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APPLICATION NOTE

Get USP <129> Equivalent Data with Maurice CE-SDS

Analyzing protein size and purity has become faster and easier with automated CE-SDS platforms. However, the suitability of such methods must be measured against compendial requirements. For IgG monoclonal antibodies, the U.S Pharmacopeia (USP) General Chapter <129> describes analytical procedures, namely size-exclusion chromatography (SEC) and capillary electrophoresis sodium dodecyl sulfate (CE-SDS), to assess therapeutic purity[1]. In this application note we demonstrate the comparability of the Maurice CE-SDS PLUS method with the USP<129> protocol for analysis of monoclonal antibodies. Using USP's IgG System Suitability Reference Standard, the USP <129> method was first run on Maurice to determine ease of method transfer, followed by optimization of the Maurice protocol for comparison with USP <129>.

A Note on Maurice

Maurice simplifies protein charge and size analysis while providing high-quality data. Offering both CE SDS and icIEF capabilities, Maurice automates CE analysis by removing the cumbersome steps required in conventional SDS-PAGE and IEF techniques. All you need to do is prepare and load your samples along with the pre-assembled cartridges. Maurice takes care of the rest. You get CE-SDS results in less than 35 minutes and data can be analyzed on either Compass for iCE software or on Waters Empower® software with the Maurice Empower Control Kit.

Materials and Methods

The monoclonal IgG System Suitability Reference Standard (PN 1445550) was obtained from the USP. The Maurice CE-SDS PLUS Application Kit (PN PS-MAK03-S) was obtained from ProteinSimple, a Bio-Techne brand and its components, along with additional reagents procured from other vendors, are listed below.

Name	Vendor	Catalog #
CE-SDS PLUS Cartridges	ProteinSimple	PN PS-MC02-SP
CE-SDS Plus 1X Sample Buffer	ProteinSimple	PN 046-567
CE-SDS Wash Solution, 20 mL	ProteinSimple	PN 046-569
Maurice CE-SDS Running Buffer – Top	ProteinSimple	PN 046-384
Maurice CE-SDS Orange Pressure Caps	ProteinSimple	PN 046-572
Separation Matrix, 15 mL	ProteinSimple	PN 046-386
Running Buffer – Top, 10/pack	ProteinSimple	PN 046-384
Running Buffer – Bottom, 12 mL	ProteinSimple	PN 046-385
Conditioning Solution 1, 20 mL	ProteinSimple	PN 046-014
Conditioning Solution 2, 20 mL	ProteinSimple	PN 046-015
2 mL Glass Reagent Vials	ProteinSimple	PN 046-017
96-well Plates, 10/pk	ProteinSimple	PN 046-021
Clear Screw Caps for Sample Vials	ProteinSimple	PN 046-138
CE-SDS Internal Standard, 2/pk	ProteinSimple	PN 046-144
CE-SDS Cartridge Cleaning Vial	ProteinSimple	PN 046-125
SDS-MW Sample Buffer	SCIEX	PN 390953
β mercaptoethanol	Millipore Sigma	PN M-3148
Iodoacetamide	Millipore Sigma	PN I6125

For method optimization with CE-SDS PLUS, the lyophilized monoclonal IgG System Suitability Reference Standard was reconstituted in the CE-SDS PLUS 1X Sample Buffer to a final concentration of 1 mg/mL and a total volume of 50 µL. 2 µL of the 25X CE-SDS Internal Standard was added to this solution, along with either 2.5 µL of 14.2 β-ME for reduced samples, or 2.5 µL of 20mM IAM for non-reduced samples. Reduced samples were then denatured at 70°C for 10 minutes and non-reduced samples at 65°C for 5 minutes. Samples were kept on ice for 5 minutes before transferring to a 96-well plate and then centrifuged for 10 minutes at 1,000 x g. Samples and batch reagents were loaded onto Maurice along with a CE-SDS PLUS cartridge. The samples were injected for 20 seconds at 4,600 V and separated at 5,750 V for 25 minutes for reduced IgG samples and 35 minutes for non-reduced IgG samples. All data were analyzed and compiled with Compass for iCE, JMP®, and GraphPad Prism 9 software.

Results

Running the USP <129> method on Maurice

The monoclonal IgG system suitability sample was run on Maurice by largely following the USP <129> protocol first, including using the recommended sample buffer. The non-reduced method yielded highly comparable results to the one highlighted in the suitability standard's reference document, as shown in **FIGURES 1A** and **1B**, where all 7 peaks were detected. Similarly, the reduced IgG run on Maurice resulted in all the expected peaks (HC, LC, and NGHC), along with a small number of species running slower than the heavy chain, which is also seen in the reference document (**FIGURES 1C** and **1D**). Such comparable data from both reduced and non-reduced experiments demonstrated that standard methods from conventional CE-SDS platforms can easily be transferred to Maurice.

