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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND RALTIGRAVIR IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple stability indicating high performance liquid chromatographic method has been developed for the simultaneous determination of Lamivudine in combination with Raltigravir using reverse phase Inertsil ODS C18 column (150 × 4.6 mm, 5µm) with UV detection at 254 nm. The mobile phase consisting of Methanol and 0.1% ortho phosphoric acid in a ratio of (70:30, v/v) and at a flow rate of 1.0 mL/min. The method was linear over the concentration range for Lamivudine 15-75 µg/mL and for Raltigravir 30-150 µg/mL. The recoveries of active pharmaceutical ingredient (API)Lamivudine and Raltigravir were found to be in the range of 99.82-100.82% and 100.71-102.43% respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing Lamivudine and Raltigravir in combined tablet dosage form.

Keywords: Lamivudine, Raltigravir, HPLC, Validation.

INTRODUCTION

Lamivudine is used for the treatment of HIV infection and hepatitis-B. Lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellular to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination.Its chemical name is described as 4-amino-1-[(2R,5S) -2 -(hydroxymethyl) -1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one[1] (Fig.1). Raltigravir is a drug used in the treatment of HIV infection and tuberculosis disorders. Raltegravir target integrates 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. It 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 [2](Fig. 2). In the fixed dose combination of Lamivudine and Raltigravir both the drugs have two different mechanisms and Advantages of this combination therapy are it effectively achieves target HIV infections of individual drug's side-effects, produces synergistic effects, increased patient compliance.

The stability indicating method is defined as validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product.

Literature survey revealed few analytical

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methods are reported for the estimation of Lamivudine and Raltegravir in single and combination with other drugs [3-9]. However, there is no RP-HPLC method has been reported for the estimation of above selected combination. The aim of the present study was to develop a simple, precise, reliable, sensitive and selective stability indicating HPLC method with UV detection for the simultaneous analysis of Lamivudine and Raltigravir in combined dosage formulation.

EXPERIMENTAL

Chemicals and reagents

The pharmaceutical grade pure samples of Lamivudine (99.77%) and Raltigravir (99.69%) obtained from Pharmatrain Research Laboratories, Hyderabad and Orthophosphoric acid buffer, Methanol, hydrochloric acid and HPLC water were purchased from E-Merck Specialties Pvt. Ltd., Mumbai.

Apparatus and chromatographic condition

The chromatographic separation was performed on a WatersAlliance HPLC,integrated with Auto Sampler and UV detector.The Analytical Inertsil ODS C18 column (150 × 4.6 mm, 5µm). The mobile phase consisted of mixture of buffer 300 ml and 700ml of Methanol and degassed in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration. Flow rate was adjusted to 1.0ml/min.

Preparation of standard solution

Accurately weighed and transferred 15 and 30mg of Lamivudine and Raltigravir working standard into a 10mL clean dry volumetric flask, containing diluent and sonicate to dissolve it completely and make volume up to the mark with the same diluent. Further pipette 1.0ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.Further pipette 3.0ml of above solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of sample solution

Accurately weighed and transfer equivalent to 15 and 30mg of Lamivudine and Raltigravir tablet powder into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the solution. Then pipette 1.0 ml of filtrate into a 10ml volumetric flask and dilute up to the mark with diluent.Further pipette 3.0 ml of above solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

20 µL of standard and sample solution was injected into the chromatographic system. Peak areas were measured and % assay was calculated.

RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines. Obtained validation parameters are presented in Table 1.

Linearity

The linearity for HPLC method was determined at five concentration levels ranging from 15-75µg/mL for Lamivudine and 30-150 µg/mL for Raltigravir**.** The calibration curve was constructed by plotting response factor against respective concentration of Lamivudine and Raltigravir. The plots of peak area Vs respective concentration were found to be linear in the range of 15-75 µg/mL and 30-150 µg/mL with coefficient of correlation (r^2) 0.999 and 0.999 for Lamivudine and Raltigravir respectively. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for Lamivudine and Raltigravir were given in Fig. 3 and Fig. 4.

