bio-techne®

Protocol

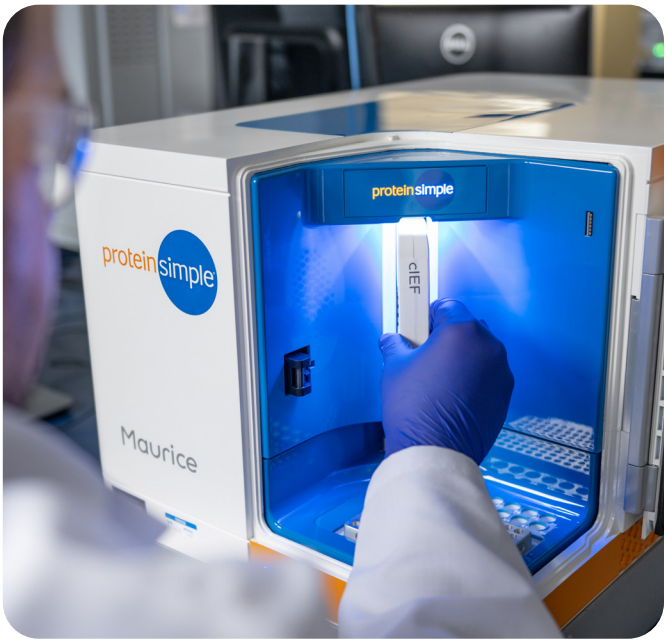
Mosunetuzumab

Introduction

Mosunetuzumab (brand name Lunsumio™) is a bispecific antibody (BsAb) used to treat follicular lymphoma. It binds to two different targets: CD20 on B-cells and CD3 on T-cells.

The simultaneous binding of Mosunetuzumab to two different targets ultimately leads to the death of cancerous B-cells[1]. Developed by Genentech, Mosunetuzumab was approved as a therapeutic by the FDA in December 2022.

This monograph demonstrates the charge and size characterization of the innovator drug and a research-grade biosimilar of Mosunetuzumab through imaged capillary isoelectric focusing (icIEF) and capillary electrophoresis sodium dodecyl sulfate (CE-SDS).



Maurice icIEF Method

Sample Preparation
The innovator drug was procured from Genentech. The Mosunetuzumab biosimar (#ICH5026) was procured from Ichlorbio. The samples were diluted to a final concentration of 0.1 mg/mL in the ampholyte solution.

Ampholyte solution
BR1 (Broad Range 3 to 10)
NR3 (Narrow Range 8 to 10.5)
5 mM arginine
These reagents are provided in the Maurice cIEF Method Development Kit (PN PS-MDK01-C)

pI markers
7.05 (PN 046-032)
9.5 (PN 046-047).

Running conditions
1 minute at 1500 V then 12 minutes at 3000 V.

Imaging
Absorbance and native fluorescence.

Data Analysis
Compass for iCE, Version 4.0.0.

Results

Figure 1 shows representative electropherograms of the innovator and biosimilar, detected with absorbance using the Maurice icIEF method. The acidic, main, and basic peaks are clearly separated, with their percent peak area and reproducibility captured in **Table 1**.

Similarly, native fluorescence (NF) detection of the samples is shown in **Figure 2**, with the results listed in **Table 2**. Advantages of NF on the Maurice system include 4X higher sensitivity than absorbance, therefore suitable for analyzing lower amounts of analyte, and generating quieter baselines, which simplify method optimization and transfer[2].

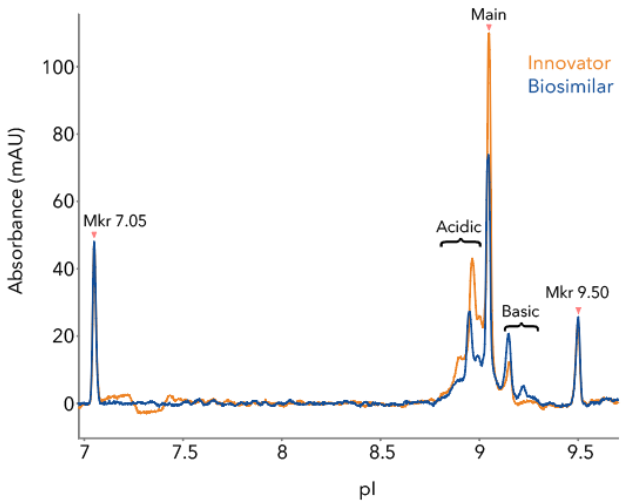


FIGURE 1. Charge profile of Mosunetuzumab with absorbance detection, resolving into acidic, main, and basic peaks. The pI markers are indicated by "Mkr".

