

Bevacizumab

Introduction

Bevacizumab is a humanized monoclonal antibody that targets vascular endothelial growth factor A (VEGFA). It is used as an agent for the treatment of a number of cancers including glioblastoma and colorectal cancer^{1,2}. Several biosimilars are currently in development².

Maurice icIEF Method

Carboxypeptidase B (CpB) treatment: Bevacizumab was diluted to 1.0 mg/mL in water prior to CpB digestion. CpB (1 mg/mL stock solution) was added at a ratio of 1:100 (CpB to sample) and incubated at 37 °C for 20 minutes and then placed on ice. CpB was obtained from Sigma-Aldrich (PN C9584).

Sample preparation: Bevacizumab was diluted to 0.2 mg/mL in the ampholyte solution.

Ampholyte solution: Pharmalytes 8–10.5 (3%) and 5–8 (1%) containing 3.2 M urea, 5 mM IDA and 10 mM arginine.

pI markers: 5.85 and 10.17.

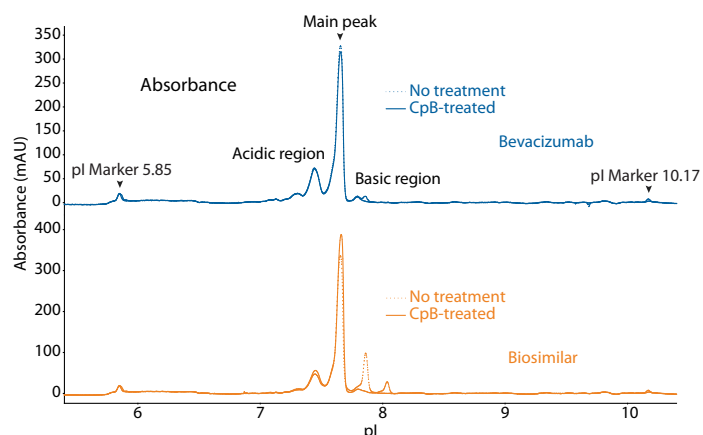
Running conditions: 1 minute at 1500 V, then 8 minutes at 3000 V.

Imaging: Absorbance and fluorescence.

Results

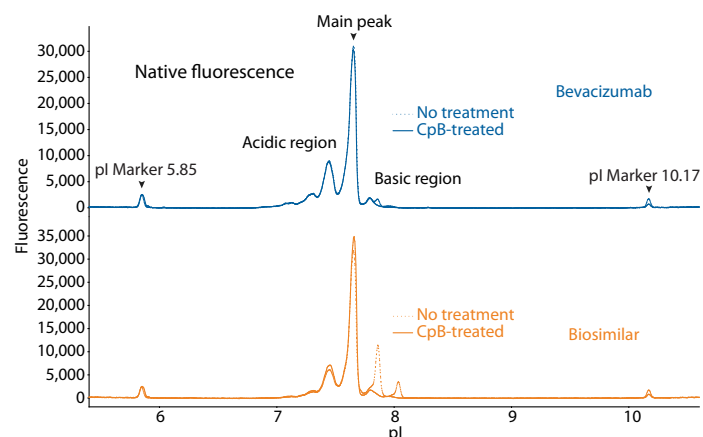
To compare bevacizumab to a biosimilar, we used the icIEF platform method described above to monitor charge heterogeneity by absorbance (**Figure 1**) and by native fluorescence (**Figure 2**) detection.

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 1, 2**), 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
Bevacizumab	No treatment	30.7	64.9	4.4	N/A
	CpB-treated	30.3	66.6	3.0	-1.4
Biosimilar	No treatment	17.2	63.3	19.5	N/A
	CpB-treated	22.1	74.8	3.1	-16.4

FIGURE 1. icIEF absorbance (top) and peak area percentages (bottom) of bevacizumab.



