Determination of Insulin in Humans with Insulin-Dependent Diabetes Mellitus Patients by HPLC with Diode Array Detection

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Insulin is a protein hormone synthesized by pancreatic β-cells and used in controlling the blood-glucose level. Insulin monomer contains 51 amino acid residues in two chains (chain A with 21 residues and chain B with 30 residues) linked together by two disulfide bridges. Since its discovery in 1921, insulin has remained a major clinical drug for treatment of diabetes mellitus [1].

A simple, rapid, precise, accurate and reliable high-performance liquid chromatographic (HPLC) method with diode array detection (DAD) has been developed and validated for the determination of insulin in human plasma.

A good chromatographic separation was achieved on a C18 column with a mobile phase consisting of acetonitrile and 0.2 M sodium sulfate (pH 2.4) 25:75 (v/v). Its flow rate was 1.2 ml min⁻¹. The elution time for insulin was approximately 13.8 min. Calibration curve of insulin in human plasma was linear in the concentration range of 0.15–25 μg ml⁻¹. Intra- and inter-day relative standard deviation for insulin in human plasma was less than 6.3 and 8.5 %, respectively. Limits of detection and quantification in human plasma were 0.10 and 0.15 μg ml⁻¹, respectively.

The study was performed on eight insulin-dependent diabetes mellitus patients (four females and four males). Their ages ranged from 21 to 60 (Mean±SD, 37.25±12.79) and their weights ranged from 61 to 75 kg (Mean±SD, 69.00±6.14). All the patients were informed for the purpose, protocol and risk of the study. Blood samples (2 ml) were collected different times (5, 15, 30, 45, 60, 90, 120, 180 and 240 min) after subcutaneous injection of 25 IU of Actrapid HM and blood samples were analyzed immediately. The peak plasma level (Cmax) is the highest observed concentration and tmax is the corresponding time of this concentration. The areas under the plasma concentration-time curves (AUC0-t) were calculated with the linear trapezoidal rule. Elimination rate constant (k-el) was calculated by the least-squares regression using the last five time points of each curve. The apparent elimination half-life (t½) was the quotient of the natural logarithm of 2 and the elimination rate constant, and clearance (CL) was determined with the formula k-el x Vd.

In this study, a simple, sensitive and reliable method consisting of liquid-liquid phase extraction and reversed-phase isocratic HPLC method with diode array detection was developed and fully validated for the determination of insulin human plasma. The method showed high selectivity, precision and accuracy for the use in pharmacokinetic study and therapeutic monitoring of insulin. Therefore, the developed HPLC method can serve as a cheap and easy tool for the evaluation of the bias of immunoassay methods used for the determination of insulin levels in human plasma.

References