Infliximab

Introduction

Infliximab targets tumor necrosis factor alpha (TNFα) and has been shown to be effective in the treatment of autoimmune diseases like Crohn's disease[1]. Several biosimilars have already been approved[2].

Maurice icIEF Method

Carboxypeptidase B (CpB) treatment: Infliximab 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: Infliximab 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 and10 mM arginine.

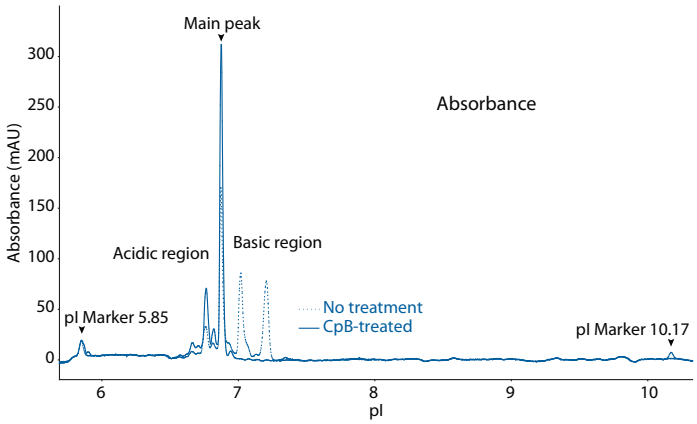
pI markers: 5.85 and 10.1.

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

Imaging: Absorbance and fluorescence.

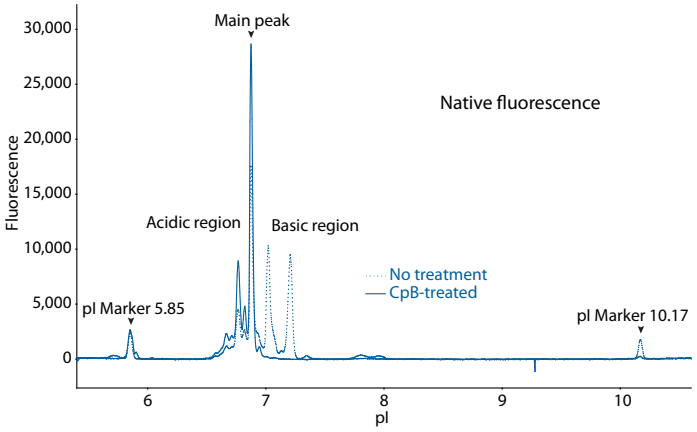
Results

icIEF analysis of infliximab is shown in **Figure 1** with absorbance detection and in **Figure 2** with fluorescence detection. Treatment with CpB the revealed presence of terminal lysine variants.



SAMPLE		ACIDIC REGION	MAIN PEAK	BASIC REGION	Δ BASIC REGION
Infliximab	No treatment	19.0	35.4	45.6	N/A
	CpB-treated	39.9	57.5	2.6	-43

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



SAMPLE		ACIDIC REGION	MAIN PEAK	BASIC REGION	Δ BASIC REGION
Infliximab	No treatment	20.8	33.1	46.2	N/A
	CpB-treated	45.8	50.9	3.3	-42.9

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

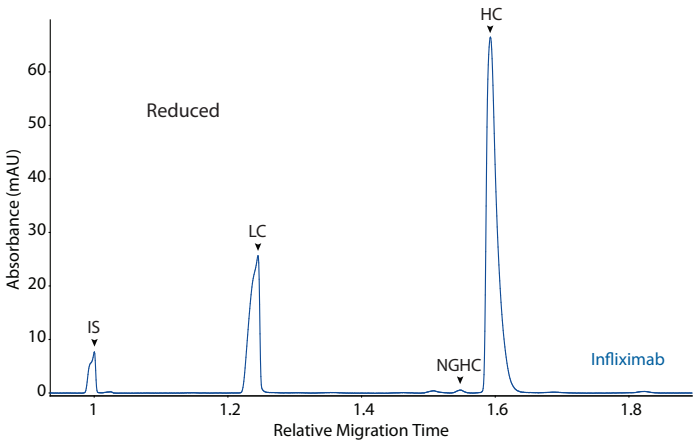
Maurice CE-SDS Method

Sample preparation: Infliximab 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.

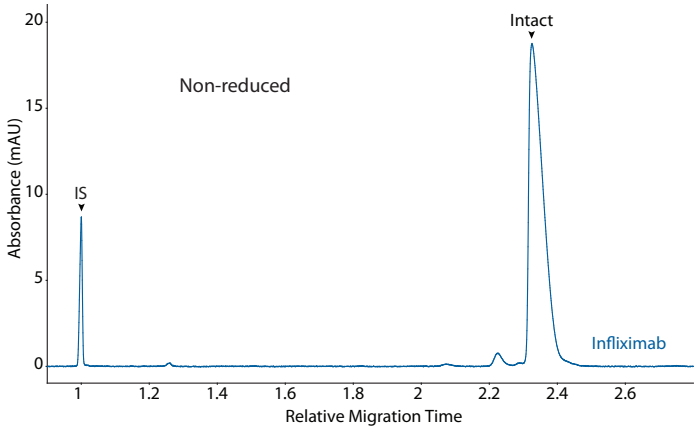
Results

Infliximab was analyzed on the CE-SDS platform method described above under reducing (**Figure 3**) and non-reducing (**Figure 4**) conditions, revealing the purity of the sample.



SAMPLE	LC	NGHC	HC	OTHER
Infliximab	30.9	0.6	68.2	0.4

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



SAMPLE	OTHER	NG	INTACT
Infliximab	3.7	0.0	96.3

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

References

1. Remicade® (infliximab): 20 years of contributions to science and medicine, R Melsheimer, A Geldhof, I Apaolaza and T Schaible, Biologics: Targets and Therapy, 2019; 13:139–178..
2. Biosimilars already approved and in development, T Dörner, J Isaacs, J Gonçalves, V Azevedo, G Castañeda-Hernández, R Strohal and I McInnes, *Considerations in Medicine*, 2017; 1:7–12.

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