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Research Article

VALIDATED REVERSE PHASE HPLC METHOD FOR DETERMINATION OF RALTEGRAVIR IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

A simple, rapid, selective, sensitive, linear, precise and accurate RP-HPLC method was developed and validated for the determination of raltegravir in bulk and in tablet dosage forms. Seperation of the drug was achieved on a reverse phase Symmetry C8 column (150mmx4.6mm I.D., 5 μm particle size) at ambient temperature using a mobile phase consisting of phosphate buffer pH 2.5 and acetonitrile in the ratio of 40:60 v/v. Isocratic elution at a flow rate of 0.6 mL/min was employed. The UV detection wavelength was 247 nm and 20 μl of sample was injected. The linearity was found in the range of 5-25 μg/ml with a correlation coefficient of 0.999. The retention time for raltegravir was 2.881 min. The % recovery was within the range between 99.36 % and 101.85 %. The method was validated as per the ICH guidelines for its sensitivity, linearity, accuracy and precision. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was successfully employed for routine quality control analysis of raltegravir in bulk samples and its pharmaceutical formulations.

Keywords: Raltegravir, RP-HPLC, UV detection, Validation.

INTRODUCTION

Raltegravir potassium (Fig. 1) is chemically, N-[(4-Fluorophenyl)methyl]-1,6-dihydro5-hydroxy-1-methyl-2-[1-methyl-1-[[(5-methyl-1,3,4-oxadiazol-2-yl)carbonyl]amino]ethyl]-6-oxo-4pyrimidinecarboxamide monopotassium salt[1]. Raltegravir is a human immunodeficiency virus integrase strand transfer inhibitor, is indicated in combination with other antiretroviral agents for the treatment of human immunodeficiency virus (HIV-1) infection. Raltegravir inhibits the catalytic activity of HIV-1 integrase, an HIV-1 encoded enzyme that is required for viral replication. Inhibition of integrase prevents the covalent insertion, or integration, of unintegrated linear HIV-1 DNA into the host cell genome preventing the formation of the HIV-1 provirus. The provirus is required to direct the production of progeny virus, so inhibiting integration prevents propagation of the viral infection[2]. Literature survey revealed that a few analytical methods have been reported for the determination of raltegravir in pure drug and in pharmaceutical dosage forms using HPLC[3-7] and LC-MS[8-11] either in single or in combined forms**.** The aim of the present work is to develop and validate a simple, fast and reliable isocratic RP-HPLC method with UV detection for the determination of raltegravir in bulk and in tablet dosage forms. Confirmation of the applicability of the developed

method was validated according to the International Conference on Harmonization (ICH) for the determination of raltegravir in bulk and in tablet dosage forms[12].

MATERIALS AND METHODS

Chemicals

Acetonitrile of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. HPLC grade water and potassium dihydrogen phosphate was purchased from Merck Specialities Pvt. Ltd., Mumbai. Raltegravir standard sample was provided by Pharma Train, Hyderabad, India.

Instrumentation and analytical conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase C8 column (150 mmx4.6 mm; 5 µm), a 2695 binary pump, a 10 µl injection loop and a 2487 dual absorbance detector and running on Waters Empower software. Isocratic elution with phosphate buffer: acetonitrile (40:60 v/v, pH 2.5 adjusted with orthophosphoric acid) was used at a flow rate of 0.6 ml/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use. The UV spectrum of raltegravir was taken using a Elico SL-159 UV-Visible spectrophotometer.

Preparation of stock and standard solutions

Accurately weigh and transfer 10 mg of raltegravir working standard into a 100 ml volumetric flask, add about 70 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter. The calibration curve was plotted with the five concentrations of the 5-25 µg/ml working standard solutions. Chromatogram was recorded thrice for each dilution. Calibration solutions were prepared daily and analyzed immediately after preparation.

Assay of raltegravir tablets

Twenty tablets of raltegravir potassium containing 400 mg of raltegravir were weighed accurately. Tablet powder equivalent to 2 mg of raltegravir potassium was transferred to a 100 ml volumetric flask, dissolve in 70 ml of mobile phase and made upto volume with mobile phase. Mix well and filter through 0.45 µm filter. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter. An aliquot of this solution was injected into HPLC system. Peak area of raltegravir was measured for the determination.

Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 5-25 µg/ml prepared in triplicates to test linearity. The peak area of raltegravir was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared raltegravir test solution in the same equipment at a concentration value of 100 % (15 µg/ml) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of raltegravir was determined and precision was reported as % RSD.

Method accuracy was tested (% recovery and % RSD of individual measurements) by analyzing sample of raltegravir at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of raltegravir recovered in the samples. Sample solution short term stability was tested at ambient temperature (20 ± 10^0C) for three days. In order to confirm the stability of both standard solutions at 100 % level and tablet sample solutions, both solutions protected from light were re-injected after 24 and 48 hrs at ambient temperature and compared with freshly prepared solutions.

RESULTS AND DISCUSSION

Selection of the detection wavelength

The UV spectra of raltegravir in 40:60 v/v mixture of phosphate buffer and acetonitrile was scanned in the region between 200 and 400 nm and shows λmax at 247 nm.

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. The drug raltegravir is non-polar. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C8 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of phosphate buffer and acetonitrile was selected as mobile phase and the effect of composition of mobile phase on the retention time of raltegravir was thoroughly investigated. The concentration of phosphate buffer and acetonitrile were optimized to give symmetric peak with short run time and the typical chromatogram was shown in Figure 2. A short run time and the stability of peak asymmetry were observed in the ratio of 40:60 % v/v of phosphate buffer and acetonitrile. It was found to be optimum mobile phase concentration.