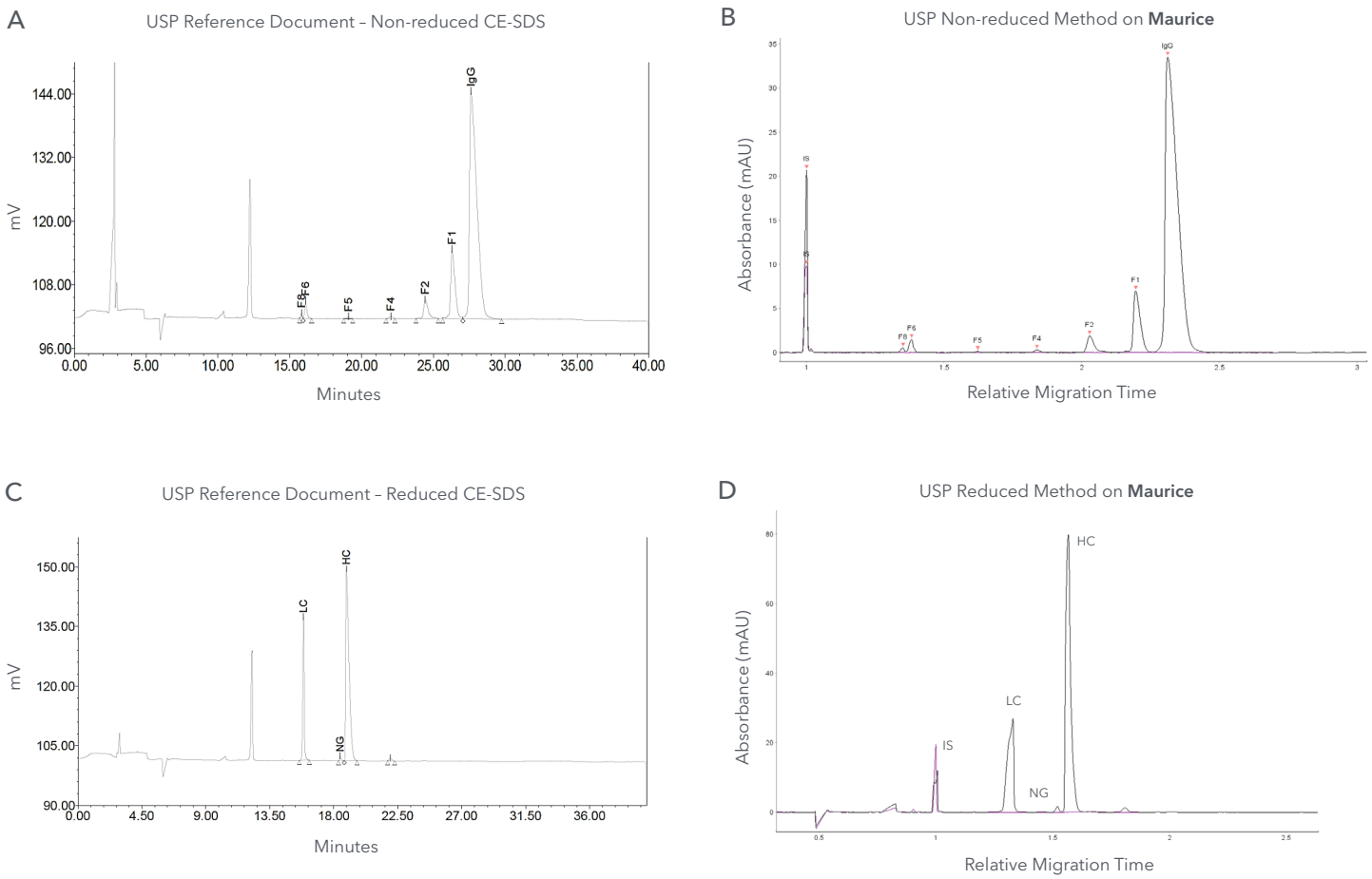

FIGURE 1. Running the USP <129> protocol on Maurice using the IgG System Suitability Reference Standard. **(A)** Non-reduced CE-SDS results in the USP reference document, provided with the IgG system suitability sample. **(B)** Results from the USP-recommended non-reduced CE-SDS method on Maurice, where results are highly comparable with those in the reference document. **(C)** Reduced CE-SDS data in the USP reference document. **(D)** The USP-recommended reduced CE-SDS method on Maurice also generates similar results as in the reference document. Note **(B)** and **(D)** feature overlays of a blank injection.

