

POWERING UP MAURICE WITH WATERS™ EMPOWER® SOFTWARE



INTRODUCTION

Maurice is your one-stop cIEF and CE-SDS platform that delivers high-resolution, reproducible identity, purity, and heterogeneity analysis of biotherapeutic proteins. The simple, automated workflow is hassle-free and reduces hands-on time to minimize potential sources of error and user-to-user variability. Maurice can be controlled using Compass for iCE software, but is also supported by Waters™ Empower® 3 Chromatography Data Software for seamless integration into your laboratory environment.

In this application note, we'll take you through the data acquisition and data analysis for a monoclonal antibody, using an Empower® controlled Maurice. We'll also introduce the new ability to view your cIEF data as a 3D spectrum, giving you a comprehensive understanding of how proteins focus in a single image.

GETTING STARTED

You'll need Empower® 3 FR2 SR2 (Win7), Empower® 3 FR4 or FR5 (Win10), Waters™ LAC/E32 server with Empower® system control license, and a Maurice instrument connected directly to the Waters™ LAC/E32 server to control your Maurice with Empower®. You'll also want to confirm that the instrument software included in the Maurice Empower® Control kit (ProteinSimple, 104-0040) is installed on your Maurice system. Check out the "Maurice with Empower® Installation Guide" for instructions on how to set everything up.

Once your Maurice is Empower® control enabled, just prepare your samples and consumables as described in the appropriate Maurice Product Insert, and create a sample set using the Sample Set Generator (FIGURE 1). More details on how to create a cIEF or CE-SDS method, monitor a batch, pause a batch to edit a method, clean or purge a cartridge, and view your data using Empower® can be found in the "Maurice Empower® Control Software User Guide".

