Supporting Information

Process Analytical Technology implementation for peptide manufacturing: Cleavage reaction of recombinant Lethal Toxin Neutralizing Factor (rLTNF) concatemer as a case study

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Experimental details

HPLC analysis

Concentrations of rLTNF concatemer and monomer in the reaction mixture samples were estimated using RP-HPLC. Separation was performed on a 1200-Series HPLC system from Agilent Technologies equipped with a quaternary pump, in-line degasser, thermostatic autosampler, and temperature-controlled column oven. Data acquisition, control and data analysis were performed using Agilent ChemStation software. The rLTNF concatemer was quantified using a TSKgel Protein C4-300 (4.6x150 mm, 3µ particle size) column from Tosoh, Japan, using a 40–100% acetonitrile gradient with water in the presence of 0.1% TFA at 40 ºC. Elution was monitored at 220 nm. The rLTNF monomer was quantified using Eclipse XDB C18 column (4.6x100 mm, 3.5µ particle size) from Agilent Technologies, USA. The column was equilibrated with 95% buffer A (0.1% TFA with water) for 10 min followed by injection of the sample. Elution was performed using 5-50% buffer B (0.1% TFA with ACN) over 15 minutes. The column was operated at a flow rate of 1 ml/min. Analysis was performed at 40 ºC and monitored at 220 nm.

Figure S1 Schematic representation of experimental work flow that was followed for ATR-FTIR monitoring of peptide manufacturing process.
Figure S2 Scheme for implementation of PAT across different stages of enzyme reaction