Khagga Bhavyasri et al /J. Pharm. Sci. & Res. Vol. 7(9), 2015, 685-689

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Development and Validation of Forced Degradation Studies of Raltegravir using RP-HPLC and Characterization of Degradants by LC-MS/MS

Khagga Bhavyasri[1*], V.Murali Balaram[2] R.Nageswarao[3] D.Rambabu[4], E.Sasikiran Goud[5], M.Ajitha[1]

[1] *Center for Pharmaceutical Sciences Department, J.N.T.University, Kukatpally, Hyderabad- 500 072, A.P, India;*
[2] *Professor, Sultan Ul-Uloom College of Pharmacy, Hyderabad, A.P, India*
[3] *Indian Institute of Chemical Sciences, Tarnaka,Hyderabad-500 072, A.P, India;*
[4] *Agilent Technologies India Pvt. Ltd, Hyderabad, A.P, India;*
[5] *Sri Krishnadevraya University, Anantapuramu.*

Abstract

Aim:
Develop and validate a simple, shorter and effective HPLC method with UV detection (213nm) and subsequent validation for forced degradation studies of Raltegravir using RP-HPLC and characterization of degradants by LC-MS/MS.

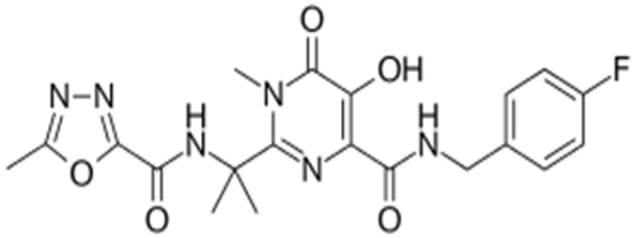
Materials and method:
The method uses isocratic the mobile phase mixture of Buffer and acetonitrile taken in the ratio in the ratio of 60:40(v/v) on Hypersil BDS, C18, 100 x 4.6 mm, 5µ m column.

Results:
The RSD for five injections was observed to 0.2 percentage and linearity range of 25-150 percentage of label claims established with 1.0 correlation. The observed result shows that the method was rapid, precise, accurate and simple. The method was validated as per ICH guidelines.

Key Words-Raltegravir, Method development, LC-MS, Hypersil BDS.

INTRODUCTION

Raltegravir Molecular formula C20H21FN6O5.Molcular weight is 444.42gr/mol. IUPAC Name *N*-(2-(4-(4-flurobenzylcarbamoyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)propan-2-yl) -5- methyl-1,3,4-oxadiazole-2-carboxamide.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. [1,2,3]. 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 [4-8] and LC-MS [9-12] 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[13].



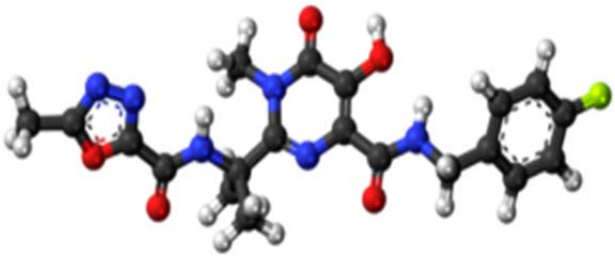


Figure: 1 Chemical structure of raltegravir

MATERIALS AND METHODS

Chemicals

Qualified standards and samples of raltegravir were obtained from local laboratories and were used without any further purification. The chemicals like Potassium dihydrogen Orthophosphate , triethylamine and Ortho phosphoric acid were purchased from Merck, Mumbai. Millipore water generated from TK water system. The analytical column used was Hypersil BDS, C18, 100 x 4.6 mm, 5µ.

Instruments

A Waters prominence HPLC system equipped with a quaternary UFLC LC-20AD pump, a DGU-20A_5 degasser, a SPD-M20A diode array detector, a SIL-20AC auto sampler, a CTO-20AC column oven and CBM-20A communications bus module was used for method development and validation studies.

Standard preparation

Accurately Weighed and transferred 10mg of raltegravir working standards into a 10 ml clean dry volumetric flask, add 0.4ml of water: methanol 20:80 v/v as diluent, sonicated for 30minutes and make up to the final volume with diluents.

Preparation of sample:

5 tablets were weighed and calculate the average weight of each tablet. Then the weight equivalent to 5 tablets were transferred into a 500mL volumetric flask, 300mL of diluent added and sonicated for 30 minutes, further the volume made up with water and filtered. From the filtered solution 0.1ml pipette out into a 10ml volumetric flask and made up to 10ml with diluents.