Method optimization with Maurice CE-SDS PLUS

Using the same batch of samples from the previous experiment, the reproducibility of the USP <129> method was evaluated over a batch of 48 injections. A slight decrease (~3%) in the intact percent peak area was observed under non-reducing conditions. To improve these results, design of experiments (DOE) was leveraged to evaluate different experimental and instrument conditions for method optimization. Varying concentrations of the CE-SDS PLUS sample buffer were evaluated in this case, along with different sample concentrations, separation times, and injection voltages (**TABLE 1**). A linear correlation was observed between sample concentration and fragmentation, as seen in **FIGURE 2A**. However, the method was found to be robust even with varying CE-SDS PLUS sample buffer concentrations, separation times, and injection voltages, as seen in **FIGURES 2B, 2C**, and **2D**.

Experimental Condition	Range
Sample concentration	0.6-1.4 mg/mL
CE-SDS PLUS Sample Buffer concentration	0.5-1.5X
Separation time	10-30 minutes
Injection voltage	4,500-5,500 V

TABLE 1. Different sample and instrument conditions examined during method optimization.

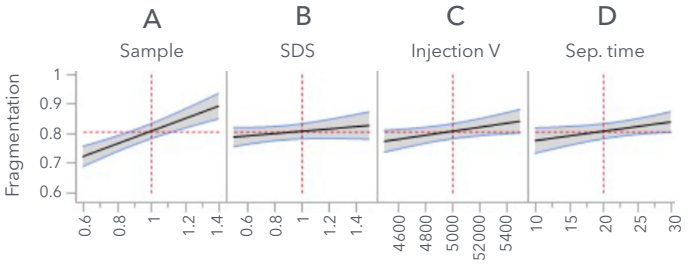

FIGURE 2. Evaluation of varying sample and instrument conditions. Sample concentration was linearly correlated with fragmentation **(A).** However, the CE-SDS PLUS method was not susceptible to changes of %SDS in the CE-SDS PLUS sample buffer **(B),** changing injection voltage **(C),** and varying separation times **(D).** These results were compiled using JMP.

Experimental Condition	Range
β-ME	0.3-1M
Denaturation time	5-15 minutes
Denaturation temperature	65-75°C

TABLE 2. Different conditions evaluated for reduced CE-SDS method optimization.

Further optimization of the reduced CE-SDS method on Maurice involved investigating parameters such as reducing agent concentration, denaturation time, and denaturation temperature. **TABLE 2** shows the range of the different conditions evaluated. Extra peaks associated with incomplete reduction of the IgG sample were observed at denaturation temperatures lower than 70°C (data not shown). However, neither increasing the concentration of β-ME nor increasing the denaturation time affected the IgG profiles. Consequently, the optimized method called for 0.71M β-ME and sample denaturation for 10 minutes at 70°C. This method was then compared with the USP-recommended reduced method. Results from both methods were remarkably similar **(FIGURE 3A)**, further corroborated by the quantification of percent peak areas shown in **FIGURE 3B**, which presents the statistical analysis (Mann-Whitney test) of the data.

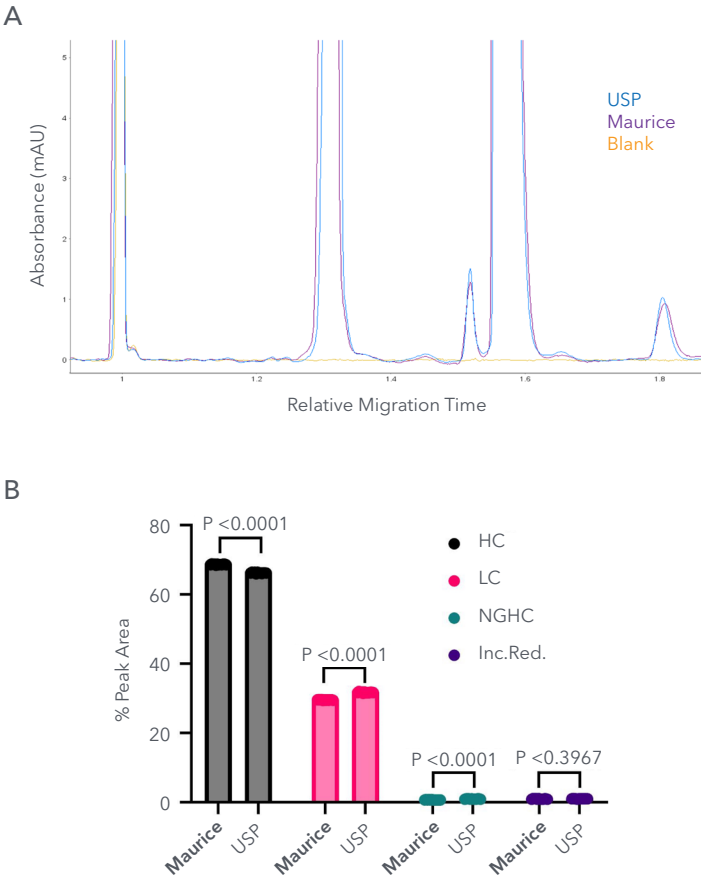

FIGURE 3. Comparison of the USP and Maurice methods under reduced conditions. **(A)** An overlay of the electropherograms generated by both methods showed that Maurice performs comparably to the USP method. **(B)** Calculation of P values using the Mann-Whitney test, which was done on GraphPad Prism (HC: heavy chain; LC: light chain; NGHC: non-glycosylated heavy chain; Inc. Red: incomplete reduction). Measuring the % peak area further established the comparability of both methods.

