Biosimilar Platform Methods on Maurice

Summary

Biopharmaceuticals represent many of the most profitable drugs sold[1]. These therapeutics currently generate over 100 billion dollars annually in global sales. The expiry of patent protection for many biological medicines presents an attractive opportunity for the development of biosimilars, which are amino-acid-copy drugs intended to offer comparable safety and efficacy to the off-patent innovator biologics. The approval of a biosimilar requires several criteria to be met, one of which is the demonstration of comparability with the innovator molecule through various analytical techniques[2].

These techniques are key to showing that biosimilars have no significant differences with the innovator in terms of quality, efficacy and safety. The complex nature of large biologics demands sensitive, quantitative and robust analytical techniques to ensure critical quality attributes (CQAs) meet the requirements mandated by global regulatory authorities. Imaged capillary isoelectric focusing (icIEF) is used extensively for examining CQAs of charge variants[3]. Obtaining matching icIEF data between the innovator and biosimilar is one way that similarity is demonstrated for a biosimilar[2].

To facilitate rapid, accurate comparisons to innovator molecules, ProteinSimple has developed a robust icIEF platform method for the charge heterogeneity analysis of a wide variety of biosimilars. This method was developed on the next-generation capillary electrophoresis system, Maurice™, which combines both icIEF and CE-SDS detection schemes in one fully automated system for protein profiling by either size or charge separation methods. The icIEF method robustness was confirmed by design of experiments (DOE) using a central composite design (CCD). In addition, the innovator and cognate biosimilar molecules were evaluated by Maurice's CE-SDS mode using platform methods.

Workflow

MATERIALS

ProteinSimple

- Instrument: Maurice
- Maurice cIEF Method Development Kit (PN PS-MDK01-C)
- Maurice CE-SDS Application Kit (PN PS-MAK02-S)
- Maurice CE-SDS Cartidges (PN PS-MC02-S)
- Maurice cIEF Cartridges (PN PS-MC02-C)

Other reagents

- Carboxypeptidase B (Sigma-Aldrich, PN C9584)
- β-mercaptoethanol (β–ME, Sigma-Aldrich, M6250)
- Iodoacetamide (IAM, Sigma-Aldrich, A3221)

Samples

- Innovator and biosimilar therapeutic mAbs

PLATFORM METHODS

icIEF

1. Create an ampholyte mix consisting of 4% Pharmalytes (3% 8–10.5 and 1% 5–8), 3.2 M urea, 5 mM IDA,10 mM arginine, and pI markers 5.85 and 10.17. The recipe in **Table 1** will make 1 mL of ampholyte mix.

2. Carboxypeptidase B (CpB), which removes terminal lysine residues, can be added before icIEF analysis to identify lysine variants in comparison with an untreated control[4]. To create CpB-treated samples, reconstitute CpB in water to a concentration of 1 mg/mL. Add CpB at a ratio of 1:100 (CpB to sample). In the examples below, samples were diluted to 1.0 mg/mL in water prior to CpB digestion. Incubate at 37 °C for 20 minutes and then place on ice.

3. Dilute samples to a final concentration of 0.2 mg/mL in the ampholyte mix. Run samples for 1 minute at 1500 V, and then 8 minutes at 3000 V.

1% MC	PHARMALYTES		8 M UREA	200 mM IDA	500 mM ARGININE	pI 5.85	pI 10.17	WATER
	5–8	8–10.5						
350 µL	12.5 µL	37.5 µL	500 µL	31.3 µL	12.5 µL	4 µL	4 µL	48 µL

TABLE 1. Recipe to create 1 mL of the icIEF ampholyte mixture. (MC) Methyl cellulose. (IDA) Iminodiacetic acid.

CE-SDS

1. Dilute samples to 1.0 mg/mL with 1X Sample Buffer.
2. Treat samples for 10 minutes at 70 °C in the presence of either 11.5 mM IAM (non-reducing) or 650 mM β–ME (reducing).
3. Inject samples for 20 seconds at 4600 V, followed by either a 25-minute separation for reduced samples or 35-minute separation for non-reduced samples at 5750 V.

