**Development of a RP-HPLC (reverse phase-high performance liquid chromatography) method for the determination of Metformin in human plasma**

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**Abstract**

**Background** : The study presents the development and validation of a simple HPLC method for the determination of metformin in human plasma, to be used for the clinical monitoring of metformin after oral administration or for bioequivalence studies.

**Aims and objectives** : To develop and validate a rapid, selective and sensitive ion-pairing HPLC-UV (high performance liquid chromatography-ultraviolet) method for the determination of metformin in human plasma, using a conventional reverse phase column.

**Method** : Ion-pair separation followed by UV (ultraviolet) detection performed on deproteinised and dichloromethane washed plasma samples was chosen for the determination of metformin. The HPLC method used a RP-C18 column (reverse phase-column C18) (LiChrocart 125-4) and analytical guard column, with an isocratic elution (1.25 mL/min) at 50°C column temperature. The mobile phase was acetonitrile:sodium phosphate buffer 10 mM (32.5:67.5 v/v) and sodium dodecylsulphate 0.3%, pH=6.0 with H3PO4 85%. The eluent was monitored at 236 nm.

**Results** : The calibration curves were linear (r > 0.9998) in the concentration ranges of 50-1600 ng/mL for metformin in plasma. The method was validated for linearity, accuracy and precision. The LLOQ (lower limit of quantification) were found to be 50.0 ng/mL. The intra- and inter-day coefficients of variation and accuracy were <15% for all concentrations of quality controls studied, and <20% LLOQ. Absolute recovery was found to be > 90% for all three concentrations of plasma quality controls studied (300-1500 ng/ml).

**Conclusions** :The proposed method was found to be rapid, precise and accurate for quantification of metformin in human plasma. The method was further used in a bioavailability and bioequivalence study.

**Keywords** : metformin, ion-pair HPLC method, plasma analysis, quantification.