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An Effective Stability Indicating RP-HPLC Method for Simultaneous Estimation of Lamivudine and Raltegravir in Bulk and Their Tablet Dosage Form

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: In the current study, asimple and specific stability indicating RP-HPLC method was developed and validated for the determination of Lamivudine and Raltegravir in bulk drug and it tablet dosage form using an UV-detector. Good separation was achieved by isocratic ally on a Zorbax SB-Phenyl (150 × 4.6 mm, 3.5 µ, 80 A°) column, using a mobile phase composition of buffer (0.1% v/v Phosporic acid in water): Acetonitrile (40:60 v/v) at a flow rate of 1.0 mL/min. The eluted analytes detected at 260 nm wavelength.
Results: Lamivudine and Raltegravir were eluted at 3.1 and 5.4 min respectively with run time 7 min. Linearity in the method was measured in the concentration range of 30 – 70 µg/mL and 60 – 140 µg/mL for Lamivudine and Raltegravirrespectively. The percentage recoveries of Lamivudine and Raltegravirwere determined to be 100.30% and 100.53%, respectively. The validation of the developed method is carried as per USFDA and ICH guidelines, and the degradants were well resolved from Raltegravir and Lamivudine peaks. The developed RP-HPLC method was highly precise, specific, sensitive, and stability indicating.

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Conclusion: The results of the analysis prove that thedeveloped RP-HPLC method is simple, economical and widely acceptable, which can be used in routine quality control tests in the industry.

Keywords: Lamivudine; raltegravir; isocratic elution; stability indicating.

1. INTRODUCTION

The antiviral therapy with combination of multi drugs is a great advancement in the treatment of human immune virus (HIV) and hepatitis B diseases. The use of multiple drug therapy, i.e., at least three or more drugs alone or in combination daily is in practice to treat the HIV effectively [1-2]. However, extensive research on multiple drug therapy revealed that a t-drug regimen consisting of Lamivudine and Raltegravir controls the HIV disease effectively.

Lamivudine chemically, is 4-amino-1-[(2R, 5S)-2-(hydroxyl methyl)-1,3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one [3-6]. It ceases DNA replication by inhibiting the reverse transcriptase enzyme competitively [7]. Chemically, Raltegravir is a (4R,12aS)-N-[(2,4-difluorophenyl)methyl]-3,4,6,8,12,12a-hexahydro-7-hydroxy-4-methyl-6,8-dioxo-2H-pyrido[1′,2′:4,5]pyrazino[2,1b][1,3]oxazine-9-carboxamide, integrase strand transfer inhibitor(INSTI) that blocks HIV replication by preventing the integration of viral DNA into the genetic material of host (human immune cells (T cells)) [8-11] (Fig 1).

A sensitive, shot run and effective analytical methodsare required for a drug to analyze individually or simultaneously in combination with other drugs in pharmaceutical industry.On the converse based on the review of literature very few analytical methods such as RP-HPLC methods and UV methods were published for the estimation of Lamivudine and Raltegravir individually [12-14]. Further, there are some RP-HPLC methods available for simultaneous estimation of Lamivudine, Raltegravir, and tinofivirdisproxil fumarate or butcaver sulfate or abacavir in triple combination [15-19]. As per FDA official news, Lamivudine and Raltegravir fixed dose film-coated tablet got an approval in April 2019 for the treatment of HIV-1. To our knowledge, there is no short run time RP - HPLC method reported for simultaneous estimation of Lamivudine and Raltegravir in bulk drug and pharmaceutical formulations. Thus, efforts were made to develop selective, sensitive and fast analytical method for the estimation of Lamivudine and Raltegravir in their combined dosage form using reverse phase high performance liquid chromatographic method.

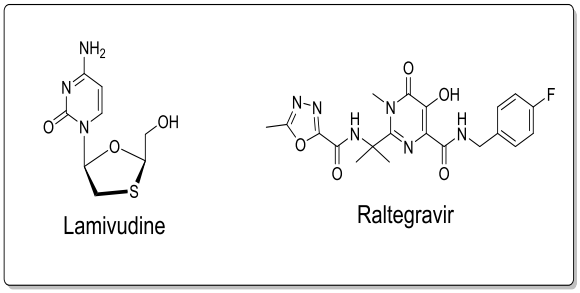

Fig. 1. Chemical structures of Lamivudine and Raltegravir

2. MATERIALS AND METHODS

2.1 Materials

HPLC grade acetonitrile, Milli-Q water, Phosphoric acid and remaining analytical grade chemicals were obtained from Merck India Limited, Mumbai, India. API of Lamivudine and Raltegravir was provided by Fortune Pharma, Hyderabad, as a gift sample.

2.2 Chromatographic Conditions

RP-HPLC experiments were performed on WATERS 2695 with 2487 PDA detector with auto sampler, data-processing, and acquisition has done by using the Empower 2 software. Effective separation attained by injecting 10 µL of the standard solution into Zorbax SB-Phenyl (150 × 4.6 mm, 3.5 µ, 80 A°) column, using a mobile phase composed of buffer (0.1% v/v Phosporic acid in water): Acetonitrile (40:60) at a flow rate of 1.0 mL/min, and the eluted analytes were detected at 260 nm wavelength. Ambient temperature 30°C was maintained in auto sampler and in the analytical column. Standard, sample and mobile phase solutions were filtered through a 0.45-µm nylon filter prior to injecting into the HPLC system (Fig. 2).

2.3 Preparation of Standard Solution

Weigh accurately working standard equivalent to 50 mg of Lamivudine and 100 mg of Raltegravir transferred into 100 mL volumetric flask, add ~30 ml of diluent (acetonitrile and water (50:50)) and dissolve, further make up the volume with diluent. 1.0 ml of above solution was transferred into 10 mL volumetric flask, again volume made with diluents to obtain concentration of 50 µg/mL, and 100µg/mL for Lamivudine and Raltegravir, respectively, said as 100% level concentrations. Prior to injecting, standard solution was filtered through 0.4 µm Nylon filter (Fig. 3).

