Application Note

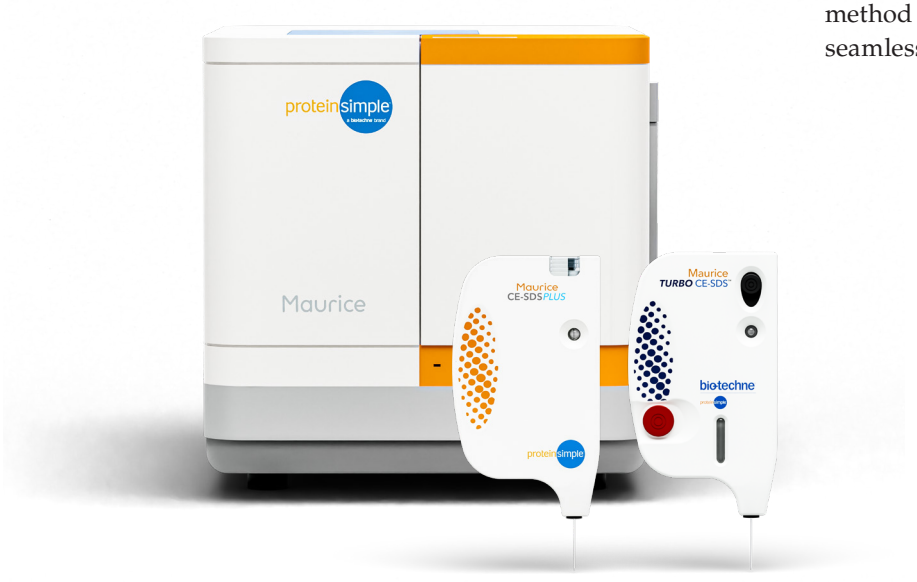
Biotherapeutic Characterization From Discovery To Release With Maurice Turbo CE-SDS and CE-SDS PLUS

High-Quality Analysis of Protein Purity with the Maurice™ System

Introduction

Different stages of drug development have their unique analytical requirements, based on sample throughput, sample types, and data quality needs, requiring the use of different instruments at each stage. One of the challenges this poses is the need for extensive method development and method transfer, particularly when moving from one phase of the development pipeline to the next. This results in an additional investment of time, capital, and labor. Since time is critical in getting therapies to patients, it is important to implement analytical tools that address these bottlenecks.

This application note describes two label-free capillary electrophoresis sodium dodecyl sulfate (CE-SDS) methods on a single instrument, Maurice, that address the needs of protein purity/size characterization from discovery to GMP release. This study presents data on purity, linearity, and reproducibility of these methods using an array of molecules, including reference standards for monoclonal antibodies from the National Institutes of Standards and Technology (NIST) and the United States Pharmacopeia (USP) and a GMP protein from Bio-Techne.



About Maurice

Maurice is a fully integrated capillary electrophoresis system that lets you analyze the size and charge heterogeneity of your biotherapeutics by enabling both CE-SDS and imaged capillary isoelectric focusing (icIEF), and both types of analyses are conducted using pre-assembled cartridges for sample processing. You can switch between methods without worrying about instrument set up or clean up – you only need to prepare your samples accordingly and insert the appropriate cartridge into the system. Maurice provides fast, high-quality, reproducible data using Compass for iCE or Waters™ Empower® software, both of which are 21 CFR Part 11 compliant.

CE-SDS on Maurice is a great alternative to SDS-PAGE as it is faster, automated, quantitative, and offers much higher resolution and sensitivity. Maurice offers two cartridges for CE-SDS analysis – the Maurice Turbo CE-SDS™ cartridge for fast and high throughput analysis (in 5.5 – 8 minutes), ideal for discovery, and the Maurice CE-SDS PLUS cartridge for superior resolution (in 25-35 minutes), ideal for analytical development and QC release testing. No matter which method you choose, method development can be achieved within a day, with seamless transfer across different stages.

Materials and Methods

All experiments were conducted on Maurice instruments. **TABLE 1** lists the materials used in this study, including the Maurice Turbo CE-SDS and CE-SDS PLUS Size Application Kits that contain all the materials and reagents required for their respective assays.

NIST mAb and USP mAb samples were diluted with the Maurice CE-SDS 1X Sample Buffer and made to a final concentration of 1mg/mL. For linearity experiments, the concentrations of NIST mAb ranged from 0.134 mg/mL to 1.07 mg/mL. IL-15 was also diluted with the CE-SDS 1X Sample Buffer to a final concentration of 0.5 mg/mL. The Maurice CE-SDS Molecular Weight Markers and Maurice CE-SDS IgG Standard were prepared according to the instructions in their respective product inserts.

Data on the Maurice CE-SDS IgG Standard is in the Supplemental Information section of this application note.

All samples were treated with either 2.5 µL of 250 mM iodoacetamide (IAM) or 2.5 µL of 14.2 M β-mercaptoethanol (β-ME) for non-reduced or reduced CE-SDS analysis, respectively. The samples were denatured for 10 minutes at 70°C, cooled on ice for 5 minutes and centrifuged. The resulting samples were analyzed on Maurice using the methods described below.

For the Turbo CE-SDS method, the samples were electrokinetically injected into the cartridge 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. For the CE-SDS PLUS method, the samples were injected for 20 seconds at 4600 V before separation by electrophoresis at 5750 V. Reduced samples were separated for 25 minutes and non-reduced samples were separated for 35 minutes. All data were analyzed using the Compass for iCE software.

Material	Vendor	Catalog #
Maurice Turbo CE-SDS Size Application Kit	ProteinSimple, a Bio-Techne brand	PS-MAK01-TS
Maurice Turbo CE-SDS Cartridge		PS-MC02-TS, PS-MC01-TS
Maurice CE-SDS PLUS Size Application Kit		PS-MAK03-S
Maurice CE-SDS PLUS Cartridge		PS-MC02-SP
Maurice CE-SDS Molecular Weight Markers		046-432
Maurice CE-SDS IgG Standard		046-039
Recombinant Human IL-15 GMP Protein	R&D Systems, a Bio-Techne brand	BT-015-GMP-01M
NIST mAb $IgG1_K$	NIST	RM8671 (Lot# 14HB-D-002)
USP mAb1	USP	1445539 (Lot# F11920)
USP mAb2		1445547 (Lot# F12970)
USP mAb3		1445595 (Lot# F12980)
Iodoacetamide (IAM)	Millipore Sigma	16125
β-mercaptoethanol (β-ME)		M-3148

TABLE 1. List of materials and reagents used in this study.