SAMPLE		ACIDIC REGION	MAIN PEAK	BASIC REGION	Δ BASIC REGION
Bevacizumab	No treatment	34.8	59.9	5.3	N/A
	CpB-treated	35.1	59.7	5.1	-0.2
Biosimilar	No treatment	21.4	57.4	21.2	N/A
	CpB-treated	27.0	67.8	5.2	-16.0

FIGURE 2. icIEF fluorescence (top) and peak area percentages (bottom) of bevacizumab.

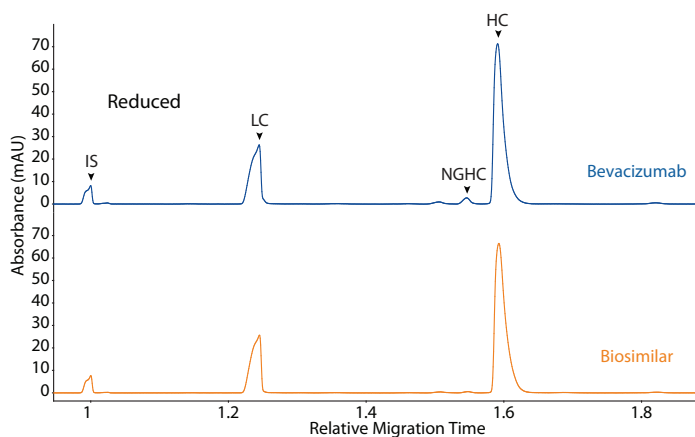
Maurice CE-SDS Method

Sample preparation: Bevacizumab was diluted to 1 mg/mL with 1X Sample Buffer prior to treatment for 10 minutes at 70 °C in the presence of either 11.5 mM IAM (non-reducing) or 650 nM β-ME (reducing).

Running conditions: Samples were injected for 20 seconds at 4600 V, followed by a 25-minute separation (reducing) or a 35-minute separation (non-reducing) at 5750 V.

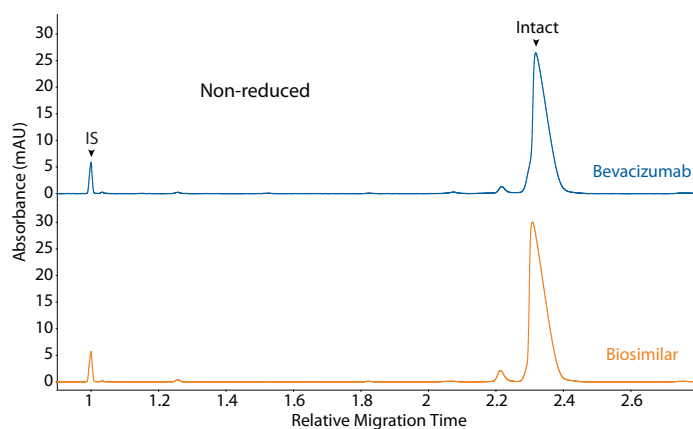
Results

Bevacizumab was analyzed on the CE-SDS platform method described above under reducing (Figure 3) and non-reducing (Figure 4) conditions, resulting in comparable purity.



SAMPLE	LC	NGHC	HC	OTHER
Bevacizumab	30.9	0.3	68.6	0.2
Biosimilar	31.5	1.9	66.1	0.5

FIGURE 3. CE-SDS reduced (top) and peak area percentages (bottom) of bevacizumab. (IS) Internal standard. (LC) Light chain. (NGHC) Non-glycosylated heavy chain. (HC) Heavy chain.



SAMPLE	OTHER	NG	INTACT
Bevacizumab	4.6	0.0	95.4
Biosimilar	5.3	0.0	94.7

FIGURE 4. CE-SDS non-reduced (top) and peak area percentages (bottom) of bevacizumab. (IS) Internal standard. (NG) Non-glycosylated.

References

1. The role of bevacizumab in the treatment of glioblastoma, R Diaz, S Ali, M Qadir, M De La Fuente, M Ivan and R Komotar, *Journal of Neurooncology*, 2015; 133(3):455–467.
2. Bevacizumab in colorectal cancer: current role in treatment and the potential of biosimilars, L Rosen, I Jacobs and R Burkes, *Target Oncology*, 2017; 12(5):559–610.