2.4 Preparation of Sample Solution

An amount of tablet (Dutrebis) powder (150 mg) equivalent to 50 mg of Lamivudine and 100 mg of Raltegravir was weighed and transferred into 100 mL volumetric flask, volume made with diluents to 100 mL. One milliliter of above solution was transferred into 10 mL volumetric flask, volume made with diluents to obtain concentration of 50 µg/mL, and 100µg/mL for Lamivudine and Raltegravir respectively. Prior to injecting, sample solution was filtered through 0.4 µm Nylon filter (Fig. 4).

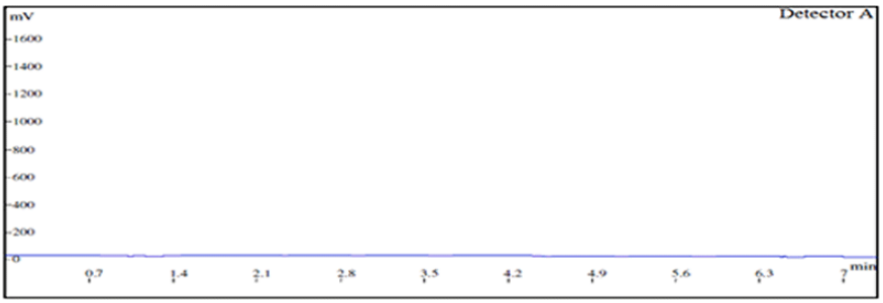

Fig. 2. Blank chromatogram

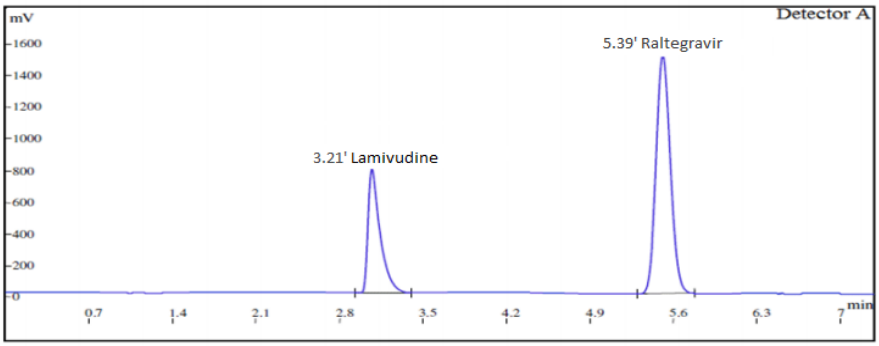

Fig. 3. Chromatogram of lamivudine and raltegravir standards

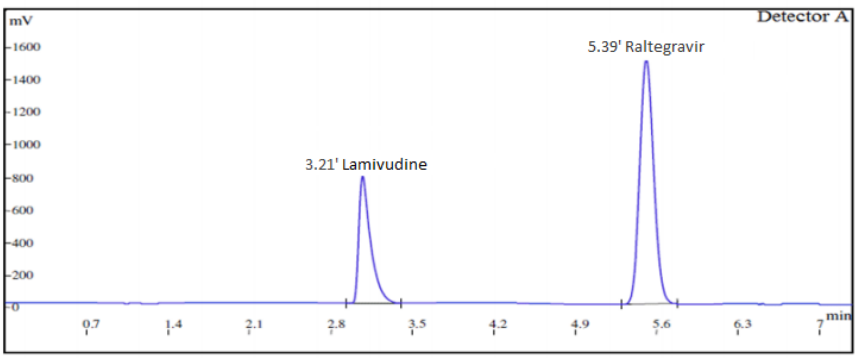

Fig. 4. Chromatogram of Lamivudine and Raltegravir samples

3. METHOD VALIDATION

3.1 System Suitability Test

The system suitability test of the current method was carried out by injecting 100% level of working standard concentration in 6 replicates, and parameters like percentage relative standard deviation (% RSD), USP tailing factors (T), USP plate count (N), and resolution (R) were evaluated for the obtained chromatograms.

3.2 Linearity

The Linearity is the ability of the method to elicit test results that are proportional to concentration of the analyte in the sample. The linearity of the present method has performed by injecting the series of working standard concentrations ranges from 30 µg/ml to 70 µg/ml of Lamivudine and 60 µg/mL to 140 µg/mL of Raltegravir into the HPLC system under optimized chromatographic conditions. Eventually, linearity graph was plotted for concentration vs peak area and determined regression coefficient (r2) value.

3.3 Precision

It is the closeness of test results obtained by the method to the true value. Usually, it was determined in the intraday and inter-day. Intraday and inter-day precision of the method were performed by injecting 100% level of working standard concentration for 6 times in a day and 3 times per day for three continuous days. Percentage RSD calculated for peak areas obtained.

3.4 Accuracy

The accuracy of the method was established by recovery of known amount sample solution spiked at three different standard concentration levels about 50, 100, and 150%, each level of solution injected in triplicate. The percentage mean recovery at three different levels of the drug solution was calculated.

3.5 Specificity

Specificity represents the ability of the method to determine or assess the intended drug in the presence of other substances without interferences. Ten microliter volume of prepared blank solution, 100% level pure working standard solution injected individually. The retention time (RT) of individual injection of standard sample solution alone and along with placebo was observed to assess any interference that has been happened with peaks of Lamivudine and Raltegravir in obtained chromatograms.

3.6 Sensitivity

The LOD and LOQ were calculated by implementation of standard deviation method, in which the following formulae were used.

LOD ¼ 3 σ=S
LOQ ¼ 10 σ=S

Where σ is the standard deviation of the intercept, and S is the slope of the linear curve.

3.7 Robustness

The robustness of the method was checked by slightly and deliberately changing the flow rate, mobile phase composition, and maximum absorption wavelength. It can be performed by evaluating the system suitability parameters after changing the HPLC flow rate (± 0.1 mL/ min) and mobile phase ratio (± 1 mL).

4. FORCED DEGRADATION STUDIES

In forced degradation studies, intentionally drug substance is exposed to conditions more intense than accelerated conditions. Chemical stability of the drug molecule can be depicted with forced degradation studies, which helps in successful development of stable formulation with appropriate storage conditions. ICH guidelines emphasized certain degradation conditions like acid hydrolysis, base hydrolysis, oxidation, thermal degradation, and photo stability in ICH Q1A, QIB, and Q2B guidelines.