Recovery

Three different samples of known concentration ranging from 15-75 µg/mL for lamivudine and 30-150 µg/mL for raltigravir were prepared and these are analyzed against standard solution**.** The result of recovery analysis of Lamivudine and Raltigravir was found to be in the range of 99.82-100.82% and 100.71 - 102.43% respectively. The obtained results are presented in Table 2.

Sensitivity

The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.96µg/mL and 6.94µg/mL for Lamivudine and 1.84µg/mL 6.96µg/mL for Raltigravir. The LOD and LOQ showed that the method is sensitive.

System suitability test

The specificity of this method was determined by complete separation of Lamivudine and Raltigraviras shown in Fig. 5 with parameters like retention time, resolution and tailing factor. Here tailing factor for peaks of Lamivudine and Raltigravir was less than 2% and resolution was satisfactory. The average retention time forLamivudine and Raltigravir were found to be2.368 and 3.380 respectively, for five replicates. The peaks obtained for Lamivudine and Raltigravir were sharp and have clear baseline separation. Analysis was also performed for activeLamivudine and Raltigravir, placebo sample (All the ingredients except active Lamivudine and Raltigravir) both at stressed and unstressed condition. After analysis it was found that there is no interference of peaks in the

Lamivudine and Raltigravir active sample. Hence the developed method was specific for the analysis of this product**.**

Precision

The method precision study was performed for five sample preparations of marketed formulations. A study was carried out for intermediate precision with the same analyst on the different days for five sample preparations of marketed formulations. The Method precision and Intermediate precision results are presented in Table 3. The assay results of tablet dosage formulation by the proposed method are presented in Table 4.

Robustness

Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust.

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of Lamivudine and Raltigravir remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the specified period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the developed method. The results of the degradation studies are presented in Table 5.

Control sample

Accurately weighed and transferred 15 and 30mg of Lamivudine and Raltigravir working standard into a 10mL clean dry volumetric flask. Diluent was added and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1.0 ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Acid degradation sample

Pipette 3.0 ml of control sample into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. The solution was filtered through 0.22µ filter and then filtrate was injected into chromatographic system. The obtained chromatogram shown in Fig. 6.

Base degradation sample

Pipette 3.0 ml of control sample into a 10ml volumetric flask and add 3 ml of 0.1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. The solution was filtered through 0.22µ filter and then filtrate was injected into chromatographic system. The obtained chromatogram shown in Fig. 7.

Peroxide degradation sample

Pipette 3.0 ml of control sample into a 10ml volumetric flask, 1 ml of 3% w/v of hydrogen peroxide was added and the volume was made up to the mark with diluent . The volumetric flask was then kept at room temperature for 15 min. The solution was filtered through 0.22µ filter and then filtrate was injected into chromatographic system. The obtained chromatogram shown in Fig. 8.

Thermal degradation sample

3 ml of control sample was taken in Petridish and kept in Hot air oven at 110^0 C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.The chromatogram shown in fig. 9.

Table 1. Analytical validation parameters (System suitability and Linearity)

Parameter	Lamivudine	Raltigravir
Linearity	15-75 µg/mL	30-150 µg/mL
Slope	4639	24312
Intercept	38129	50932
RSQ(r^2)	0.999	0.999
LOD	1.96	1.84
LOQ	6.94	6.96
Theoretical Plates	2749	3765
Tailing Factor	1.64	1.47
Retention Time (min)	2.368	3.380

Table 2A. Accuracy data for Lamivudine

% Level	Amount Added(µg)	Amount recovery(µg)	% Recovery	Mean Recovery
50%	22.5	22.0	99.82%	99.15%
100%	45	45.71	100.06%	
150%	67.5	68.0	100.82%	

*Average of three determinations

Table 2B. Accuracy data for Raltigravir

%Level	Amount Added(µg)	Amount recovery(µg)	% Recovery	Mean Recovery
50%	45.0	45.0	100.71%	101.42%
100%	90.0	92.05	102.43%	
150%	135.0	135.32	100.71%	

