CE-SDS Analysis of a NISTmAb Reference Standard Using Both Maurice and the SCIEX PA 800/PA 800 Plus

Introduction

Regulatory agencies require that all biopharmaceutical companies monitor critical quality attributes for any monoclonal antibody therapeutic product. CE-SDS is a commonly used method as it provides purity and identity information required by the FDA.

Maurice and Maurice S. systems totally transform conventional CE-SDS separation and analysis, streamlining the entire process.[1] Maurice S. runs CE-SDS applications, and Maurice lets you analyze samples two ways using either CE-SDS or cIEF. There's no optic calibration needed, and all steps of the assay happen in a ready to go CE-SDS cartridge. Maurice automates everything too, so potential sources of error are minimized. That means you'll get really high quality data all the time. Clean-up between batches takes less than six minutes, with absolutely no cross-contamination between batches.

In this application note, we'll show you how Maurice data compares against SCIEX PA 800 systems for reduced and non-reduced CE-SDS separation of a reference monoclonal antibody from the National Institute of Standards and Technology (NIST).

NIST monoclonal antibody

The NIST monoclonal antibody (NISTmAb) reference material (RM 8671) is a recombinant humanized IgG1κ that contains a high abundance of N-terminal pyroglutamination, C-terminal lysine clipping, and heavy chain glycosylation. There's also a low abundance of post-translational modifications like methionine oxidation, deamidation, and glycation.[2] The NISTmAb is a 150 kDa homodimer expressed in murine suspension culture that's undergone industry-standard downstream processing to ensure its purity. Each lot comes with its own unique Report of Investigation where physicochemical attributes were evaluated using size exclusion chromatography (SEC), capillary sodium dodecyl sulfate electrophoresis (CE-SDS) and capillary zone electrophoresis (CZE).

Running the NISTmAb on Maurice

We prepared a 1 mg/mL NISTmAb reduced sample by combining 5 µL of 10 mg/mL mAb stock with 40.5 µL Maurice 1X Sample Buffer, 2 µL Maurice 25X Internal Standard, and 2.5 µL 14.2 M β-mercaptoethanol. Non-reduced samples were prepared by mixing 5 µL of 10 mg/mL mAb stock with 40.5 µL of Maurice 1X Sample Buffer, 2 µL Maurice 25X Internal Standard and 2.5 µL of freshly prepared 250 mM iodoacetamide. All samples were denatured at 70 °C for 10 minutes and stored on ice for 5 minutes. Samples were vortexed and spun down, then 50 µL was transferred to a sample vial and centrifuged at 1000 x g for 10 minutes. Sample vials were placed in Maurice (PN 090-000) along with a CE-SDS cartridge containing a new CE-SDS Running Buffer Top vial and the batch was run using Compass for iCE.

All samples were electrokinetically injected into a pre-conditioned capillary for 20 seconds and then reduced samples were separated for 25 minutes and non-reduced samples for 35 minutes. NISTmAb size variants were detected using absorbance at 220 nm. Triplicate data was analyzed using Compass for iCE (version 1.1.5).

Running the NISTmAb on SCIEX PA 800 and PA 800 Plus

We ran the NISTmAb on a SCIEX ProteomeLab PA 800 (PN 14403) running Karat Software (version 8.0) in parallel with Maurice using similar batch conditions (**Table 1**). 50 µL of 1 mg/mL of the NISTmAb was prepared using standard conditions recommended by SCIEX. Reduced and non-reduced samples were prepared 2.5 mL β-mercaptoethanol or 250 mM iodoacetamide, respectively, before incubating for 10 minutes at 70 °C. Samples were injected into a manually assembled, pre-conditioned capillary for 20 seconds then reduced samples were separated for 30 minutes and the non-reduced samples for 35 minutes. Triplicate data was analyzed using Waters Empower® 3 software (version 7.30.00.00).

In the NIST Report of Investigation, 1 mg/mL NISTmAb was analyzed on a SCIEX PA 800 Plus fitted with a photodiode array (PDA) multi-wavelength UV detector and characterization values were reported.2 Equivalency between the PA 800 and the PA 800 Plus has already been determined at SCIEX (data not published). Samples were separated in a manually assembled cartridge and then electrokinetically injected into a pre-conditioned capillary for 20 seconds then separated for 35 minutes and detected using absorbance at 220 nm.

