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Development and validation of analytical method for the estimation of Lamivudine and Dolutegravir sodium in dosage form

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Abstract

A simple, precise, accurate, rapid and sensitive HPLC method was developed for simultaneous estimation of Lamivudine and Dolutegravir sodium in tablet formulation. The chromatographic separation was attained on a Waters C18 column (150×4.6mm, particle size 5µ) in isocratic mode using Agilent 1260 Infinity-||, high performance thin layer chromatography system (HPLC) with UV detector. The mobile phase comprised of buffer (pH adjusted to 3.0 using orthophosphoric acid), acetonitrile and methanol (55:35:10 v/v). water (pH adjusted to 3.3 with orthophosphoric acid) and acetonitrile (58:42 v/v). The flow rate and injection volume were 1.1ml/min and 20 µL respectively and the detection was carried out at 260 nm using an UV detector. The developed method was validated as per ICH Q2B guidelines for linearity, accuracy, precision, robustness, limit of detection and limit of quantification. The linearity for Lamivudine and Dolutegravir sodium was found to in the range of 18-90µg/ml and 3-15µg/ml respectively with correlation coefficient (r^2) > 0.99. The assay of marketed formulation was found to be 99.75% and 99.09% for Lamivudine and Dolutegravir sodium respectively. The recoveries for Lamivudine and Dolutegravir sodium was found to be 99.63% & 99.75% at 80% level, 99.37% and 99.78% at 100% level and 100.15% and 100.47% at 120% level respectively.

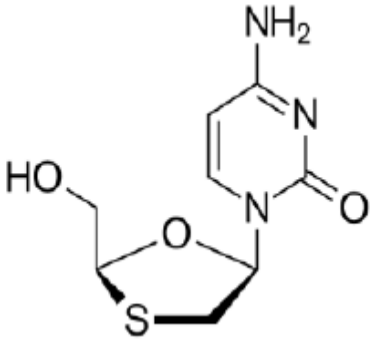
Keywords: Lamivudine, Dolutegravir Sodium, Validation, Simultaneous

INTRODUCTION

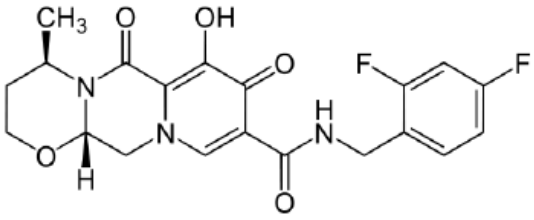
Lamivudine (LAM) having IUPAC name as (2R,cis)-4-amino-1-(2hydroxymethyl-1,3-oxathiolan-5-yl)- (1H)-pyrimidin-2-one is a synthetic nucleoside analogue (Figure 1). This molecule has been approved for cure of chronic hepatitis B at lower doses than for management of HIV/AIDS. The principle mode of action involves the inhibition of HIV-1 reverse transcriptase (RT) via DNA chain termination after integration of the nucleoside analogue into viral DNA. It also helps in advancing the seroconversion of e-antigen positive hepatitis B and also progresses histology staging of the liver. [1, 2] It is usually available in combination with other antiretroviral drugs like zidovudine and abacavir. Dolutegravir (DOLU) having IUPAC name as 4-{[(2S, 4R)-1-(4-Biphenylyl)-5-ethoxy-4-methyl-5-oxo-2-pentanyl] amino}-4-oxobutanoic acid (Figure 1) interferes the important step for the HIV replication cycle. DOLU inhibits HIV integrase by binding to the integrase active site and obstructing the strand transfer step of retroviral DNA integration. [3, 4]

The literature review proposed that there are numerous methods reported for spectrophotometric [5-7] and chromatographic [7-9] estimation of LAM and DOLU individually but at present no literature reveals chromatographic method for simultaneous determination of these drugs in combination. Thus, an HPLC method for simultaneous determination of LAM and DOLU was commended which would be advantageous to the present work.