4.1 Acidic Degradation Solution

0.2 mL of 1 N HCl is added to 1.0 mL of stock solution and was reflux for 3 hours at 70 °C in water bath, cool the solution for 24 h at room temperature. Later the solution was neutralizing with 1 N NaOH and done the further dilution to get a solution having 50 µg/mL of Lamivudine and 100 µg/mL of Raltegravir (Fig. 5).

4.2 Alkali Degradation Solution

0.2 mL of 1 N NaOHis added to 1.0 mL of stock solution and was reflux for 3 hours at 70°C in

water bath, cool the solution for 24 h at room temperature. Later the solution was neutralizing with 1 N HCl and done the further dilution to get a solution having 50 µg/mL of Lamivudine and 100 µg/mL of Raltegravir (Fig. 6).

4.3 Oxidative Degradation Solution

1.0 mL of 3% Hydrogen peroxide and 1.0 mL of stock solution taken into 100 ml of Volumetric flask and was reflux for 3 hours at 70 °C in water bath, cool the solution for 24 h at room temperature. Later, make up to10 mL with diluent to obtain concentration of 50 µg/mL and 100 µg/mL for Lamivudine and Raltegravir respectively (Fig. 7) [20].

4.4 Thermal Degradation Solution

100 mL of standard stock solution taken into 500 mL of Volumetric Flask and kept it in heating chamber for 24 h at 80°C/75% RH. Further above solution is diluted to 10 mL to obtain concentration of 50 µg/mL and 100 µg/mL for Lamivudine and Raltegravir respectively (Fig. 8).

4.5 Photolytic Degradation

1 mL of sample stock solution filtrate was transferred to 100 mL of Volumetric Flask; it was kept for 12 hours in UV-light. Then volume was made up to mark with Diluent and mixed well and injected. The representative chromatogram is shown in Fig. 9.