Linearity

NIST mAb was serially diluted from 1.07 mg/mL down to 0.134 mg/mL and these samples were analyzed on Maurice with both Turbo CE-SDS and CE-SDS PLUS to determine and compare the linearity of both cartridges. Overlays of NIST mAb profiles at different concentrations under non-reduced conditions are shown in **FIGURES 1A** and **1B** for the Turbo CE-SDS and CE-SDS PLUS cartridges respectively. Each run with Turbo CE-SDS was completed in **8 minutes**, and with CE-SDS PLUS in **35 minutes**, however, both datasets are reported using the relative migration time. A gradual increase in peak intensity with an increase in sample concentration was observed with both methods. Notably, the CE-SDS PLUS data showed higher peak intensities compared to Turbo CE-SDS, but this difference is expected owing to the different capillary lengths of the two cartridges. The Turbo CE-SDS cartridge has a shorter capillary, enabling it to process samples faster and is thus ideal for high-throughput applications. In contrast, the CE-SDS PLUS cartridge has a longer capillary, allowing for longer separation times and therefore enhanced resolution, more suitable for the needs of QC labs. From each method in this experiment, the average peak areas of the monomer were plotted against different concentrations of the NIST mAb sample, as shown in **Figures 2A** and **2B**. Excellent R^2 values (~1.0) were obtained for Turbo CE-SDS and CE-SDS PLUS respectively, showing that both methods are linear and comparable for non-reduced conditions.

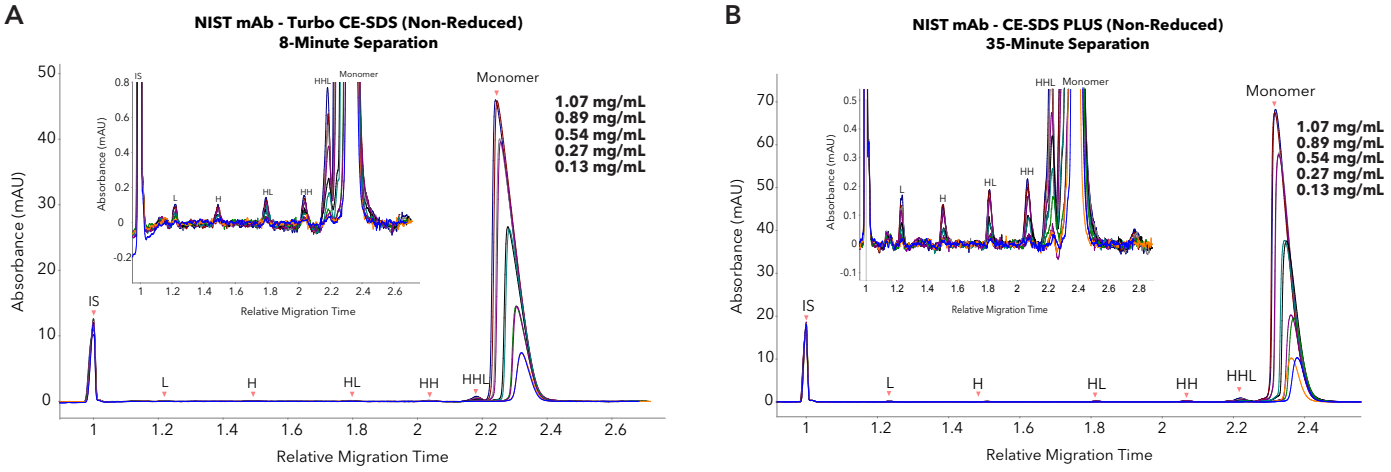


FIGURE 1. Linearity of NIST mAb on both Turbo CE-SDS and CE-SDS PLUS cartridges on Maurice under non-reduced conditions. Five different concentrations of NIST mAb were analyzed with **A**. Turbo CE-SDS and **B**. CE-SDS PLUS cartridges. The observed increase in peak intensity correlates well with an increase in sample concentration for both methods. The insets clearly show all the peaks detected with these CE-SDS cartridges. (IS: Internal Standard; L: light; H: heavy; HL: heavy-Light; HH: heavy-heavy; HHL: heavy-heavy-light).

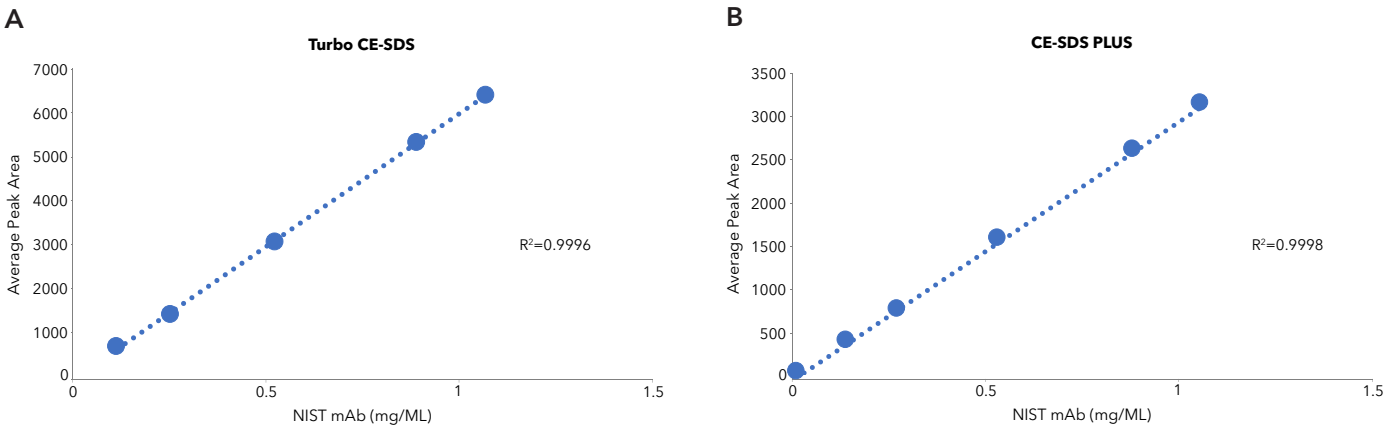


FIGURE 2. Graphical representation of the dilutional linearity of A. the Turbo CE-SDS and B. CE-SDS PLUS cartridge under non-reduced conditions. The average main (monomer) peak area was plotted against different sample concentrations. Both methods showed excellent linearity with R^2 values of 0.9996 and 0.9998 respectively.

The same range of NIST mAb concentrations was also used to evaluate the linearity of both CE-SDS cartridges under reduced conditions, where each injection with Turbo CE-SDS was separated in **~5.5 minutes** and separated in **25 minutes** with CE-SDS PLUS. **FIGURES 3A** and **3B** show the overlaid profiles from the Turbo CE-SDS and CE-SDS PLUS methods, respectively, where an increase in peak intensity was clearly observed with an increase in NIST mAb concentration. The average peak areas of the heavy chain (HC) and light chain (LC) were plotted against different sample concentrations tested, as shown in **Figures 4A** and **4B**, resulting in R^2 values approaching 1 and once again demonstrating excellent linearity of both cartridges.