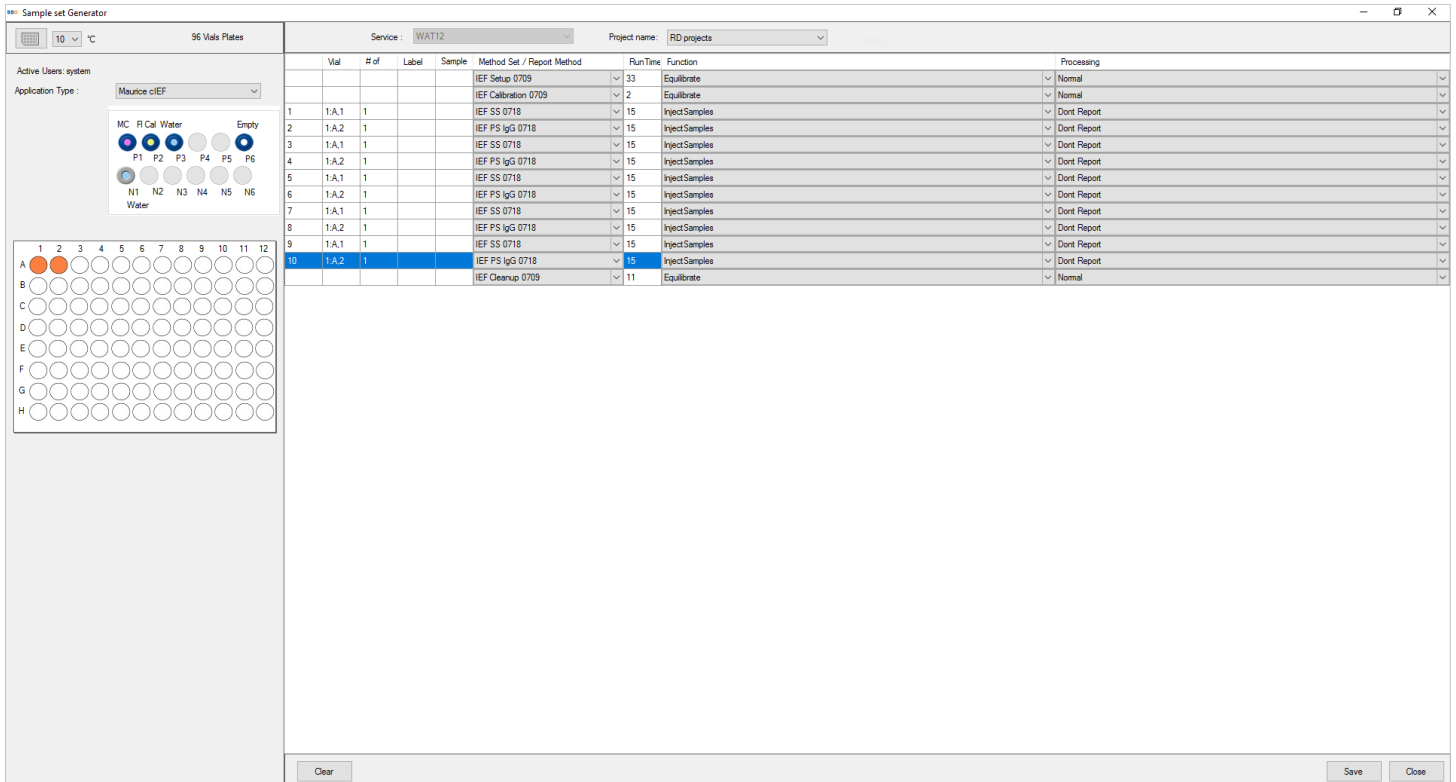


FIGURE 1. Maurice cIEF method created using Empower® software.

