Electronic Supplementary Material (ESI) for Analytical Methods.

Stability and Biosimilarity Assessment of Infliximab using Orthogonal Testing Protocol and Statistically-guided Interpretation of Peptide Mapping

Lamiaa A. Hassan[a], Sara M. Shatat[a], Basma M. Eltanany[b], Medhat A. Al-Ghobashy [b,c,*]&Samah S. Abbas [b]

[a] National Organization for Research and Control of Biologicals, Egypt

[b] Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Egypt

[c] Bioanalysis Research Group, School of Pharmacy, Newgiza University, Egypt

[*]**Correspondence:**

Dr. Medhat A. Al-Ghobashy, Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt.

E-mail: medhat.alghobashy@cu.edu.eg

Keywords:

Peptide Mapping; Principal Component Analysis; Infliximab; Biosimilars

Supporting Data 1: Bioanalytical Techniques

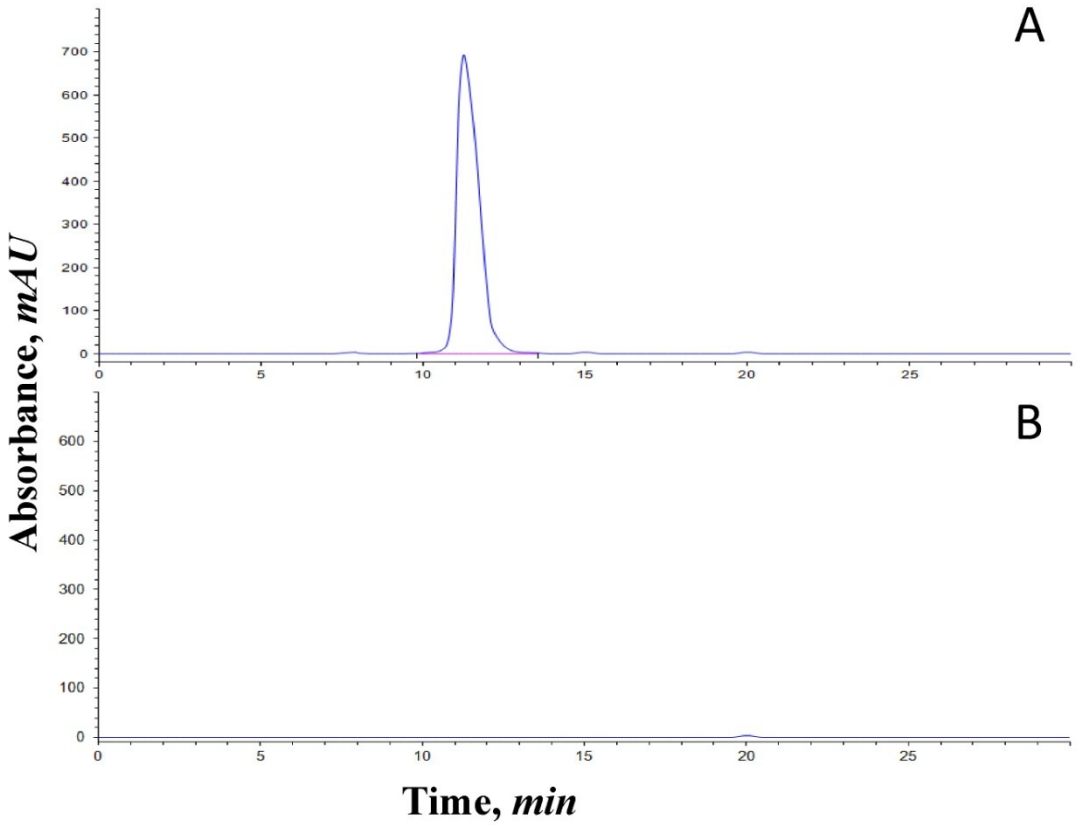

Fig. S1: SE-HPLC chromatograms of A) Infliximab standard and B) Formulation buffer. The analysis was carried out using YMC-Pack-Diol 300analytical column, a mobile phase of 0.4M sodium perchlorate monohydrate in 40mM phosphate buffer (pH6.8 ± 0.05), flow rate 0.8 mL min^{-1} and a detection wavelength of 214 nm.