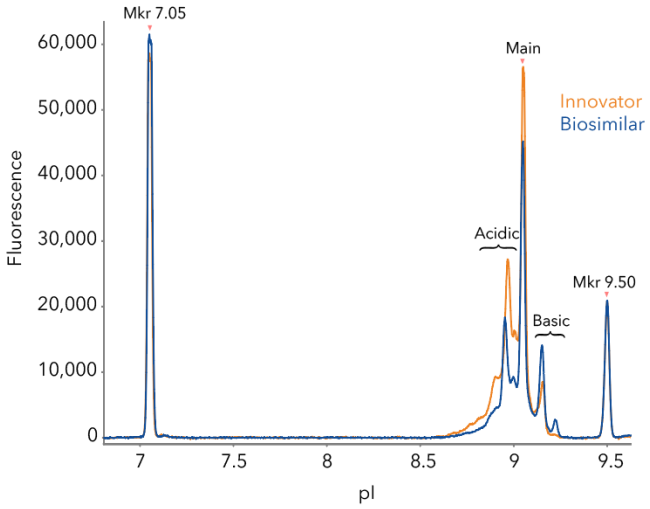


FIGURE 2. Charge profile of the innovator and biosimilar with native fluorescence detection, resolving into acidic, main, and basic peaks. The baselines observed are quieter compared to the results from absorbance detection.

Percent Peak Area (Absorbance), n=3						
	Innovator			Biosimilar		
	Acidic	Main	Basic	Acidic	Main	Basic
Average	49.03	43.60	7.37	37.6	42.57	19.87
Standard Deviation	0.15	0.10	0.06	0.10	0.25	0.21
% RSD	0.31	0.23	0.78	0.27	0.59	1.05

TABLE 1. Comparative icIEF results for percent peak area between the innovator and biosimilar detected with absorbance. Results are displayed for three consecutive injections of each sample. An overall relative standard deviation (%RSD) of ≤1.05 demonstrates excellent reproducibility of this method.

Percent Peak Area (Native Fluorescence), n=3						
	Innovator			Biosimilar		
	Acidic	Main	Basic	Acidic	Main	Basic
Average	52.10	39.77	8.13	39.93	43.33	16.73
Standard Deviation	0.61	0.59	0.12	0.31	0.35	0.06
% RSD	1.17	1.47	1.42	0.77	0.81	0.35

TABLE 2. Comparative icIEF results for percent peak area between the innovator and biosimilar detected with native fluorescence. Results are displayed for three consecutive injections of each sample, with an excellent %RSD value of ≤1.47.

Maurice CE-SDS Method

Sample Preparation
The innovator drug and a research-grade biosimilar of Mosunetuzumab were each diluted to 1 mg/mL using the Maurice 1X CE-SDS PLUS Sample Buffer (PN 046-567).

The Maurice CE-SDS 25X Internal Standard (IS, 4%, PN 046-144) was added to all samples, followed by the addition of 5% (V/V) of either iodoacetamide (IAM, 250 mM) for non-reduced analysis, or β-mercaptoethanol (β-ME, 14.2 M) for reduced analysis.

All samples were then heated for 10 minutes at 70°C, cooled on ice for five minutes and finally subjected to centrifugation.

Running conditions
Injection for 20 seconds at 4600 V, then separation at 35 minutes (non-reduced) or 25 minutes (reduced) at 5750 V.

Cartridge
CE-SDS PLUS (PN PS-MC02-SP)

Detection
Absorbance (220nm)

Data Analysis
Compass for iCE, Version 4.0.0

Results

Figure 3 shows representative electropherograms of the innovator and biosimilar analyzed with Maurice CE-SDS under non-reduced conditions, with the RSD value listed in **Table 3**.

Figure 4 shows the purity of both samples under reduced conditions along with the separation of the individual heavy chains (HCs) of the BsAb.

Quantitation results are shown in **Table 4** on the next page. Both methods were highly reproducible, with %RSDs under 2%.