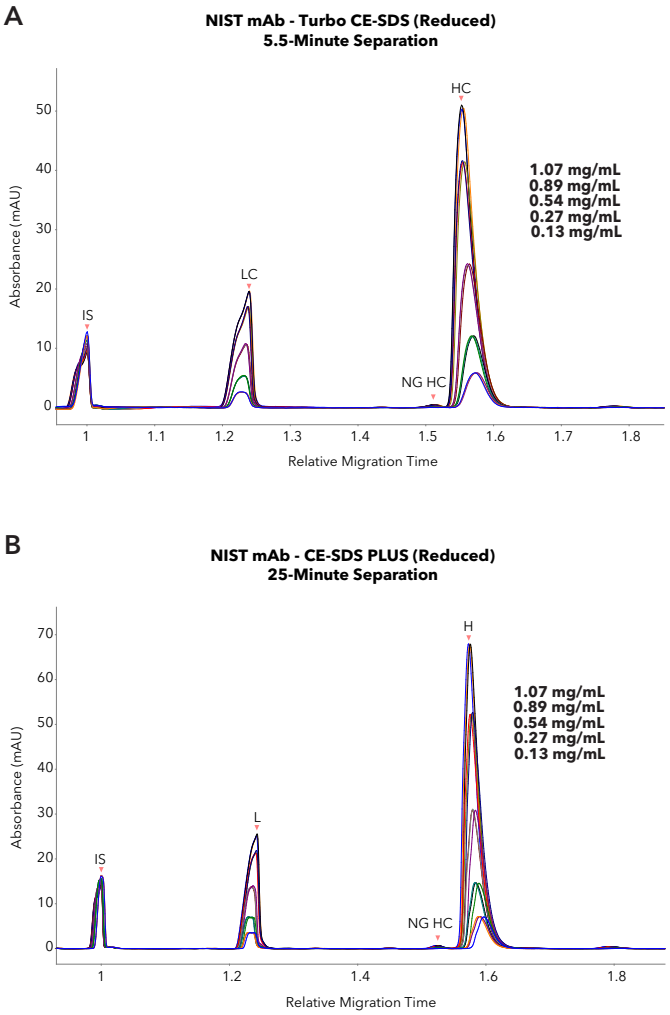


FIGURE 3. Dilutional linearity of both CE-SDS cartridges under reduced conditions using NIST mAb. Five different concentrations of NIST mAb were analyzed with **A**. Turbo CE-SDS and **B**. CE-SDS PLUS cartridges. An increase in peak intensity is clearly seen with an increase in sample concentration, with well resolved peaks (IS: Internal Standardl; LC: light chain: NG HC: non-glycosylated heavy chain; HC: heavy chain).

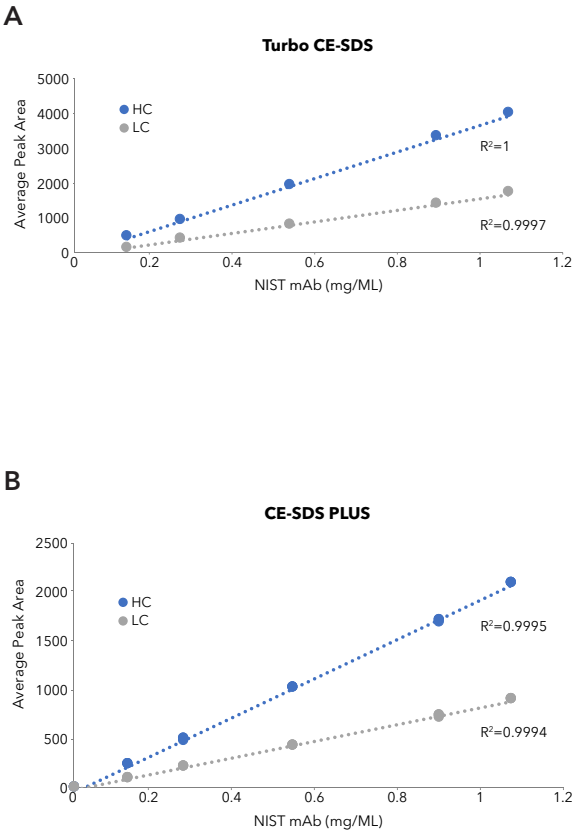


FIGURE 4. Graphical representation of the dilutional linearity of the Turbo CE-SDS and CE-SDS PLUS cartridge under reduced conditions. The average peak areas of the HC and LC were plotted against varying NIST mAb concentrations, resulting in R^2 values of **A.** 1 (HC) and 0.9997 (LC) for Turbo CE-SDS, and **B.** 0.9995 (HC) and 0.9994 (LC) for CE-SDS PLUS, confirming that both methods meet the required linearity.

Purity

Purity is an important critical quality attribute of biotherapeutics that must be routinely evaluated in different stages of development. While each Maurice CE-SDS cartridge provides certain benefits in specific development stages, it is important to establish that they both perform comparably for purity analysis. **Figures 5A** and **5B** show replicate injections of NIST mAb samples with the Turbo CE-SDS and CE-SDS PLUS cartridges respectively, with each method run for the same amount of time under non-reduced conditions.

The Turbo CE-SDS cartridge, by virtue of being fast, can analyze a higher number of samples in a given amount of time compared to CE-SDS PLUS. The purity of the monomer was ≥98.4% with both methods, as summarized in **Tables 2** and **3**. Furthermore, the relative standard deviation (%RSD) was 0.13 for the Turbo CE-SDS method, showing how reproducible it is. Similarly, **Figures 6A** and **6B** compare the purity of a NIST mAb sample analyzed under reduced conditions with Turbo CE-SDS and CE-SDS PLUS, with the results shown in **Tables 4** and **5**.

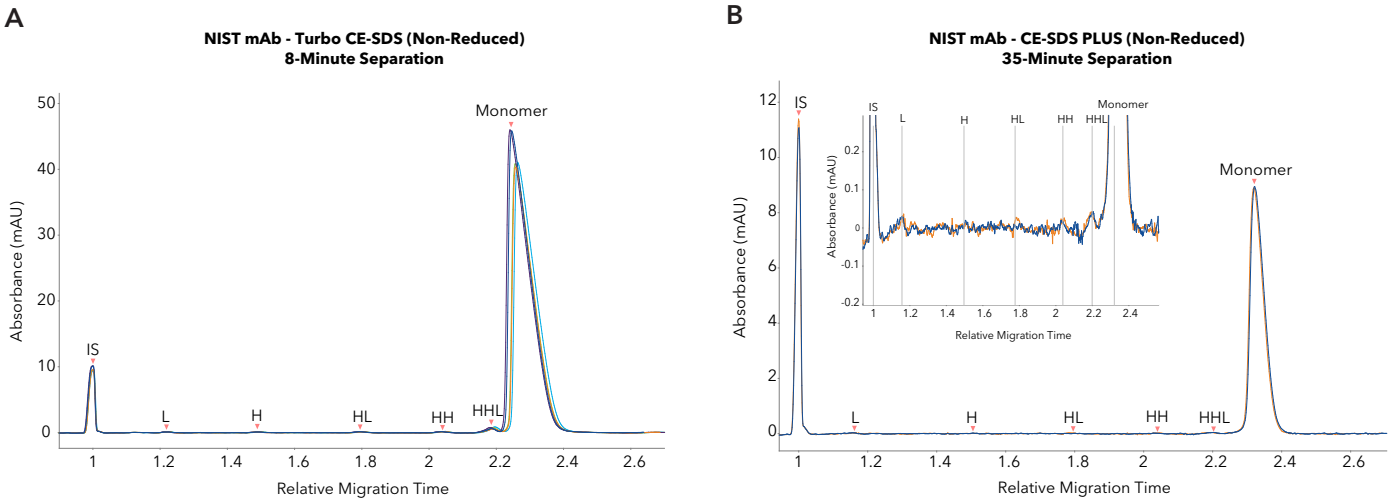


FIGURE 5. Purity assessment of NIST mAb with Turbo CE-SDS and CE-SDS PLUS under non-reduced conditions. Both cartridges were run for the same amount of time, with **A.** five sample injections detected using Turbo CE-SDS and **B.** two sample injections analyzed with CE-SDS PLUS. The main peak was found to be of high purity, with other peaks detected in lower amounts as shown in the inset.