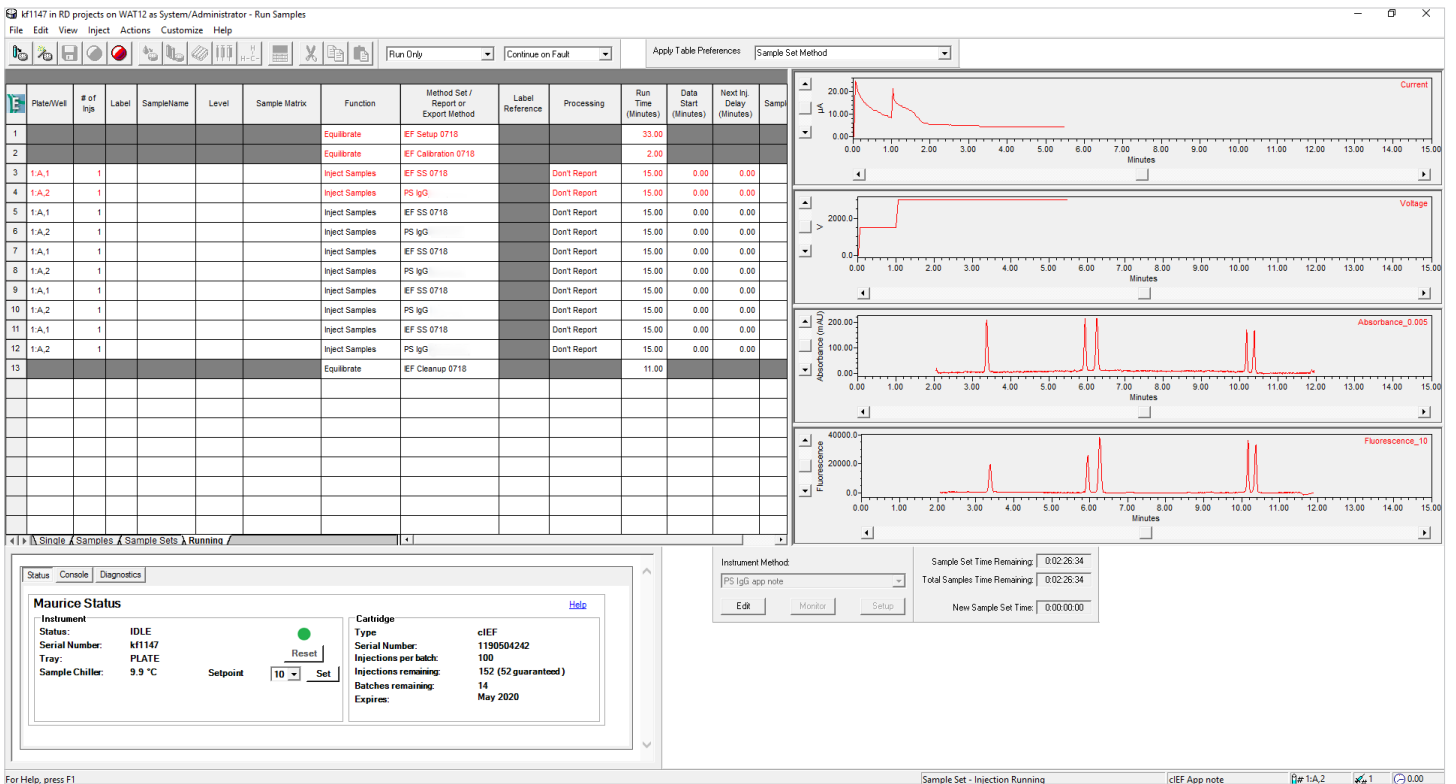


FIGURE 2. cIEF separation of the System Suitability monitored in real time using Empower® software. Focusing voltage, current, absorbance can be monitored in real time, and fluorescence images are available for viewing for completed injections.

RUNNING cIEF METHODS

MONITORING AND VIEWING cIEF DATA

Samples analyzed using a Maurice cIEF method were detected using absorbance and, for higher or elevated sensitivity, native fluorescence. The Maurice cIEF System Suitability (ProteinSimple, 046-044) was prepared as described by the Product Insert and run using the default System Suitability method. Additionally, 0.1 mg/mL of the IgG Standard (ProteinSimple, 046-039) was prepared with pI markers 4.05 and 8.40 in 2% 3-10 Pharmalyte/2% 5-8 Pharmalyte, 10 mM iminodiacetic acid, 10 mM arginine, and 2 M urea. The IgG standard was run as 10 replicate injections for each batch and focused for 1 minute at 1500 V followed by 8 minutes at 3000 V. The samples were run on both a Maurice controlled

by Empower® and a Maurice controlled by Compass for iCE. The sample separation on the Maurice controlled by Empower® was monitored in real time in the Empower® "Run Samples" window (FIGURE 2).

The focus series can also be visualized spatially using a 3D spectral plot (FIGURE 3). This allows you to see when peaks reach optimal focusing in one image instead of comparing a series of 2D image slices taken during the separation every 10 seconds. Plots appear with time on the x-axis, absorbance units on the y-axis, and pixel position on the z-axis.

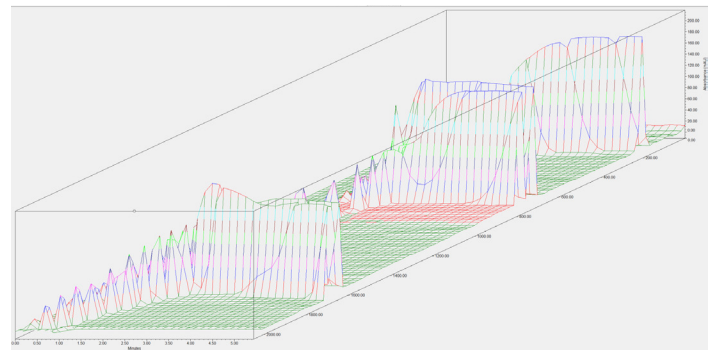
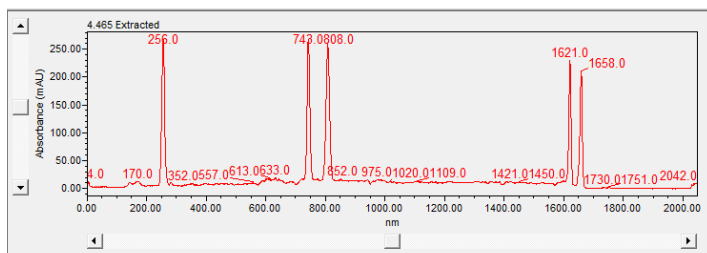


FIGURE 3. Spectrum data for the System Suitability in 2D (left) and 3D (right). The 3D data help you see when a peak reaches focus in one 3D image, instead of comparing a series of 2D images take every 10 seconds during the sample separation.

