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Development and Validation of RP- HPLC Method for the Determination of Raltegravir In Human Plasma

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ABSTRACT

A rapid, specific and accurate high performance liquid chromatographic method for the determination of Raltegravir in human plasma using metronidazole as internal standard has been developed and validated. The separation was performed on a Phenomenex C_{18} (5µm; 250 X 4.6mm) column. The composition of the mobile phase is 60:40 (v/v) of 10 mM phosphate buffer (pH 3.5± 0.05) and acetonitrile. The peaks were detected by UV-Visible detection at a wavelength of 268 nm. The extraction process involved a simple liquid-liquid extraction technique using methyl-t-butyl ether. The method showed good linearity in the range of 40.0-4003.9 ng mL^{-1} with a sensitivity (limit of detection) of 40.0 ng mL^{-1} using 200 µL of K_2EDTA plasma. The mean recovery of Raltegravir from all the quality control samples is 78.71% with a coefficient of variation of 1.5% and recovery of internal standard was 73.2%. The intra-day accuracy at three levels of quality control samples ranged from 96.8 - 102.2% with a precision of 3.5 to 5.8%. The inter-day accuracy ranged from 94.3-103.5% with a precision of 2.7-7.7%. The peaks were well separated from the plasma interferences. The method is successfully validated as per FDA guidelines in human plasma containing K_2EDTA as an anti-coagulant.

Keywords: Raltegravir, antiretroviral, HIV-1 integrase inhibitor, HPLC.