INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

UV – SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF RALTEGRAVIR IN BULK AND TABLET DOSAGE FORM

B. Siddartha[1*] and I. Sudheer Babu[2]

[1]Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy, Maisammaguda, Dhulapally, Secunderabad, Telangana State, India.
[2]Sir C.R. Reddy College of Pharmaceutical Sciences, Eluru, Andhra Pradesh, India.

ABSTRACT

A simple, precise and accurate UV Spectrophotometric method has been developed and validated for estimation of Raltegravir in bulk and tablet dosage form. In this method Raltegravir shows λmax at 290nm using 0.1N NaOH as a solvent and calibration graphs were plotted over the concentrations ranging from 10 to 60µg/ml of Raltegravir with correlation coefficient 0.999. The proposed method was validated as per ICH Q2 (R1) guidelines for precision, linearity, accuracy and recovery. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.311µg/ml and 0.941µg/ml respectively by simple UV spectroscopy. The proposed method was validated.

Keywords: Raltegravir, UV-Spectroscopy, Validation, 0.1N NaOH.

INTRODUCTION

Raltegravir is an antiretroviral drug produced by Merck & Co., used to treat HIV infection. Raltegravir is chemically N-[(4-fluorophenyl)methyl]-5-hydroxy-1-methyl-2-{2-[(5-methyl-1,3,4-oxadiazol-2-yl) formamido] propan-2-yl}-6-oxo-1,6-dihydropyrimidine-4-carboxamide. The molecular formula is $C_{20}H_{21}FN_6O_5$ and molecular weight is 444.42 g/mol. Raltegravir targets integrase, an HIV enzyme that integrates the viral genetic material into human chromosomes, a critical step in the pathogenesis of HIV. The drug is metabolized away via glucuronidation.

In literature survey reveals various UV-spectroscopic, RP-HPLC, HPTLC and LC-MSmethods[2-8]. In present study, simple, economical, accurate, reproducible analytical methods with betterdetection range for estimation of Raltegravir in its pure form and its pharmaceutical formulations were developed. The developed method was validated as per ICH guidelines[9].

EXPERIMENTAL

MATERIALS AND METHODS

The spectrophotometric measurements were carried out using a Shimadzu UV-1700 UV/Vis spectrophotometer with 1cm matched quartz cell and Shimadzu ELB 300 analytical balance, Raltegravir pure drug (99.95%) was obtained as a gift sample. All chemicals and reagents used were of analytical grade.

Preparation of Standard solution

Standard drug of Raltegravir was proposed by dissolving 25mg pure Raltegravir in 0.1N NaOH and transferred into 250ml volumetric flask to obtain 100µg/ml of stock solution. The standard solution of Raltegravir having concentration of 40µg/ml was scanned in UV range (200-400nm) in 1.0 cm cell against in solvent as blank and spectrum was obtained.

Determination of λmax

40µg/ml of Raltegravir was prepared and scanned in UV range of 200-400nm and spectrum was obtained. The λmax was found to be at 334nm wavelength where absorbance was found maximum at this wavelength. Hence it is considered as absorbance maxima (λmax) shown in Figure-1.

Preparation of calibration curve

Standard stock solution was suitably diluted with 0.1N NaOH to obtain concentrations

ranging from 10-60μg/ml. Absorbance of these solutions was measured at 334nm. Calibration curve was obtained by plotting graph between concentration and absorbance shown in Figure-2.

Preparation of test solution

20 Tablets were weighed and its average weight was determined. An accurately weighed tablet powder equivalent to 25mg of Raltegravir transferred into 250ml volumetric flask dissolved in 0.1N NaOH, sonicated for 10min and volume was made up to the mark. Solution was filtered using whattman filter paper (No.41) to obtain 100μg/ml stock solution.

VALIDATION

Linearity

The absorbances were observed from 10 to 60μg/ml and were shown in Table-1. Linearity was obtained between 10 to 60μg/ml. Concentration graph was plotted for concentration and absorbance. The equation of calibration curve obtained was y = 0.0017x - 0.005. The correlation coefficient (r) was 0.999 shown in Figure-2.

Accuracy

To determine the accuracy of the method recovery was performed by standard addition method. To pre-analyzed sample known amount of standard Raltegravir was spiked in different concentrations. The recovery was performed at three levels 50%, 100% and 150% of standard Raltegravir. Solutions were analyzed and percentage recovery was calculated from calibration curve shown in Table-2

Precision

Repeatability

Six concentrations of 40μg/ml were prepared and the absorbances were read. The % RSD was calculated and shown in Table-3.

Intraday and Interday Precision

The concentration of 10μg/ml, 20μg/ml and 30μg/ml of Raltegravir (on label claim basis) was taken. The absorbance of the final solution was read after 0hr, 12hr and 24hr in 1.0 cm cell at selected wavelength. Similarly the absorbance of the same solutions was read on 1st, 2nd and 3rd day. All the solutions are prepared triplicate and analyzed. The results were recorded in Table-4 &5.

