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DEVELOPMENT AND VALIDATION OF A GRADIENT HPLC METHOD FOR THE QUANTIFICATION OF INSULIN IN RAT PLASMA: APPLICATION TO PHARMACOKINETIC STUDIES

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ABSTRACT

A simple, specific, rapid and sensitive reversed phase high performance liquid chromatography method was developed to determine insulin in rat's plasma. The method entails direct injection of rat plasma sample after deproteinization using acetonitrile - propanol (1:1). The mobile phase comprise a mixture of water and acetonitrile containing 0.1% v/v trifloroacetic acid (TFA) with gradient analysis by increasing the proportion of mobile phase of water from 25% v/v to 40% v/v within 20 minutes. A phenomenon C18 column (5µm 4.6 x 250 mm column) was used for the chromatographic separation which delivered at a flow rate of 0.6 mL/min at 210nm wavelength. Under these conditions the method was validated with respect to linearity, recovery, specificity, accuracy, precision, and stability. The method was proven to be linear over the concentration range of 0.39 -50.00µg/ml with mean correlation coefficient of 0.9999. The mean extraction recovery was 99.60%, while the coefficient of variation of within-day and between-day measurements was all less than 8%. The limit of quantification (LOQ) and limit of detection (LOD) of the method were 0.39 µg/ml and 0.13 µg/ml, respectively. The shorter run time (minute), specificity, sensitivity and reproducibility of this method were found to be satisfactory and deemed suitable for routine determination of insulin in rat.

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