The paper mainly focuses on developing a simple, rapid and accurate HPLC method for the estimation of LAM and DOLU and the developed method was validated as per ICH guidelines. [10]



Lamivudine



Dolutegravir sodium

Figure 1: Chemical Structures of Lamivudine (LAM) and Dolutegravir sodium (DOLU)

MATERIALS AND METHODS

Chemicals

All HPLC grade solvents such as acetonitrile (ACN) and methanol of Merck Life Science were procured from research lab fine chem industries, Mumbai. The drugs

Lamivudine (LAM) and Dolutegravir sodium (DOLU) were procured from Cipla Limited Mumbai. The marketed formulation Dovato of ViiV Healthcare having label claim for LAM and DOLU as 300mg and 50 mg was used for the analysis.

Chromatographic Conditions

The chromatographic separation was performed using Agilent 1260 Infinity-||, high performance thin layer chromatography system (HPLC) with UV detector, LC solutions software. A Waters C18 column (150×4.6mm, particle size 5µ) maintained at ambient temperature was used for the chromatographic separation. The mobile phase optimized for the chromatographic separation consisted of buffer (pH adjusted to 3.0 using orthophosphoric acid), acetonitrile and methanol (55:35:10 v/v). The mobile phases were degassed using an ultrasonic water bath and filtered through membrane filter 0.45µ. The flow rate was maintained at 1ml/min and injection volume was fixed 20 µL. The run time for the analysis was 5 mins and the detection was carried out at 260 nm using an UV detector.

Preparation of standard stock solution

Accurately weigh about 60.0 mg of LAM and 10mg DOLU and transfer them into 100 mL volumetric flask, add 80 ml of the mobile phase to it. The resulting solution was sonicated for 15 minutes and volume was made upto the mark and filtered through the membrane filter 0.22µ. This filtrate was then used as standard stock solution having concentration 600µg/mL of LAM & 100µg/mL DOLU.

Preparation of Sample solution

Accurately 20 tablets were weighed and triturated in a motor pestle. The tablet powder equivalent to 300 mg LAM and 50mg DOLU was accurately weighed and transferred to 100mL volumetric flask. To this 80 mL of mobile phase was added and sonicated for 15 min. The final volume was made up to 100mL with mobile phase and the solution was filtered through the membrane filter 0.22µ. This filtrate was further diluted to yield concentration of 600µg/mL of LAM & 100µg/mL DOLU.

Validation of HPLC Method

The recommended HPLC method was validated as per the International Conference on Harmonization (ICH) guidelines for system suitability, specificity, precision, linearity, accuracy and robustness.

System Suitability

The standard solutions of LAM and DOLU having concentrations 600µg /ml and 100µg /ml respectively were injected into the HPLC system. The chromatographic parameters like Rt values and peak areas were calculated for the standard solutions and the values acquired established the suitability of the system for the analysis.

Specificity

The blank solution, standard (STD) solutions and sample solutions of LAM and DOLU having concentrations 600µg /ml and 100µg /ml respectively were injected in duplicates. The chromatograms obtained for the blank, standard and sample solutions were compared to determine the specificity of the method.

System precision

The average peak areas of standard solutions of LAM and DOLU having concentrations 600µg /ml and 100µg /ml respectively were determined by injecting six replicate injections and analyzed by the established method. The results for system precision were expressed in terms of percent relative standard deviation (% RSD).

Method precision

The samples solutions of LAM and DOLU having concentrations 600µg /ml and 100µg /ml respectively were injected in six replicates and analyzed as per established method. The % assay for both the analytes were calculated and results were expressed in terms of % RSD.

Intermediate precision

Intermediate precision was determined by analyzing six different samples of concentrations of 600µg /ml and 100µg /ml of LAM and DOLU respectively. The results of the two independent analysis performed on same and different days were compared and the % assay for both analytes was calculated for intra-day and inter-day precision and expressed in terms of % RSD.

Linearity

The linearity of peak area responses for LAM and DOLU was calculated by evaluating the working standard solutions after sequential dilution to yield 18-90µg/ml and 3-15µg/ml of LAM and DOLU respectively. The graph of concentration versus peak area was plotted to determine the linearity. The correlation coefficient, y-intercept and slope of the regression were calculated.