CE-SDS Results with Reduced Samples

We compared our results running the reduced NISTmAb on Maurice with the PA 800 in house and with the PA 800 Plus data published by NIST in the Report of Investigation (**Figure 1**).[2] All three systems baseline resolved the non-glycosylated heavy chain (NGHC) and the heavy chain (HC) of the NISTmAb, and detected very small NGHC and thioester peaks. Visually, the peak height of the light chain (LC) in the PA 800 and PA 800 Plus was higher with a smaller full width, half max compared to Maurice, but quantitation of peak areas proved overall areas were comparable (**Table 2**). Percent

BATCH CONDITION	MAURICE REDUCED SAMPLES	PA 800 REDUCED SAMPLES	MAURICE NON-REDUCED SAMPLES	PA 800 NON- REDUCED SAMPLES
Sample concentration	1 mg/mL	1 mg/mL	1 mg/mL	1 mg/mL
Sample buffer	Maurice CE-SDS 1X Sample Buffer (ProteinSimple)	SDS Sample Buffer (SCIEX)	Maurice CE-SDS 1X Sample Buffer (ProteinSimple)	SDS Sample Buffer (SCIEX)
Reducing/ alkylating agent	0.7 M β-mercaptoethanol	0.7 M β-mercaptoethanol	250 mM iodoacetamide	250 mM iodoacetamide
Denaturing conditions	10 minutes at 70 °C	10 minutes at 70 °C	10 minutes at 70 °C	10 minutes at 70 °C
Cartridge	Pre-assembled cartridge	Manually assembled cartridge	Pre-assembled cartridge	Manually assembled cartridge
Sample injection	20 seconds, 4600 V	20 seconds, 5000 V	20 seconds, 4600 V	20 seconds, 5000 V
Separation time	25 minutes, 5750 V	25 minutes, 15 kV	35 minutes, 5750 V	35 minutes, 15kV
Analysis software	Compass for iCE	Empower	Compass for iCE	Empower

TABLE 1. Similar batch conditions were run on Maurice and the PA 800 using reduced and non-reduced conditions.

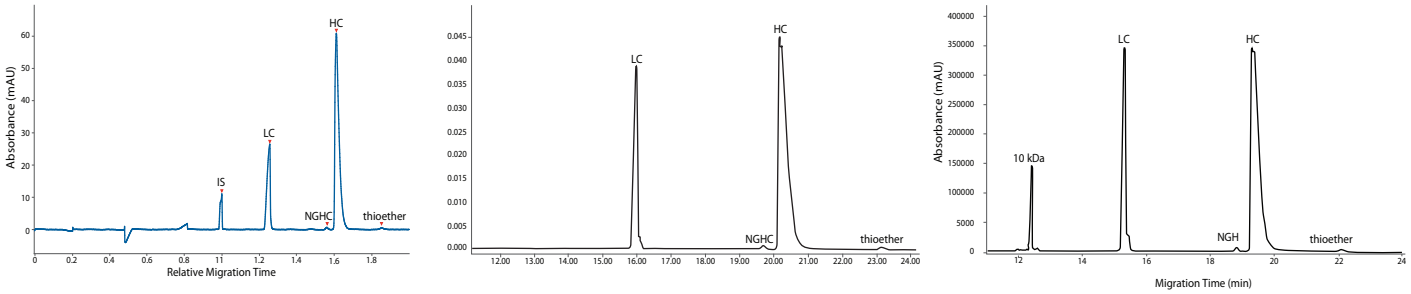

FIGURE 1. Maurice (left), the PA 800 (middle), and the PA 800 Plus (right, run by NIST[2]) all baseline-resolved the 1 mg/mL NISTmAb (RM 8971). All systems resolved and detected the light chain (LC), non-glycosylated heavy chain (NGHC), heavy chain (HC) and thioether peak. Maurice and the PA 800 Plus both also resolved a 10 kDa internal standard (IS) which wasn't included in the PA 800 batch.

peak areas between Maurice and the PA 800 were within 4.6% of each other for the major peaks, and within 0.2% for the minor species across all three systems. Relative abundance for each peak of the NISTmAb on Maurice was very precise as peaks with greater than 10% relative abundance had CVs ≤ 0.3%. The NGHC and thioether relative peak abundance percentages came in less than 1% with CVs ≤ 4.7% — showing system precision even for peaks with relatively low abundance. The PA 800 provided comparable quantitation but had slightly higher CVs for the lower abundance peaks at 6.0% and came in at 10.3% for the non-glycosylated heavy chain and thioether peak, respectively.

