International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Development and Validation of New RP-UPLC Method For Simultaneous Determination of Lamivudine and Dolutegravir in Combined Dosage Form

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A B S T R A C T

A reverse phase ultra performance liquid chromatographic method (RP-UPLC) was developed for the determination of Lamivudine and Dolutegravir in combined and tablet dosage forms. Separation was carried out by using Waters- UPLC system equipped with auto sampler, PDA detector, Inspire C18 (2.1 x 50 mm, 1.8 µm) column, used phosphate buffer (P^H-6.0) - acetonitrile in gradient mode at a flow rate of 0.25 ml/min used as mobile phase and detection was carried out at 257 nm at ambient temperature. The active pharmaceutical ingredient was extracted from tablet dosage form using a mixture of acetonitrile and water (50:50) as diluent. The calibration graphs were linear and the method showed excellent recoveries in tablet dosage form. The developed UPLC method was validated and meets the requirements delineated by the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision and robustness. The intra-day and inter-day variation was found be less than 1%. The method was reproducible and selective for the estimation of lamivudine and dolutegravir.

Keywords: UPLC, Lamivudine, Dolutegravir.

1. Introduction

Lamivudine[1] is a reverse transcriptase inhibitor and also analogue of zalcitabine, in which a sulfur atom replaces the 3' carbon of the pentose ring. Nomenclature:-4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one Molecular formula is $C_8H_{11}N_3O_3S$. Molecular weight is 229.2. It is an amorphous white, solid, freely soluble in water and boiling alcohol[2]. Its structural formula is shown in figure 1.

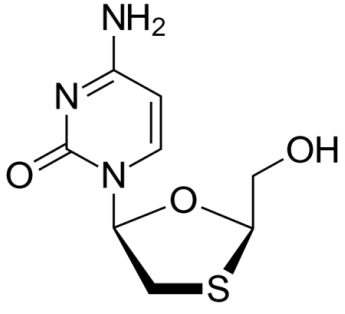

Figure 1: Chemical Structure of lamivudine

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Dolutegravir[3] is an orally bioavailable integrase strand-transfer inhibitor (INSTI), with activity against human immunodeficiency virus type 1 (HIV-1) infection. Chemical name of drug is (3S,7R)-N-[(2,4-difluorophenyl)methyl]-11-hydroxy-7-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclo[8.4.0.0^{3,8}]tetradeca-10,13-diene-13-carboxamide and having molecular formula $C_{20}H_{19}F_2N_3O_5$, molecular weight 419.385 g.mol-1. It prevents integrase[4] from binding to retroviral deoxyribonucleic acid (DNA), and blocks the strand transfer step, which is essential for the HIV replication cycle. The main metabolite found in blood plasma is the ether glucuronide and therefore, it is inactive. Its structural formula is shown in figure 2.

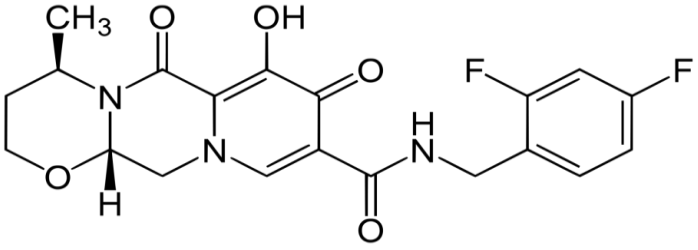

Figure 2: Chemical Structure of Dolutegravir

The literature survey revealed that there are a few UPLC and spectroscopic methods available for the determination of lamivudine and dolutegravir in combination with other drugs. In view of non-availability of UPLC methods for the selected combination, as per literature, author has aimed to develop a new UPLC method for simultaneous estimation of lamivudine and dolutegravir in combined pharmaceutical dosage form [5-7].

2. Experimental

Chemicals and reagents: Lamivudine and dolutegravir pure drugs were made available from NamanidhiPharma, Pune, Maharashtra, India. Ortho phosphoric acid, methanol and Acetonitrile were obtained from Merck. All chemicals and reagent used were of HPLC grade, and Milli-Q-water was used throughout the experiment.

Equipments: The Waters UPLC system with a PDA detector was used for method development and validation. The output signal was monitored and processed by using Empower software.