Different concentrations of the alkylating agent (IAM), different denaturation times, and temperatures were evaluated for non-reduced CE-SDS method optimization. Among the three parameters, the concentration of IAM appeared to be the most significant factor, as seen in **FIGURE 4A**, where a higher concentration correlated with lower fragmentation. Shorter denaturation times at lower temperatures were also found to cause lesser fragmentation (~15% reduction in peak areas of fragments, **FIGURE 4B**). Therefore, the optimized CE-SDS PLUS method called for treating IgG samples with 20 mM IAM and subjecting them to denaturation at 65°C for 5 minutes.

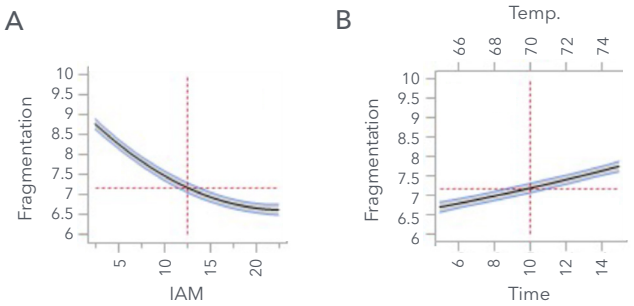

FIGURE 4. Non-reduced method optimization with varying IAM concentrations, denaturation times, and temperatures. Higher IAM concentrations caused less fragmentation **(A)**, as did lower denaturation temperatures for shorter durations **(B)**. These results were compiled using JMP.

Compared to the USP <129> method, the optimized non-reduced Maurice method was found to result in lower fragmentation. **FIGURE 5A** shows an overlay of the results obtained from both methods. Not only was a reduction in fragmentation observed with the Maurice method, but finer details, e.g., a doublet in fragment 2 (F2) were seen. Statistical analysis (Mann-Whitney test) of this data is shown in **FIGURE 5B**. The degree of fragmentation from each of these methods was quantified and showed that the Maurice method led to nearly a 20% decrease in fragmentation.

Compared to the percent peak area of the intact IgG from the USP method, referred to earlier during the assay reproducibility assessment, the modified method showed a significantly smaller change over the 48-injection batch. Therefore, while the USP method itself was reproducible, changing the sample buffer and modifying the denaturation conditions helped further minimize changes over a long batch **(FIGURE 6)**.

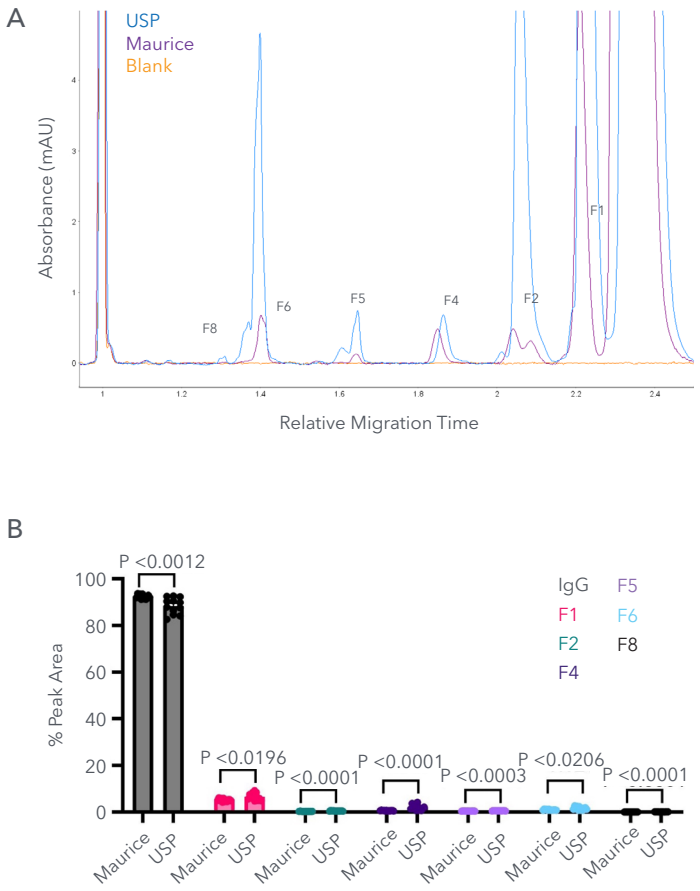

FIGURE 5. Comparison of USP and Maurice non-reduced methods. (A) An overlay of the electropherograms from both methods and a blank clearly shows a significant reduction in fragmentation with the Maurice method. **(B)** Calculation of P values using the Mann-Whitney test, which was done on GraphPad Prism. Quantification of the % peak areas confirms that the Maurice non-reduced method results in at least 20% reduction of fragmentation (IgG: intact peak; F1: fragment 1, F2: fragment 2 etc.).