Assay

The assay & % purity was performed by taking ISENSTRESS with label claim 400mg. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form. The results were shown in Table-6.

Ruggedness

It was carried out by analyzing the sample by three different days and estimation of drug by proposed methods. Results of studies are shown in Table-8.

RESULTS AND DISCUSSIONS

Attempt has been made to develop rapid, sensitive, economic, precise and accurate analytical method for Raltegravir in pure and pharmaceutical dosage form. The proposed method is based on UV Spectrophotometric absorption in UV region using 0.1N NaOHas solvent. Maximum absorbance was found to be at 334nm. LOD and LOQ were found to be 0.311μg/ml and 0.941μg/ml(Table-7). Beer's law was obeyed in concentrations ranging from 10 to 60μg/ml. The correlation coefficient values were above 0.999which shows that absorbance was linear with concentration. The optical characteristics such as Beer's law limit, correlation coefficient, slope, intercept, molar absorptivity, scandell's sensitivity were calculated and validated (Table-9). Precision of the method was confirmed by Intraday and Interday analysis, %RSD values were found to be less than 2.0. The percent recovery was found to be nearly 100% indicating reproducibility and accuracy of the methods. Hence the proposed method could be effectively adopted for routine quality control of Raltegravir in bulk and formulated tablet dosage form.

ACKNOWLEDGEMENTS

The authors are thankful to Malla Reddy College of Pharmacy, for providing the facilities to carry out the work.

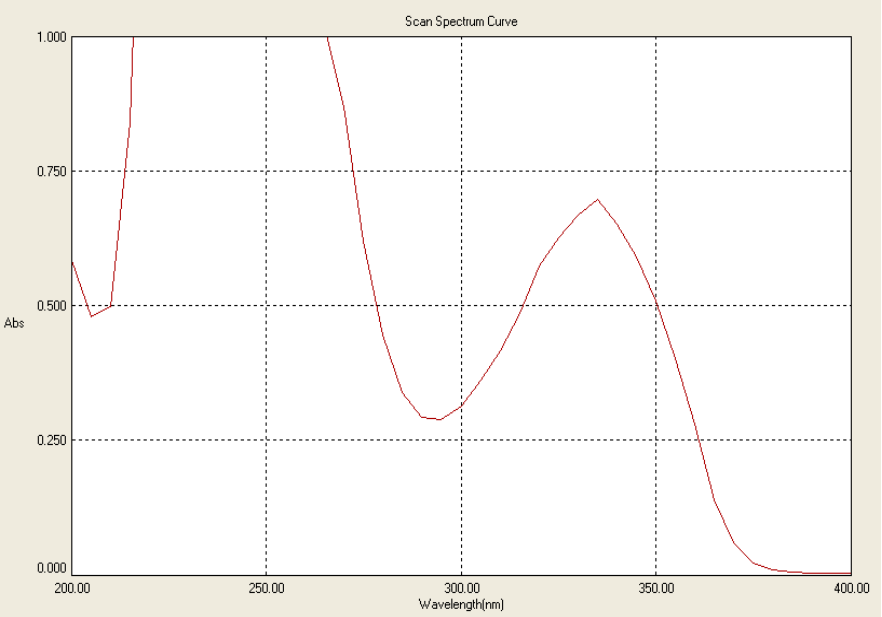

Fig. 1: UV Spectrum of Raltegravir in 0.1N NaOH at λmax = 334nm

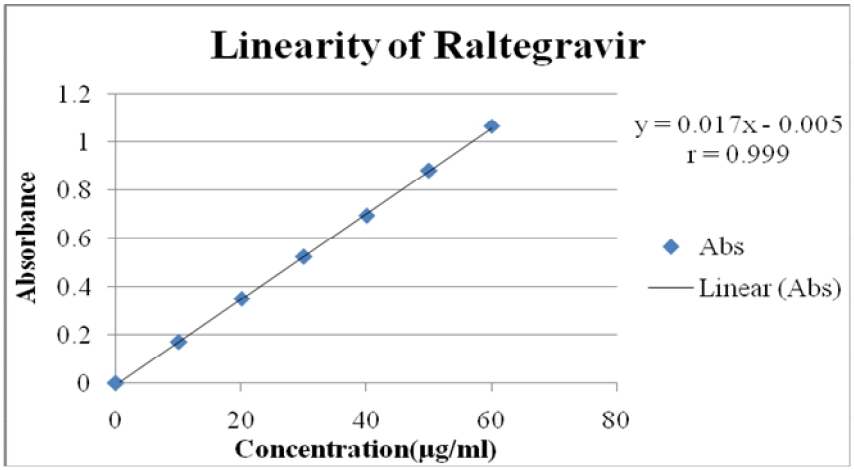

Fig. 2: Calibration curve of Raltegravir in 0.1N NaOH showing linearity relationship

Table 1: Calibration data for analysis of 0.1N HCl in 0.1N HClat λmax = 290nm

Concentration (µg/ml)	Mean Absorbance (± SD)
10	0.171 (0.0006)
20	0.349 (0.0010)
30	0.523 (0.0012)
40	0.694 (0.0010)
50	0.871 (0.0025)
60	1.045 (0.0017)