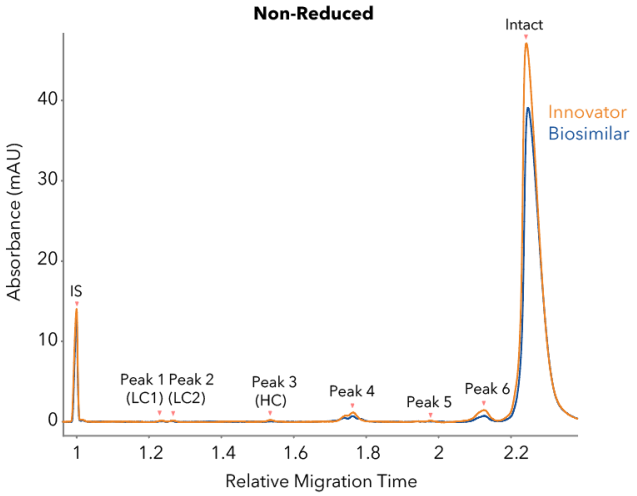


FIGURE 3. Non-reduced CE-SDS analysis of the innovator and biosimilar. The method is sensitive enough to detect minor peaks in both samples.

Percent Peak Area (Intact Peak), n=4			
Sample	Average	Standard Deviation	% RSD
Innovator	94.70	0.18	0.19
Biosimilar	96.75	0.51	0.52

TABLE 3. Results from four different injections each of the innovator and biosimilar under non-reduced conditions. Excellent %RSD values confirm the method's reproducibility.

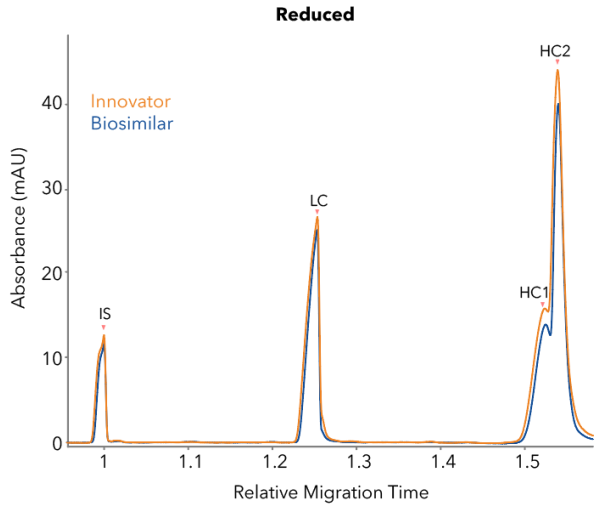


FIGURE 4. Reduced CE-SDS analysis of Mosunetuzumab. The abundant peaks are the light chain (LC) and heavy chains (HC1 and HC2), which are expected for CE-SDS runs under reduced conditions.

Percent Peak Area (n=4)						
	Innovator			Biosimilar		
	HC2	HC1	LC	HC2	HC1	LC
Average	48.03	18.58	33.38	45.58	20.73	33.70
Standard Deviation	0.25	0.13	0.19	0.34	0.36	0.28
% RSD	0.52	0.68	0.57	0.75	1.73	0.84

TABLE 4. Results from four different injections each of the innovator and biosimilar under reduced conditions, with %RSD values ≤1.73 overall, highlighting the reproducibility of the reduced method.

Conclusion

The Maurice™ system was used to characterize both the innovator and a research-grade biosimilar of Mosunetuzumab. Through analysis with icIEF and CE-SDS on the same instrument, distinct charge profiles and separation of peaks were observed, demonstrating the Maurice system's strength in assessing qualitative differences between innovator and biosimilar molecules.

References

1. **Hosseini I, Gadkar K, Stefanich E, Li CC, Sun LL, Chu YW, Ramanujan S.** Mitigating the risk of cytokine release syndrome in a Phase I trial of CD20/CD3 bispecific antibody mosunetuzumab in NHL: impact of translational system modeling. NPJ Syst Biol Appl. 2020 Aug 28;6(1):28. doi: 10.1038/s41540-020-00145-7. PMID: 32859946; PMCID: PMC7455723.
2. **Application Note:** Improving Charge Variant Analysis with Maurice Native Fluorescence, *ProteinSimple, a Bio-Techne brand.*

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