Chromatographic conditions

The chromatographic column used was Hypersil BDS column with dimensions of 100 mm X 4.6 mm with 5µm particle size. The column temperature was maintained at 30^{o}C and detection was monitored at a wavelength of 213nm. Injection volume was 10 µl and the mobile phase flow was set at 1.0mL/min. The water, methanol in the ratio 20:80 v/v was used as diluents for preparation of solutions.

METHOD VALIDATION

The developed method for determination of raltegravir was validated for system suitability along with method selectivity, specificity, linearity, range, precision, accuracy, range, ruggedness, robustness according to the ICH guidelines.

Method validation parameters

The system suitability was conducted using standard preparation and evaluated by injecting five replicate injections. Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present. Performed the specificity parameter of the method by injecting Diluent, placebo into the chromatographic system and evaluated by show any peak at the retention time of analyte. Performed the linearity with raltegravir in the range of 25 to 150% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. Also performed precision at higher level by injecting six times into the chromatographic system.

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation of series of measurements. The system precision was conducted using raltegravir and evaluated by making six replicate injections. The Accuracy of the method by recoveries of raltegravir sample solutions at different concentration levels ranging from 50 to 150%.The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

RESULTS AND DISCUSSION:

Optimization of chromatographic conditions:

Method development includes selection of appropriate chromatographic conditions/factors like detection wave length, selection and optimization of stationary and mobile phases. The wavelength of 213 nm was selected due to it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify raltegravir. Preliminary development trials were performed with various ODS and BDS columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Hypersil BDS, C18, 100 x 4.6 mm, 5µm column there a significant improvement in the peak shapes with 1.0 tailing factor.

System suitability:

The RSD from five replicate injections of standard preparation was 0.2 %, tailing factor for raltegravir peak was 1.0 and theoretical plates obtained 5050.

Selectivity:

Performed the specificity parameter of the method by injecting diluent, standard preparation sample preparation and placebo preparation into the chromatographic system and recorded the retention times. Specificity study of the method proved no peak observed at retention time of raltegravir. Specificity results of raltegravir given in the below Table-1.The selectivity chromatograms shown in the Figures 2 and 3

Table 1: Selectivity results of raltegravir

S.No.	Sample	Retention time
1	Blank	-
2	Placebo	-
3	Standard	2.820
4	Sample	2.823

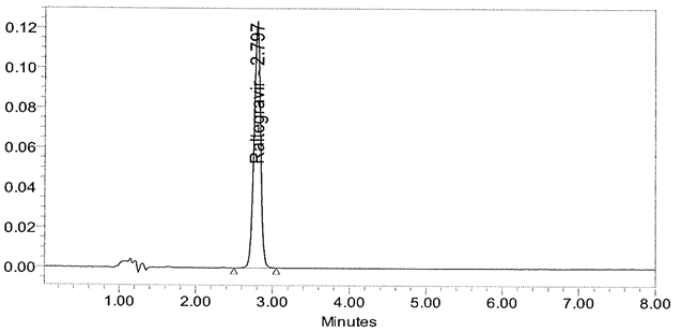


Fig: 2 Chromatogram of raltegravir standard

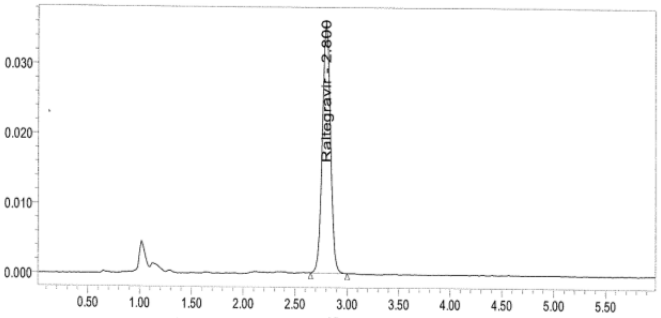


Fig: 3 Chromatogram of raltegravir sample

Linearity:
To demonstrate the linearity with raltegravir standard in the range of 25 to 150% of specification limit**.** Correlation coefficient of raltegravir was 0.999.The linearity results shown in the below Table -2

Table 2: Linearity results of raltegravir

S.No.:	Concentration in ppm	Area response
1.	25	190265
2.	50	377693
3.	75	575376
4.	100	758196
5.	125	945056
6.	150	1126400

Accuracy:
Accuracy study found that the mean % of recovery was more than 97.0% and less than 103.0% at each level 50 to 150% of concentration levels, hence method is accurate. The accuracy results are given Table-3.