Percent Peak Area - Turbo CE-SDS (Non-Reduced)						
Injection	**Monomer**	**HHL**	**HH**	**HL**	**H**	**L**
7	98.5	1	0.1	0.1	0.1	0.1
16	98.7	0.8	0.1	0.1	0.1	0.1
46	98.7	0.8	0.1	0.1	0.1	0.1
55	98.6	0.9	0.1	0.1	0.1	0.1
87	98.4	1.1	0.1	0.1	0.1	0.2
Average	**98.58**	0.92	0.1	0.1	0.1	0.12
%RSD	**0.13**	14.17	0.00	0.00	0.00	37.27

TABLE 2. Purity of NIST mAb evaluated for five sample injections with Turbo CE-SDS (non-reduced).

Percent Peak Area - CE-SDS PLUS (Non-Reduced)						
Injection	**Monomer**	**HHL**	**HH**	**HL**	**H**	**L**
8	**99.4**	0.2	0.2	0.1	0.1	0.1
10	**99.4**	0.2	0.1	0.1	0.1	0.1

TABLE 3. Purity of NIST mAb evaluated for two sample injections with CE-SDS PLUS (non-reduced).

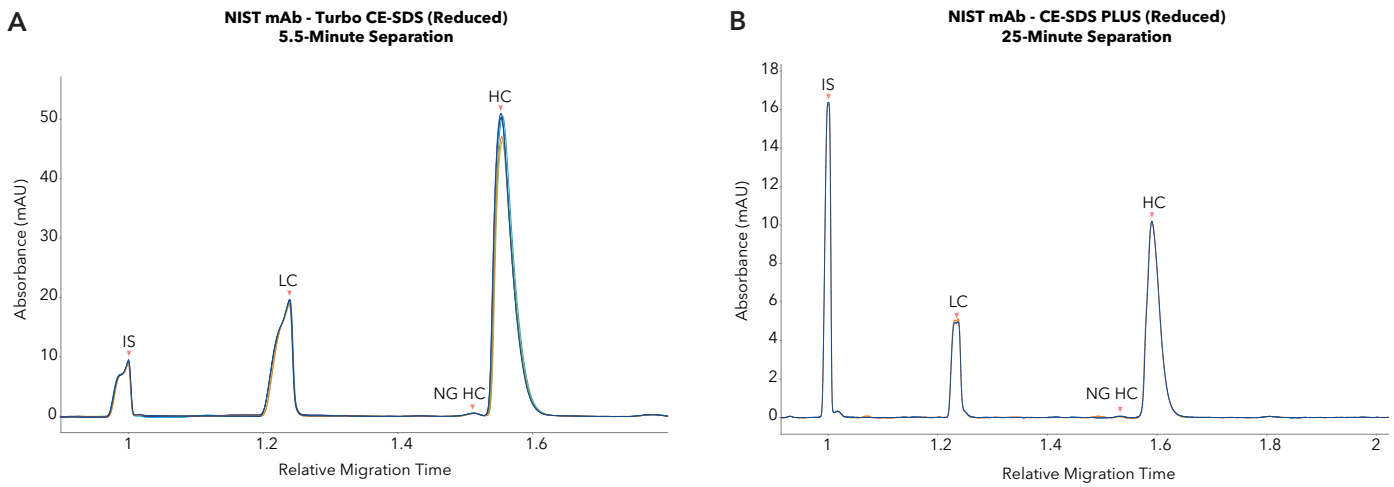


FIGURE 6. Purity assessment of NIST mAb with Turbo CE-SDS and CE-SDS PLUS under reduced conditions. An overlay of profiles from **A.** five sample injections using Turbo CE-SDS and **B.** two sample injections analyzed with CE-SDS PLUS.

Percent Peak Area - Turbo CE-SDS (Reduced)			
Injection	**HC**	**LC**	**NG HC**
27	69	30.5	0.5
36	68.8	30.7	0.5
66	68.5	31	0.5
75	68.1	31.4	0.5
93	68.8	30.7	0.5
Avg	**68.64**	**30.86**	**0.5**
%RSD	**0.457**	**1.14**	**0**

TABLE 4. Purity of NIST mAb evaluated for five sample injections with Turbo CE-SDS (reduced).

Percent Peak Area - CE-SDS PLUS (Reduced)			
Injection	**HC**	**LC**	**NG HC**
19	69.8	29.9	0.3
21	69.1	30.8	0.1

TABLE 5. Purity of NIST mAb evaluated for two sample injections with CE-SDS PLUS (reduced).

Data Comparability

This section further establishes data comparability between the two Maurice CE-SDS cartridges using two different classes of molecules. The first class consists of USP mAb standards (USP mAb1, mAb2, and mAb3), which are common reference standards used for the development of IgG-based therapeutics[1-3]. A study published previously has shown that the Maurice CE-SDS PLUS method is comparable to the method outlined in the USP General Chapter <129>, which describes analytical procedures for determining therapeutic purity[4,5]. The second class is a cytokine (GMP-grade IL-15) used in the development of cellular therapies. **FIGURES 7A** and **7B** are representative electropherograms of USP mAb1 analyzed under non-reduced conditions with Turbo CE-SDS and CE-SDS PLUS. In addition to showing comparability, the analysis of replicate sample injections demonstrates reproducibility of each method, with all peaks identified. Profiles of the other USP mAb standards are shown in the Supplemental Information section, which shows comparative data between Turbo CE-SDS and CE-SDS PLUS for both non-reduced and reduced conditions. A comparison between the two cartridges used for non-reduced CE-SDS is graphically represented in **FIGURE 8A**, where the percent peak area of the monomer is quantified for all three USP mAb standards. Similarly, **FIGURE 8B** is a graphical representation of the results generated for all three USP mAb standards under reduced conditions, quantifying the percent peak areas of the LC and HC. Both datasets show how well the results compare for each method, further indicating the ease of method transfer. Percent peak area and RSD values are listed in **TABLE 6**. **FIGURES 9A** and **9B** show the profiles of IL-15 analyzed with Turbo CE-SDS and CE-SDS PLUS under non-reduced conditions, and **FIGURES 10A** and **10B** demonstrate the sample profiles under reduced conditions. The purity obtained was ≥97.6% overall, with details listed in **TABLE 7**.