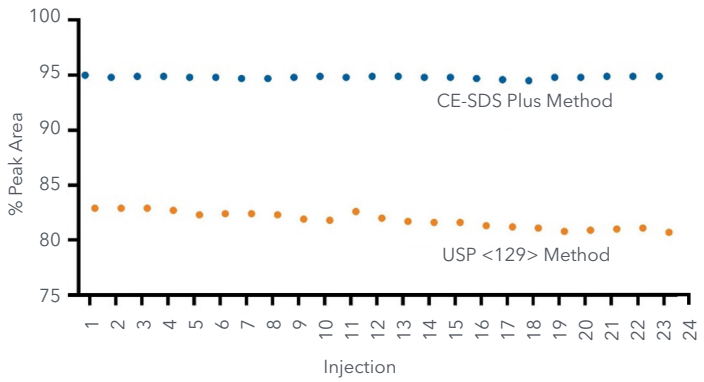

FIGURE 6. Evaluation of assay reproducibility. Although the USP <129> method showed good reproducibility, a small decay (~3%) in the percent peak area of the intact peak was observed over a 48-injection batch. However, the optimized method using the CE-SDS PLUS sample buffer improved the reproducibility.

Linearity of the optimized methods

The linearity of reduced and non-reduced optimized methods was evaluated using the IgG System Suitability Reference Standard. The sample concentrations ranged from 0.18 mg/mL up to 2 mg/mL. A 48-injection batch was run, with each concentration run in triplicate. **FIGURES 7A** and **7B** show the linearity of non-reduced and reduced methods, respectively. R^2 values >0.99 were observed across the entire concentration range for the non-reduced Maurice method, thus providing excellent linearity up to 2 mg/mL. The reduced method also showed good linearity up to 2 mg/mL, but the best R^2 values (>0.97) were observed for samples ranging from 0.26-1.2 mg/mL. **TABLE 3** lists the R^2 values of the heavy chain, light chain, and non-glycosylated heavy chain.

Peak	R^2
HC	0.9737
LC	0.9918
NGHC	0.9775

TABLE 3. R^2 values for peaks from the reduced Maurice method.