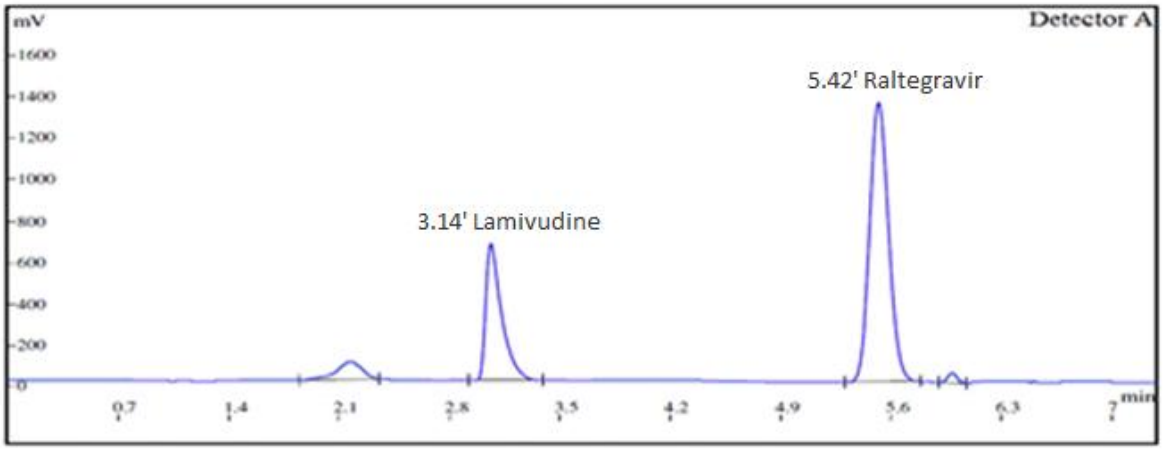

Fig. 5. Acidic stress degradation chromatogram of Lamivudine and Raltegravir

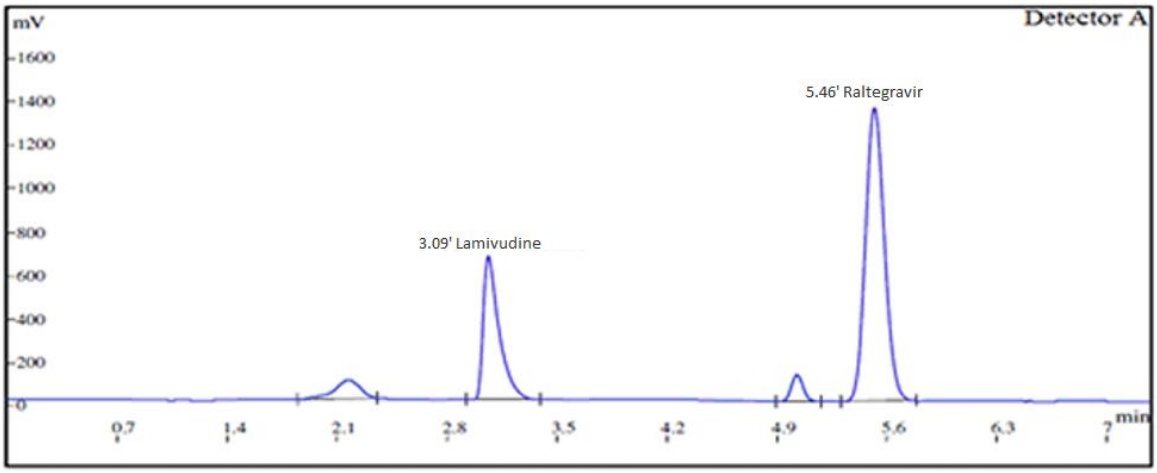

Fig. 6. Alkaline stress degradation chromatogram of Lamivudine and Raltegravir

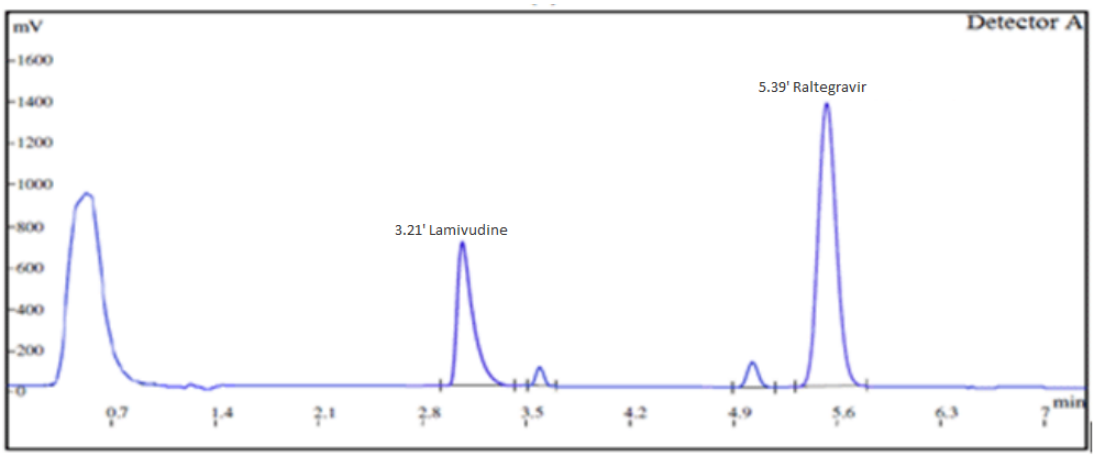

Fig. 7. Oxidative stress degradation chromatogram of Lamivudine and Raltegravir

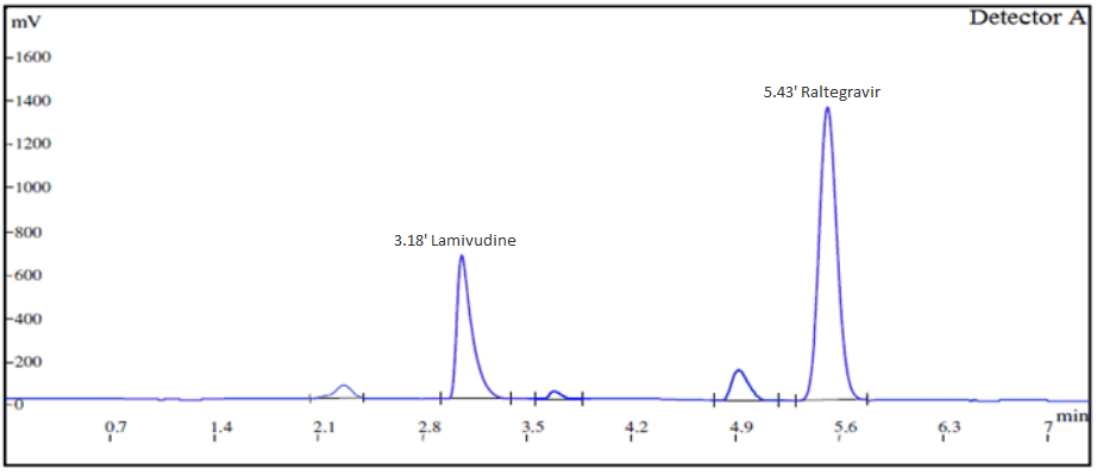

Fig. 8. Thermal stress degradation chromatogram of Lamivudine and Raltegravir

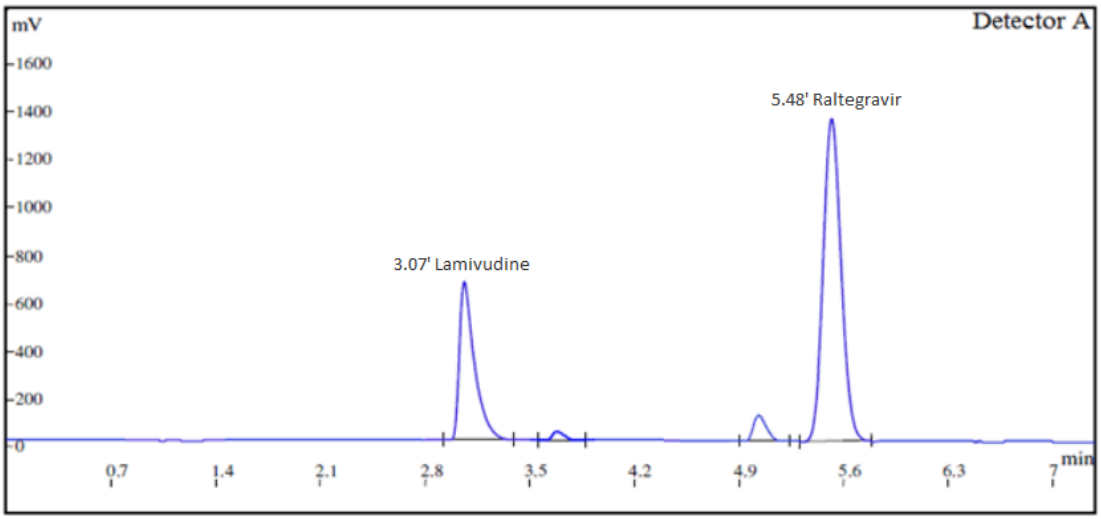

Fig. 9. Photolytic stress degradation chromatogram of Lamivudine and Raltegravir

4.6 Assay

Assay of the Lamivudine and Raltegravircan be determined by injecting standard and sample solutions at the concentration about 50 µg/mL and 100 µg/ mL respectively. The preparation of sample and standard solutions was mentioned prior the in methods section.

4.7 Results

As per solubility studies, we found that Lamivudine was freely soluble in water, acetonitrile and partially soluble in methanol. Raltegravir was freely soluble in methanol and water. Based on the solubility data, water and acetonitrile in (50: 50) ratio selected as diluent to prepare sample and standard solutions.

5. METHOD OPTIMIZATION

Method optimization was successfully completed by applying trial and error method in such a way to obtain a chromatogram with good accepted number of USP plates,efficiency,resolution (R), and tailing factor. In this way, numerous trials have been done by altering flow rate, mobile phase composition and columns.Eventually, the methodwith Zorbax SB-Phenyl (150 × 4.6 mm, 3.5 µ, 80 A°) column, mobile phase composition of buffer (0.1% v/v Phosporic acid in water) :Acetonitrile (40:60) and a flow rate of 1.0 mL/min was selected as optimized method. The results obtained in the trial and error method were mentioned in Table 1; trial 7 selected as optimized conditions and optimized chromatogram shown in Fig. 10 [20].

