

**Figure 5**

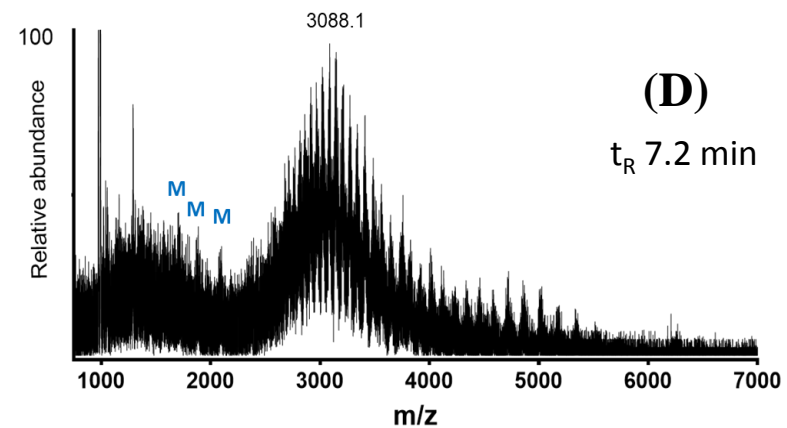
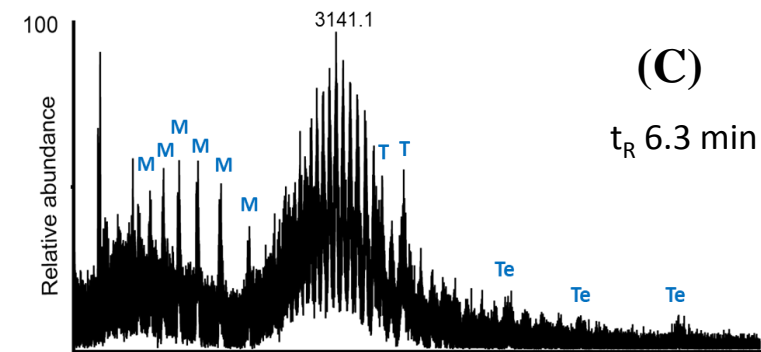
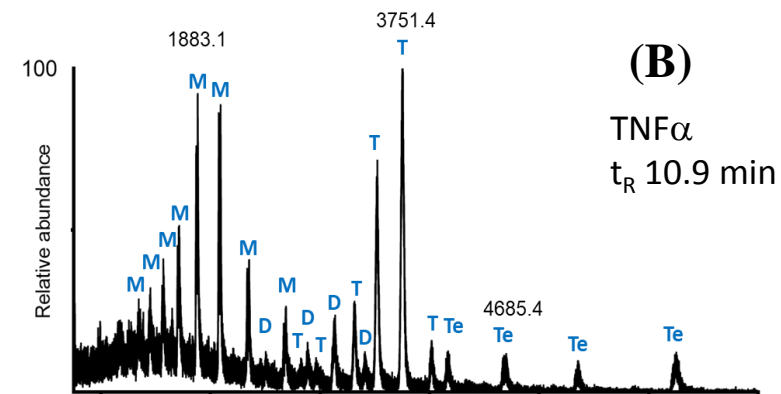
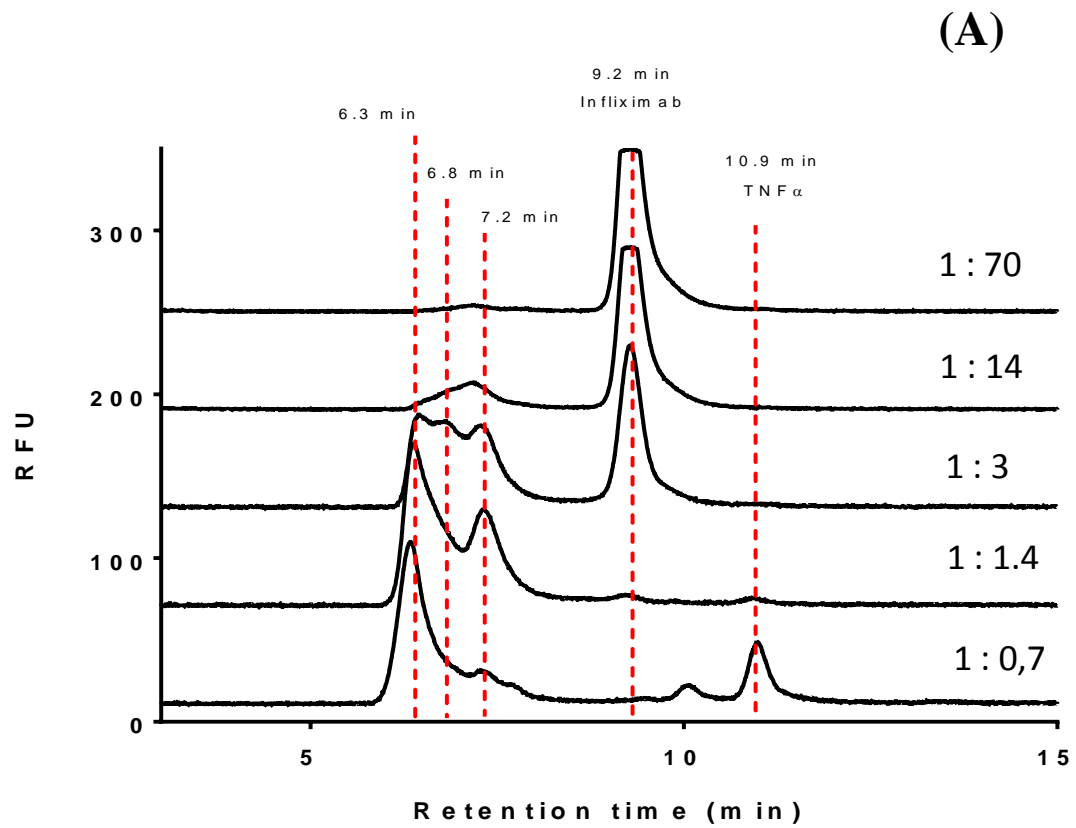


Figure 5: TNF $\alpha$ /Infliximab analyzed by SEC-FLD-MS; (A) Chromatograms of TNF $\alpha$ /Infliximab mixtures at different ratios obtained by SEC-FLD.

Online SEC- MS analysis, mass spectra ; (B) TNF $\alpha$ , (C) TNF $\alpha$ /Infliximab complex at 6.3 min and (D) TNF $\alpha$ /Infliximab complex at 7.2 min.

M, D, T, Te are monomeric, dimeric, trimeric and tetrameric forms of TNF $\alpha$ , respectively.

Mobile phase: ammonium bicarbonate 150 mM pH 6.5 (10% IPA). Column: BioSEC-3, (4.6 mm  $\times$  300 mm, 300 Å, particle diameter 3  $\mu$ m); Flow rate: 300  $\mu$ L.min<sup>-1</sup>; FLD:  $\lambda_{ex}$  = 280 nm,  $\lambda_{em}$  = 350 nm.

MS conditions: MS condition: drying gas. 250 °C – 6 L min<sup>-1</sup>, sheath gas 150 °C – 6L min<sup>-1</sup>, nebulizer pressure 25 psig, transferred capillary, nozzle, fragmentor and skimmer voltages were set at 2500 V, 2000 V, 300 V and 80 V respectively