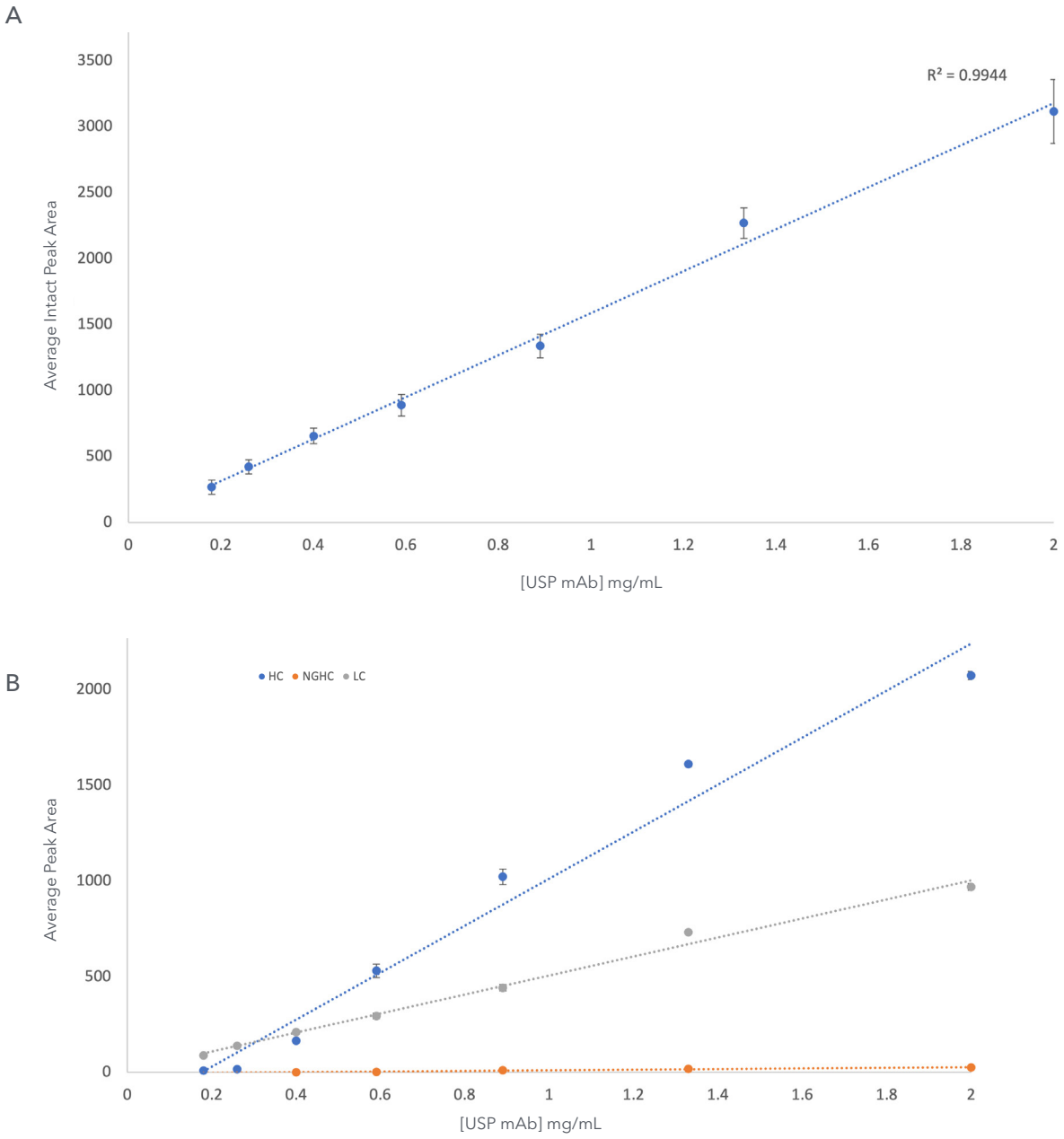

FIGURE 7. Dilutional linearity of the optimized Maurice methods. **(A)** The non-reduced method showed excellent linearity across the entire sample concentration range tested. **(B)** Good linearity was observed even in the reduced method, but sample concentrations between 0.26-1.2 mg/mL resulted in optimal R^2 values.

Additional data comparability

Finally, three USP performance standards were analyzed on Maurice using the optimized methods and the results were compared with those from USP <129>. Overall, the results from Maurice demonstrated high agreement with the compendial ones **(FIGURES 8A-8C)**. Notably, certain differences were observed for mAb3 by using the two methods **(FIGURE 8C)**.

Specifically, compared to the USP method, the optimized Maurice method showed significantly lower levels of fragmentation for mAb3 under non-reducing conditions. These data further confirm that CE-SDS methods can be easily transferred to Maurice, requiring little to no method optimization.

