Cetuximab

Introduction

Cetuximab targets the epidermal growth factor receptor (EGFR). It is used as a therapeutic agent in the treatment of a number of cancers including colorectal, head and neck cancer[1,2]. Cetuximab was approved by the FDA in 2004, and its US patent expired in 2016.

Maurice icIEF Method

Carboxypeptidase B (CpB) treatment: Cetuximab 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: Cetuximab 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 10.17.

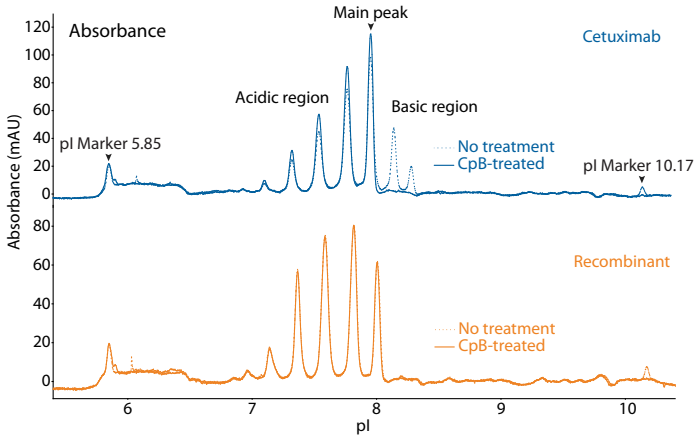
Running conditions: 1 minute at 1500 V, then 8 minutes at 3000 V.

Imaging: Absorbance and fluorescence.

Recombinant: The cetuximab biosimilar was obtained from R&D Systems, PN MAB9577.

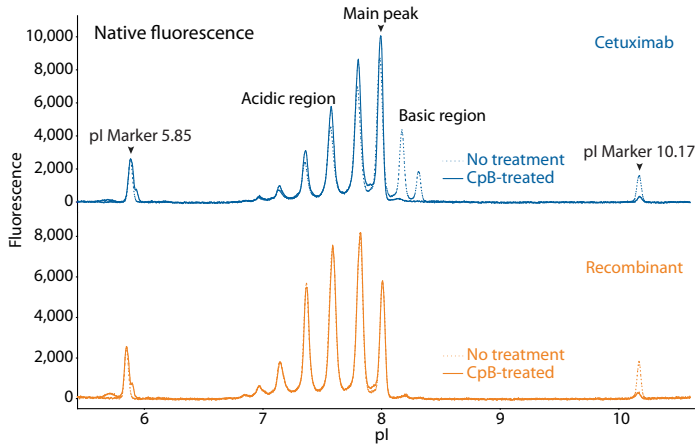
Results

To compare cetuximab to a recombinant, we used the icIEF platform method described above to monitor charge heterogeneity by absorbance (**Figure 1**) and by native fluorescence detection (**Figure 2**). These analyses revealed noteworthy differences between the molecules. For example, the innovator has two peaks in the basic region that disappear upon treatment with CpB (**Figure 1, 2**), suggesting that the innovator has terminal-lysine-containing variants that are absent in the recombinant.



SAMPLE		ACIDIC REGION	MAIN PEAK	BASIC REGION	Δ BASIC REGION
Cetuximab	No treatment	54.3	28.6	17.1	N/A
	CpB-treated	70.0	30.0	0.0	-17.1
Recombinant	No treatment	81.4	16.9	1.7	N/A
	CpB-treated	81.8	17.0	1.3	-0.4

FIGURE 1. icIEF absorbance (top) and peak area percentages (bottom) of cetuximab and a recombinant.



SAMPLE		ACIDIC REGION	MAIN PEAK	BASIC REGION	Δ BASIC REGION
Cetuximab	No treatment	56.2	27.7	16.1	N/A
	CpB-treated	70.7	28.2	1.1	-15.0
Recombinant	No treatment	83.6	15.7	0.7	N/A
	CpB-treated	83.0	16.5	0.5	-0.2

FIGURE 2. icIEF fluorescence (top) and peak area percentages (bottom) of cetuximab and a recombinant.

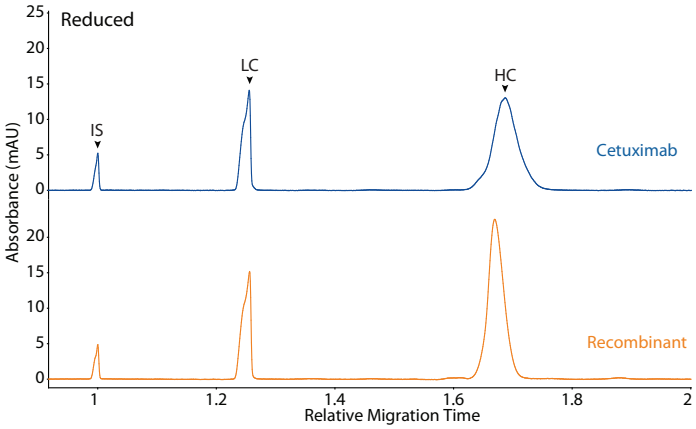
Maurice CE-SDS Method

Sample preparation: Cetuximab 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.

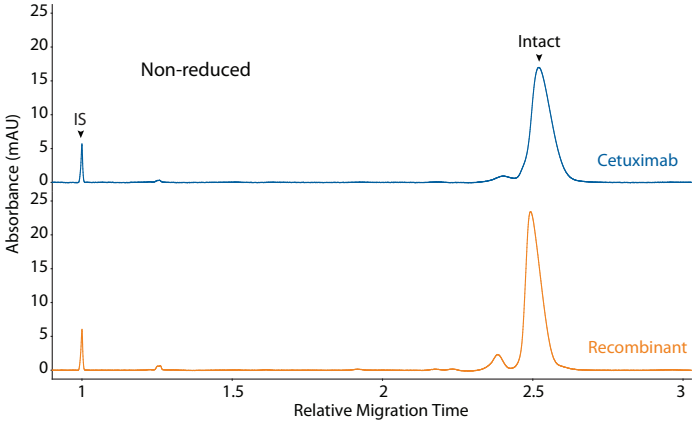
Results

Cetuximab and a recombinant were analyzed on the CE-SDS platform method described above under reducing (**Figure 3**) and non-reducing (**Figure 4**) conditions. These analyses revealed differences in peak area percentages, for example the heavy chain under reducing conditions and the intact mAb under non-reducing conditions.



SAMPLE	LC	NGHC	HC	OTHER
Cetuximab	30.0	0.2	69.4	0.3
Recombinant	30.1	0.9	67.7	1.3

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



SAMPLE	OTHER	NG	INTACT
Cetuximab	1.5	4.0	94.5
Recombinant	3.5	7.7	88.7

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

References

1. Cetuximab: from bench to bedside, B Vincenzi, A Zoccoli, F Pantano, O Venditti and S Galluzzo, *Current Cancer Drug Targets*, 2010; (10)1:80–95.
2. New thearpies in head and neck cancer, R Santuray, D Johnson and J Grandis, *Trends Cancer*, 2019; 4(5):385–396..

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