Belimumab Protocol

Introduction

Belimumab (brand name Benlysta®) is a monoclonal antibody used to treat systemic lupus erythematosus (SLE). It binds to soluble B lymphocyte stimulator (BLyS), preventing it from promoting B cell survival and differentiation, which leads to a decrease in abnormal B cell production and consequently, a reduction of SLE-related symptoms. Developed by GlaxoSmithKline, Belimumab was approved by the FDA in March 2011 as the first targeted biologic therapy for SLE patients in over 50 years[1,2].

The protocol presented here details the analysis of Belimumab for charge heterogeneity and size characterization by imaged capillary isoelectric focusing (icIEF) and capillary electrophoresis sodium dodecyl sulfate (CE-SDS) respectively, on the Maurice™ system. Data are shown for both the innovator drug and a research-grade biosimilar.

Maurice icIEF Method

Sample Preparation

The Belimumab innovator drug was procured from GlaxoSmithKline. The biosimilar (#ICH5080) was obtained from Ichorbio. Each sample was diluted to a final concentration of 0.1 mg/mL in the prepared ampholyte solution.

Ampholyte Solution

4% Pharmalytes (4:1 of 8-10.5: 3-10) and 20% SimpleSol (PN 046-575).
These reagents are provided in the Maurice cIEF Method Development Kit (PS-MDK01-C).

pI Markers

7.05 (PN 046-032)
9.50 (PN 046-047)

Running Conditions

1 minute at 1500 V, then 12 minutes at 3000 V.

Cartridge

Maurice cIEF (PS-MC02-C)

Data Analysis

Compass for iCE, Version 4.0.0.

Results

Figure 1A shows the representative electropherograms of the innovator and biosimilar samples analyzed for charge heterogeneity with absorbance detection. While five peaks were detected in both samples, a clear shift in pI values was observed in the biosimilar, indicating the need for further analysis of individual peaks to ascertain the cause for this shift. Quantitative data on these peaks is presented in **Table 1**. Another published study describes the charge heterogeneity analysis and charge variant fraction collection of the Belimumab innovator and biosimilar on the MauriceFlex™ system, followed by LC-MS characterization, which revealed different structural modifications between the two samples[3]. **Figure 1B** shows the charge heterogeneity of both samples detected with native fluorescence, a key feature of the Maurice system that offers 4X the sensitivity and quieter baselines, allowing the use of lower amounts of samples for analysis[4]. Five key peaks in both samples were detected, with their percent peak areas listed in **Table 2**.

FIGURE // 01

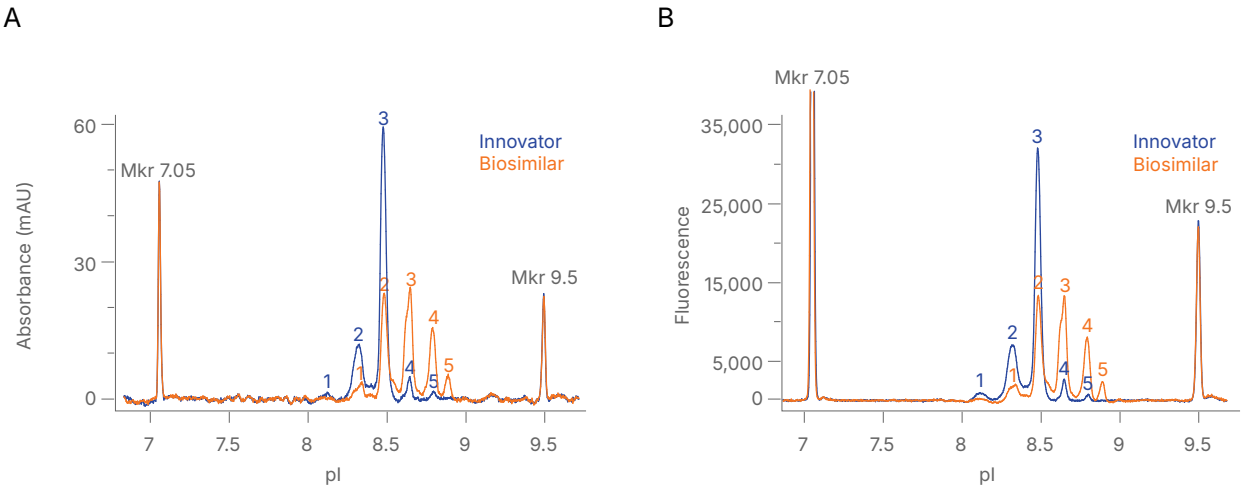

Figure 1. Charge profiles of Belimumab on the Maurice system. A. An overlay of the profiles of the innovator and biosimilar with absorbance detection and B. native fluorescence detection. Both modes of detection revealed five peaks for each sample, with a shift in pI values for the biosimilar. For example, peak 1 of the biosimilar corresponds to peak 2 of the innovator. The pI markers are indicated by "Mkr".

TABLE // 01

Percent Peak Area - Absorbance (n=3)

		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Innovator	Average	1.7	24.30	68.30	4.20	1.47
	Standard Deviation	0.10	0.35	0.61	0.17	0.06
	% RSD	5.88	1.43	0.89	4.12	3.94
Biosimilar	Average	6.37	32.50	35.37	20.43	5.33
	Standard Deviation	0.15	0.00	0.15	0.25	0.15
	% RSD	2.40	0.00	0.43	1.23	2.86

Table 1. Comparison of percent peak areas between the innovator and biosimilar samples detected with absorbance. Results are listed for three consecutive sample injections, showing an overall %RSD of <4%.