Results

icIEF METHOD ROBUSTNESS

To determine the robustness of the icIEF platform method, a multivariate test of method conditions was conducted. A design of experiments (DOE) was developed and analyzed with JMP® software using a central composite design (CCD). The three variables chosen for this analysis are focus time, basic ampholyte concentration, and cathodic blocker concentration (**Table 2**). These variables were chosen because they are known to have the greatest overall effect from previous studies, DOE analysis, and troubleshooting experience.

CCD DOE	FOCUS TIME (MINUTES)	PHARMALYTE 8–10.5 (%)	ARGININE (mM)
Axial High	9.29	4.29	15.14
High	9	4	14
Center	8	3	10
Low	7	2	6
Axial Low	6.71	1.71	4.86

TABLE 2. Central composite design (CCD) to test the robustness of the icIEF platform method.

Two mAbs with non-overlapping isoelectric points and separation patterns, infliximab and rituximab, were mixed together and tested under each condition. Even at the extremes of all three parameters, the focusing pattern of the mAbs are similar to that at the target condition (**Figure 1**).

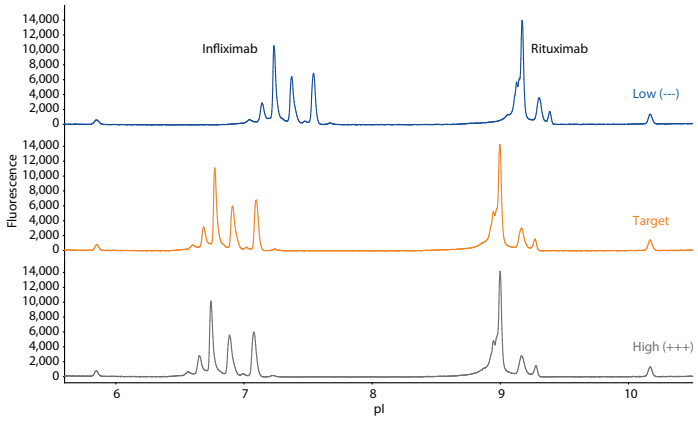


FIGURE 1. Electropherograms resulting from the icIEF platform method applied to a mixture of infliximab and rituximab at the low (–––), target, and high (+++) values of the three critical method parameters of focus time, basic ampholyte concentration, and cathodic blocker concentration.

Surface response analysis shows that the percent main peak (%MP) does not vary significantly in response to the variation of these parameters (**Figure 2**).

To provide assurance that the upper and lower pI markers are retained, the 17 DOE runs were repeated twice. Under no circumstance did the pI markers move outside the window of detection (**Figure 3**). Taken together, these results show that the method is robust and reproducible for the analysis of different therapeutic monoclonal antibodies.