*Average of three determinations

Table 3. Method precision and Intermediate precision of Lamivudine and Raltigravir

Drug	Concentration (µg/mL)	Method precision		Intermediate precision	
		SD	%RSD	SD	%RSD
Lamivudine	45	4503.36	1.57	2916.331	1.24
Raltigravir	90	5523.308	1.30	2514.806	0.70

Table 4. Assay result of tablet dosage formulation

	Label Claim (mg)	% Assay
Lamivudine	150	99.77
Raltigravir	300	99.69

Table 5. Forced degradation studies of Lamivudine and Raltigravir

Stress Conditions	Degradation Time	Peak Area		% Degradation	
		Lamivudine	Raltigravir	Lamivudine	Raltigravir
Control	-	220864	367882	-	-
Acid	6 hour	188706	337789	14.56	8.18
Base	6 hour	193521	303392	12.38	17.53
Peroxide	6 hour	185349	349046	16.08	5.12
Thermal	24 hours	201537	345226	8.76	6.16

Fig 1. Chemical structure of lamivudine

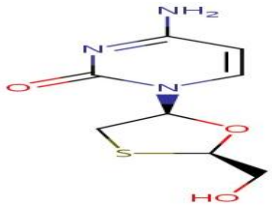


Fig 2. Chemical structure of raltigravir

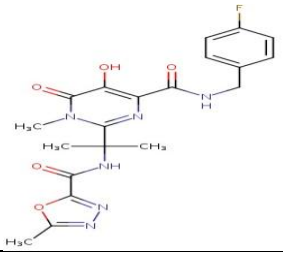


Fig 3. Calibration curve for Lamivudine

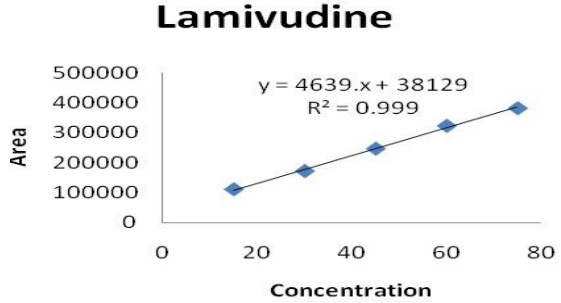


Fig 4. Calibration curve for Raltigravir

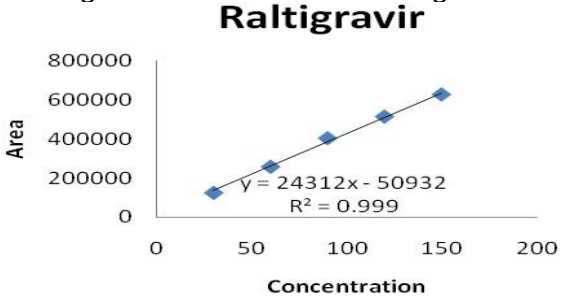


Fig 5. Typical chromatogram of Lamivudine and Raltigravir

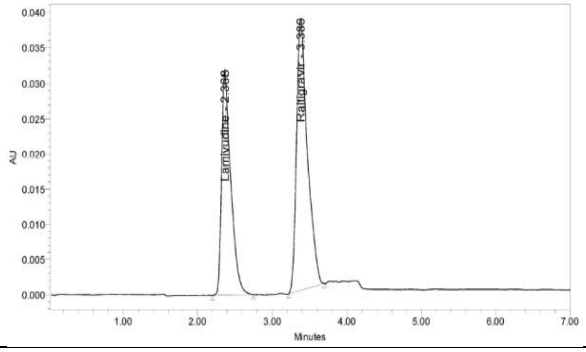


Fig 6. Acid degradation chromatogram of Lamivudine and Raltigravir