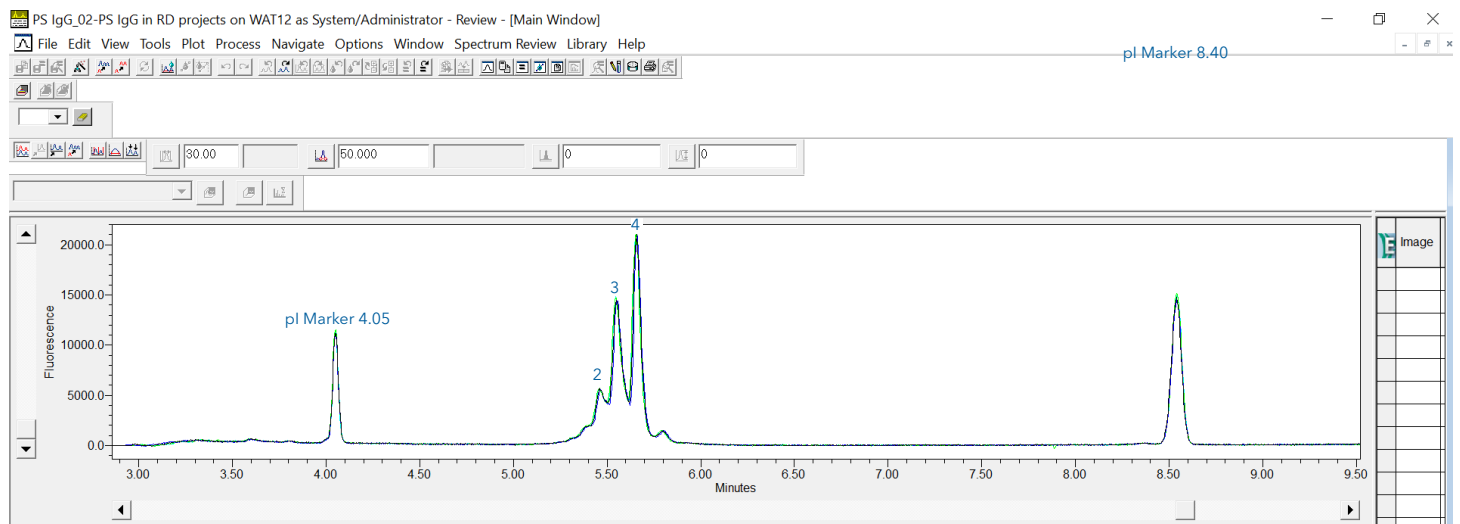


FIGURE 4. An overlay of three consecutive cIEF injections of the IgG Standard visually demonstrates the data reproducibility for samples run on an Empower® controlled Maurice.

INJECTION	EMPOWER® % COMPOSITION (FLUORESCENCE)			COMPASS FOR ICE % COMPOSITION (FLUORESCENCE)		
	PEAK 2	PEAK 3	PEAK 4	PEAK 2	PEAK 3	PEAK 4
1	13.76	36.19	43.48	13.88	36.82	43.72
2	13.82	36.25	42.90	13.95	36.61	43.40
3	12.94	37.47	44.08	14.10	36.35	43.76
4	13.44	36.67	42.54	14.00	37.47	43.75
5	13.98	36.58	43.33	13.69	37.86	44.38
6	13.56	36.11	42.60	13.78	37.57	43.75
7	13.13	37.07	44.00	13.81	36.72	44.70
8	13.75	35.45	42.89	14.17	36.43	43.14
9	12.88	36.87	42.93	13.87	36.94	43.87
10	13.59	36.74	44.05	14.43	37.78	43.36
Mean	13.48	36.54	43.28	13.97	37.06	43.78
% RSD	2.83	1.55	1.38	1.57	1.52	1.06

TABLE 1. Data summary of IgG Standard peaks with a percent composition >10%, run on either an Empower® controlled Maurice or Compass for iCE controlled Maurice. The data was analyzed with Empower® and proved the data was consistent between both software platforms, and within expected run-to-run and sample prep variance, with percent peak compositions all within 0.5%, and reproducible with CVs < 2.9%.

