# 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 and 10 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 revealed the presence of terminal lysine variants.
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.

References