Table 3: Accuracy results

S.No.:	Level in %	% Mean Recovery
1.	50	100.13
2.	100	101.11
3.	150	100.30

Precision:
The precision of test method was validated by assaying six samples prepared on raltegravir and calculate relative standard deviation of Assay results. The precision results are given Table-4

Table: 4 Precision results

S.No	Area of Raltegravir
1	99.90
2	99.68
3	100.09
4	101.23
5	99.54
6	100.89
Average	100.22
SD	0.6831
% RSD	0.68

LOD and LOQ
The limit of detection and limit of quantification of test method was validated based on signal to noise ratio method. The LOD and LOQ data given table 5.

Table 5: LOD and LOQ establishment data

3.3. Degradation of Raltegravir
The degradation behavior of LV under various stress conditions was investigated by LC.

Oxidation:
To 1 ml of stock solution of raltegravir, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 40µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation
To 1 ml of stock solution Raltegravir, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 40µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Parameter	Concentration in ppm
LOD	0.066
LOQ	2.001

Alkali Degradation Studies
To 1 ml of stock solution Raltegravir, 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 40µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

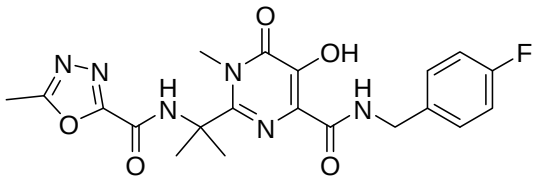
Thermal/ Dry Heat Degradation Studies:
The standard drug solution was placed in oven at 105^0c for 6 hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 40µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability Studies:
The photochemical stability of the drug was also studied by exposing the 100µg/ml solution to UV light by keeping the beaker in UV chamber for 7 days or 200 Watt hours/m^2 in photo stability chamber For HPLC study, the resultant solution was diluted to obtain 40µg/ml and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

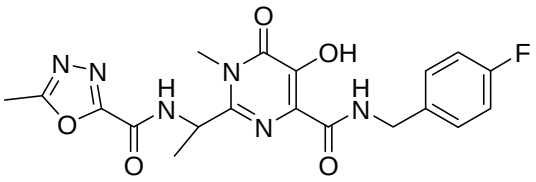
Mass spectral fragmentation:
The analysis of the degradation products was carried by LC and LC-MS. Raltegravir was subjected to LC-MS/MS with atmospheric pressure chemical ionization (APCI) to know the fragmentation pattern of drug. The MS^2 analysis of the precursor ion (m/z 704) of the drug given below with molecular structure and molecular weight.

CONCLUSION:

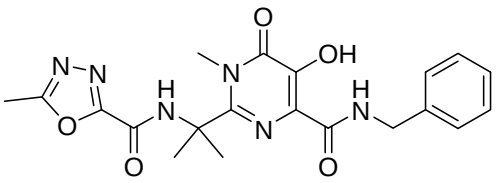
A validated stability indicating assay LC-PDA method was developed to study the degradation behavior of Raltegravir under hydrolysis (acid, base and neutral), oxidation, thermal and UV conditions. LC-MS/MS characterization of degradation products was carried out and pathways of decomposition were proposed. The drug was found to be degraded extensively in all conditions except oxidation due to presence of carbamate and urea linkage, which were susceptible to hydrolysis