REPRODUCIBLE AND EQUIVALENT CIEF RESULTS

The data generated both in Empower® and Compass for iCE were analyzed to evaluate data reproducibility. An overlay of three consecutive injections of the IgG Standard in Empower® software (FIGURE 4) and quantification of the data demonstrated data consistency, with CVs all under 2.9% for 10 injections (TABLE 1). The quantitation also established the equivalency in data generated using an Empower® controlled Maurice and a Compass for iCE

controlled Maurice as the percent peak composition for peaks with a percent composition above 10% were all within 0.5%, well within expected run-to-run and sample prep variance. (TABLE 1). The observed pI between data collected on both systems further proved the data equivalency as the average pI for all peaks were within 0.02 pI units (TABLE 2).

AVERAGE pI, (N=10)		
PEAK	EMPOWER® (% CV)	COMPASS FOR ICE (% CV)
1	5.42 (0.18%)	5.40 (0.18%)
2	5.46 (0.08%)	5.46 (0.04%)
3	5.55 (0.09%)	5.55 (0.04%)
4	5.66 (0.08%)	5.66 (0.04%)
5	5.80 (0.14%)	5.79 (0.07%)

TABLE 2. IgG Standard pI values, with %CVs in parenthesis, reported in runs controlled by either Empower® or Compass for iCE. Data was analyzed using Empower®.