Table 1. Different trials

Trail	Column	Buffer	Mobile Phase	Flow rate ml/min	Observation
01	X-terra MSC18 (150 X 4.6 mm, 5µm)	0.05% Phosphoric acid in water	Buffer: ACN (50:50)	1.0	Peaks tailing were observed.
02	X-terra MSC18 (150 X 4.6 mm, 5µm)	0.05% Phosphoric acid in water	Buffer: ACN (30:70)	1.0	Peaks resolution and symmetry were not good
03	X-Bridge C18 (150 X 4.6 mm, 3µm)	0.05% TFA in water	Buffer: ACN (50:50)	1.0	Asymmetrical peak shapes were observed
04	Luna C18 (150 X 4.6 mm, 5µm)	0.05% TFA in water	Buffer: ACN (50:50)	1.0	Resolution not sufficient
05	Zorbax SB-Phenyl (150 × 4.6 mm, 3.5 µ, 80 A°)	0.05% TFA in water	Buffer: ACN (50:50)	1.0	Poor resolution with good peak shape
06	Zorbax SB-Phenyl (150 × 4.6 mm, 3.5 µ, 80 A°)	0.05% Phosphoric acid in water	Buffer: ACN (50:50)	1.0	Good peak shape with minimum resolution
07	Zorbax SB-Phenyl (150 × 4.6 mm, 3.5 µ, 80 A°)	0.05% Phosphoric acid in water	Buffer: ACN (40:60)	1.0	Optimum resolution with good peak shape.

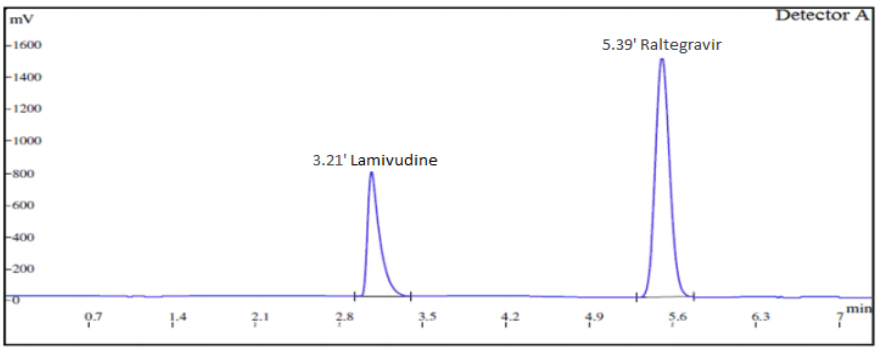

Fig. 10. Optimized chromatogram of the method

6. METHOD VALIDATION RESULTS

6.1 System Suitability

100% level concentration of both drugs was prepared and injected into the HPLC system. The USP tailing factor (≤ 2), % RSD (≤ 2) and USP plate count (> 2000) values shown in Table 2 were satisfying the acceptance criteria as per Q2 specifications of ICH guidelines.

6.2 Linearity

The linearity was performed for the prepared standard solutions of lamivudine and raltegravirat various concentration range of 30 to 70 µg/mL and 60 to 140 µg/mL respectively (Fig. 11). It was determined by constructing calibration curve between peak area and concentration (Table 3, Figs. 12 & 13). The computed regression coefficient (R2) value found to be 0.999 and 0.999 for Lamivudine and Raltegravir, respectively, and manifests the linearity of the method within the ICH guidelines limit [20].

6.3 Accuracy

Percentage mean recovery of the Lamivudine and Raltegravir at three different concentration levels that were observed as 100% ± 2 illustrates the acceptance of the method as per Q2 specifications of ICH guidelines. Results were shown in Table 4.

Table 2. Results of system suitability parameters of 100% level standard solution

Injection	Lamivudine				Raltegravir			
	RT	Peak area	USP plate count (N)	USP tailing (T)	RT	Peak area	USP plate count (N)	USP tailing (T)
1	3.21	17,00,250	8300	0.94	5.47	33,50,465	6778	1.10
2	3.11	17,12,321	8340	0.92	5.46	33,59,786	6712	1.11
3	3.12	17,09,951	8430	0.94	5.46	33,58,697	6698	1.08
4	3.11	17,16,132	8641	0.94	5.46	33,52,365	6746	1.07
5	3.11	17,11,862	8531	0.93	5.47	33,49,325	6812	1.09
6	3.13	17,01,021	8475	0.95	5.46	33,55,897	6653	1.10
Mean	3.13	17,08,590	8452.8	0.93	5.46	33,54,423	6733.16	1.09
SD	0.0392	6483.915	125.39	0.0103	0.0051	4361.015	57.44	0.0147
%RSD	1.2517	0.3795	1.4834	1.1026	0.094521	0.1300	0.8531	1.3483

SD standard deviation, %RSD relative standard deviation, RT retention time, Acceptance limit % RSD (≤ 2), USP tailing factor (≤ 2), and USP plate count (> 2000)

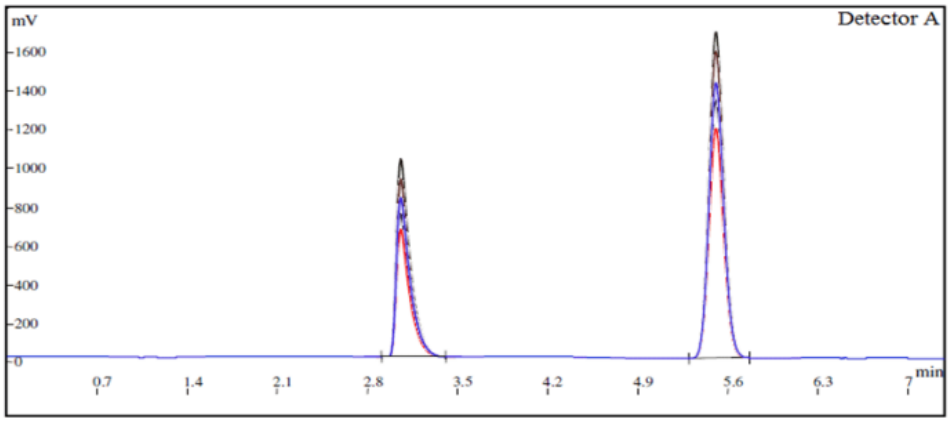

Fig. 11. Overlain Chromatogram of Lamivudine and Raltegravir (Linearity)