A previous study[3] compared interim NISTmAb material (RM 8670) on the PA 800 Plus using UV and LIF detection. When we ran the same interim material on Maurice, the data across all three systems was again comparable (**Table 3**). All peaks were within 3.0% of each other and minor peaks were within 0.2%, demonstrating Maurice CE-SDS data is comparable to the PA 800 Plus CE-SDS methods.

	LC %PEAK AREA	NGHC %PEAK AREA	HC %PEAK AREA	THIOETHER %PEAK AREA	%GLYCAN OCCUPANCY
MAURICE					
Average	30.1%	0.4%	69.1%	0.4%	99.4%
Std. Dev.	0.08	0.02	0.07	0.02	0.03
%CV	0.3%	4.7%	0.1%	4.7%	0.03%
PA 800					
Average	25.5%	0.5%	73.6%	0.5%	99.0%
Std. Dev.	0.14	0.03	0.08	0.05	0.08
%CV	0.5%	6.0%	0.1%	10.3%	0.1%
PA 800 Plus*					
Average	NA	NA	NA	0.3%	99.4%
Std. Dev.	NA	NA	NA	NA	NA
%CV	NA	NA	NA	NA	NA

TABLE 2. Comparable reduced NISTmAb (RM 8671) quantitation between Maurice, the PA 800 (UV absorbance), and the PA 800 Plus (UV absorbance). Data for Maurice and the PA 800 were very reproducible with CVs for Maurice under 4.7% and CVs for the PA 800 under 10.3%. *Data generated externally by NIST.[2]

SYSTEM	LC %PEAK AREA	NGHC %PEAK AREA	HC %PEAK AREA	THIOETHER %PEAK AREA	%GLYCAN OCCUPANCY
Maurice	29.8%	0.6%	69.1%	0.4%	99.4%
PA 800 Plus UV*	32.3%	0.6%	66.1%	0.4%	99.3%
PA 800 Plus LIF*	30.4%	0.6%	67.6%	0.6%	99.2%

TABLE 3. Comparable reduced interim NISTmAb (RM 8670) quantitation between Maurice and the PA 800 Plus using UV absorbance detection and LIF detection. *Data from an external publication.[3]

CE-SDS Results with Non-Reduced Samples

We also got comparable results when running the non-reduced NISTmAb on Maurice and the PA 800 in house, and compared to the PA 800 Plus data published by NIST in the Report of Investigation[2] (**Figure 2**). All three systems were able to detect low-abundance molecular fragments that correspond to the LC, HC, HC:LC, HC:HC, HC:HC:LC, and clip along with the intact mAb monomer. The percent of lower molecular weight (LMW) species, including the clip, was comparable with less than a percent difference between Maurice and the PA 800. Monomer purity was also comparable at within 1% across all systems used. Data reproducibility on Maurice for the LMW species was slightly better on Maurice with a CV of 9.0% compared to 12.0% on the PA 800 and reproducibility for the monomer was equivalent with a CV of 0.2% on both the PA 800 and Maurice (**Table 4**).

MAURICE	%LMW AREA	%MONOMER PURITY
Average	2.5%	97.5%
Std. Dev.	0.2	0.2
%CV	9.0%	0.2%
PA 800	**%LMW AREA**	**%MONOMER PURITY**
Average	1.8%	98.2%
Std. Dev.	0.2	0.2
%CV	12.0%	0.2%
PA 800 PLUS*	**%LMW AREA**	**%MONOMER PURITY**
Average	NA	98.5%
Std. Dev.	NA	NA
%CV	NA	NA

TABLE 4. Comparable reduced NISTmAb (RM 8671) quantitation between Maurice, the PA 800 (UV absorbance), and the PA 800 Plus (UV absorbance). Data for Maurice and the PA 800 were very reproducible with CVs in the single digits. *Data generated externally by NIST.[2]