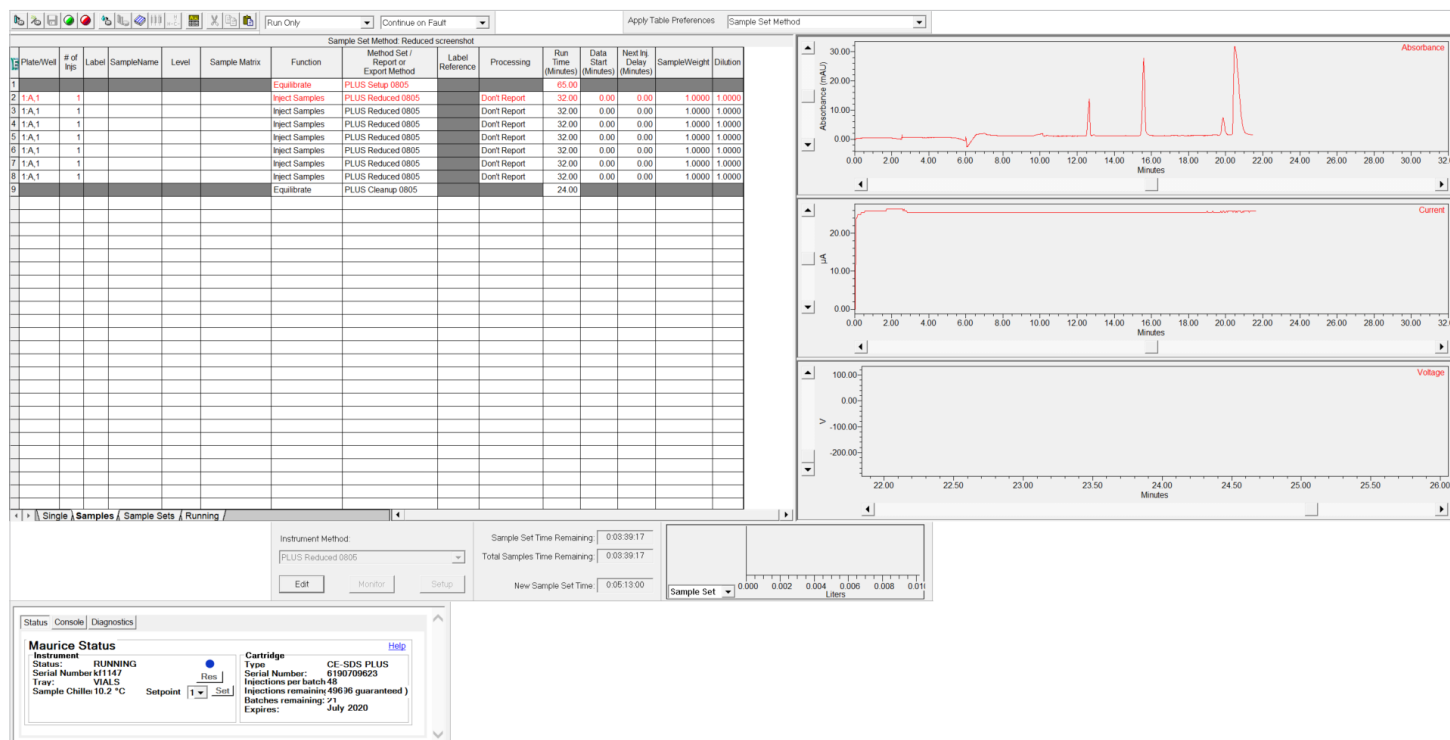


FIGURE 5. CE-SDS separation of the ProteinSimple IgG Standard monitored in real time using Empower® software.

RUNNING CE-SDS METHODS

MONITORING AND VIEWING CE-SDS DATA

1.0 mg/mL of reduced ProteinSimple IgG Standard was prepared as recommended by the ProteinSimple CE-SDS IgG Standard Product Insert and run as 10 replicates on an Empower® controlled Maurice and a Compass for iCE controlled Maurice. The samples were separated for 25 minutes at 5750 V and sample separations were monitored in real time in the Empower® "Run Samples" window (FIGURE 5).

REPRODUCIBLE AND EQUIVALENT CE-SDS RESULTS

An overlay of three consecutive injections of the IgG Standard using Empower® software visually establishes the data reproducibility achieved with Maurice (FIGURE 6). Quantification of the data further confirms the data consistency, as all CVs were under 1.0% when samples were run via Empower or Compass for iCE (TABLE 3).