Table 3. Peak areas of linearity standard solutions of Lamivudine and Raltegravir

Lamivudine		Raltegravir	
Concentration (µg/mL)	**Peak area**	**Concentration (µg/mL)**	**Peak area**
30	1028543	60	1998432
40	1340002	80	2642371
50	1700452	100	3350698
60	2049861	120	3946321
70	2374647	140	4673684
R^2	0.999	R^2	0.999

R^2 regression coefficient

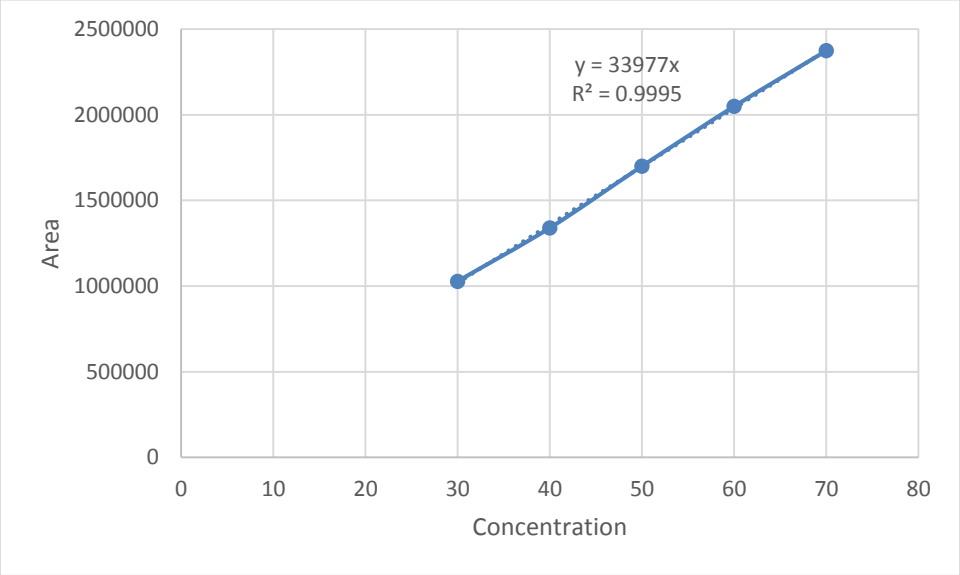

Fig. 12. Linearity curve of Lamivudine

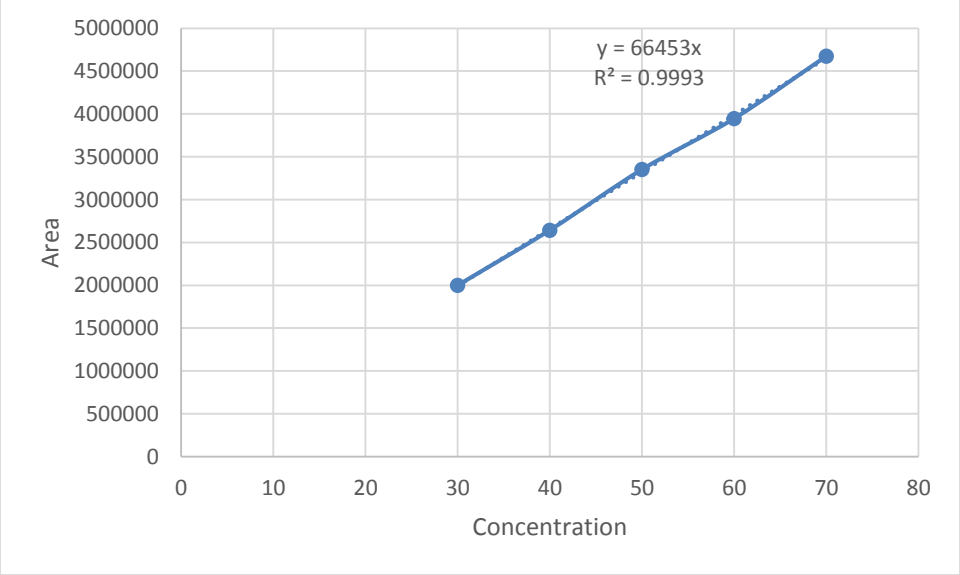

Fig. 13. Linearity curve of Raltegravir

Table 4. Results of percentage of recovery

%Level	Amount of Added	Amount of Recovered	% mean recovery
Lamivudine			
50	25	24.98	99.92
	25	25.11	100.44
	25	25.14	100.56
100	50	50.61	101.22
	50	49.89	99.78
	50	49.91	99.82
150	75	75.43	100.57
	75	75.01	100.01
	75	75.23	100.31
Raltegravir			
50	50	50.21	100.42
	50	50.43	100.86
	50	50.37	100.74
100	100	100.41	100.41
	100	100.32	100.32
	100	100.64	100.64
150	150	150.81	100.54
	150	150.94	100.63
	150	150.37	100.25

At each percentage level mean percentage recovery in the acceptable limit of 98 to 102%

6.4 Precision

We found to be 0.45 and 0.77 percentage RSD values by injecting 100% level working standard solution of Lamivudine and Raltegravir respectively (Table 5), and depicts the precision of the method.

6.5 Sensitivity

The LOQ and LODfound as 11µg/mL and3.65µg/mL for Lamivudine and 5µg/mL and 1.6µg/mL for Raltegravir, respectively, which indicates that method has good sensitivity.

6.6 Robustness

The robustness was unaffected when small, deliberate changes like in mobile phase ratio, flow rate, and absorption maximum and all values obtained in acceptable limits as per ICH guidelines and results are summarized in Table 6.