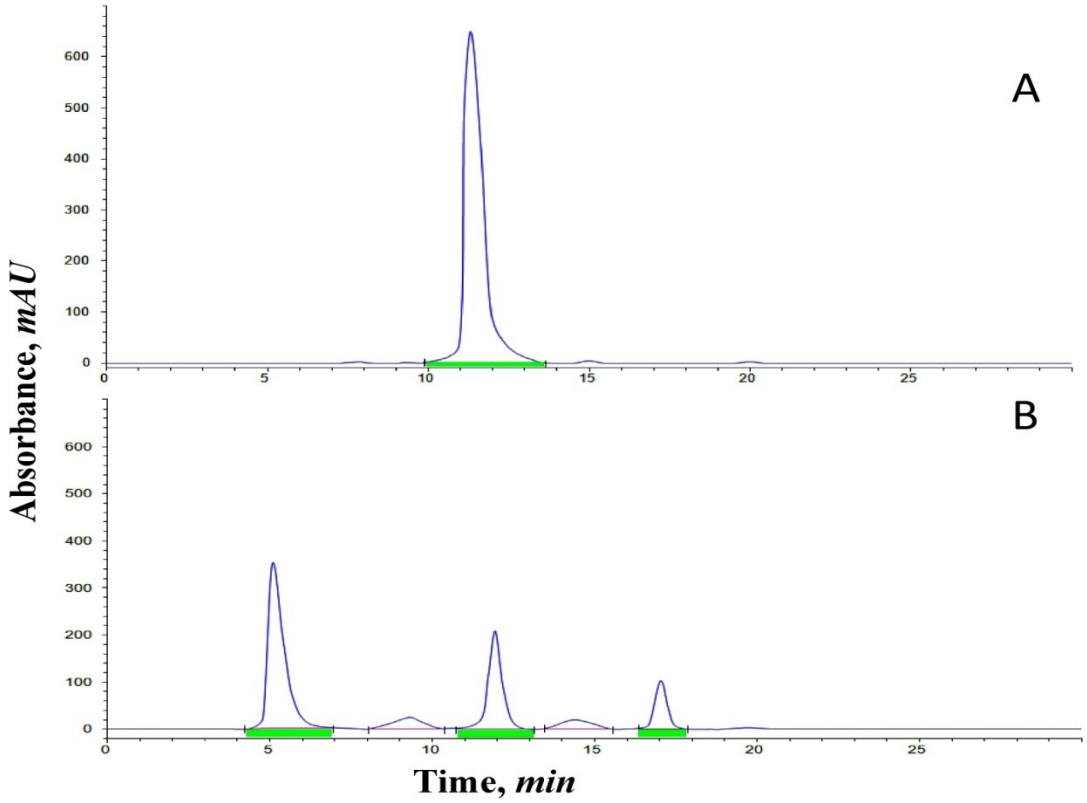

Fig. S2: SE-HPLC chromatograms showing peak purity of A) standard Infliximab and B) degraded sample (4 weeks at 37°C at pH 4.0). The analysis was carried out using YMC-Pack-Diol 300analytical column, a mobile phase of 0.4M sodium perchlorate monohydrate in 40mM phosphate buffer (pH6.8 ± 0.05), flow rate 0.8 mL min^{-1} and a detection wavelength of 214 nm.