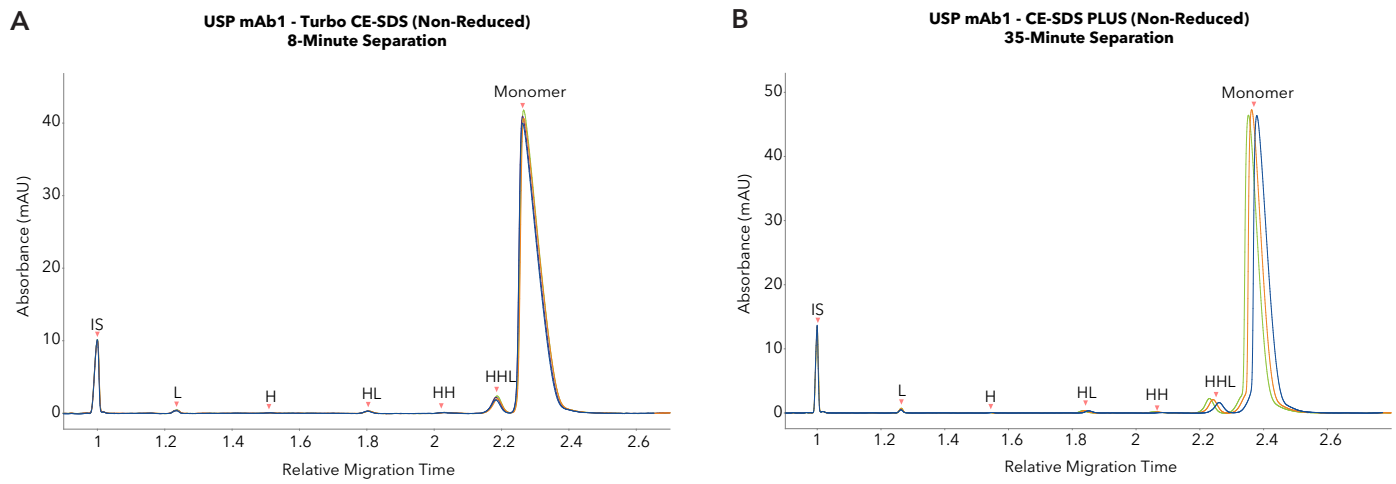


FIGURE 7. Analysis of USP mAb1 under non-reduced conditions with A. Turbo CE-SDS, where profiles of 4 sample injections are overlaid, and B. CE-SDS, where data for 3 sample injections are shown.

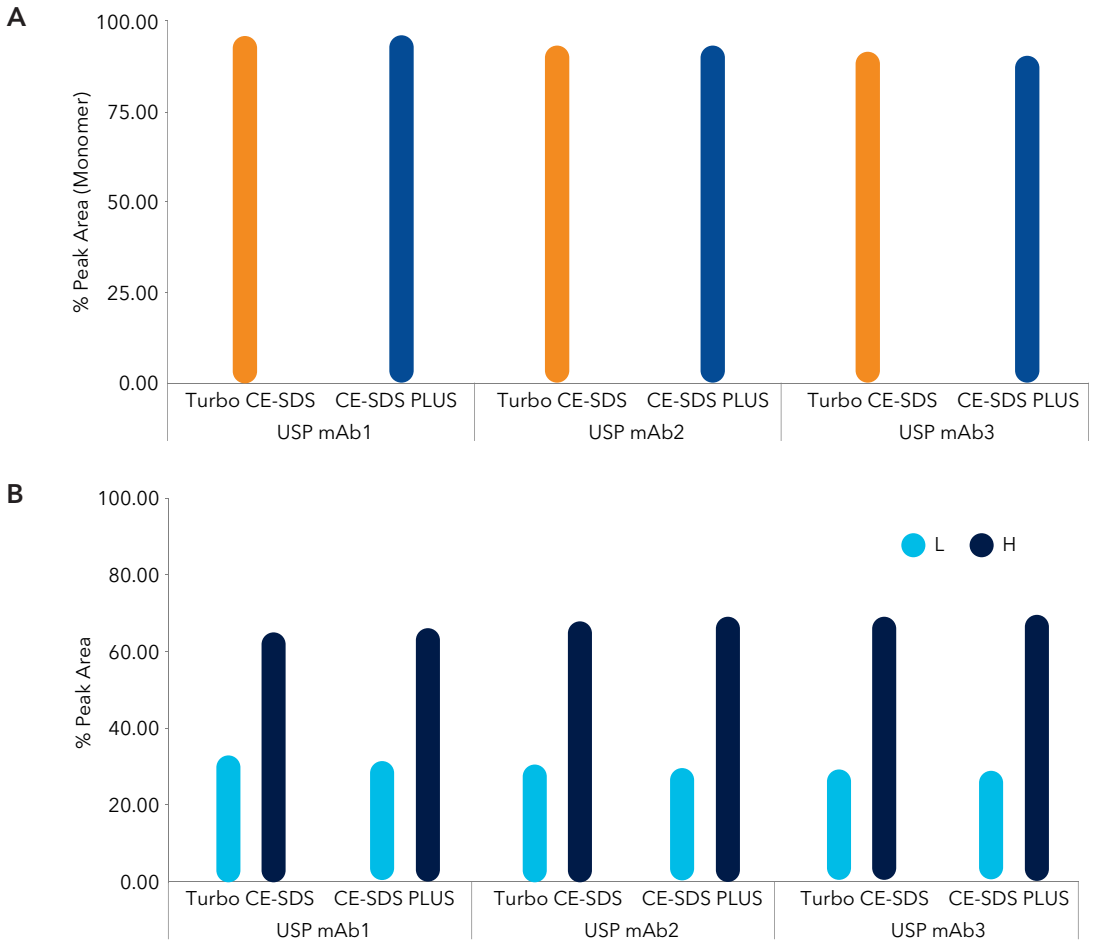


FIGURE 8. Quantitation of percent peak areas for USP mAb1, mAb2, and mAb3. A. Graphical representation of the percent peak area of the monomer from non-reduced conditions, and B. graphical representation of the LC and HC percent peak area from reduced conditions. The data between the two methods are comparable for all three USP mAb standards.

Sample	Cartridge	Reduced				Non-Reduced	
		LC		HC		Monomer	
		%Peak Area	%RSD	%Peak Area	%RSD	%Peak Area	%RSD
USP mAb1	Turbo CE-SDS	33.87	1.47	65.60	0.71	96.03	0.26
	CE-SDS PLUS	33.09	0.37	66.36	0.18	95.80	0.68
USP mAb2	Turbo CE-SDS	31.01	0.57	68.34	0.36	93.08	0.05
	CE-SDS PLUS	30.65	0.43	68.63	0.14	92.77	0.61
USP mAb3	Turbo CE-SDS	29.78	1.49	68.90	0.55	91.53	0.59
	CE-SDS PLUS	29.17	0.30	69.27	0.10	90.30	1.92

TABLE 6. Summary of the results from the analysis of three USP mAb standards. Percent peak area and RSD values are shown for LC, HC, and the monomer, determined from reduced and non-reduced CE-SDS analysis respectively.