7. FORCED DEGRADATION RESULTS AND DISCUSSION

Forced degradation conditions such as acidic, basic, oxidative, reduction, thermal, hydrolysis, and photolytic stresses were attempted as per the International Conference of Harmonization (ICH) guidelines Q1A (R2). There is an effect of assay results. The results are shown in Tables 7 & 8.

7.1 Percentage Assay

Lamivudine and Raltegravir assay percentage in tablets were found as 100% ± 15. Hence it indicates that the analyzed tablets have purity within the acceptance specification as per ICH guidelines. Results were shown in Table 9.

7.2 Discussion

Few methods were reported for the estimation of Lamivudine and Raltegravir in different combination [12–14]. In the present method, raltigravir was eluted at 5.46 min and Lamivudine was eluted at 3.13 min with a run time of 7 min. The developed procedure using reverse phase HPLC with UV-detector and the bestmobile phase composition was found to bebuffer (0.1% v/v Phosporic acid in water): Acetonitrile (40:60 v/v). The flow rate of the solvent system was kept at 1.0 mL/ min. TheLinearity was obtained in over the concentration range of30 – 70 µg/mL and 60 – 140 µg/mL for Lamivudine and Raltegravir respectively (R2=0.999) with a LOD and LOQ of 0.05 µg/mL and 0.1 µg/mL, respectively.

Table 5. Results of intraday and inter-day precision of 100% level solution

Precision		Lamivudine		Raltegravir	
Intra day	**Sample Name**	**RT**	**Peak Area**	**RT**	**Peak Area**
	Injection 1	3.14	1708060	5.41	3307641
	Injection 2	3.18	1716375	5.49	3343891
	Injection 3	3.10	1700431	5.46	3289458
	Injection 4	3.15	1710321	5.47	3293785
	Injection 5	3.19	1723211	5.42	3304987
	Injection 6	3.20	1708314	5.49	3349872
	Mean	3.16	1711119	5.456667	3314939
	SD	0.037417	7822.246	0.034448	25720.07
	%RSD	1.184069	0.457142	0.631302	0.775884
Inter-day	**Sample Name**	**RT**	**Peak Area**	**RT**	**Peak Area**
	Injection 1	3.13	1708945	5.44	3299451
Day 1	Injection 2	3.16	1710264	5.43	3309762
	Injection 3	3.15	1707968	5.43	3308758
	Injection 1	3.12	1706843	5.46	3317628
Day 2	Injection 2	3.12	1714697	5.44	3271847
	Injection 3	3.14	1713548	5.49	3263543
	Injection 1	3.13	1716954	5.43	3289785
Day 3	Injection 2	3.15	1709658	5.45	3258746
	Injection 3	3.11	1706427	5.47	3289644
	Mean	3.134444	1710589	5.448889	3289907
	SD	0.016667	3674.985	0.020883	21217.44
	%RSD	0.531726	0.214837	0.383257	0.644925

SD standard deviation, %RSD relative standard deviation, RT retention time.

Table 6. Results of robustness of 100% level solution

Variation of parameter		Lamivudine					Raltegravir				
		RT	Peak area	USP plate count	USP tailing factor	% assay	RT	Peak area	USP plate count	USP tailing factor	% assay
Mobile phase ratio	39:61	3.11	1710258	8643	0.91	100.02	5.42	3341672	6687	1.09	99.98
	40:60	3.14	1711483	8580	0.93	100.10	5.39	3362839	6630	1.11	100.04
	41:59	3.17	1712346	8612	0.97	100.41	5.47	3312484	6641	1.17	100.13
Flow rate (±0.1 ml)	0.9	3.37	1714872	8742	1.01	99.87	5.64	3374621	6740	1.24	100.43
	1.0	3.14	1719682	8690	0.96	100.42	5.41	3369481	6638	1.10	100.17
	1.1	3.93	1719989	8583	0.97	100.17	5.20	3358532	6618	1.06	100.32

RT retention time; slight change in method parameter could not affect the USP plate count and tailing factor.

Table 7. Retention time of degradant product of Lamivudine and Raltegravir (Stress Degradation Study)

Conditions	Retention time (Rt) (minute)				
	Lamivudine	Raltegravir	Degradant 1	Degradant 2	Degradant 3
Acid Degradation	3.14	5.42	2.14	6.02	-
Base Degradation	3.09	5.46	2.14	5.09	-
Oxidation Degradation	3.21	5.39	3.56	5.03	-
Thermal Degradation	3.18	5.43	2.30	3.59	4.98
Photolytic Degradation	3.07	5.48	3.60	5.11	-

Table 8. Results of forced degradation studies

Stress Type	Stress Conditions	Lamivudine		Raltegravir	
		% Assay	% Degradation	% Assay	% Degradation
Control Sample	Sample itself	98.9	NA	100.5	NA
Acid Degradation	1 N HCl, 0.2 mL at 70°C for 3 hr	87.2	11.5	85.9	14.5
Base Degradation	1N NaOH 0.2 mL at 70°C for 3 hr	80.9	17.6	87.2	13.2
Oxidation Degradation	1mL 3% H_2O_2 at 70°C for 2 hr	88.3	10.2	88.1	13.2
Thermal Degradation	At 80°C for 24 hr	87.2	11.3	86.9	13.5
Photolytic Degradation	At UV light for 12 hr	86.8	11.7	87.1	13.3

Table 9. Results of % assay of the tablet dosage form

Drug	Peak Name	RT	Peak area	USP tailing	USP plate count	Label claim (mg)	% Assay
Lamivudine	Standard	3.109	1708060	0.98	8340	150	99.5
	Test	3.113	1691240	0.99	8695		
Raltegravir	Standard	5.418	3307641	1.01	6712	300	99.64
	Test	5.413	3301975	1.01	6953		

Average weight of the tablet 500 mg, % purity of Lamivudine standard (API) 99.5, and % purity of Raltegravirstandard (API) 99.6.

Validation performed according to the ICH guidelines where the results are fast, accurate, robust, specific and linear [21]. The stability-indicating assay was done in the same manner as many reported methods [22,23]. The degradation studies results are summarized Table 8.