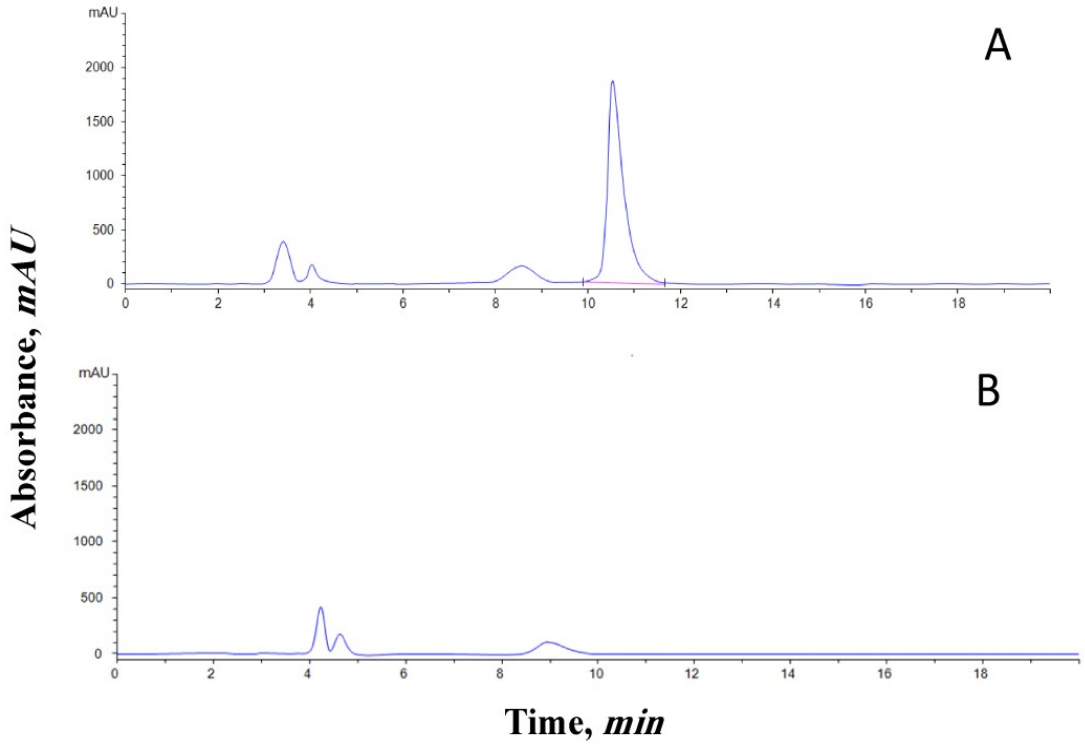

Fig. S3: RP-HPLC chromatograms of A) Infliximab standard and B) Formulation buffer. The analysis was carried out using Zorbax SB-C8 column, a mobile phase of solvent A (0.1 % TFA in water) and solvent B (0.1 % TFA inacetonitrile), flow rate 1.0mL min^{-1} and a detection wavelength of 214 nm.

