60.7
			6	30.9	8.5	60.6
			7	30.8	8.6	60.6
			8	30.6	8.6	60.7
			Average	30.6	8.7	60.7
			Std. Dev.	0.4	0.3	0.3
			%RSD	1.4	3.3	0.5

TABLE 7. Summary of %peak area data and statistics across multiple instruments, multiple cartridges, and multiple users demonstrates Maurice's quantitation is incredibly robust.

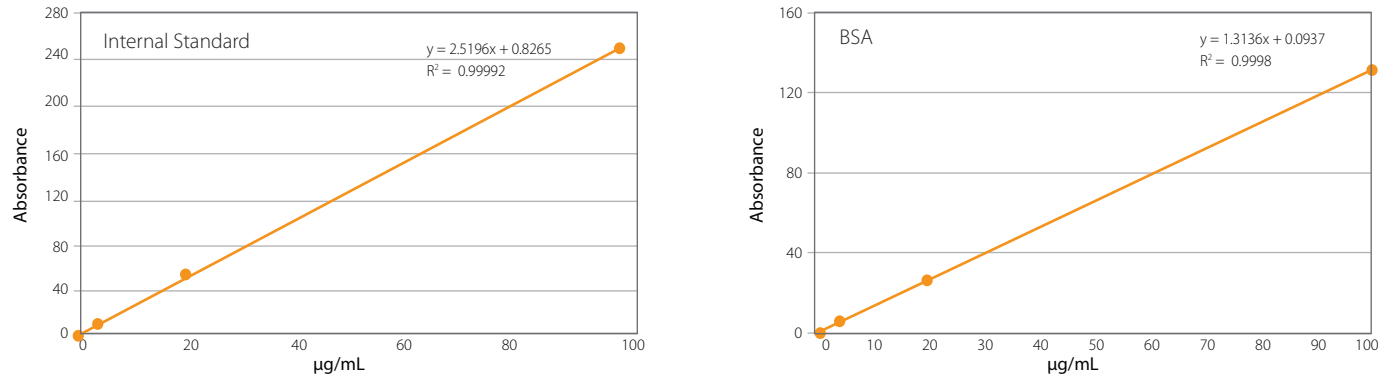

FIGURE 11. Maurice's CE-SDS application had a dynamic range of at least 2 logs with an R^2 of 0.9998 or greater when using either the Internal Standard (left) or BSA (right) as the model system.

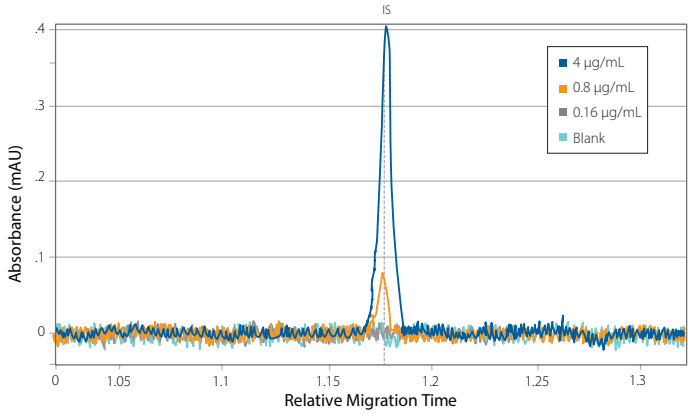

FIGURE 12. A serial dilution (0.16-100 µg/mL) of the 10 kDa Internal Standard determined a CE-SDS application LOD of 0.21 µg/mL and an LOQ of 0.71 µg/mL. Samples were diluted in 1X Sample Buffer and then reduced with βME before heating at 70 °C for 10 minutes.

from 100 to 0.16 µg/mL, and baseline noise was calculated using the area adjacent to the peak at the highest internal standard concentration. The LOD was calculated by dividing three times the standard deviation of the noise by the dilution curve slope, and the LOQ was calculated by multiplying the standard deviation of the noise by 10 and dividing by the dilution curve slope. This resulted in an Internal Standard LOD and LOQ of 0.21 µg/mL and 0.71 µg/mL, respectively.

Conclusion

Maurice's CE-SDS application lets you monitor purity of your IgGs in a simple, quick, and robust format. After you've prepped your samples, all it takes is 10 minutes to get your batch going. And Maurice sets up your batch, runs your samples, and Compass for iCE automatically analyzes all the data for you. Plus it'll minimize the number of failed injections with its handy reinjection feature.

We tested peak separation on seven proteins and showed that separation is linear with an R^2 of 0.9924 when plotting migration time against molecular weight. Maurice's CE-SDS application is also linear across 2 logs of protein concentration, gives you the sensitivity you need at <1.0 µg/mL, and precision numbers come in at 2.5% or less every time. You'll also get baseline resolution of non-glycosylated and glycosylated heavy chains in just 2 minutes with fantastically reproducible RSDs under 2.4%. Not to mention peak percentages and relative migration times stayed consistent from the beginning of the batch to the end with RSDs at 1.4% and 0.2%. And you'll get that the same great data across different IgG concentrations, different instruments, different cartridges and different analysts. Can't beat that!

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