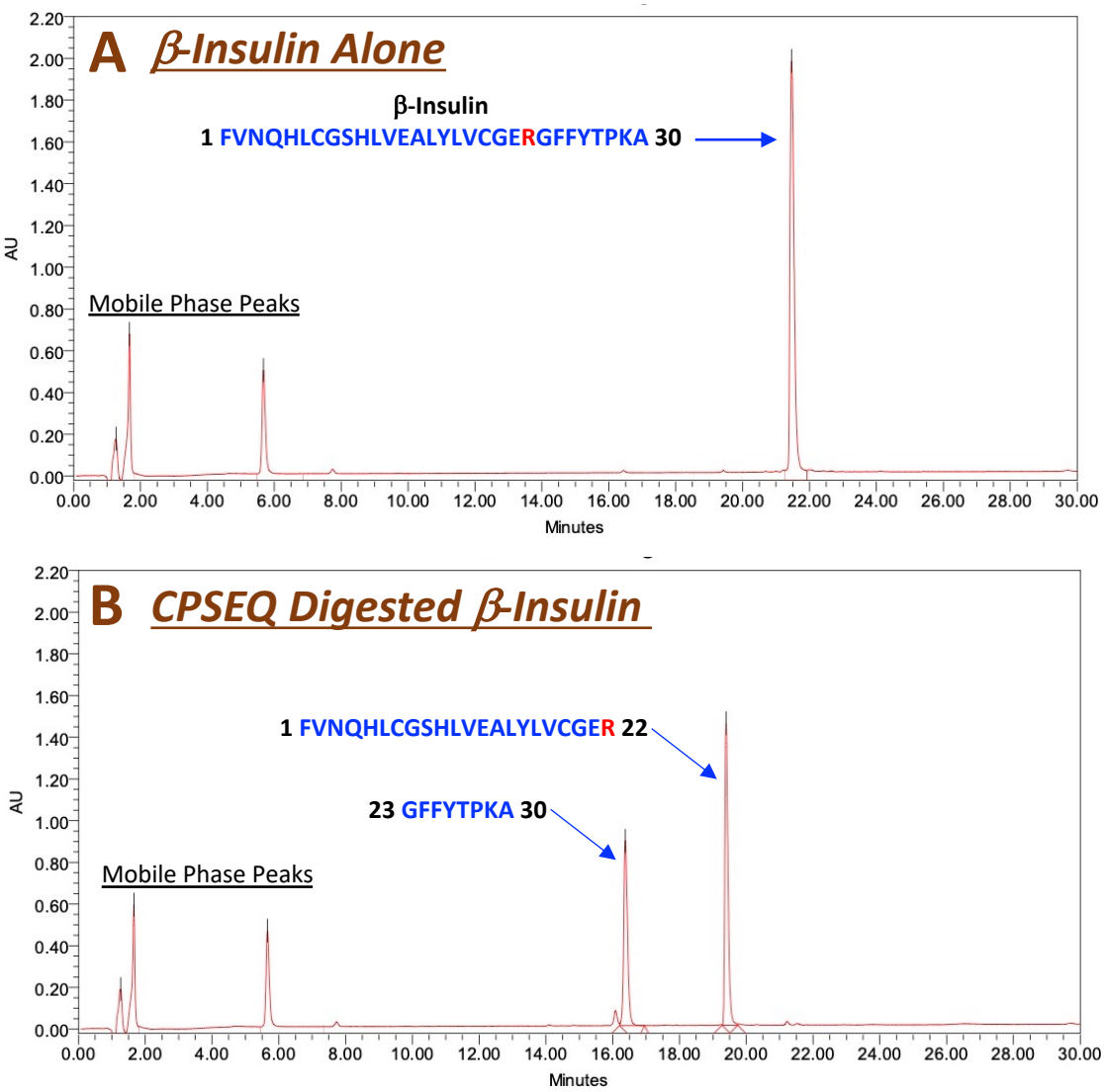
Reverse Phase HPLC of Undigested β-Insulin and Clostripain (CPSEQ) Digested β-Insulin



50 μg β-insulin was incubated with buffer (Panel A) or 0.45 units (~2.5 μgP) CPSEQ (Panel B) in 50 mM Tris-HCl, pH 7.5 with 4.6 mM $CaCl_2$ for 30 minutes at 37°C and resolved by reverse phase HPLC on a C18 column.

Clostripain (CPSEQ) is a sulfhydryl proteinase produced by *Clostridium histolyticum*. It is specific for the carboxyl peptide bond of arginine (**R**). β-Insulin is a 30 amino acid peptide containing a single arginine residue at position 22 and elutes as a single peak at 21.5 minutes. Digestion with clostripain results in cleavage at R22 generating two smaller peptides of 22 and 8 amino acids in length that elute at 19.4 and 16.4 minutes, respectively.