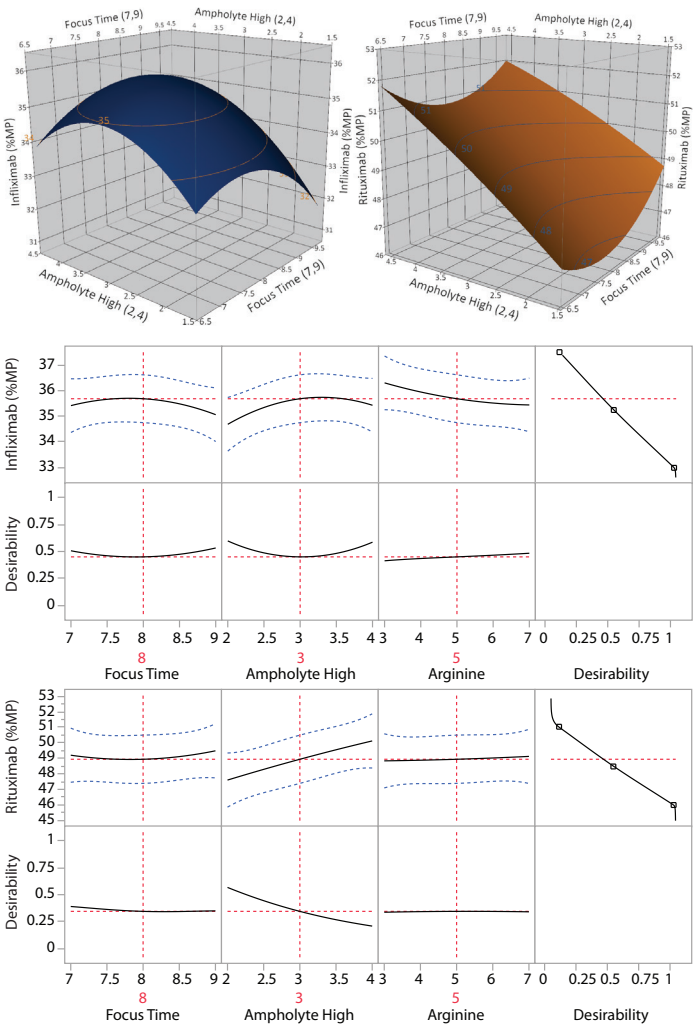


FIGURE 2. Robustness of the icIEF platform method. The percent main peak (%MP) of infliximab and rituximab are stable despite significant changes to critical method parameters. The samples in this analysis were not CpB-treated.

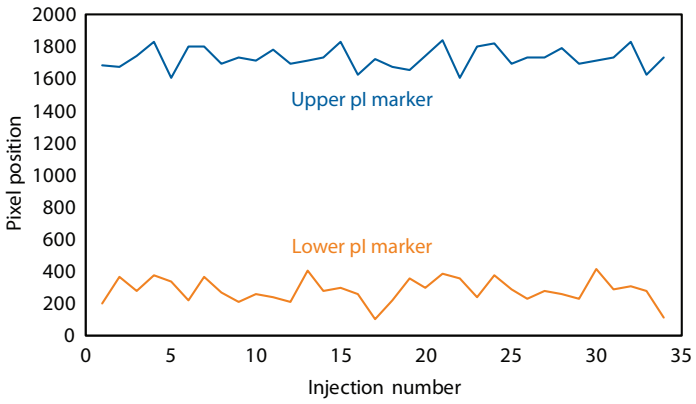


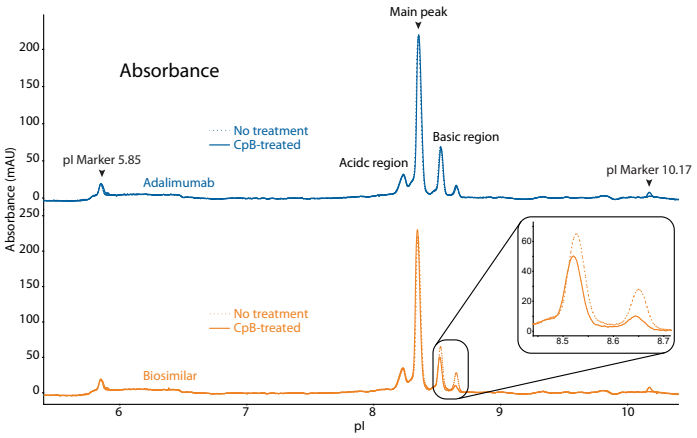
FIGURE 3. The lower and upper pI markers are retained throughout two consecutive repeats of the DOE (34 injections total). Pixels 0–2000 correspond to a window of detection between approximately pI 5–11 using this method.

ADALIMUMAB (HUMIRA®)

Adalimumab (trade name Humira®) is a biological drug used to treat rheumatoid arthritis, Crohn's disease and psoriasis. Adalimumab reduces inflammatory responses by inhibiting TNFα binding to its receptor. It was the best selling biopharmaceutical until its patent expired in 2016, reaching $16 billion in global sales annually[5]. Several biosimilars are already on the market.