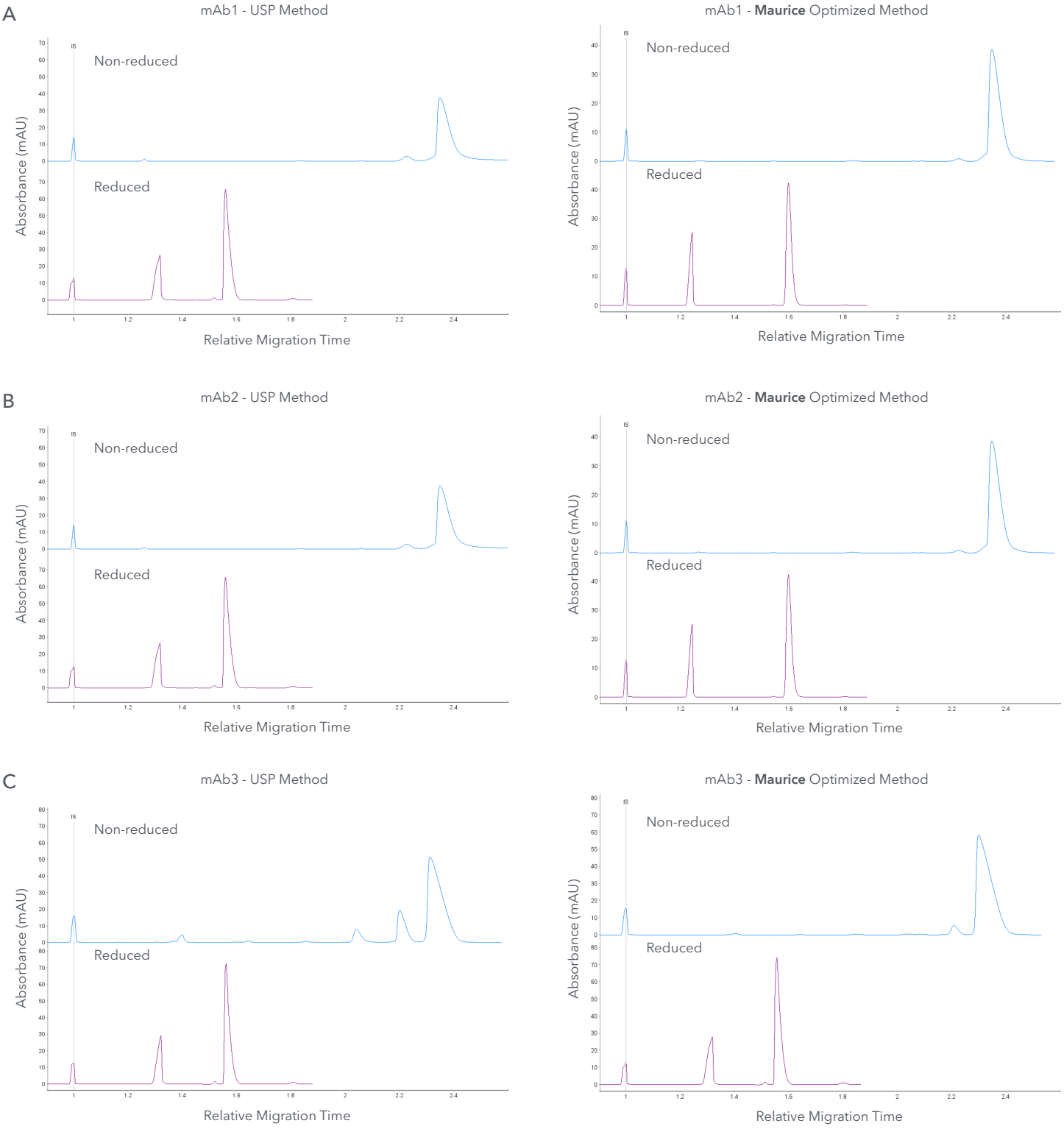

FIGURE 8. Comparison of the USP <129> (left) and optimized Maurice methods (right) in the analysis of different USP standards. **(A)** Reduced and non-reduced analysis of mAb1 with both methods yielded highly comparable results. **(B)** Results of mAb2 analysis using both methods were also in agreement with each other. **(C)** The reduced mAb3 sample generated comparable results from both methods, but lower levels of fragmentation were observed with the non-reduced optimized Maurice method.

Conclusion

Adopting analytical techniques that follow compendial guidelines and meeting their criteria doesn't have to be difficult, even if you're using different platforms. The study described in this application note demonstrated the ease of adopting the USP <129> protocol on Maurice. The highly comparable results of multiple USP standards generated are proof that method transfer is fast and easy. The method was also found to be reproducible, with a small decay in percent peak area observed over a batch of 48 injections. In addition to an already successful transfer, optimizing the method with Maurice's CE-SDS PLUS buffer further improved the reproducibility and was also found to decrease fragmentation in non-reduced experiments. Thus, Maurice lets you get reliable USP <129>-suitable results from your CE-SDS experiments without wasting precious time on method optimization.

References

1. United States Pharmacopeia. (2017). General Chapter <129>. Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies. Retrieved from http://go.usp.org/l/323321/2018-10-16/xxzsl

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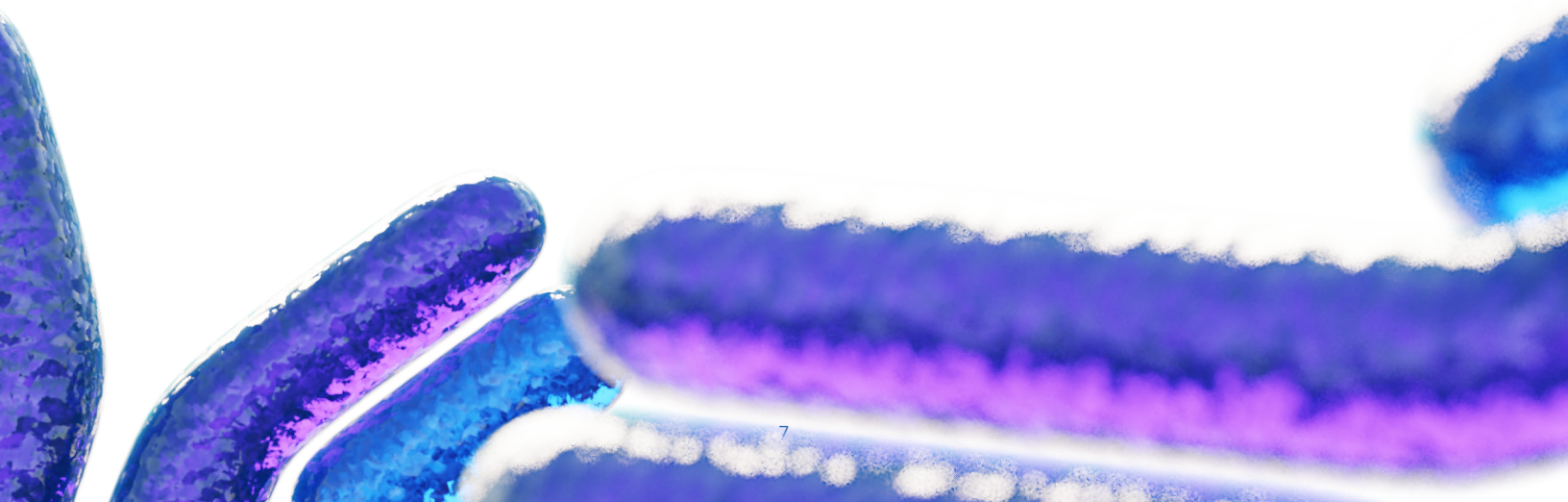