Accuracy

The accuracy of the method was calculated by recovery studies. The known amount of standards LAM and DOLU at 80%, 100%, and 120% levels were added to the pre-analyzed sample and the analysis was performed in replicates. Tablet powder equivalent to 300 mg LAM and 50mg DOLU was accurately weighed in nine different volumetric flasks and to this standard LAM and DOLU 80mg, 100mg and120mg were added. The samples were dissolved in mobile phase using sonicator for 15 mins and further filtered using membrane filter 0.22µ. The resulting solutions were then analyzed using HPLC. The % recovery values were calculated at all levels.

Robustness

The effects of changes in chromatographic conditions were determined conferring to ICH guidelines to establish robustness of the method. The change in flow rate, composition of mobile phase and pH were considered. The retention time of LAM and DOLU were determined and % RSD for each changing chromatographic condition was calculated.

Limit of Detection (LOD) and Limit of quantification (LOQ)

LOD defined as the lowest concentration of the analyte which can be detected but not necessarily quantified. LOD was determined by calculating the noise ratio of the instrument and the lowest peak areas of the samples LAM and DOLU. The LOD was calculated using the formula,

$$\text{LOD} = \frac{3\sigma}{S}$$

Where, σ – standard deviation

S – Slope of calibration curve

LOQ defined as the lowest concentration of the analyte which can be detected as well as quantified with accuracy. LOQ was determined by calculating the noise ratio of the instrument and the peak areas of the samples LAM and DOLU. The LOQ was calculated using the formula.

$$LOQ = \frac{10\ \sigma}{S}$$

Where, σ – standard deviation

S – Slope of calibration curve

RESULTS AND DISCUSSION

System Suitability

The system suitability of the method was determined from the results obtained after analysis as mentioned in Table 1, which represents the suitability of the method for analysis of LAM and DOLU in combination.

Specificity

The chromatograms of the blank, standard (STD) and sample solutions were obtained and compared. The chromatograms depicted absence of co-eluting peaks at the respective retention time for LAM and DOLU (Figure 2-3). Therefore, the method was found to be specific.

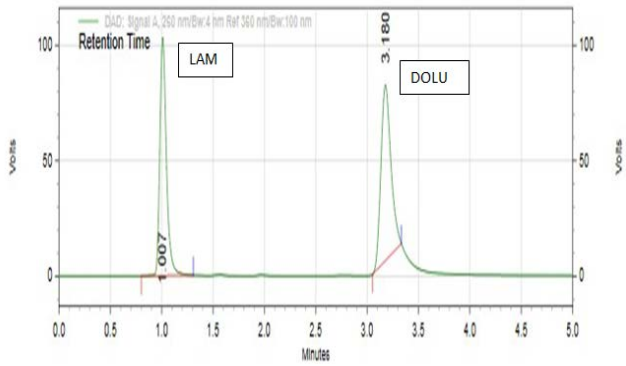


Figure 2: Chromatogram of STD Lamivudine and Dolutegravir sodium solution

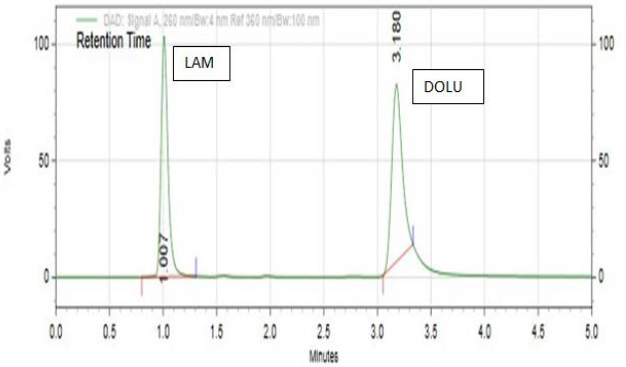


Figure 3: Chromatogram of sample solution

System precision

The average peak areas for standards of LAM and DOLU were determined and the results for system precision were expressed in terms of % RSD (Table 2). The %RSD values for LAM and DOLU were found to be 0.48% and 0.47% respectively.

Method precision

The % assay for both the analytes LAM and DOLU was determined and the %RSD values were found to be within the acceptable criteria <2.0% (Table 3).