Chromatographic condition: The mobile phase used was phosphate buffer (pH 6.0) and acetonitrile in the gradient mode employing flow rate at 0.25 ml/min. The analytical column Inspire C18(2.1 x 50 mm, 1.8 µm). The detection was carried out at a wavelength of 257 nm with a run time of 3min. Was used Water and Acetonitrile in the ratio of 50:50 v/v used as diluent.

Preparation of solutions:

Preparation of orthophosphate buffer solution (P^H 6.0): 1.36 gm potassium dihydrogen phosphate was weighed and transferred into a 1000 mL flask and 400mL of Milli-Q water was added and mixed well. Then volume was made upto 1000mL, sonicated for five minutes and cooled to room temperature. The pH of buffer solution was adjusted to 6.0±0.05 with Sodium hydroxide solution and then filtered through a 0.45 µ membrane filter.

Preparation of mobile phase: A mixture of the above phosphate buffer (pH 6.0) and acetonitrile was used as the mobile phase and used in the gradient mode as following

Time (min)	% of buffer	% of acetonitrile
0.0	80	20
1.0	40	60
2.1	80	20

The diluent
A 50:50 v/v mixture of water and acetonitrile was prepared and used as the diluent in the preparation of drug dilutions.

Preparation of standard solution: Accurately weighed and transferred 300 mg of lamivudine and 50 mg of dolutegravir pure drugs into a 100 ml clean dry volumetric flasks add diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent (Stock solution). Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1.5 ml of the above stock solution into a 10ml volumetric flask and make up to the mark with diluent.

Preparation of sample solution: Twenty tablets were weighed to get the average weight and then grind.An amount of powder equivalent to about 300 mg lamivudine and 50 mg dolutegravirinto a 100 ml clean dry volumetricflasks add diluent and sonicate to dissolve it completely and make volume up to the mark with the diluents. Filter the solution and further pipette 1 ml of the above filtrate into a 10ml volumetric flask and dilute up to the mark with

diluent. Further pipette 1.5 ml of the above stock solution into a 10ml volumetric flask and make up to the mark with diluent.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 0.25 ml/min for 30 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 4 µL of standard into Inspire C18 (2.1 x 50 mm, 1.8 µm)column,the mobile phase of composition 0.1% orthophosphoric acid buffer and acetonitrile in the gradient mode was allowed to flow through the column at a flow rate of 0.25 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in Table 1 and figure 3.

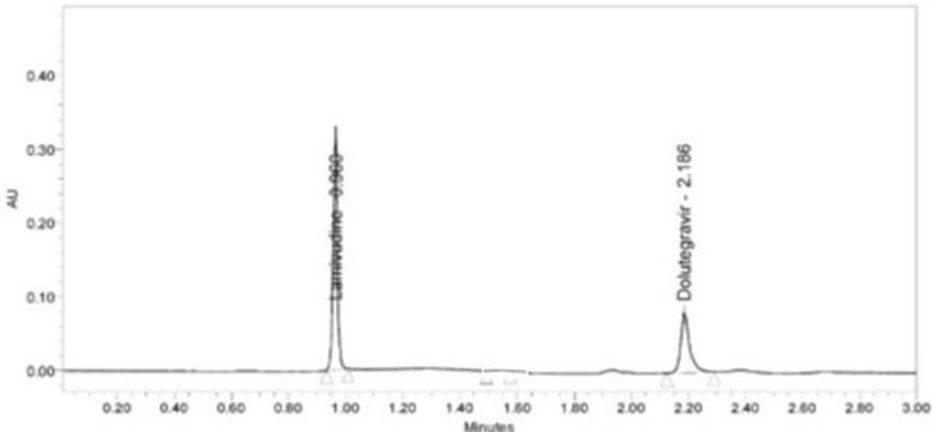

Figure 3: Standard chromatogram

Table 1: System Suitability Parameters

Parameters	Lamivudine	Dolutegravir
Retention time (min)	0.960	2.186
USP Plate count	12962.64	24826.53
USP Tailing	1.06	1.32

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine lamivudine and dolutegravir simultaneously in their tablet dosage form. The result obtained for lamivudine and dolutegravir was comparable with the corresponding labeled amounts and they were shown in Table-2 and figure-4.

