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Development and Validation Method for Quantitative Determination of Rifampicin in Human Plasma and Its Application in Bioequivalence Test

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Abstract : Rifampicin is a semisynthetic antibiotic derivative of rifamycin B produced by Streptomyces mediterranei. RIF has been used worldwide as first line drug-prescribed throughout tuberculosis therapy. This study aims to develop and to validate an HPLC method couple with a UV detection for determination of rifampicin in spiked human plasma and its application for bioequivalence study. The chromatographic separation was achieved on an RP-C18 column (LachromHitachi, 250 x 4.6 mm., 5µm), utilizing a mobile phase of phosphate buffer/acetonitrile (55:45, v/v, pH 6.8 ± 0.1) at a flow of 1.5 mL/min. Detection was carried out at 337 nm by using spectrophotometer. The developed method was statistically validated for the linearity, accuracy, limit of detection, limit of quantitation, precise and specifity. The specifity of the method was ascertained by comparing chromatograms of blank plasma and plasma containing rifampicin; the matrix and rifampicin were well separated. The limit of detection and limit of quantification were 0.7 µg/mL and 2.3 µg/mL, respectively. The regression curve of standard was linear ($r > 0.999$) over a range concentration of 20.0 – 100.0 µg/mL. The mean recovery of the method was 96.68 ± 8.06 %. Both intraday and interday precision data showed reproducibility (R.S.D. 2.98% and 1.13 %, respectively). Therefore, the method can be used for routine analysis of rifampicin in human plasma and in bioequivalence study. The validated method was successfully applied in pharmacokinetic and bioequivalence study of rifampicin tablet in a limited number of subjects (under an Ethical Clearance No. KE/FK/6201/EC/2015). The mean values of Cmax, Tmax, AUC(0-24) and AUC(o-∞) for the test formulation of rifampicin were 5.81 ± 0.88 µg/mL, 1.25 hour, 29.16 ± 4.05 µg/mL. h. and 29.41 ± 4.07 µg/mL. h., respectively. Meanwhile for the reference formulation, the values were 5.04 ± 0.54 µg/mL, 1.31 hour, 27.20 ± 3.98 µg/mL.h. and 27.49 ± 4.01 µg/mL.h. From bioequivalence study, the 90% CIs for the test formulation/reference formulation ratio for the logarithmic transformations of Cmax and AUC(0-24) were 97.96-129.48% and 99.13-120.02%, respectively. According to the bioequivamence test guidelines of the European Commission-European Medicines Agency, it can be concluded that the test formulation of rifampicin is bioequivalence with the reference formulation.

Keywords : validation, HPLC, plasma, bioequivalence
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