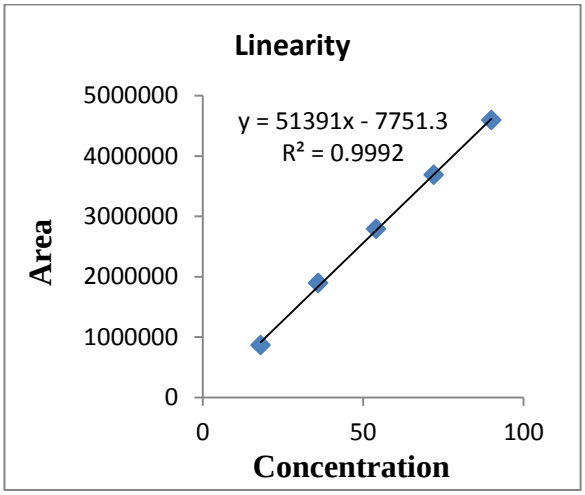


Figure 4: Linearity of Lamivudine

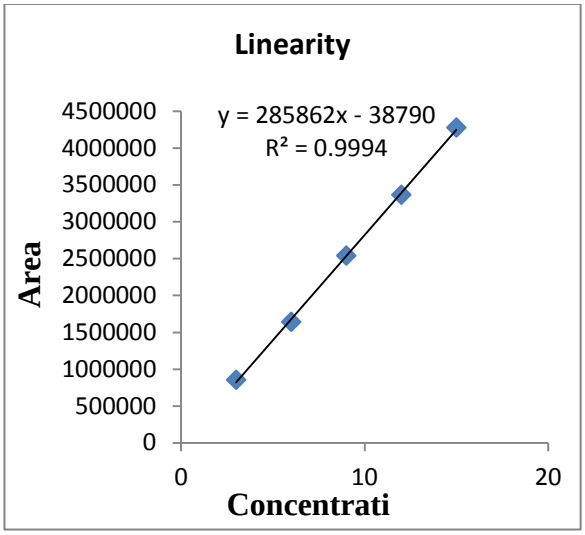


Figure 5: Linearity of Dolutegravir sodium

Table 1: System suitability

Parameters	Retention Time (Rt)	Peak Area
Lamivudine (LAM)	1.020	3685286
Dolutegravir Sodium (DOLU)	3.313	3366163

Table 2: System Precision

Inject. No	Peak Area of Lamivudine	Peak Area of Dolutegravir Sodium
1	3695391	3363615
2	3654332	3326973
3	3694967	3361734
4	3704795	3366965
5	3695975	3366854
6	3685124	3366967
Mean	3688430.667	3358851.33
SD	17829.9264	15766.86
% RSD	0.48	0.47

Table 3: Method Precision

Sample No.	% Assay of Lamivudine	% Assay of Dolutegravir Sodium
1	100.37	99.47
2	99.62	98.55
3	98.89	99.56
4	100.25	99.73
5	99.28	99.19
6	100.09	98.05
Mean	99.75	99.09
SD	0.58701	0.65627
% RSD	0.59	0.66

Table 4: Intermediate Precision (Inter-day)

Sr. No.	% Assay of Lamivudine		% Assay of Dolutegravir Sodium	
	Assay (% w/w, Analysis-Day 1)	Assay (% w/w, Analysis-Day 2)	Assay (% w/w, Analysis-Day 1)	Assay (% w/w, Analysis-Day 2)
1	98.20	98.75	99.20	98.25
2	97.99	99.24	99.69	99.41
3	98.63	98.74	100.04	98.85
4	98.09	97.52	99.73	99.75
5	99.82	98.05	99.91	98.25
6	97.92	99.2	98.40	100.12
Average	98.44	98.58	99.50	99.11
% RSD	0.73	0.68	0.61	0.79
Overall % RSD	0.71		0.70	

Table 5: Intermediate Precision (Intra-day)