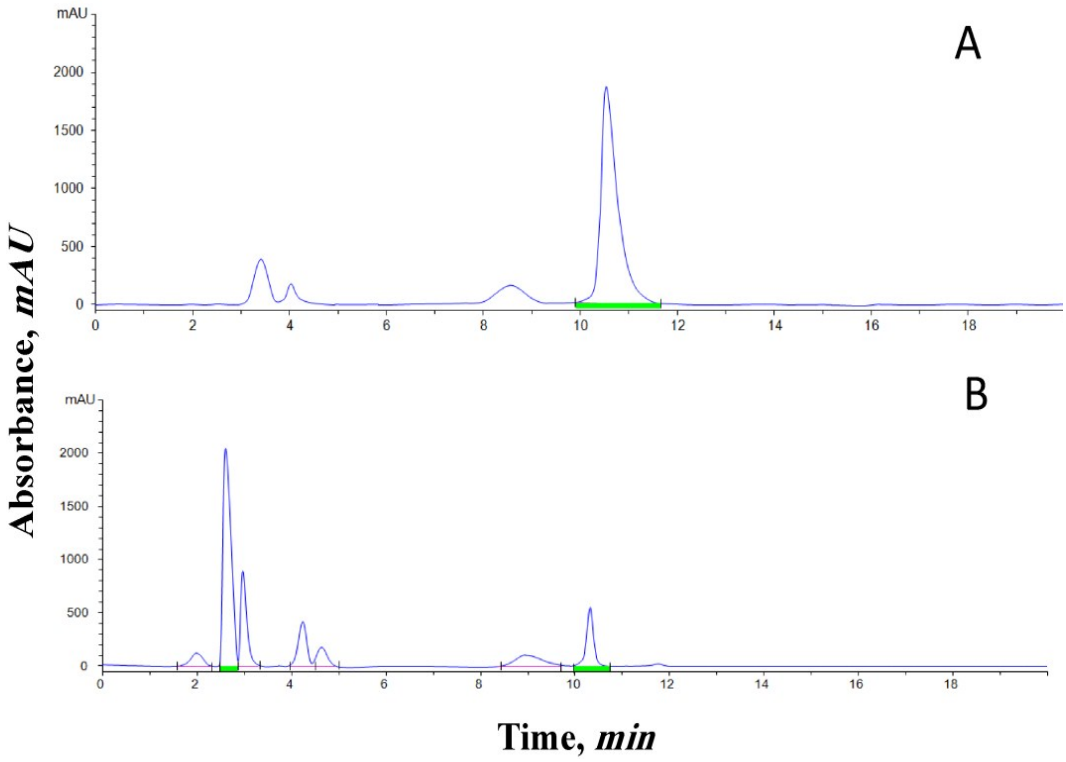

Fig. S4: RP-HPLC chromatograms showing peak purity of A) Infliximab standard and B) oxidized sample.The analysis was carried out using Zorbax SB-C8 column, a mobile phase of solvent A (0.1 % TFA in water) and solvent B (0.1 % TFA in acetonitrile), flow rate 1.0 mL min^{-1} and a detection wavelength of 214 nm.