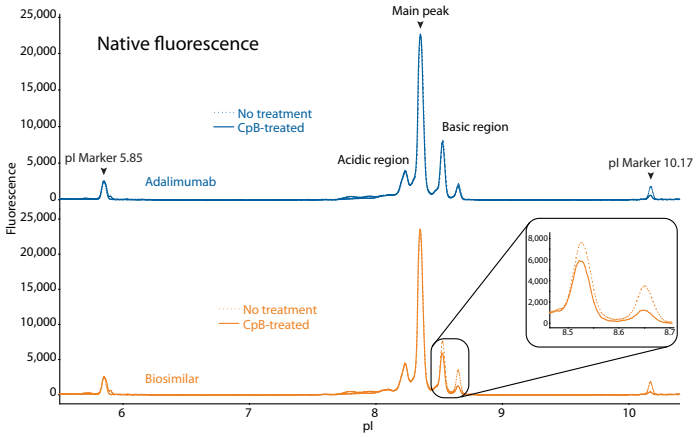
To compare adalimumab to a biosimilar, we used the icIEF platform method described above to monitor charge heterogeneity by absorbance (**Figure 4**) and by native fluorescence (**Figure 5**) detection.

These analyses revealed that the innovator and biosimilar are similar in charge heterogeneity and purity. To evaluate the presence of lysine variants, the antibodies were treated with CpB before analysis on icIEF. Treatment with CpB had a more dramatic effect on the basic region of the biosimilar than on the innovator molecule (**Figure 4, 5**), indicating that the biosimilar may have more terminal-lysine-containing variants in the final sample preparation.



SAMPLE		ACIDIC REGION	MAIN PEAK	BASIC REGION	Δ BASIC REGION
Adalimumab	No treatment	17.9	58.9	23.1	N/A
	CpB-treated	20.8	57.6	21.5	-1.6
Biosimilar	No treatment	19.7	56.0	24.3	N/A
	CpB-treated	24.0	59.0	17.0	-7.3

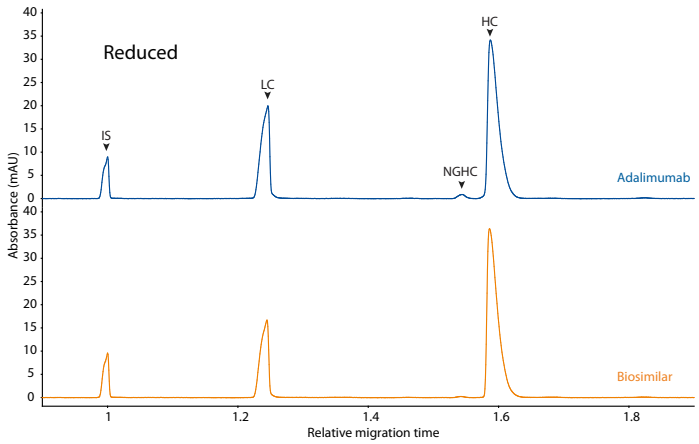
FIGURE 4. icIEF absorbance (top) and peak integrations (bottom) of adalimumab and a biosimilar. All numbers represent percent peak area.



SAMPLE		ACIDIC REGION	MAIN PEAK	BASIC REGION	Δ BASIC REGION
Adalimumab	No treatment	16.4	63.8	19.8	N/A
	CpB-treated	16.2	61.6	22.2	2.4
Biosimilar	No treatment	16.8	58.8	24.4	N/A
	CpB-treated	17.9	64.5	17.6	-6.8

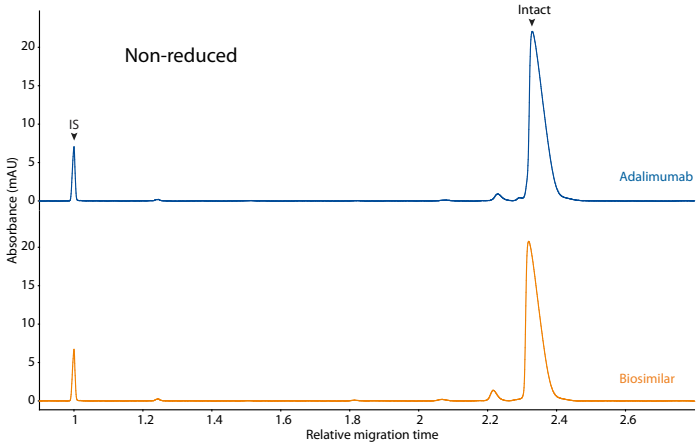
FIGURE 5. icIEF fluorescence (top) and peak integrations (bottom) of adalimumab and a biosimilar. All numbers represent percent peak area.