Table 2: Recovery data of Raltegravir in 0.1N HCl

Ingredient	Amount of drug from formulation	Amount of standard added	Percentage added	Amount added	Amount found	% Recovery (Mean ± RSD)*
Raltegravir	20µg	10µg	50%	10	9.96	99.61± 0.90
Raltegravir	20µg	20µg	100%	20	19.75	98.76± 0.44
Raltegravir	20µg	30µg	150%	30	29.94	99.81± 0.38

*n=3 (Average of 3 determinations)

Table 3: Precision data of Udenafil in 0.1N HCl

S.No	Concentration(µg/ml)	Absorbance
1	40µg/ml	0.695
2	40µg/ml	0.693
3	40µg/ml	0.693
4	40µg/ml	0.695
5	40µg/ml	0.695
6	40µg/ml	0.691
Mean		0.694
Stddev		0.0016
%RSD		0.24

Table 4: Results of Inter-day Precision of Raltegravir in 0.1N HCl

Parameter	% Recovery Estimated (Mean + RSD)*		
	10µg/ml	20µg/ml	30µg/ml
At 0 hr	99.78 ± 0.58	99.59 ± 0.17	99.88 ± 0.11
At 12 hr	99.20 ± 0.58	99.97 ± 0.44	100.13 ± 0.29
At 24 hr	100.17 ± 0.89	99.97 ± 0.60	99.81 ± 0.33

*n=3 (Average of 3 determinations)

Table 5: Results of Intraday Precision of Raltegravir in 0.1N HCl

Parameter	% Recovery Estimated (Mean + RSD)*		
	10µg/ml	20µg/ml	30µg/ml
Day-1	99.86 ± 0.33	99.95 ± 0.24	99.79 ± 0.11
Day-2	100.14 ± 0.43	100.24 ± 0.22	99.99 ± 0.16
Day-3	100.45 ± 0.19	100.11 ± 0.26	99.89 ± 0.24

*n=3 (Average of 3 determinations)

Table 6: Results of analysis of laboratory samples (Assay)

Sample	Label	Amount found	% Purity ± %RSD*
ISENSTRESS(Brand-1)	400mg	399.42	99.81± 0.14

*n=3 (Average of 3 determinations)

Table 7: Lowest Limit of detection and Lowest Limit of quantification

LOD (µg/ml)	LOQ (µg/ml)
0.311	0.941

Table 8: Results of Ruggedness of Raltegravir in 0.1N NaOH

Ruggedness	% RSD*
Analyst – 1	0.30
Analyst – 2	0.22

*n=3 (Average of 3 determinations)

Table 9: Validation Parameters

Parameters	Results
Beer's law limit (µg/ml)	10-60
Absorptivity (1mole^{-1}, cms^{-1})	7.599 x 10^3
Sandell's sensitivity (µg/cm2/0.001)	0.0585
Correlation coefficient	0.999
Regression equation	Y = 0.0017x - 0.005
Limit of detection	0.311
Limit of quantification	0.941
Precision (% RSD)	0.0016

REFERENCES

1. http://www.drugbank.ca/drugs/DB06817.
2. Girija B Bhavar. Simple Spectrophotometric Method for Estimation of raltegravir Potassium in Bulk and Pharmaceutical Formulations. Journal of Applied Pharmaceutical Science. 2013;3(10):147-150.
3. Stefania Notari. Simultaneous Determination of Maraviroc and raltegravir in Human Plasma by HPLC-UV, IUBMB Life. 2009;61(4):470–475.
4. Sudha T and Raghupathi T. Reverse Phase–High Performance Liquid Chromatography and Ultra Violet Spectrophotometric Method for The Estimation of raltegravir Potassium in Bulk and in Tablet Dosage form. Global Journal of Medical Research. 2011;11(2):1-9.
5. Lakshmana Rao A and Raghu Ram MS. Validated reverse phase HPLC method for determination of raltegravir in pharmaceutical preparations. International Journal of Research in Pharmacy and Chemistry. 2012;2(1):217-221.
6. Rambabu Kuchi K, Balamurali Krishna and Sambasiva Rao. New RP - HPLC Method Development and validation for Analysis of Antiviral drug raltegravir. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011;2(1):132-135.
7. Mary C Long, Chantelle Bennetto-Hood and Edward P Acosta. A sensitive HPLC–MS–MS method for the determination of raltegravir in human plasma. Journal of Chromatography B. 2008;867(2):165-171.
8. Sudha T and Shanmuga sundram. Development and validation of RP-HPLC and HPTLC Chromatographic methods of analysis for the quantitative estimation of raltegravir potassium in pharmaceutical dosage form. Research Journal of Pharmacy and Technology. 2011;4(11):1746-1750.
9. ICH, Q2(R1), Harmonized Tripartite Guideline, Validation of Analytical procedures Text and methodology, International Conference on Harmonization(ICH), Geneva, Nov. 2005.