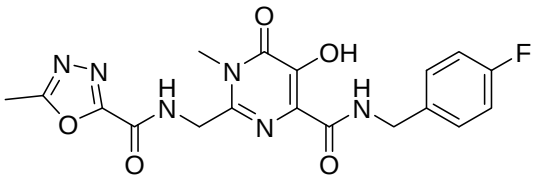
$C_{20}H_{21}FN_6O_5$
Mol. Wt.: 444.42



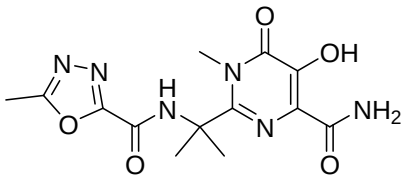
$C_{19}H_{19}FN_6O_5$
Mol. Wt.: 430.39



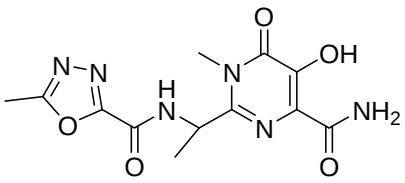
$C_{20}H_{22}N_6O_5$
Mol. Wt.: 426.43



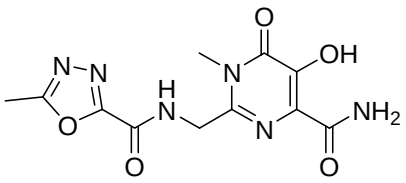
$C_{18}H_{17}FN_6O_5$
Mol. Wt.: 416.36



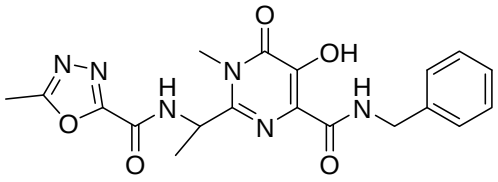
$C_{13}H_{16}N_6O_5$
Mol. Wt.: 336.3



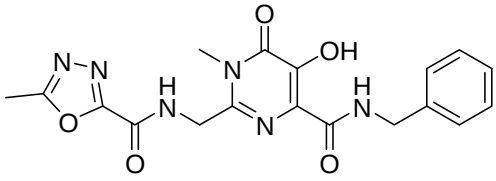
$C_{12}H_{14}N_6O_5$
Mol. Wt.: 322.28



$C_{11}H_{12}N_6O_5$
Mol. Wt.: 308.25



$C_{19}H_{20}N_6O_5$
Mol. Wt.: 412.4



$C_{18}H_{18}N_6O_5$
Mol. Wt.: 398.37

ACKNOWLEDGEMENT

The author thankful to Center for Pharmaceutical Sciences Department, J.N.T.U, Hyderabad for encouragement.

REFERENCES

1. Savarino A "A historical sketch of the discovery and development of HIV-1 integrase inhibitors". Expert Opin Investig Drugs 2006: **15** (12):1507 - 22.
2. U.S. Food and DrugAdministration (FDA). June 25, 2009. Retrieved 2009-11-15.
3. "Isentress Drug Approval Package". U.S. Food and Drug Administration (FDA). February 2008,22, 11-15.
4. Rezk NL, Angela NW and Kashuba DM. An accurate and precise high performance liquid chromatography method for the rapid quantification of the novel HIV integrase inhibitor raltegravir in human blood plasma after solid phase extraction. Anal Chim Acta, 2008; 628:204-213
5. Poirier JM, Robidou P and Jaillon P. Quantification of the HIV-integrase inhibitor raltegravir in human plasma by high performance liquid chromatography with fluorescence reduction. J Chromatogr B. 2008; 867:277-281.
6. Notari S, Tommasi C, Nicastri E, Bellagamba R, Tempestilli M and Percillo LP. Simultaneous determination of maraviroc and raltegravir in human plasma by HPLC-UV method. Inter Union Biochem Mol Biol. 2009;61:470-475.
7. Talameh JA, Rezk NL and Kashuba ADM. Quantifying the HIV-1 integrase inhibitor raltegravir in female genital tract secretions using high performance liquid chromatography with ultraviolet detection. J Chromatogr B. 2010; 878:92-96.

8. Furlan V, Taburet AM, Cruz MD, Barrail-Tran A and Goldwirt L. Quantification of raltegravir in human plasma by high performance liquid chromatography with photodiode array detection. J Chromatogr B. 2010; 878:456-460.
9. Acosta EP, Long MC and Bennetto-Hood C. A sensitive HPLC-MS-MS method for the determination of raltegravir in human plasma. J Chromatogr B. 2008; 867:165-171.
10. Decosterd LA, Fayet A, Beguin A, Zanolari B, Cruchon S and Guignard N. A LCtandem MS assay for the simultaneous measurement of new antiretroviral agents: raltegravir, maraviroc, darunavir and etravirine. J Chromatogr B. 2009; 877:1057- 1069.
11. Heine RT, Hillebrand MJ, Rosing H, Gorp ECV, Mulder JW, Beijnen JH and Huitema AD. Quantification of the HIV-intergrase inhibitor raltegravir and detection of its main metabolite in human plasma, dried blood spots and peripheral blood mononuclear cell lysate by means of high performance liquid chromatography tandem mass spectrometry. J Pharm Biomed Anal. 2009; 49:451-458.
12. Wang LZ, Lee LS, Thuya WL, Soon GH, Kong LR, Nye PL, Lee EJ, Flexner C and Goh BC. Simultaneous determination of raltegravir and raltegravir glucuronide in human plasma by liquid chromatographytandem mass spectrometric method. J Mass Spectrom. 2011;46:202-208.
13. ICH Harmonized Tripartite Guidelines, Text on Validation of Analytical Procedures: Text and Methodology. Q2B. Geneva, 1996.