Validation of method

Linearity

Five points calibration graphs was constructed covering a concentration range 5-25 µg/ml (Three independent determinations were performed at each concentration). Linear relationships between the peak area signal of raltegravir the corresponding drug concentration was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 1.

Precision

The validated method was applied for the assay of commercial tablets containing raltegravir. Sample was analyzed for six times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented in good agreement with the labeled content. Assay results, expressed as the percentage of label claim, and was found to be 101.35 showing that the content of raltegravir in tablet formulations confirmed to the content of requirements (95-105 %) of the label claim. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day on three consecutive days (n=3) indicated a % RSD of 0.14. This indicates good method precision.

Accuracy

The data for accuracy were expressed in terms of percentage recoveries of raltegravir in the real samples. The mean recovery data of raltegravir in real samples were within the range of 99.36 % and 101.85 %. The mean % RSD was 100.44 % satisfying the acceptance criteria for the study. It was proved that there is no interference due to excipients used in tablet formulation. Hence the accuracy of the method was confirmed. The results are furnished in Table 2.

Stability

The stability of raltegravir in standard and sample solutions containing determined by storing the solutions at ambient temperature (20 ± 10^{0}C). The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 98 %. This denotes that raltegravir is stable in standard and sample solutions for at least 2 days at ambient temperature.

System suitability

The system suitability parameter like capacity factor, asymmetry factor, tailing factor, HETP and number of theoretical plates were also calculated. It was observed that all the values are within the limits and the results are shown in Table 3. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of raltegravir in tablet formulation. The results are furnished in Table 4.

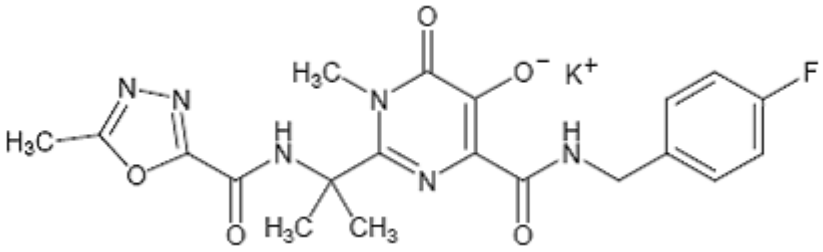

Fig. 1: Chemical Structure of Raltegravir

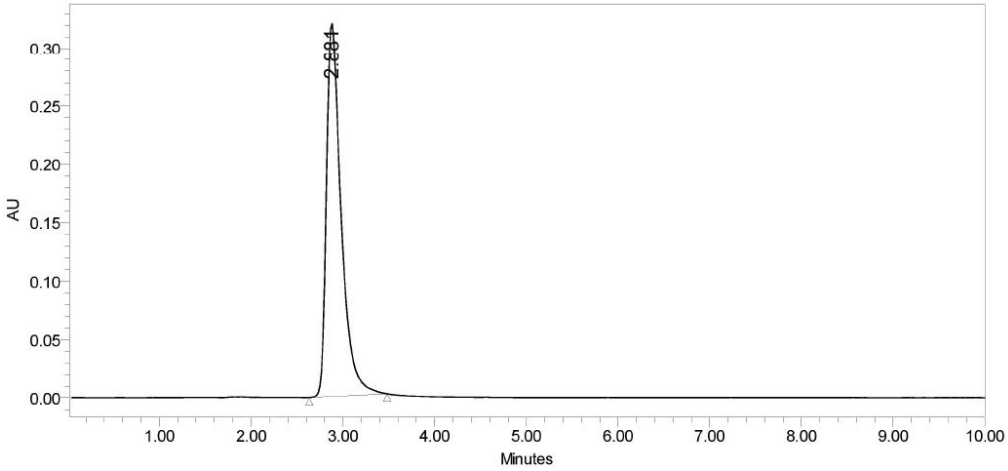

Fig. 2: Typical Chromatogram of Raltegravir

Table 1: Linearity of Raltegravir

S. No.	Linearity Level	Concentration	Area
1	I	5 µg/ml	1250028
2	II	10 µg/ml	2405987
3	III	15 µg/ml	3665123
4	IV	20 µg/ml	4828439
5	V	25 µg/ml	6297338
		Correlation Coefficient	0.999

Table 2: Recovery Studies of Raltegravir

% Concentration	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50 %	1847066	5.4	5.5	101.85 %	100.44 %
100 %	3588814	10.0	10.1	100.1 %	
150 %	5275022	15.7	15.6	99.36 %	

Table 3: System Stability Parameters of Raltegravir

Parameters	Values
λmax (nm)	247
Beer's law limit (µg/ml)	5-25
Correlation coefficient	0.999
Retention time (min)	2.881
Theoretical plates	2663
Tailing factor	1.64
Limit of detection (µg/ml)	0.006
Limit of quantification (µg/ml)	0.020

Table 4: Assay Studies of Raltegravir

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Formulation 1	2	2.02	101.35

CONCLUSION

A validated RP-HPLC method has been developed for the determination of raltegravir in bulk sample and tablet dosage form. The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of raltegravir and can be reliably adopted for routine quality control analysis of raltegravir in its tablet dosage forms.

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