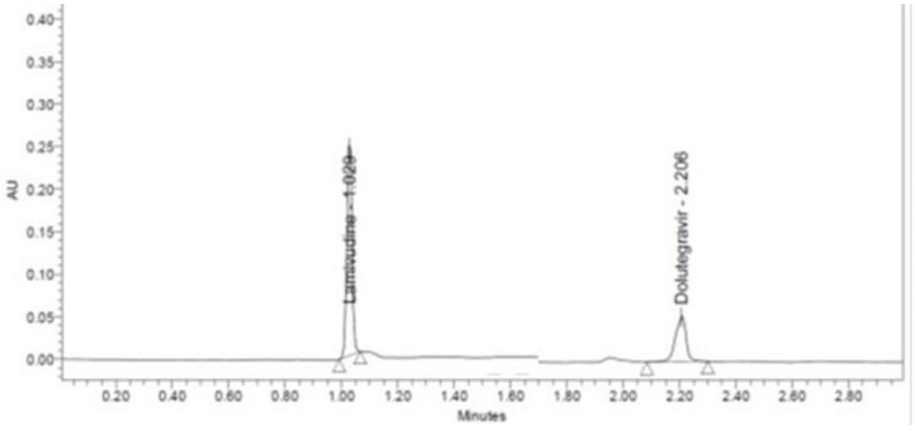

Figure 4: Sample chromatogram

Table 2: Assay Results for Lamivudine Anddolutegravir

	Label Claim (mg)	% Assay
Lamivudine	300	101.1± 0.56
Dolutegravir	50	99.86±0.48

3. Method Validation

The method was validated in accordance with ICH guidelines.[8]

Linearity: Linearity of the method was studied by injecting five concentrations of the drugs intriplicate prepared in the range of 60-180 µg/ml for lamivudine and 10-30 µg/ml for dolutegravir into the UPLC system. Linear graphs were plotted by using the peak areas against concentration in µg/ml from which the correlation coefficients, slopes and *Y*-intercepts of the calibration curves were determined. The results were shown in Table 3 and figure 5 & 6.

Table 3: Linearity results for lamivudine and dolutegravir

Parameters	lamivudine	dolutegravir
Concentration range(µg/ml)	60-180	10-30
Correlation coefficient	0.999	0.999
Intercept	1200.8	1200.2
Slope	1391.8	3554.1

