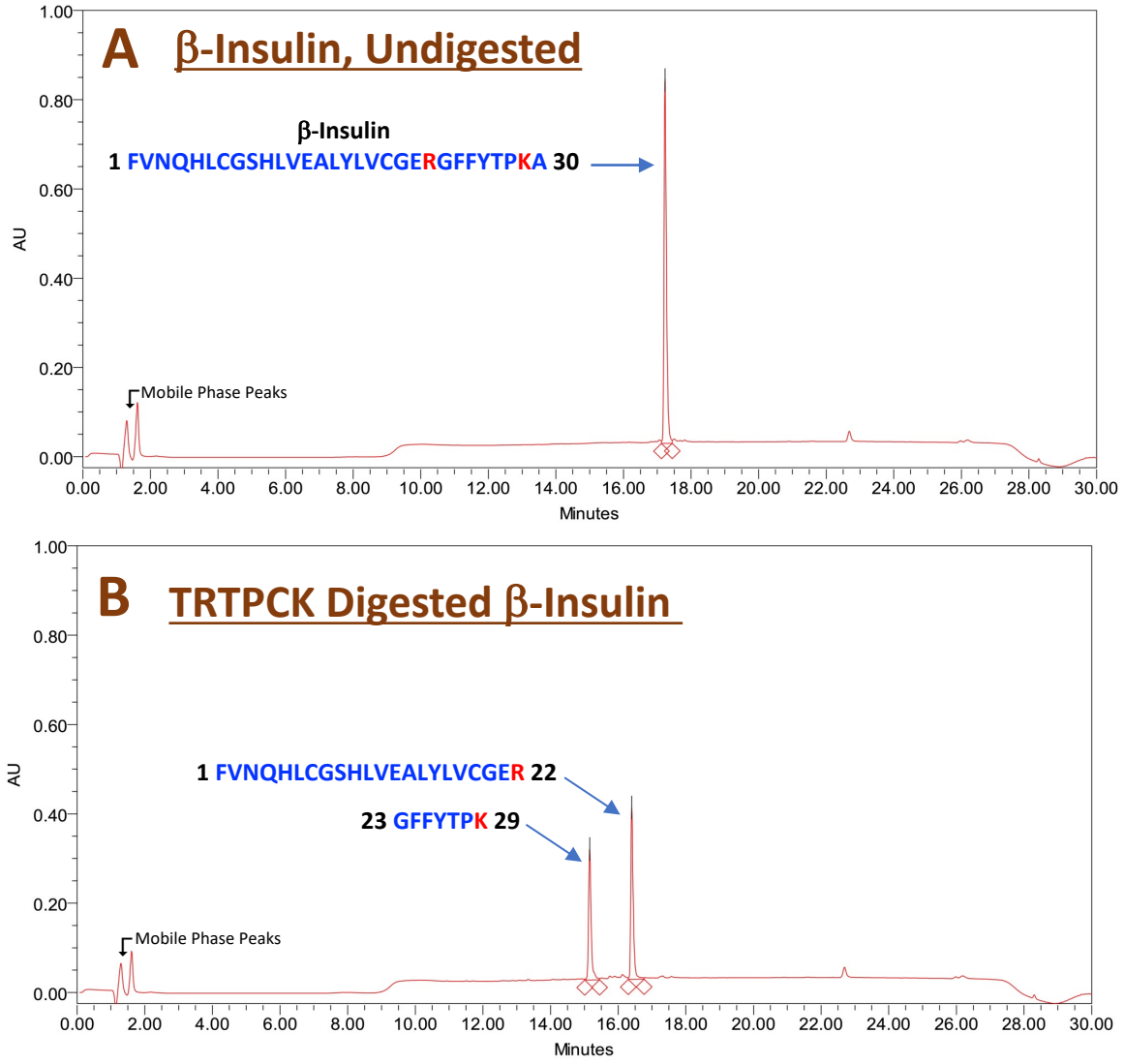
# Reverse Phase HPLC of Undigested and Trypsin (TRTPCK) Digested β-Insulin




**50 μg β-insulin was incubated with buffer for 2 hours (Panel A) or with 25 μgP TRTPCK (~7.0 units) in 50 mM Tris-HCl, 1 mM $CaCl_2$ for 2 hours (Panel B) at 37°C and resolved by reverse phase HPLC on a C18 column**

**Trypsin is a serine protease purified from bovine pancreas and is specific for the carboxy peptide bond of lysine (K) and arginine (R) residues. TRSEQZ is a chemically modified form of trypsin designed to withstand autolysis.**

**β-Insulin is a 30 amino acid peptide containing one arginine residue at position 22 and one lysine residue at position 29 and elutes as a single peak at 17.2 minutes (Panel A). Digestion with TRSEQZ for 2 hours results in complete digestion at R22 and K29 generating two smaller peptides, 22 and 7 amino acids in length, that elute at 16.3 and 15.1 minutes, respectively (Panel B).**

**Under sub-optimal conditions, cleavage at K29 may be incomplete and can result in an additional small peak consisting of the terminal 8 amino acids located on the base of the descending shoulder of the first peak.**

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