

ISOCRATIC SEPARATION OF INSULIN ANALOGS USING NOVA-PAK™ C₁₈ COLUMN

The hormone insulin¹, required for the treatment of diabetes, is a polypeptide consisting of two chains, A (21 residues) and B (30 residues), linked by a pair of disulfide bridges (see Figure 1). Human insulin has a threonine (Thr) C-terminal residue on the B-chain; porcine insulin has an alanine (Ala) residue in place of this threonine. Des-alanine insulin is produced by reacting porcine insulin with carboxypeptidase Y to remove the C-terminal alanine residue. This is a technique utilized in the production of semi-synthetic human insulin.

Carlsberg Biotechnology of Copenhagen, Denmark, producer of enzymes used in peptide synthesis, has developed a separation of these three closely related peptides on NOVA-PAK™ C₁₈ columns (see Figure 2). They tried several other columns and found that only the NOVA-PAK™ column would separate porcine insulin from Des-Ala-insulin, a tribute to the unique selectivity of the NOVA-PAK™ column packing. They utilized a Waters gradient LC consisting of two M6000A pumps, U6K, M441 detector, M720 System Controller, and M730 data Module.

FIGURE 1

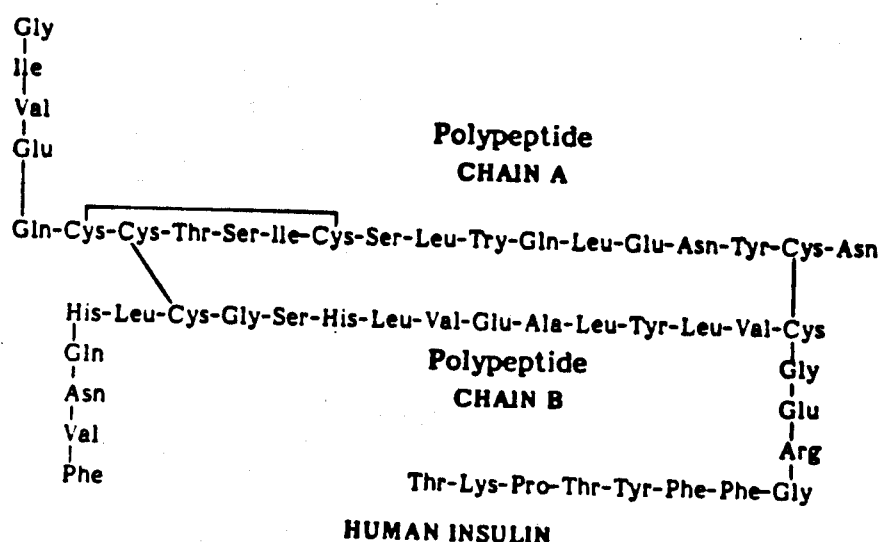
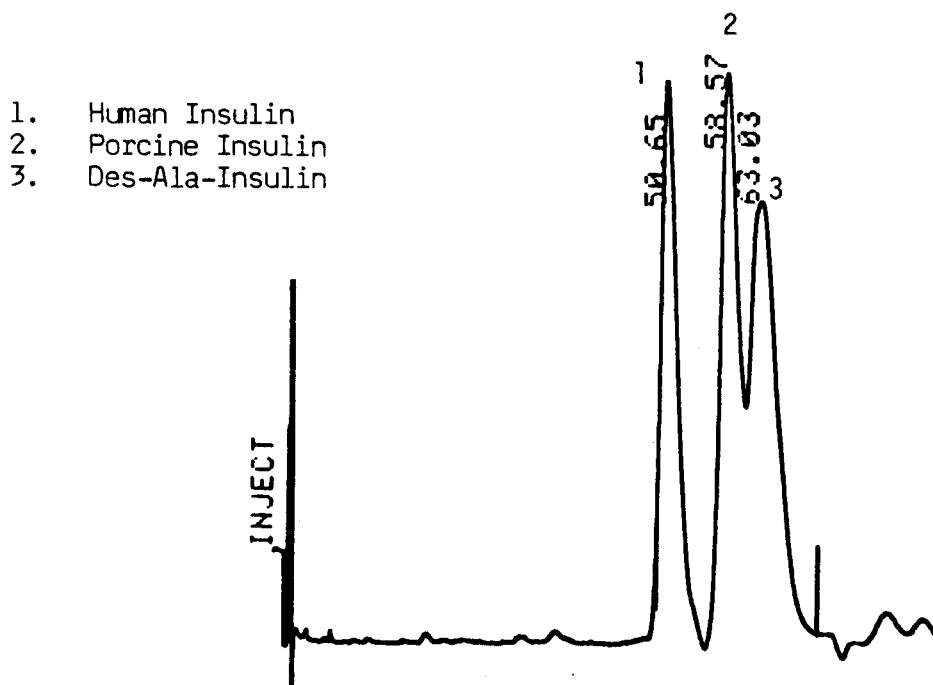


Fig. 1. Structure of Insulin.

FIGURE 2



CONDITIONS:

Column: NOVA-PAK™ C₁₈, 3.9 X 150 mm
Buffers: Stock Solution - 100 mM H₃PO₄,
50mM NaClO₄, 20 mM Triethylamine, pH 3.0
A - 75% stock, 25% CH₃CN
B - 50% stock, 50% CH₃CN
Dial-A-Mix @ 9% B
Flow Rate: 1 ml/min
Chart Speed: 0.1 cm/min
Detection: 214 nm @ 0.02 AUFS
Injection Volume: 75 µl