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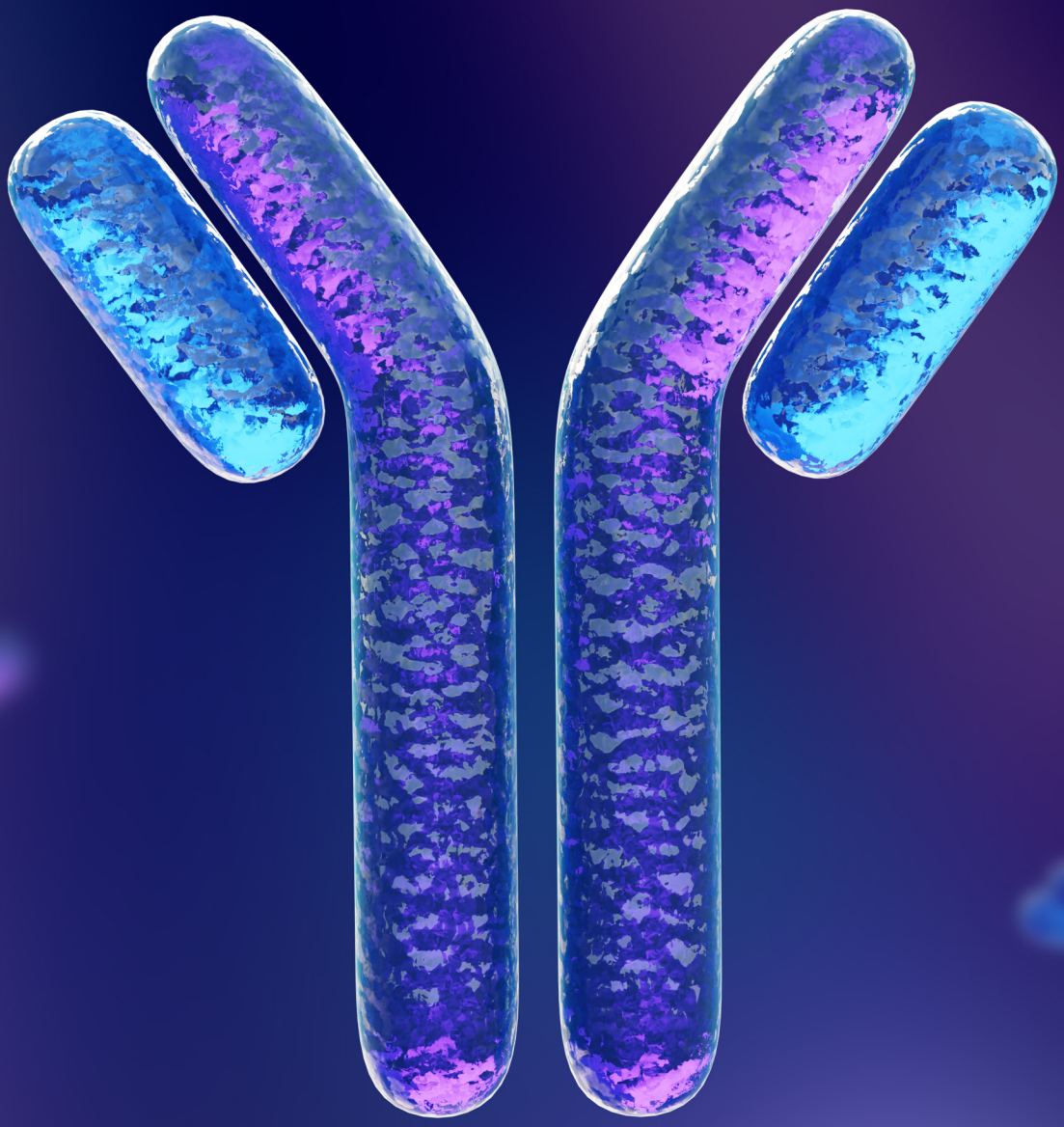

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