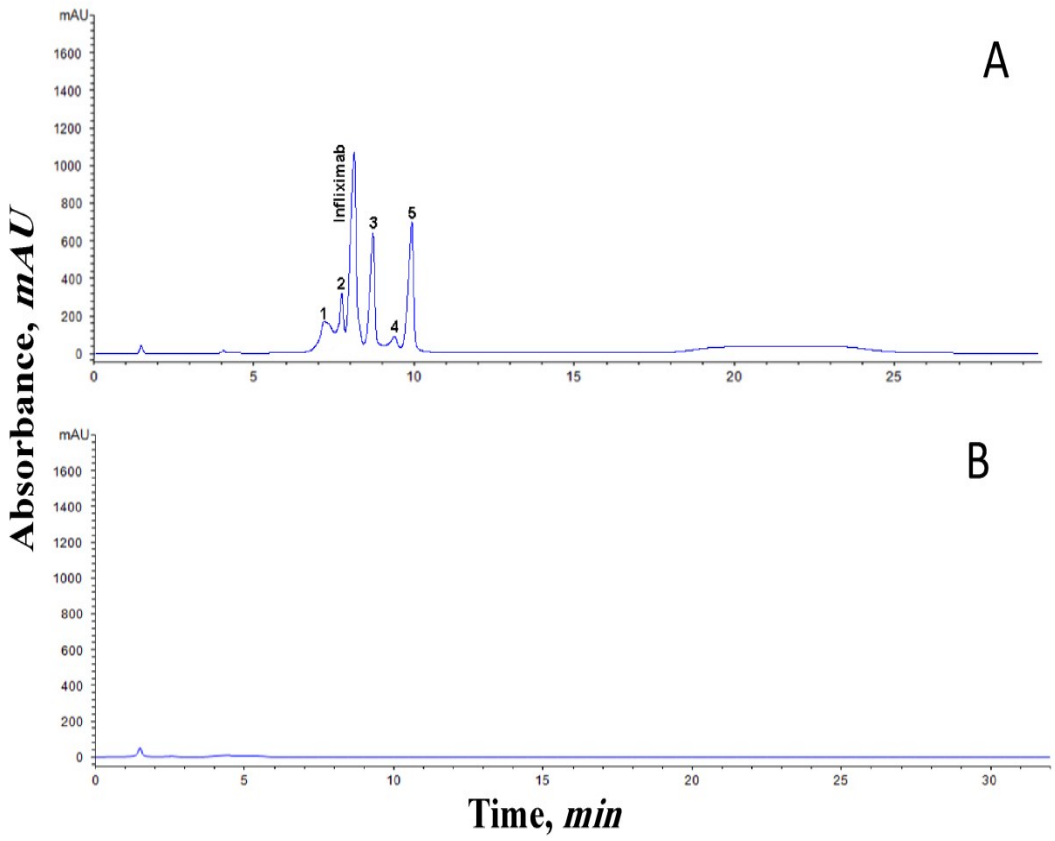

Fig. S5:CEX-HPLC chromatogram of A) Infliximab standard and B) formulation buffer. Peak 1, 2: acidic isoforms, Infliximab: main isoform, peak 3, 4&5: basic isoforms. The analysis was carried out using Pro Pac WCX-10 analytical column, gradient elution using mobile phase of solvent A 10mM sodium phosphate, (pH 7.25±0.05) and solvent B 10 mM sodium phosphate, 1M sodium chloride, (pH7.25±0.05), flow rate 0.8 mL min^{-1} and detection wavelength of 214 nm.

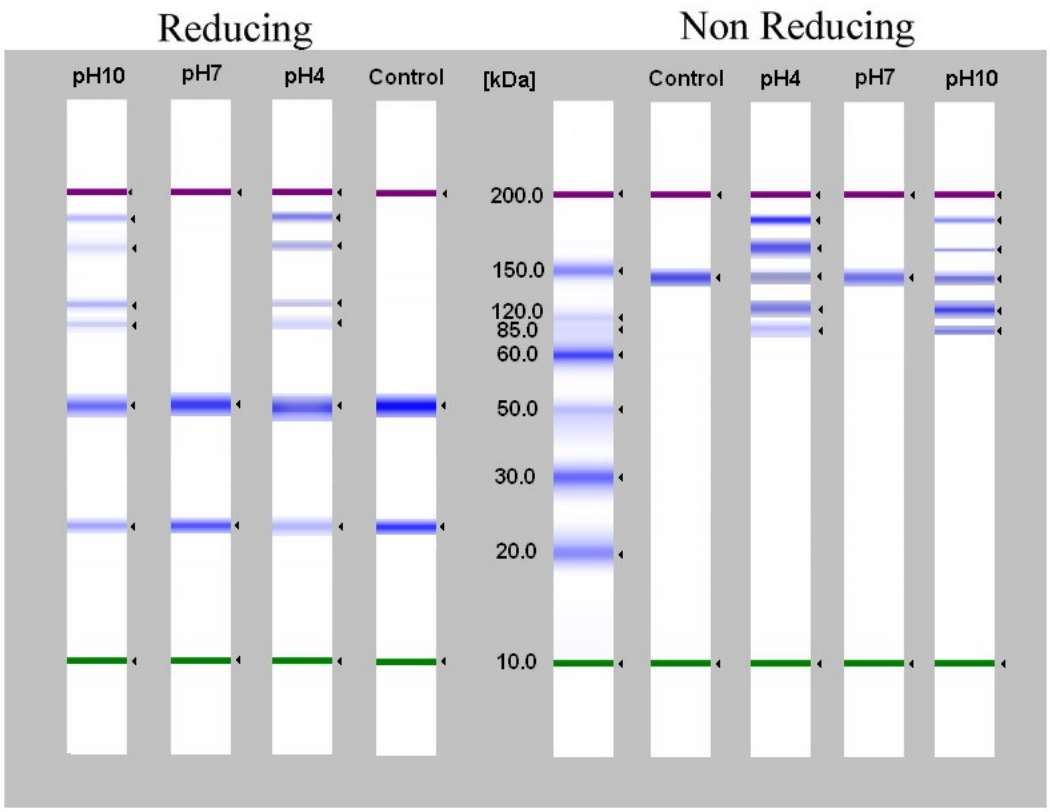

Fig. S6: Stained gels showing the effect of pH on the stability of Infliximab (Control) using Tape Station electrophoresis under reducing and non-reducing conditions after incubation for 4 weeks at 37 °C.