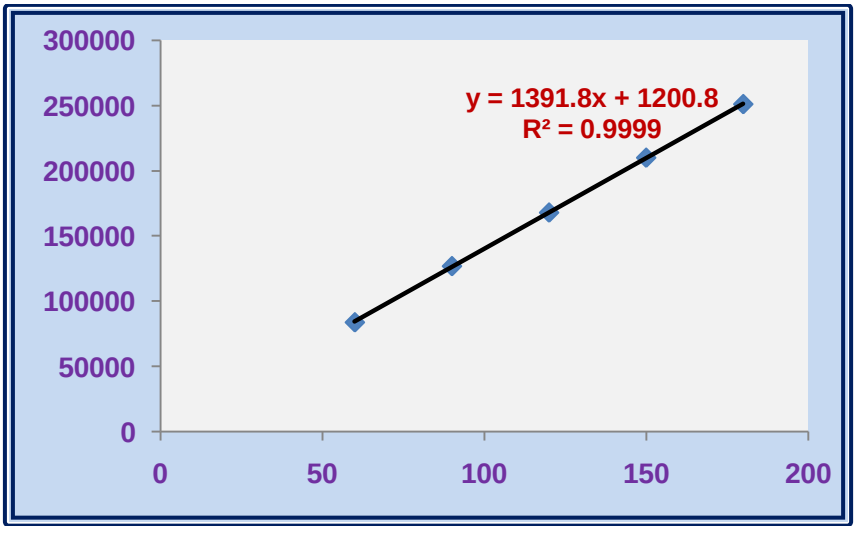

Figure 5: Linearity graph for lamivudine and dolutegravir

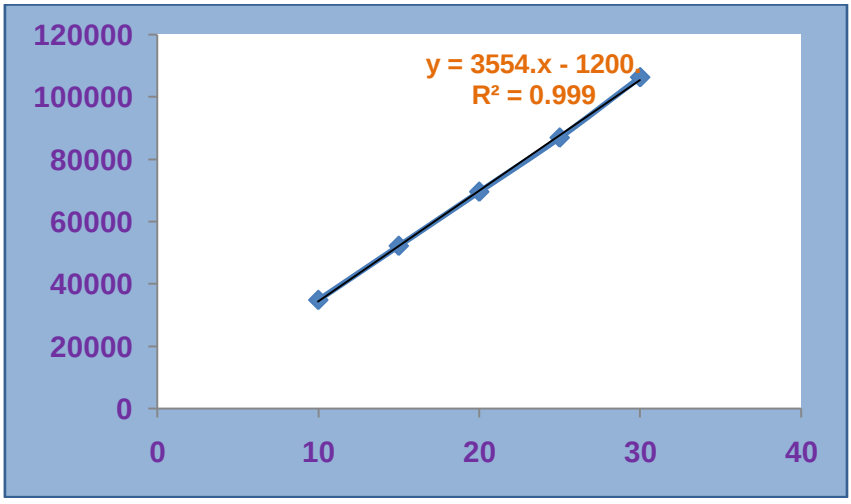

Figure 6: Linearity graph for dolutegravir

Accuracy: The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet sample. Percent recovery was calculated by comparing the area with pre analysed sample. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated by subtracting the total area from pre analysed sample area. The results were shown in Table-4.

Table 4: Accuracy results for lamivudine and dolutegravir

Pre analysed amount (µg)		Spiked amount (µg)		% recovered	
Lamivudine	**Dolutegravir**	**Lamivudine**	**Dolutegravir**	**Lamivudine**	**Dolutegravir**
60	10	30	5	102.09	102.07
60	10	30	5	101.95	101.98
60	10	30	5	101.99	102.02
60	10	60	10	100.93	100.68
60	10	60	10	101.11	100.73
60	10	60	10	100.86	100.51
60	10	90	15	99.01	101.45
60	10	90	15	98.96	101.49
60	10	90	15	98.98	101.33
			MEAN	100.65	101.36
			SD	1.33	0.61
			%RSD	**1.32**	**0.60**

Precision: For the precision study, repeatability study was carried out for short time interval under the same chromatographic conditions. For the intermediate precision study, repeatability study was carried out in different day under the same chromatographic conditions. The sample was injected in six replicate for intermediate precision and six replicate for precision. The peak area for injections was recorded. The mean and % relative standard deviation (%RSD) was calculated. From the data obtained the developed UPLC method was found to be precise. The Precision results were shown in Table-5 and Intermediate Precision in Table-5 and Table-6.

Table 5: Precision results for lamivudine and dolutegravir

Injection	Lamivudine	Dolutegravir
Injection 1	169232	66532
Injection 2	169138	65738
Injection 3	168798	65936
Injection 4	169286	66518
Injection 5	169136	65734
Injection 6	168976	65578
Average	169094.3	66006
Standard deviation	179.52	417.78
% RSD	0.11	0.63

Table 6: Intermediate Precision results for lamivudine and dolutegravir

Lamivudine			
S. No.	**Average area (n=6)**	**USP Plate Count**	**USP Tailing**
Day 1	168886	11646.66	1.09
Day 2	168137	10940.27	0.90
Overall average	168511.5	11293.47	0.99
SD	529.62		
% RSD	0.31		
Dolutegravir			
Day 1	67323	24131.82	1.32
Day 2	66198	24791.22	1.35
Overall average	66760.5	24461.52	1.33
SD	795.49		
% RSD	1.2		

Robustness: Robustness of the method was checked by making deliberate changes in chromatographic conditions like mobile phase ratio (±10%), and flow rate (0.25 ml/min). It was observed that there were no marked changes in system suitability parameters, which demonstrated that the developed UPLC method is robust.

Limit of detection and Limit of quantification:

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by standard deviation of response and slope method.

4. Conclusion

In the present work a new, accurate, precise and robust UPLC method was developed and validated for simultaneous estimation of lamivudine and dolutegravir in pharmaceutical dosage form in accordance with the ICH Guidelines. The method gives good resolution between both the compounds with a short analysis time (3 min). Linearity is observed in the concentration range of 60-180µg/ml for lamivudine and 10-30 µg/ml for dolutegravir. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be useful for the routine analysis of the lamivudine and dolutegravir in combined dosage form without any interference from excipients.

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