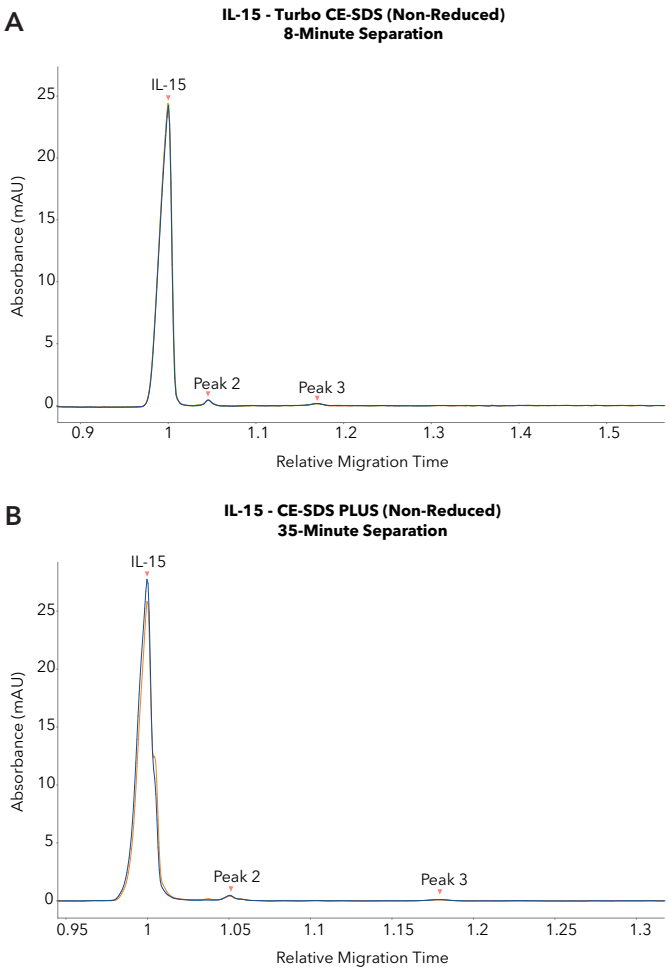


FIGURE 9. Analysis of IL-15 under non-reduced conditions with A. Turbo CE-SDS and B. CE-SDS PLUS. Each method is reproducible, and the data between the two are comparable.

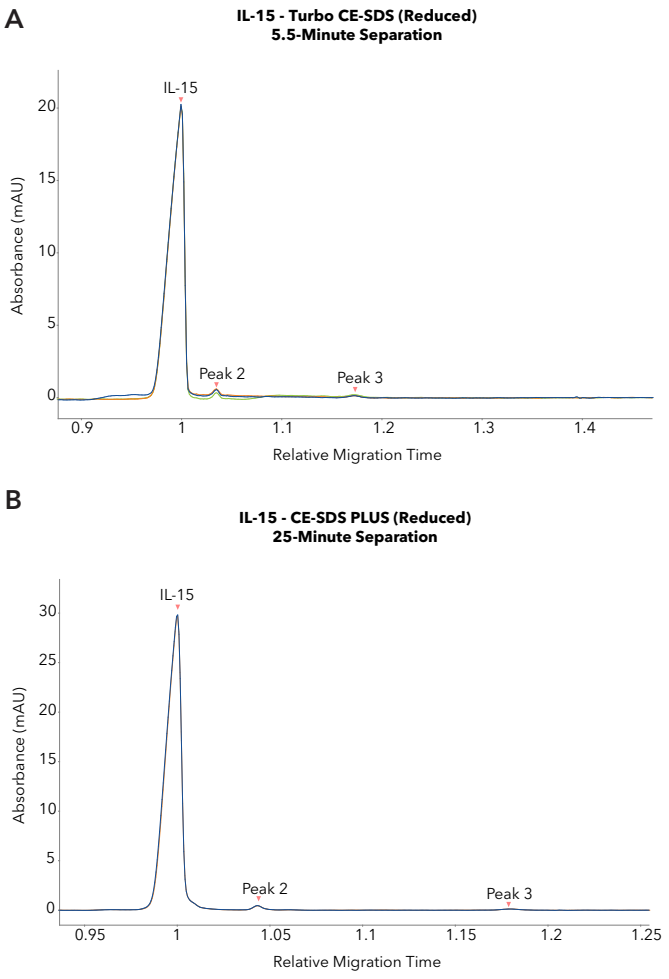


FIGURE 10. Analysis of IL-15 under reduced conditions with A. Turbo CE-SDS and B. CE-SDS PLUS, demonstrating excellent data comparability.

	Non-reduced		Reduced	
	% Purity	% RSD	%Purity	% RSD
Turbo CE-SDS (n=3)	98.4	1.82	97.64	1.31
CE-SDS PLUS (n=2)	97.7	4.91	98.35	1.43

TABLE 7. Purity of IL-15 samples determined from both CE-SDS cartridges under non-reduced and reduced conditions.

Conclusion

The data generated in this study establish that both the Turbo CE-SDS and CE-SDS PLUS cartridges are reliable options for analyzing monoclonal antibodies and GMP proteins on the Maurice system. Both methods provide high-quality data while also performing comparably, with minor differences. Excellent assay performance with respect to reproducibility, linearity, and sample purity is crucial for implementation across different development phases and has been demonstrated for both Turbo CE-SDS and CE-SDS PLUS. Both have also been used for the analysis of AAV and lentiviral vectors for cell and gene therapy applications, published in another study[3]. While the choice between the two cartridges depends on the specific analytical requirements of a given stage, the ease of use, speed of method development, and software all remain the same, ensuring smooth integration at any stage, in any lab. To learn more about CE-SDS with Maurice, visit our website.

References

1. Certificate – USP mAb 001, Monoclonal IgG1
2. Certificate – USP mAb 002, Monoclonal IgG1
3. Certificate – USP mAb 003, Monoclonal IgG1
4. Application Note: Get USP <129> Equivalent Data with Maurice CE-SDS
5. United States Pharmacopeia (2017). *General Chapter, Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies.* Retrieved from http://go.usp.org/1/323321/2018-10-16/xxzsl
6. Application Note: Characterize Your Viral Vectors from Discovery to GMP Release with Maurice™