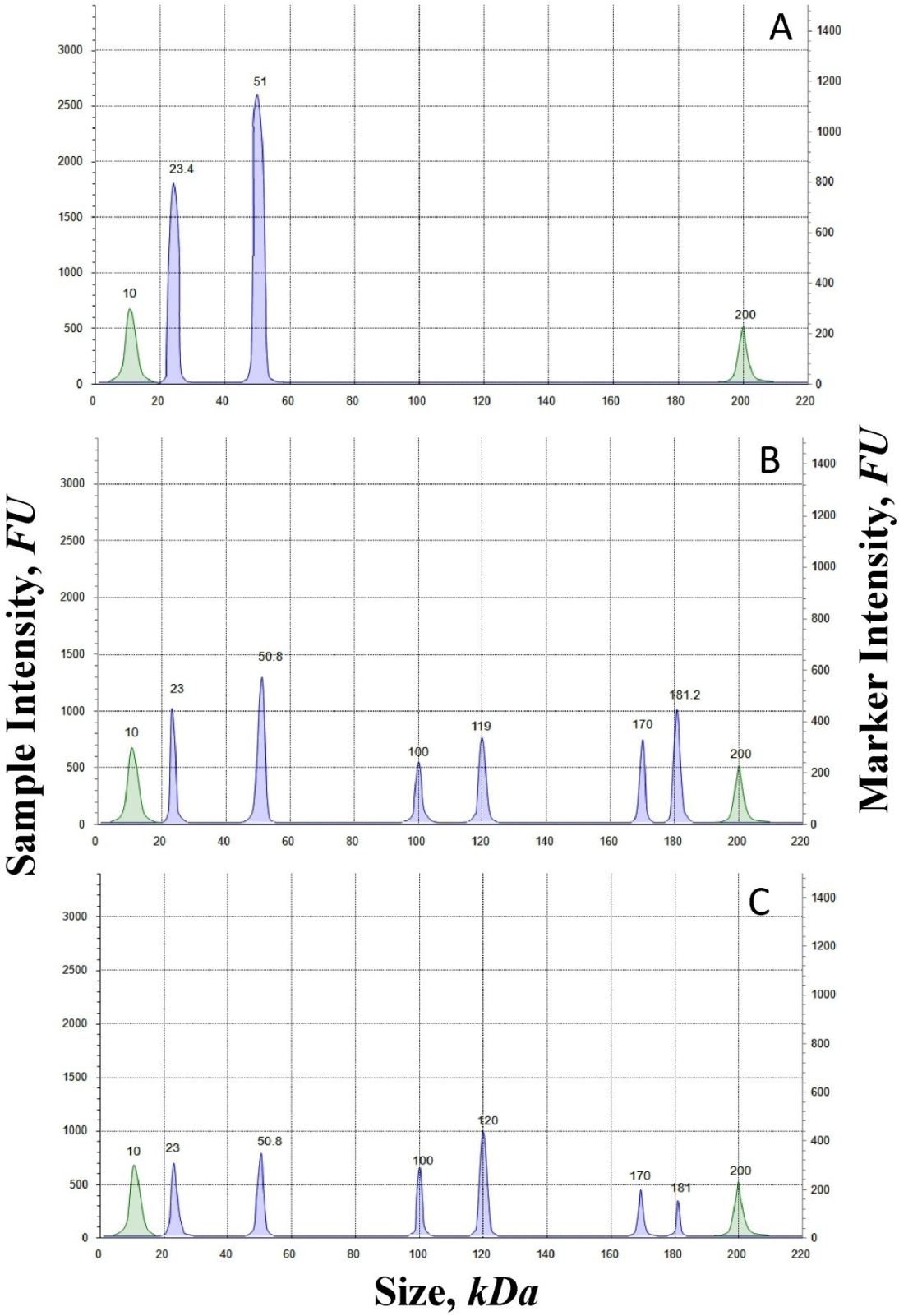

Fig. S7: Effect of pH under reducing conditions, using Tapestation electrophoresis (A) control, (B) pH 4.0, (C) pH 10.0 after incubation for 4 weeks at 37 °C.