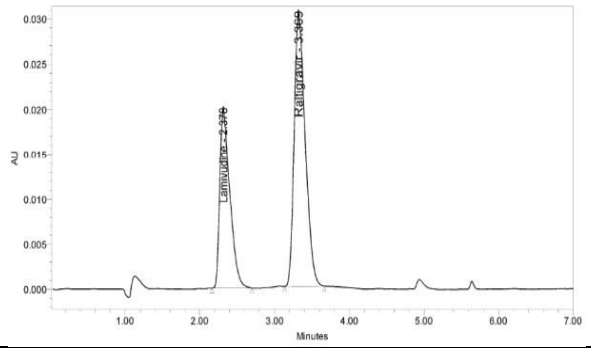


Fig 7. Base degradation chromatogram of Lamivudine and Raltigravir

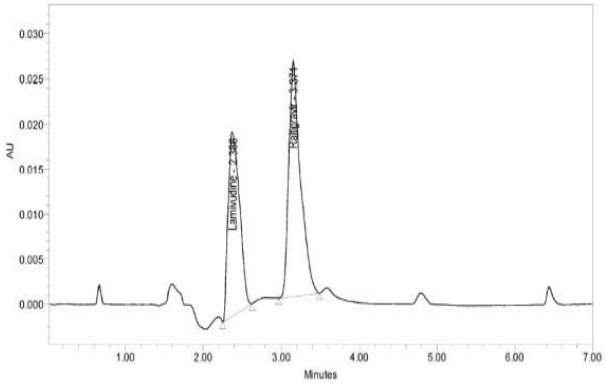


Fig 8. Peroxide degradation chromatogram of Lamivudine and Raltigravir

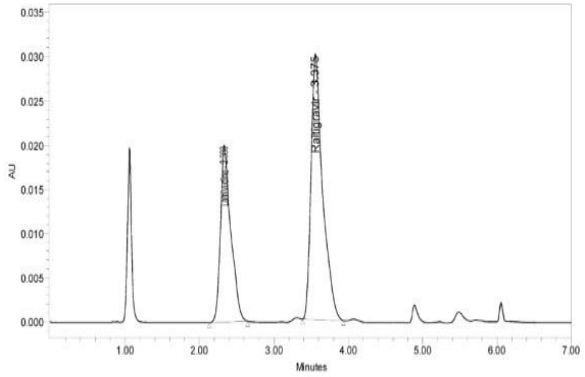
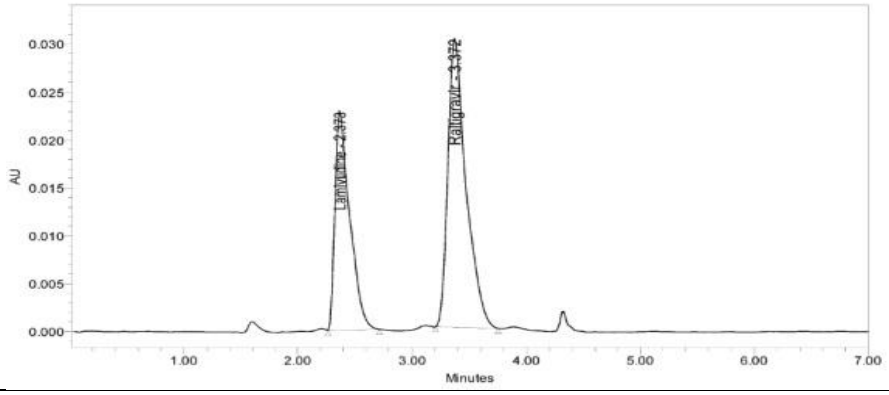


Fig 9. Thermal degradation chromatogram of Lamivudine and Raltigravir.



CONCLUSION

This study presents a simple and validated stability indicating HPLC method for simultaneous estimation of Lamivudine and Raltigravir in the presence of degradation products. The developed method is specific, accurate, precise and robust. The method was linear response in stated range and is accurate and precise. All the degradation products formed during forced decomposition studies were well separated from the analyte peaks demonstrating that the developed method was specific and stability indicating**.** The method could be applied with success even to the analysis of marketed products of lamivudine and raltigravir combined tablet formulation, as no interference was observed due to excipients or other components.

ACKNOWLEDGEMENT

Nil

CONFLICT OF INTEREST

No interest

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