8. CONCLUSION

A specific, precise, accurate, stability-indicating, isocratic RP-HPLC method was developed for the estimation of Lamivudine and Raltegravir in bulk and tablet dosage form. The compound was evaluated by forced degradation pertaining to

several stress conditions. The developed methodseparates both of the drugs and its degradation products successfully and quantifies the active contents at minute concentration levels. The developed methodfully validated according to ICH guidelines for all the parameters which were found within acceptance criteria. Hence, the proposed method can be adapted to regular analysis in pharmaceutical industry.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Corado KC, Caplan MR, Daar ES, Two-drug regimens for treatment of naive HIV-1 infection and as maintenance therapy. Drug Des Devel Ther. 2018;12:3731–3740.
   DOI: 10.2147/DDDT.S140767
2. Commissioner of FDA (2019) The FDA. Available:http://www.fda.gov.
   Accessed 7 Aug 2019.
3. Ananda KK, Anusha K, Kokilavani V, Sangeetha VP, Saranya R, Jambulingam M, Ramesh J. Method development and validation of uv-spectroscopic method for the estimation of lamivudine as an active pharmaceutical ingredient and in tablet dosage form. Int J Pharm Health Care Res. 2017;5:129–137.
4. Sibel NO, Bengi U, Ramesh R. rapid hplc assay for lamivudine in pharmaceuticals and human serum. J. Liq. Chrom. & Rel. Technol. 2002;25(9):1447–1456.
   Available:https://doi.org/10.1081/JLC-120004759.
5. Kalpesh V, Prabodh S. Anil J, Tushar D, Swapnil P, Pallavi M. Development and validation of UV- spectroscopic method for estimation of lamivudine in tablet dosage form. Int J Curr Pharm Res. 2017;9:86-89.
6. Mandloi DK, Tyagi PK, Rai VK, Dey S, Ashada RK, Mohanraj P. Method development and validation of RP-HPLC in the application of In-vitro dissolution study of lamivudine in bulk drug and tablet formulation. J Chem Pharm Res. 2009; 1(1):286-296.
7. Palumbo E. Lamivudine for chronic hepatitis B: a brief review. Braz J Infect Dis. 2008;12(5):355–357.
   DOI: 10.1590/s1413-86702008000500002
8. Lakshmana Rao A, Raghu Ram MS. Validated RP-HPLC method for determination of raltegravir in pharmaceutical preparations. Int J Res Pharm Chem. 2012;2(1):217-221.
9. Mary CL, Chantelle BH, Edward PA. a sensitive HPLC-MS method for the determination of raltegravir in human plasma. J. Chrom. B. 2008;867:165–171.
   DOI: 10.1016/j.jchromb.2008.03.022
10. Sudha T, Raghupathi T. RP-HPLC and ultraviolet spectrophotometric method for the estimation of raltegravir potassium in bulk and in tablet dosage form. Global. J. Med .Res. 2011;11:8-16.
11. Rambabu K, Balamurali K, Sambasiva, R, New rp-hplc method development and validation for analysis of antiviral drug raltegravir. Int. J. Res. Pharm. Biomed Sci 2011;2:132-135.
12. Veena DS, SanjayJD,Optimization of an RP-HPLC method for simultaneous estimation of lamivudine and raltegravir in the binary mixture by using design of the experiment. Eurasian J Anal Chem, 2017; 12:179-195.
    DOI: 10.12973/ejac.2017.00162a
13. Honeesha N, Gowri Sankar D. Simultaneous RPHPLC method development and validation for lamivudine and raltegravir in bulk dosage forms. Am J Pharm Tech Res. 2015;5:249-256.
14. Juluri KDT, Govindarajan R. RP-HPLC method development and validation for simultanious estimation and forced

degradation studies of lamivudine and raltegravir in solid dosage form. Int J App Pharm. 2018;10:242-248. DOI:https://doi.org/10.22159/ijap.2018v10i6.28027

15. Nachiket D, Prachi PK, Ganesh SS. a review on development and validation of rp-hplc method for simultaneous estimation of lamivudine, tenofovir disproxil and dolutegravir in bulk and combined dosage. World Journal of Pharmaceutical Research. 2020;9:847-854.
16. Mastanamma S, Jyothi JA, Saidulu P, Varalakshmi M Development and validation of RP-HPLC method for the simultaneous estimation of Lamivudine, Tenofovir Alafenamide and Dolutegravir bulk and their combined dosage form. Pharm Methods. 2018;9:49–55.
17. Kalpana T, Rajeswari DTR, Ganji RR Development and validation of analytical method for determination of Dolutegravir sodium, Lamivudine and tenofovir disoproxil fumarate using reverse phase high performance liquid chromatography. Pharma Chem. 2017;9:117–127.
18. Ashok G, Mondal DS. Development and validation of stability indicating method for the simultaneous estimation of batcaver sulfate, Lamivudine and Dolutegravir sodium in pharmaceutical dosage forms by RP-HPLC. Saudi J Med Pharm Sci. 2018; 4:289–296.
19. Pal N, Avanapu SR, Ravikumar P (2016) Simultaneous HPLC method development and validation for estimation of Lamivudine, abacavir and Dolutegravir in combined dosage form with their stability studies. Asian J Chem. 2016;28:273–276.
20. Godela RGS. An effective stability indicating RP-HPLC method for simultaneous estimation of Dolutegravir and Lamivudine in bulk and their tablet dosage form. Futur J Pharm Sci. 2020;6:9. Available:https://doi.org/10.1186/s43094-020-00026-0
21. ICH. ICH topic Q2(R1) validation of analytical procedure: text and methodology; 2005.
22. Adel M, Mamdouh R, Hayam M, Mostafa A. Comparative study of multi-variate and uni-variate determination of zolmitriptan in the presence of its degradation products. Anal Chem Lett. 2015;5(2):73–84.
23. Bagal D, Nagar A, Joshi A. Development and validation of stabilityindicating RP-HPLC method for estimation of Dalfampridine in bulk drug and tablet dosage form. Future J Pharm Sci. 2021; 7:87.

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