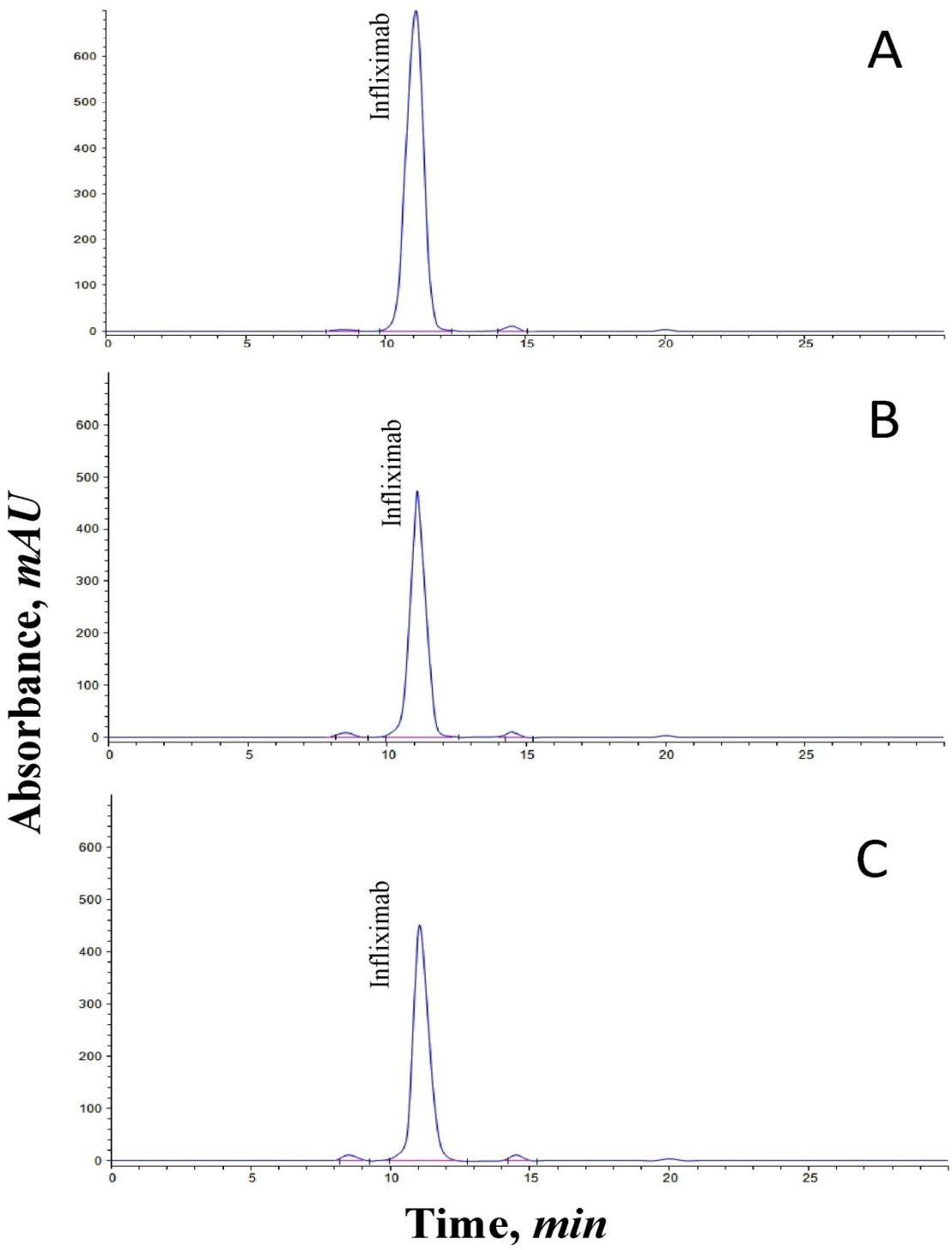

Fig. S8: Effect of agitation using SE-HPLC,A) 10, B) 30 and C) 60 minutes showing high molecular weight aggregates and low molecular weight fragments. The analysis was carried out using YMC-Pack-Diol 300analytical column, a mobile phase of 0.4M sodium perchlorate monohydrate in 40mM phosphate buffer (pH6.8 ± 0.05), flow rate 0.8 mL min^{-1} and a detection wavelength of 214 nm.