Sr. No.	% Assay of Lamivudine		% Assay of Dolutegravir Sodium	
	Assay (% w/w, Analysis-1)	Assay (% w/w, Analysis-2)	Assay (% w/w, Analysis-1)	Assay (% w/w, Analysis-2)
1	97.85	98.54	98.12	99.21
2	98.08	97.85	99.54	99.54
3	99.71	99.45	99.24	97.37
4	99.28	99.35	98.95	98.55
5	98.92	98.42	98.56	99.23
6	99.65	97.87	97.33	97.65
Average	98.92	98.58	98.62	98.59
% RSD	0.80	0.70	0.82	0.92
Overall % RSD	0.75		0.87	

Table 6: Linearity of Lamivudine and Dolutegravir sodium

Lamivudine		Dolutegravir Sodium	
Concentration of LAM (ppm)	Average Peak area of LAM	Concentration of DOLU (ppm)	Average Peak area of DOLU
18	866592	3	852673
36	1895275	6	1637337
54	2792957	9	2537475
72	3685286	12	3366163
90	4596813	15	4276189
r^2=0.9992		r^2=0.9994	

Table 7: Recovery study for Lamivudine and Dolutegravir sodium

Recovery level	Lamivudine		Dolutegravir Sodium	
	% Recovery	Average % Recovery	% Recovery	Average % Recovery
80% - 1	98.83	99.63	99.20	99.75
80% - 2	98.71		99.60	
80% - 3	98.33		99.87	
100% - 1	99.32	99.37	99.32	99.78
100% - 2	99.70		99.70	
100% - 3	99.17		100.32	
120% - 1	103.94	100.15	100.14	100.47
120% - 2	99.67		100.07	
120% - 3	101.02		101.21	

Table 8: Robustness parameter

Sr. No	Parameters	Working parameter	Changes
1	Flow	1.0 ml/min	0.9 ml/min
2	pH	3.0	3.3
3	Mobile phase composition (Buffer:ACN:Methanol)	55:35:10	55:25:20

Table 9: Robustness for Lamivudine and Dolutegravir Sodium

Robustness parameter		% RSD for LAM	% RSD for DOLU
Flow (mL/min)	0.9	0.23	0.27
	1.0	0.10	0.28
pH	3.0	0.22	0.26
	3.3	0.32	0.29
Mobile phase composition (Buffer: ACN: Methanol)	55:35:10	0.21	0.11
	55:25:20	0.24	0.13

Intermediate precision

Inter-day

The %RSD for inter-day analysis performed on different days were found to be within limits and the overall % RSD for % of LAM and DOLU were found to be 0.71% and 0.70% respectively. (Table 4).

Intra-day

The %RSD for intra-day analysis performed on same day were found to be within limits and the overall % RSD for % of LAM and DOLU were found to be 0.75% and 0.87% respectively (Table 5).

Linearity

Lamivudine and Dolutegravir sodium were found to be linear in the range of 18-90µg/ml and 3-15µg/ml respectively. Linearity graphs of concentration versus peak areas were plotted (Figure 4-5) and correlation coefficient, y-intercept and slope of the regression were determined (Table 6). The Correlation coefficient (r^2) for LAM and DOLU were found to be >0.99.

Accuracy

The % average recovery of LAM and DOLU was calculated and were found to be within acceptance criteria of 98-102%, specifying the accuracy of the method (Table 7).

Robustness

The changes in the flow rate, pH and mobile phase composition had no effect chromatographic analysis of LAM and DOLU as described in Table 9. The RSD of peak area responses for LAM and DOLU standard solutions analyzed in replicates were found to be less than 2.0 % (Table 8-9). Thus, the method was found to be robust.

Limit of Detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ for LAM were found to be 2.3µg/ml and 9.2µg/ml respectively. While the LOD and LOQ for DOLU was found to be 2.1µg/ml and 9.7µg/ml respectively.

CONCLUSION

In the present study, a rapid, accurate and precise HPLC was developed and validated for simultaneous estimation of Lamivudine and Dolutegravir sodium from marketed pharmaceutical formulations. The developed method was found to be precise, accurate and robust. Thus, developed method may be found as suitable and efficient for repetitive analysis of combined dosage forms having Lamivudine and Dolutegravir sodium.

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