Adalimumab and its biosimilar were also analyzed on the CE-SDS platform method described above under reduced (**Figure 6**) and non-reduced (**Figure 7**) conditions, resulting in comparable purity.



SAMPLE	LC	NGHC	HC	OTHER
Adalimumab	30.3	0.3	68.5	0.9
Biosimilar	31.5	1.9	66.1	0.5

FIGURE 6. CE-SDS reduced (top) and peak integrations (bottom) of adalimumab and a biosimilar. (IS) Internal standard. (LC) Light chain. (NGHC) Non-glycosylated heavy chain. (HC) Heavy chain. All numbers represent percent peak area.



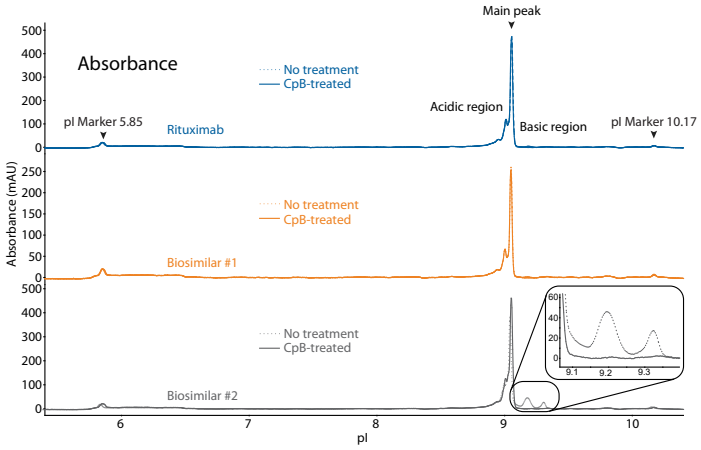
SAMPLE	OTHER	NG	INTACT
Adalimumab	3.7	0.0	96.4
Biosimilar	5.5	0.0	94.5

FIGURE 7. CE-SDS non-reduced (top) and peak integrations (bottom) of adalimumab and a biosimilar. (IS) Internal standard. (NG) Non-glycosylated. All numbers represent percent peak area.

RITUXIMAB (RITUXAN®)

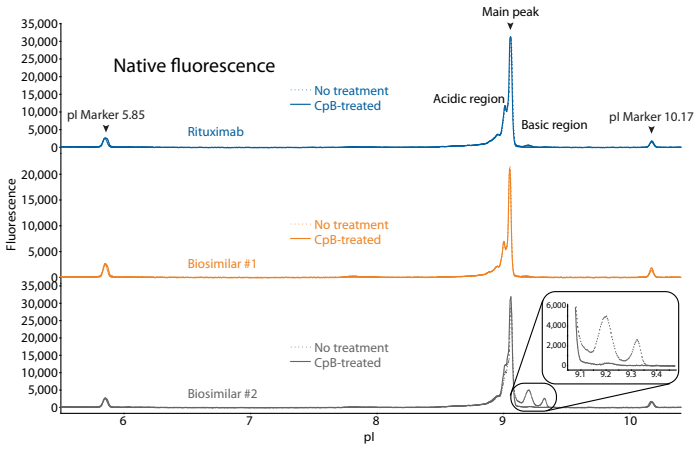
Rituximab (trade name Rituxan®) is a biological drug used to treat certain cancers and autoimmune diseases. It is one of the earliest antibody based therapies available for cancer treatment (approved in 1997), and is considered an essential medicine by the World Health Organization[6].

To compare rituximab to its biosimilars, we used the icIEF platform method described above to monitor charge heterogeneity by absorbance (**Figure 8**) and by native fluorescence (**Figure 9**) detection. These analyses revealed that the innovator and biosimilar #1 are similar in charge heterogeneity and purity. Biosimilar #2, however, had a larger peak area percentage in the basic region (**Figure 8, 9**). To evaluate if this large peak area percentage in the basic region is due to the presence of lysine variants, the antibodies were treated with CpB before analysis on icIEF. Indeed, treatment with CpB had a dramatic effect on the peak area in the basic region of biosimilar #2, resulting in a >17% decrease, while it had very little effect on the basic region of the others (≤2% decrease in peak area). This indicates that biosimlar #2 contains terminal-lysine-containing variants that are not present in the other samples. Furthermore, it is reasonable to speculate that the two basic peaks in the untreated sample of biosimilar #2 are two different terminal-lysine-containing variants because these two peaks disappear following CpB treatment. Because lysine modification can affect antibody function in vivo[7], these variants are important to monitor during antibody process development.