TABLE // 02

Percent Peak Area - Native Fluorescence (n=3)

		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Innovator	Average	3.30	26.33	65.37	4.03	0.97
	Standard Deviation	0.10	0.06	0.21	0.15	0.06
	% RSD	3.03	0.22	0.32	3.79	5.97
Biosimilar	Average	8.10	35.27	34.63	18.03	3.97
	Standard Deviation	0.17	0.45	0.47	0.06	0.06
	% RSD	2.14	1.28	1.36	0.32	1.46

Table 2. Comparison of percent peak areas between the two samples detected with native fluorescence. Results are listed for three consecutive sample injections, with an overall % RSD of <6.

Maurice CE-SDS Method

Sample Preparation

The innovator drug and a research-grade biosimilar of Belimumab were each diluted to 1 mg/mL using the Maurice 1X CE-SDS PLUS Sample Buffer (PN 046-567). Buffer exchange was with 0.25X Phosphate-Buffered Saline (PBS). 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 nM) 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

Maurice CE-SDS PLUS (PS-MC02-SP)

Detection

Absorbance (220 nm)

Data Analysis

Compass for iCE, Version 4.0.0

Results

Figure 2A shows an overlay of the innovator and biosimilar samples analyzed with CE-SDS under non-reduced conditions. The profiles were found to be largely comparable. Percent peak area and RSD values are listed in **Table 3**. Similarly, **Figure 2B** presents data from reduced CE-SDS analysis, where the biggest difference between the two samples is apparent from the light chain peaks; the biosimilar's LC peak splits into two, indicated by LC and LC′. While more research is required to determine the exact cause, such an observation could be attributed to clipping at specific amino acid sites[3]. Quantitation results are shown in **Table 4**.

FIGURE // 02

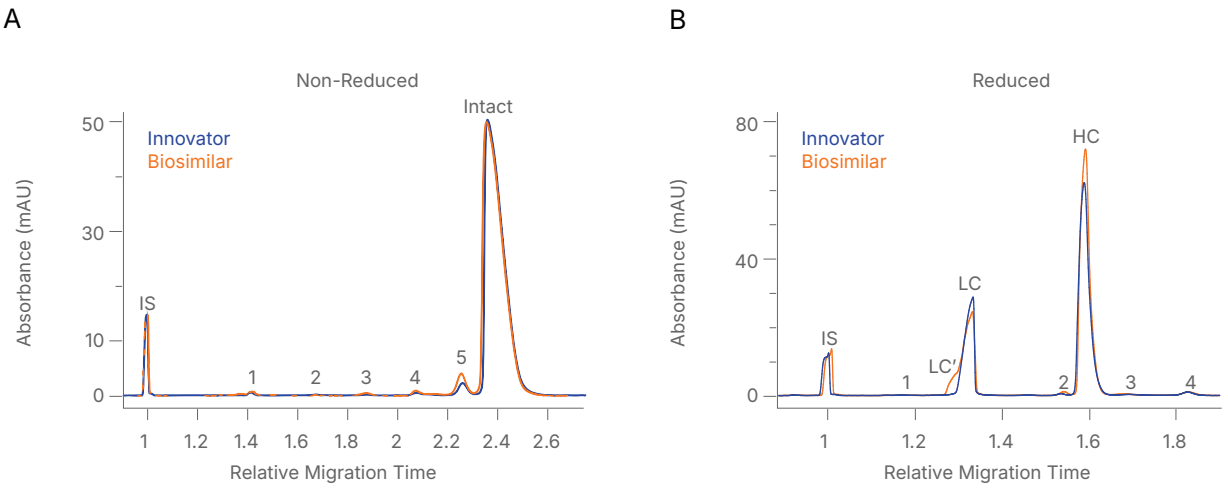

Figure 2. Size separation of Belimumab using CE-SDS on the Maurice system. A. An overlay of the innovator and biosimilar samples analyzed under non-reduced conditions. B. An overlay of both samples under reduced conditions, revealing differences in the biosimilar LC.

TABLE // 03

Percent Peak Area for Intact Peak (n=4)

Sample	Average	Standard Deviation	%RSD
Innovator	96.4	0.0	0.0
Biosimilar	94.4	0.0	0.0

Table 3. Quantitation of the intact peak of the Belimumab innovator and biosimilar, calculated from four consecutive injections of each sample. RSD values of 0% demonstrate outstanding reproducibility of this method.

TABLE // 04

Percent Peak Area (n=4)

	Innovator		Biosimilar		
	HC	LC	HC	LC	LC'
Average	64.68	33.83	63.13	29.70	4.93
Standard Deviation	0.10	0.10	0.13	0.32	0.05
% RSD	0.15	0.28	0.20	1.06	1.02

Table 4. Quantitation of the LC and HC peaks of the innovator and biosimilar samples, with an overall %RSD value of ≤ 1.06.

Conclusion

This protocol outlines the charge heterogeneity and size characterization of Belimumab, a therapeutic monoclonal antibody. Using icIEF and CE-SDS on the Maurice system, the innovator drug and a research-grade biosimilar were analyzed and compared. The results reveal differences in charge heterogeneity and size variants (mainly light chain) heterogeneity between the two samples. Overall, the Maurice system offers a reliable and sensitive method for the characterization of protein therapeutics and biosimilars, thus enhancing analytical development and quality control in the biopharmaceutical industry.

REFERENCES

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2. https://go.drugbank.com/drugs/DB08879
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4. Application Note: Improving Charge Variant Analysis with Maurice Native Fluorescence, ProteinSimple, a Bio-Techne brand.

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