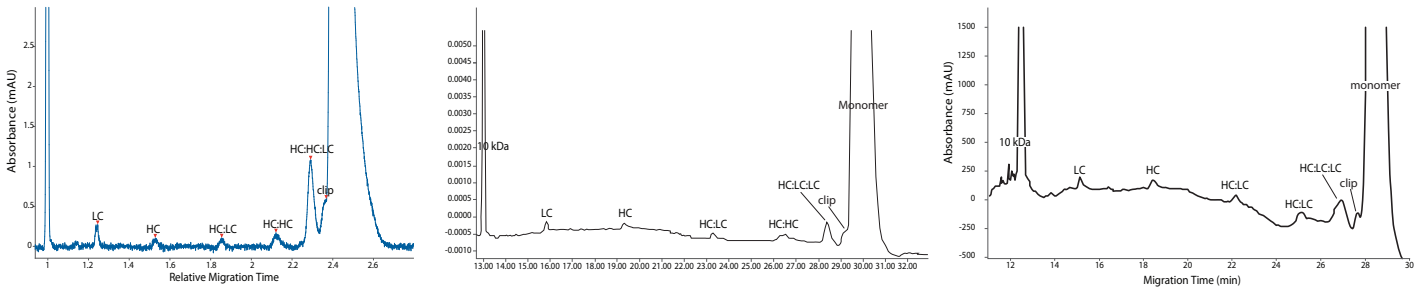

FIGURE 2. Maurice (left), the PA 800 (middle), and the PA 800 Plus (right, run by NIST) all detected five low molecular weight antibody fragments, one unidentified clip, and the intact monomer in 1 mg/mL of non-reduced NISTmAb (RM 8671).

SYSTEM	%LMW AREA	%MONOMER PURITY
Maurice	2.2%	97.8%
PA 800 Plus UV*	2.2%	97.8%
PA 800 Plus LIF*	2.0%	97.9%

TABLE 5. Comparable non-reduced interim NISTmAb (RM 8670) quantitation between Maurice and the PA 800 Plus using UV absorbance detection and LIF detection. *Data from an external publication.[3]

When we compared the interim NISTmAb results on Maurice with data published in the ACS symposium series[3], data equivalency is even more apparent with peak percentages all within 0.2%, even for the LMW species (**Table 5**).

Conclusion

CE-SDS separation and analysis with Maurice lets you assess the purity and identity of therapeutic monoclonal antibodies using a much simpler workflow that results in highly-robust, high-quality data. It also takes less than 10 minutes of hands-on time to set up a batch on Maurice compared to the PA 800/PA 800 Plus which requires a lot of manual steps like building cartridges, cleaning interfaces, and optimizing detectors that are also sources of error.

In this study, we compared CE-SDS results from Maurice with those from the SCIEX PA 800 and PA 800 Plus systems. We ran the NISTmAb, a humanized IgG1κ monoclonal released by the National Institute of Standards and Technology (NIST), on both Maurice and the SCIEX PA 800 and then also compared it to data generated by NIST on the PA 800 Plus. Quantitation across all three systems was comparable with peak percentages within 4.5% and 1% for the reduced and non-reduced NISTmAb (RM 8671), respectively. Data for the reduced and non-reduced monoclonal was also very reproducible with <0.5% CVs for peaks with greater than 10% peak area. Peaks with less than 10% peak area were slightly more reproducible on Maurice with single-digit CVs for all peaks.

We also compared reduced and non-reduced interim NISTmAb (RM 8670) on Maurice with previously published SCIEX PA 800 data using both UV and LIF detection. Data was again equivalent with percent peak areas all within 3% of each other across all three systems. So if you're looking for a more simplified way to do CE-SDS analysis with the same or better data quality you're getting now, try Maurice!

References

1. Sizing-up IgG with Maurice's CE-SDS Application, ProteinSimple Application Note.
2. National Institute Report of Investigation for Reference Material 8671, Lot # 14HB-D-002.
3. Separation methods and orthogonal techniques, DA Michels, AY Ip, TM Dillon, K Brorson, S Lute, B Chavez, KM Prentice, LJ Brady, and KJ Miller, *State-of-the Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization Volume 2. Biopharmaceutical Characterization: The NISTmAb Case Study*, 2015; 237-284.

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

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