SAMPLE		ACIDIC REGION	MAIN PEAK	BASIC REGION	Δ BASIC REGION
Rituximab	No treatment	36.1	62.7	1.2	N/A
	CpB-treated	37.1	62.9	0.0	-1.2
Biosimilar #1	No treatment	37.8	61.6	0.6	N/A
	CpB-treated	38.4	61.6	0.0	-0.6
Biosimilar #2	No treatment	27.9	54.0	18.0	N/A
	CpB-treated	35.1	64.7	0.2	-17.8

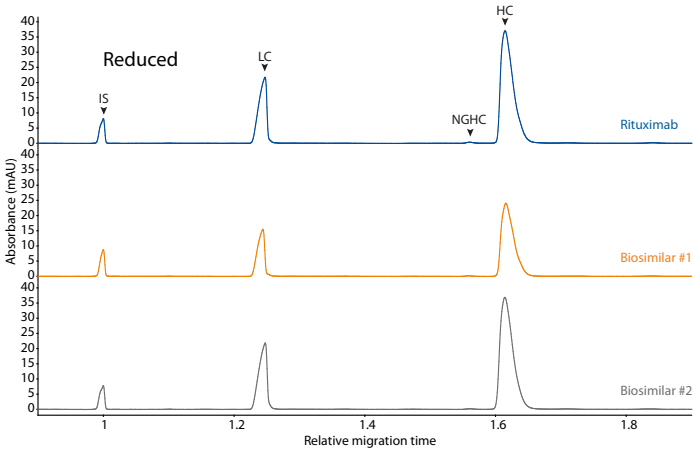
FIGURE 8. icIEF absorbance (top) and peak integrations (bottom) of rituximab and two biosimilars. All numbers represent percent peak area.



SAMPLE		ACIDIC REGION	MAIN PEAK	BASIC REGION	Δ BASIC REGION
Rituximab	No treatment	43.0	54.8	2.2	N/A
	CpB-treated	44.5	55.3	0.2	-2.0
Biosimilar #1	No treatment	41.7	56.7	1.6	N/A
	CpB-treated	41.6	57.9	0.5	-1.1
Biosimilar #2	No treatment	31.0	50.4	18.6	N/A
	CpB-treated	40.5	58.8	0.7	-17.9

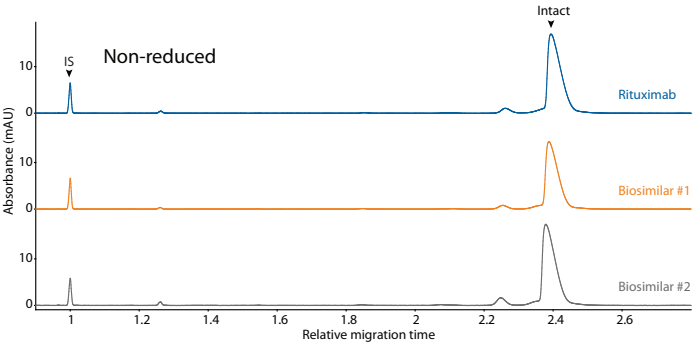
FIGURE 9. icIEF fluorescence (top) and peak integrations (bottom) of rituximab and two biosimilars. All numbers represent percent peak area.

Rituximab and its biosimilars were also analyzed on the CE-SDS platform method under reduced (**Figure 10**) and non-reduced (**Figure 11**) conditions, resulting in comparable purity.