Supplemental Information

Maurice CE-SDS IgG Standard

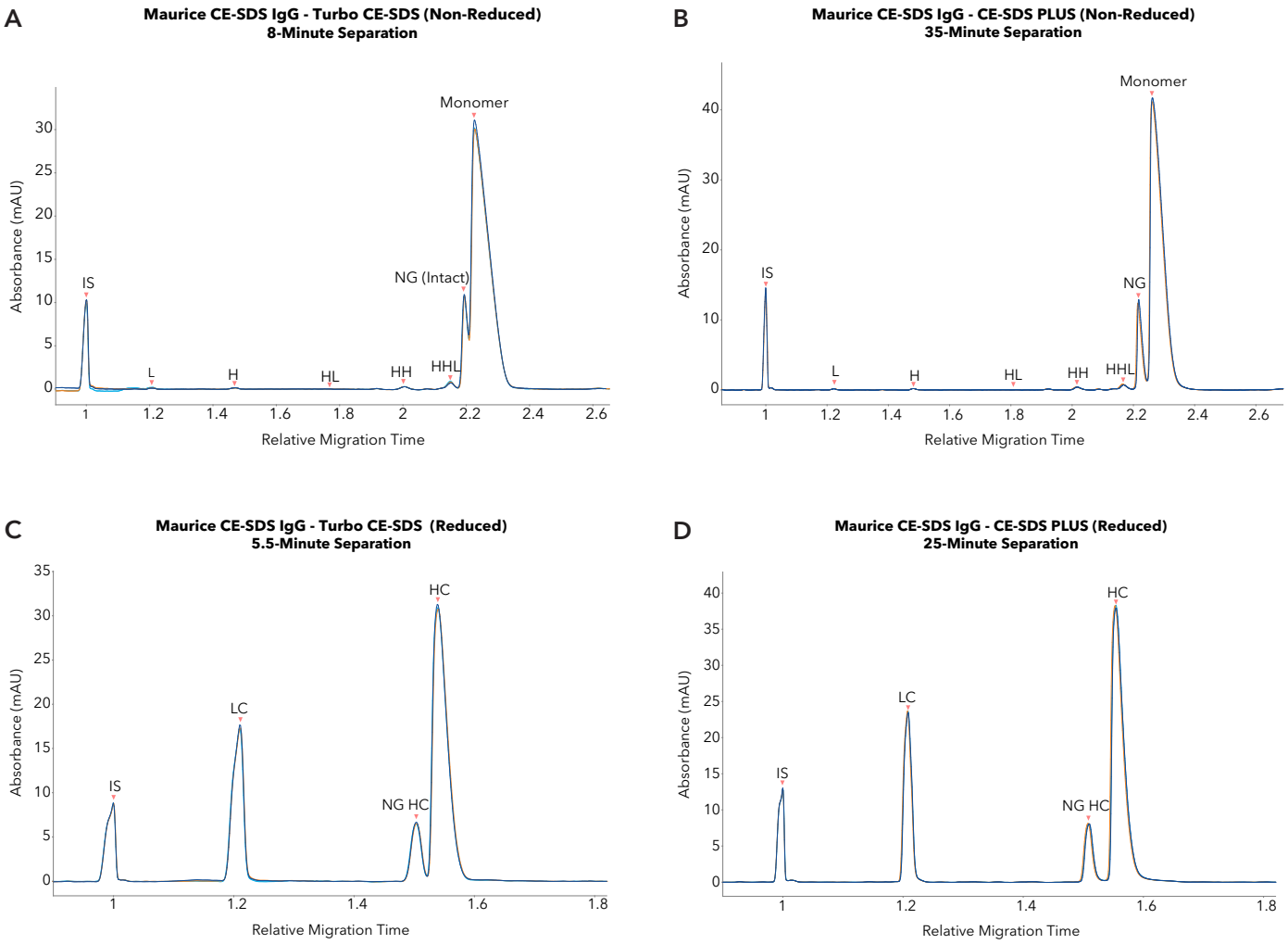


FIGURE 1. Analysis of Maurice CE-SDS IgG Standard with Turbo CE-SDS and CE-SDS PLUS under non-reduced conditions (A and B) and reduced conditions (C and D).

USP mAb Standards

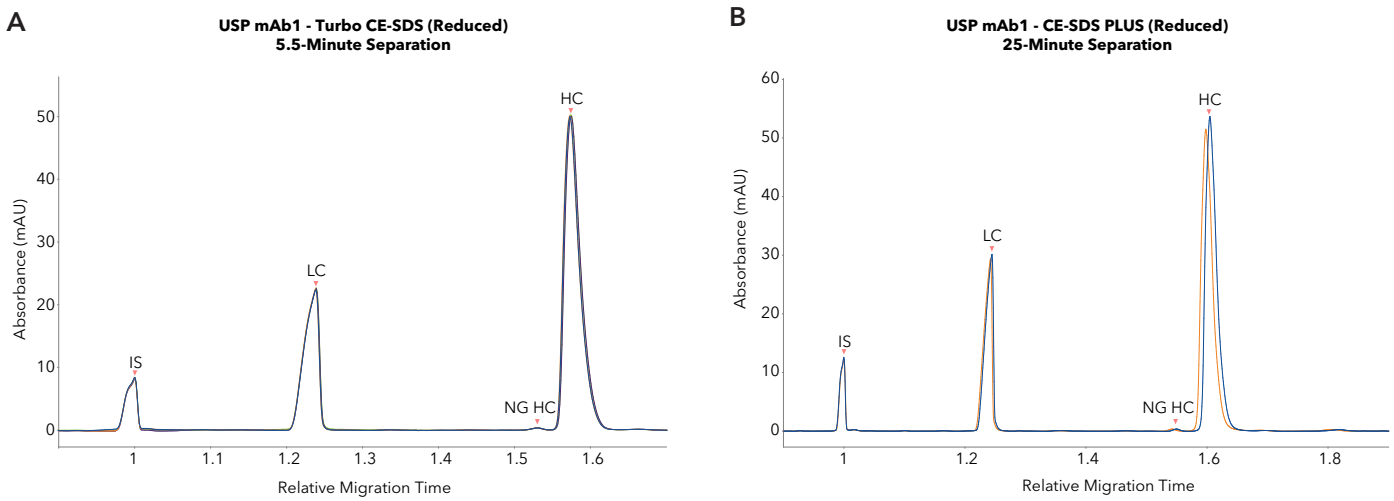


FIGURE 2. Analysis of USP mAb1 under reduced conditions with A. Turbo CE-SDS and B. CE-SDS PLUS