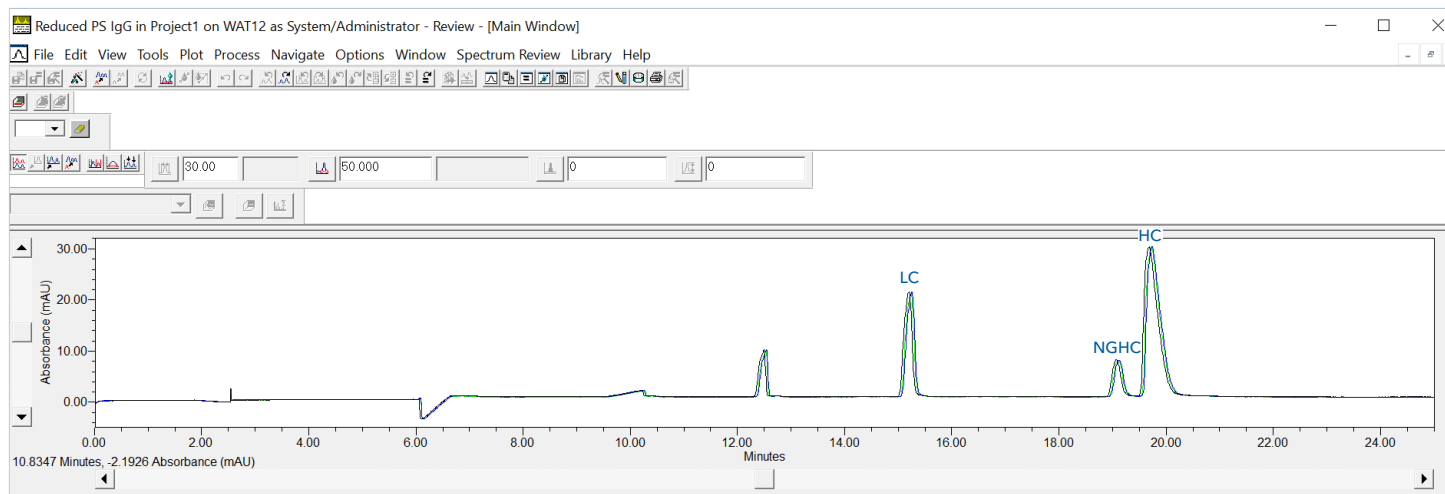


FIGURE 6. An overlay of three consecutive CE-SDS injections of reduced IgG Standard demonstrates the data reproducibility for samples run on an Empower® controlled Maurice.

INJECTION	EMPOWER®			COMPASS FOR iCE		
	LC % PEAK AREA	NGHC % PEAK AREA	HC % PEAK AREA	LC % PEAK AREA	NGHC % PEAK AREA	HC % PEAK AREA
1	24.09	8.96	58.51	24.55	8.87	57.92
2	24.05	9.00	58.53	24.40	8.88	58.09
3	24.15	8.98	58.35	24.30	8.92	58.18
4	24.14	8.93	58.44	24.36	8.90	58.22
5	24.01	8.95	58.36	24.31	8.88	58.26
6	24.07	8.99	58.48	24.31	8.87	58.29
7	24.09	8.98	58.49	24.39	8.87	58.11
8	24.05	8.97	58.45	23.97	8.92	58.66
9	23.76	8.86	59.04	24.35	8.91	58.20
10	24.08	8.99	58.54	24.24	8.88	58.33
Mean	24.05	8.96	58.52	24.32	8.89	58.23
% RSD	0.46	0.46	0.33	0.61	0.23	0.33

TABLE 3. Data summary for the % peak area of reduced ProteinSimple IgG Standard run on either an Empower® controlled Maurice or Compass for iCE controlled Maurice. The data was analyzed with Empower® and is reproducible with CVs < 0.7%, and equivalent between both software platforms with percent peak areas all within 0.3%.

Using Empower® to analyze runs controlled by either software demonstrates data equivalency as the percent peak areas for the reduced light chain (LC), non-glycosylated heavy chain (NGHC), and heavy chain (HC) were all within 0.3% (TABLE 3). All the data were reproducible, with CVs all less than 0.7%.

CONCLUSION

Characterizing biotherapeutics with Maurice is simple and straight-forward. All you have to do is prepare your samples and reagents, load a cIEF or CE-SDS cartridge depending on the application you're running, and set-up your method parameters. Maurice does everything else, giving you precise and reliable results. Your cIEF and CE-SDS data is ready in just 10 or 35 minutes per injection, respectively, so you'll have protein drug quality and identity answers quickly.

Maurice can now be operated using Waters™ Empower® software, which provides seamless control of Maurice systems with all key functions preserved and with full regulatory compliance, including 21 CFR Part 11 controls, for industry-leading security and data integrity. In this study, we characterize the ProteinSimple IgG Standard using methods set-up in either Empower® or Compass for iCE. All cIEF and CE-SDS data was analyzed in Empower® and then evaluated for data reproducibility and quantification between a Maurice controlled by Compass for iCE or Empower®.

Quantification of the cIEF and CE-SDS data using Empower® showed excellent robustness as CVs were less than 2.9% and 0.7%, respectively. When the injections were performed using Compass for iCE, the results were very consistent to those generated when using Empower®, confirming you'll get the same answer no matter which software platform you use to run Maurice. The runs from each software platform reported pIs that were within 0.02 units of each other and cIEF percent peak compositions that were within 0.5% when analyzing cIEF data. CE-SDS results were also equivalent as the calculated LC, NGHC, and HC percentages were all within 0.3% of each other.

Maurice has always given you the flexibility to easily transition between cIEF and CE-SDS methods. Now, with Empower® control, you also have the option to operate and analyze your cIEF and CE-SDS data all within Empower®, with the data reproducibility you've come to expect and the confidence that you'll always get equivalent results.



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