SAMPLE	LC	NGHC	HC	OTHER
Rituximab	32.6	0.4	66.7	0.3
Biosimilar #1	32.4	0.2	67.1	0.2
Biosimilar #2	32.8	0.2	66.8	0.2

FIGURE 10. CE-SDS reduced (top) and peak integrations (bottom) of rituximab and two biosimilars. (IS) Internal standard. (LC) Light chain. (NGHC) Non-glycosylated heavy chain. (HC) Heavy chain. All numbers represent percent peak area.



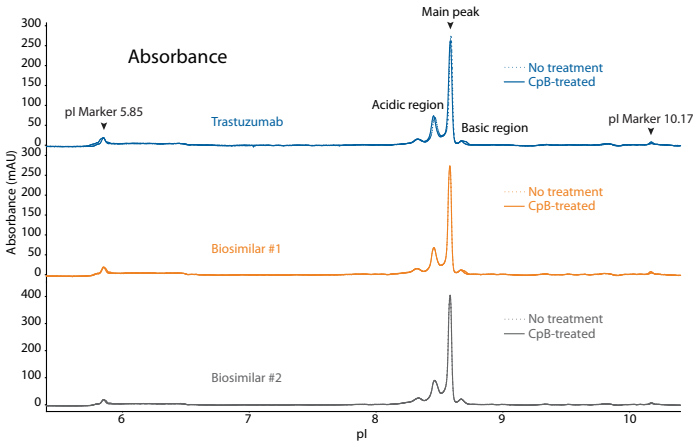
SAMPLE	OTHER	NG	INTACT
Rituximab	6.3	0.0	93.6
Biosimilar #1	6.3	0.0	93.8
Biosimilar #2	9.7	0.0	90.2

FIGURE 11. CE-SDS non-reduced (top) and peak integrations (bottom) of rituximab and two biosimilars. (IS) Internal standard. (NG) Non-glycosylated. All numbers represent percent peak area.

TRASTUZUMAB (HERCEPTIN®)

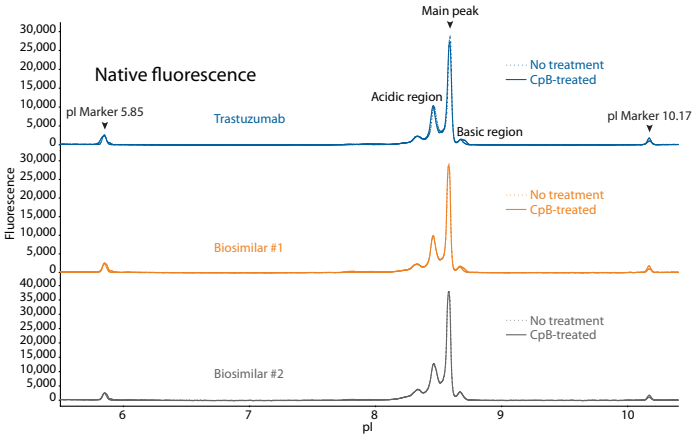
Trastuzumab (trade name Herceptin®) is a biological drug used to treat HER2-positive breast cancer, which accounts for 20-30% of all breast cancers[8]. It is one of the earliest antibody based therapies available for cancer treatment. It was approved by the FDA in September 1998 and the first biosimilar was approved in December 2017.

To compare trastuzumab to its biosimilars, we used the icIEF platform method described above to monitor charge heterogeneity by absorbance (**Figure 12**) and by native fluorescence (**Figure 13**) detection. These analyses revealed that the innovator and biosimilars are similar in charge heterogeneity and purity. To evaluate the presence of lysine variants, the antibodies were treated with CpB before analysis on icIEF. Treatment with CpB conferred a relatively small (<2%) decrease in the basic region peak area percentage (**Figure 12, 13**), suggesting that these samples do no contain a significant amount of terminal-lysine variants.