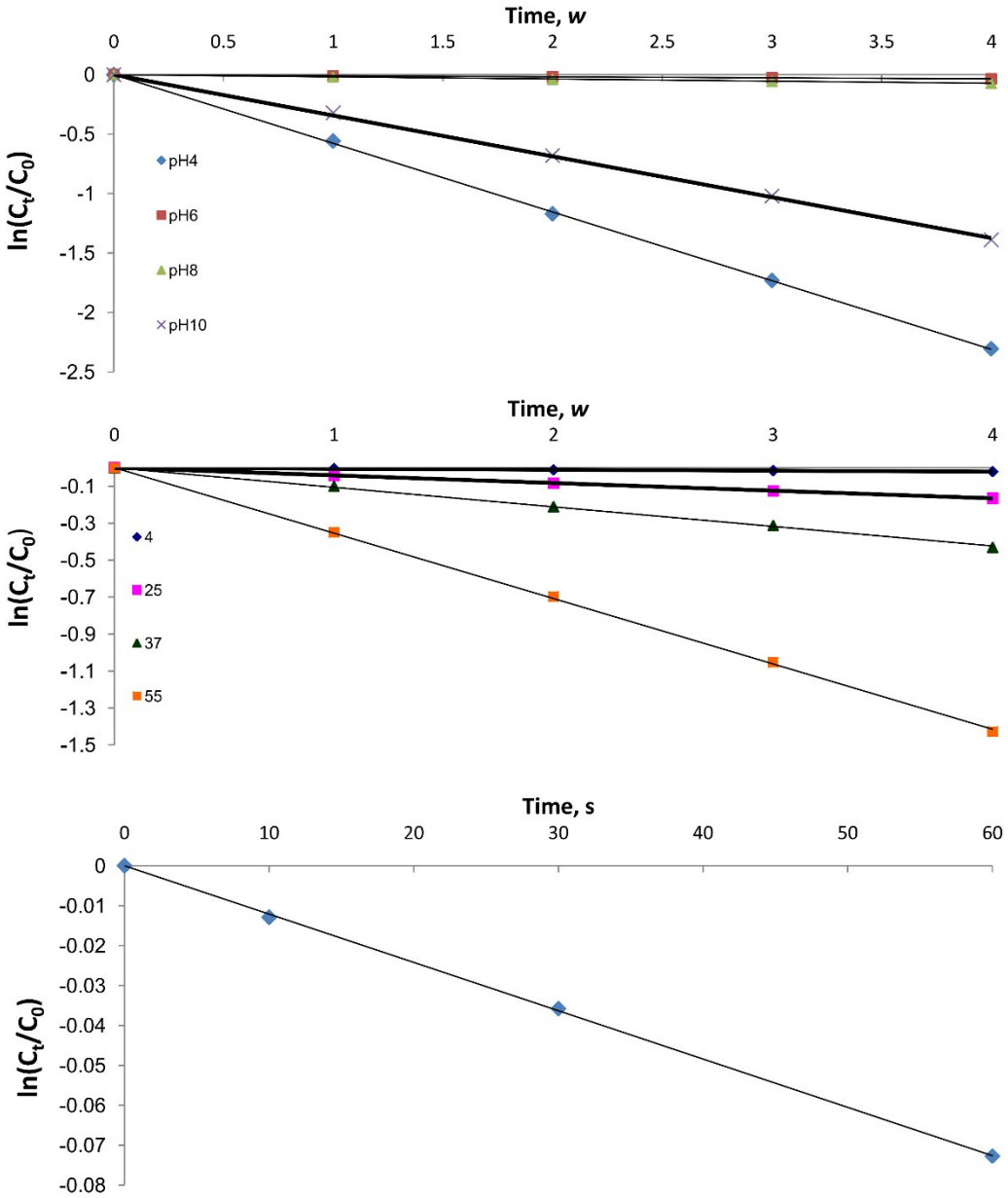

Fig. S9: Plots showing the first order kinetics model representing the degradation of Infliximab under the effect of A) pH, B) temperature and C) mechanical agitation at different time points.