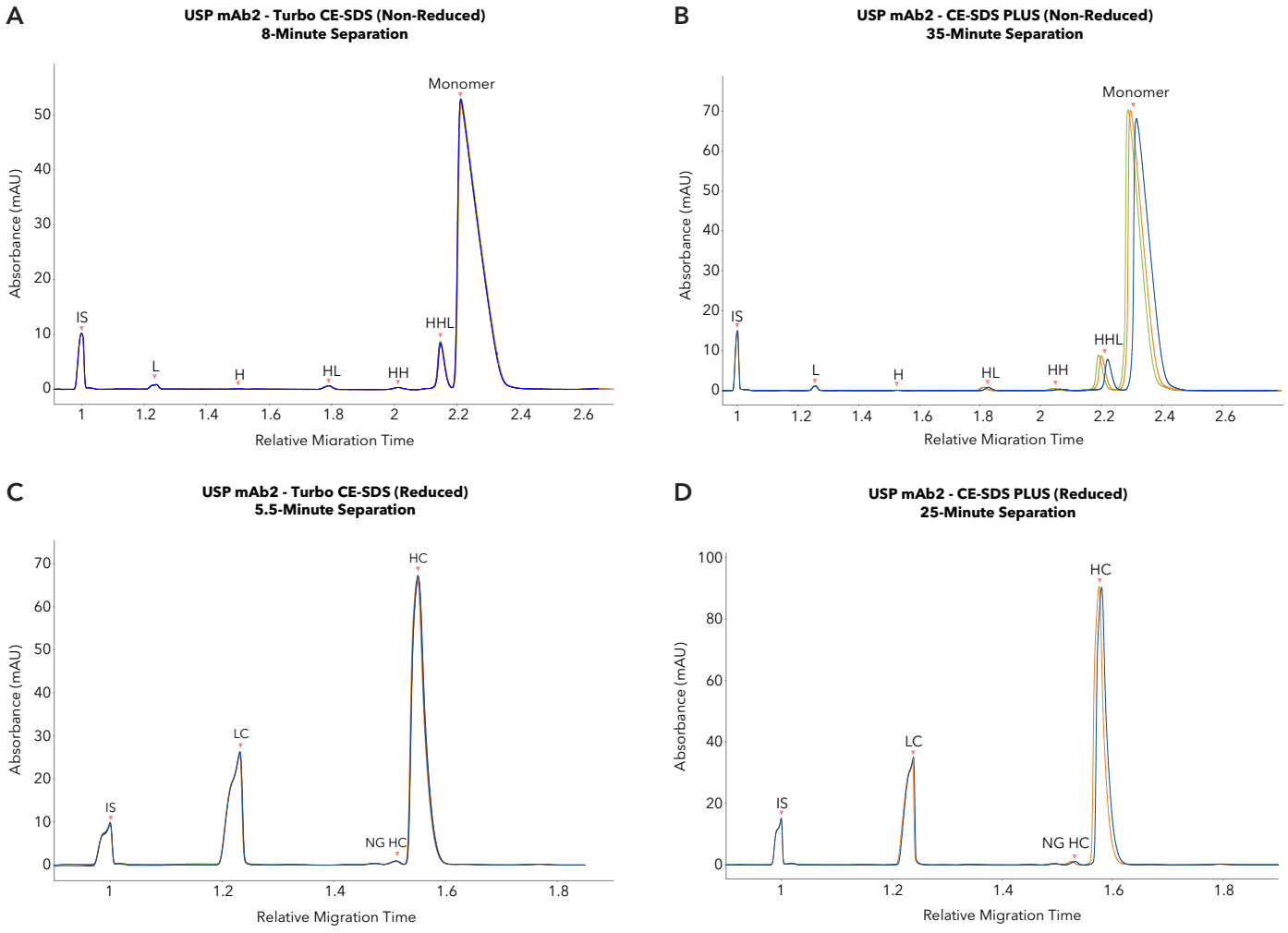


FIGURE 3. Analysis of USP mAb2 with Turbo CE-SDS and CE-SDS PLUS under non-reduced conditions (A and B) and reduced conditions (C and D).

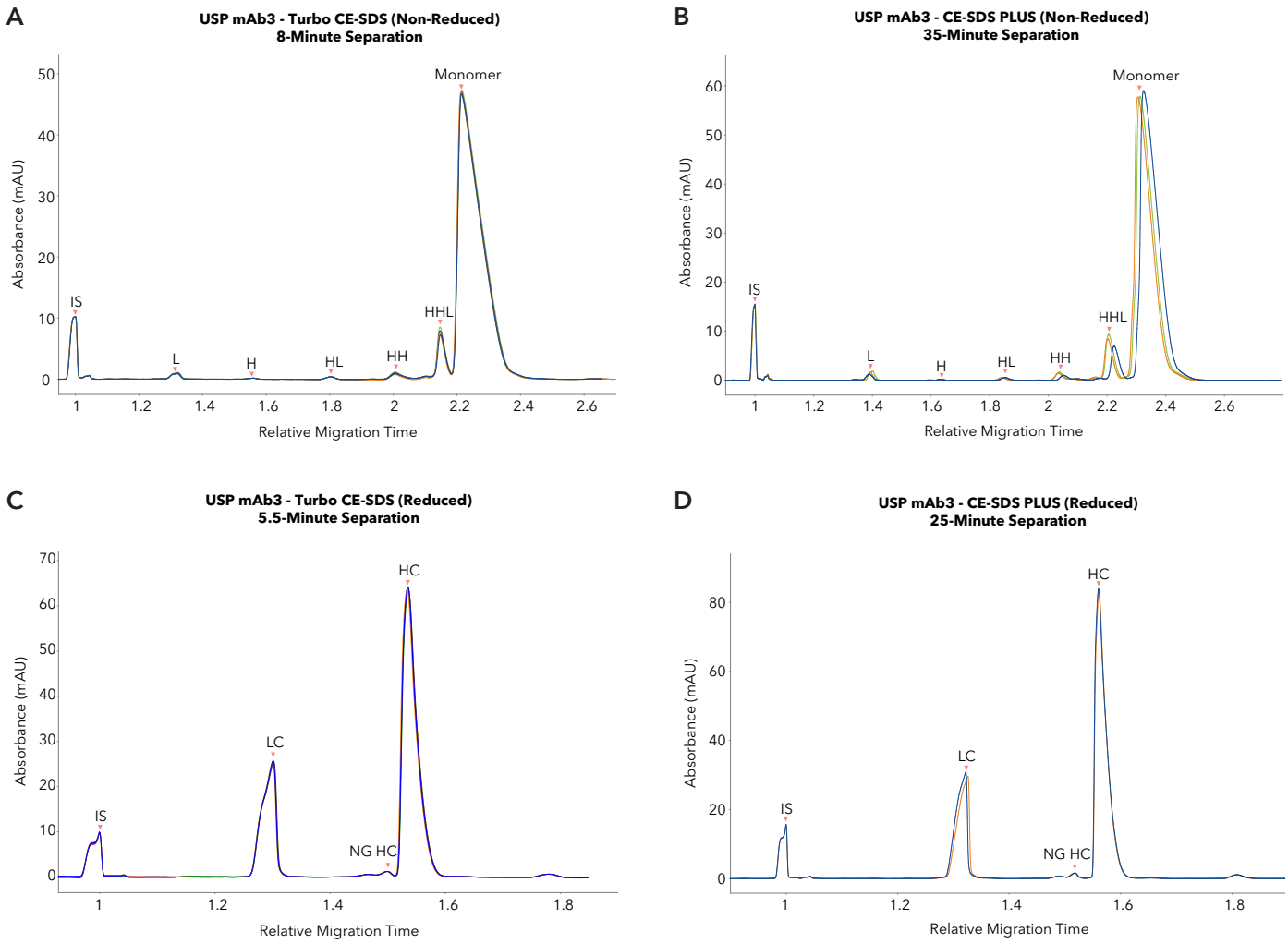


FIGURE 4. Analysis of USP mAb3 with Turbo CE-SDS and CE-SDS PLUS under non-reduced conditions (A and B) and reduced conditions (C and D).