SAMPLE		ACIDIC REGION	MAIN PEAK	BASIC REGION	Δ BASIC REGION
Trastuzumab	No treatment	35.9	59.4	4.7	N/A
	CpB-treated	36.7	60.0	3.3	-1.4
Biosimilar #1	No treatment	35.8	59.5	4.8	N/A
	CpB-treated	36.3	60.2	3.5	-1.3
Biosimilar #2	No treatment	36.3	58.9	4.8	N/A
	CpB-treated	36.2	59.1	4.7	-0.1

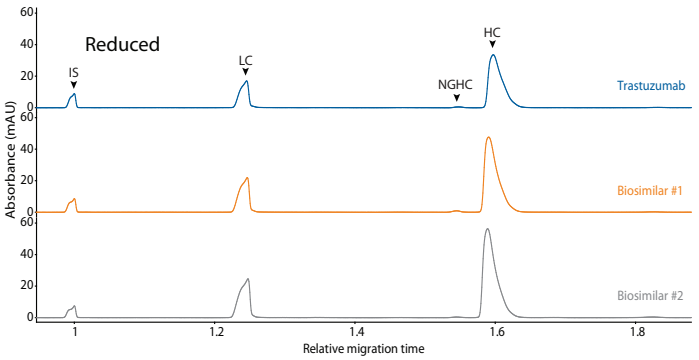
FIGURE 12. icIEF absorbance (top) and peak integrations (bottom) of trastuzumab and two biosimilars. All numbers represent percent peak area.



SAMPLE		ACIDIC REGION	MAIN PEAK	BASIC REGION	Δ BASIC REGION
Trastuzumab	No treatment	40.8	54.6	4.6	N/A
	CpB-treated	40.6	56.5	2.9	-1.7
Biosimilar #1	No treatment	41.5	53.6	4.9	N/A
	CpB-treated	41.0	55.4	3.5	-1.4
Biosimilar #2	No treatment	49.5	45.7	4.9	N/A
	CpB-treated	49.7	45.4	4.9	0.0

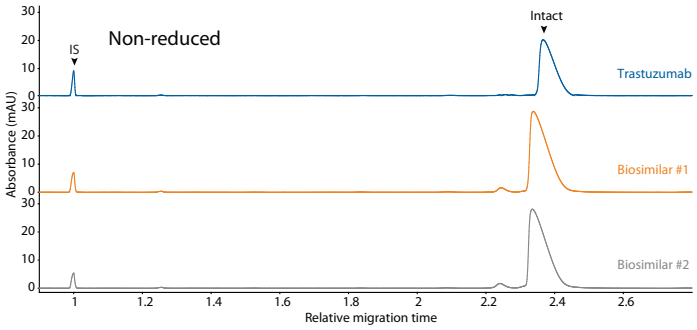
FIGURE 13. icIEF fluorescence (top) and peak integrations (bottom) of trastuzumab and two biosimilars. All numbers represent percent peak area.

Trastuzumab and its biosimilars were also analyzed on the CE-SDS platform method under reduced (**Figure 14**) and non-reduced (**Figure 15**) conditions, resulting in comparable purity.



SAMPLE	LC	NGHC	HC	OTHER
Trastuzumab	29.5	0.5	69.7	0.3
Biosimilar #1	29.8	0.8	69.2	0.3
Biosimilar #2	30.2	0.3	69.1	0.5

FIGURE 14. CE-SDS reduced (top) and peak integrations (bottom) of trastuzumab and two biosimilars. (IS) Internal standard. (LC) Light chain. (NGHC) Non-glycosylated heavy chain. (HC) Heavy chain. All numbers represent percent peak area.



SAMPLE	OTHER	NG	INTACT
Trastuzumab	3.1	0.0	96.9
Biosimilar #1	3.9	0.0	95.6
Biosimilar #2	4.3	0.0	95.7

FIGURE 15. CE-SDS non-reduced (top) and peak integrations (bottom) of trastuzumab and two biosimilars. (IS) Internal standard. (NG) Non-glycosylated. All numbers represent percent peak area.

Summary

Platform methods are beneficial for their ease-of-use, consistency and broad application. Here, we provide icIEF and CE-SDS platform methods to characterize three major commercial therapeutic mAbs and their biosimilars on Maurice. The icIEF platform method developed here is highly robust because multivariate analysis showed that major perturbations to three critical variables in the method did little to change the main peak area percentage and retention of the pI markers, thus these platform methods can be applied to various biosimilars. Taken together, Maurice takes the complexity out of therapeutic protein analysis, making it fast, reliable and versatile.

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