Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

https://doi.org/10.36673/AJRCPS.2021.v09.i04.A24

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DOLUTIGRAVIR AND LAMIVUDINE BY RP-HPLC

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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 (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 20µL respectively and the detection was carried out at 260nm 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 (r2) > 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% and 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, Antiviral, Linearity, LOD and LOQ.

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INTRODUCTON

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 No.1). This molecule has been approved for cure of chronic hepatitis B at lower doses than for management of HIV/AIDS. The principal 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]. 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 No.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"[2].

EXPERIMENTAL WORK

MATERIAL 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 Vii V Healthcare having label claim for LAM and DOLU as 300mg and 50mg 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 20µL. The run time for the analysis was 5 mins and the detection was carried out at 260nm using an UV detector"[3,4].

Preparation of standard stock solution

Accurately weigh about 60.0mg of LAM and 10mg DOLU and transfer them into 100mL volumetric flask, add 80ml 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 and 100µg/mL DOLU.

Preparation of Sample solution

Accurately "20 tablets were weighed and triturated in a motor pestle. The tablet powder equivalent to 300mg LAM and 50mg DOLU was accurately weighed and transferred to 100mL volumetric flask. To this 80mL 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 and 100µg/mL DOLU"[5-7].

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)"[8-12].

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"[13-24].

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,

$LOD = 3\sigma/S$

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

$LOD = 10\sigma/S$

Where, σ – standard deviation

S – Slope of calibration curve"[25,26].

RESULTS AND DISCUSSION

System Suitability

The system suitability of the method was determined from the results obtained after analysis as mentioned in Table No.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 No.2-3). Therefore, the method was found to be specific.

Discussion

After a number of trials with mobile phases of different composition, Acetonitrile, Phosphate buffer pH 3.0 in the ratio 50:50v/v was selected as mobile phase because of better resolution, more no. of Theoretical plates and symmetric peaks. Dolutegravir and lamivudine were found to show appreciable absorbance at 260nm when determined spectrophotometrically and hence it was selected as the detection wavelength.

Concentration range of 50-250µg/ml for Dolutegravir and Lamivudine were found to be linear with correlation coefficients 0.9986 and 0.9998 for Dolutegravir and Lamivudine respectively.

The limits of detection for Dolutegravir and Lamivudine were found to be 2.0µg/ml and 1.19µg/ml respectively and the limit of quantitation were 6.6µg/ml and 3.92µg/ml respectively. Values were represented in tabulated

The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of the commercial formulations of the selected combination with a percentage purity of 99.04% for DOLUTI and 99.8% for LAMI. The typical chromatogram for assay of marketed formulations was shown in Figure No.5 and values obtained were given in Table No.4.

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatograms confirm the presence of Dolutegravir and Lamivudine at 2.4min and 4.3min respectively without any interference. The parameters were tabulated

Accuracy of the method was verified by performing recovery studies by standard method.

The percent recovery of the standard added to the pre- analysed sample was calculated and it was found to be 98.13% to 98.89% for Dolutegravir and 99.6 to 101% for Lamivudine. This indicates that the method was accurate. Values obtained were tabulated.

The proposed method was found to be precise and reproducible with %RSD of 0.87 and 0.40 for DOLUTI and LAMI respectively. %RSD was reported in table. The method was found to be robust after changing the conditions like detection wavelength (± 2nm) and flow rate (±0.2ml). %RSD was calculated for each variation and reported. Values obtained were tabulated.

Dolutegravir and 99.6 to 103% for Lamivudine. This indicates that the method was accurate. Values obtained were tabulated.

Table No.1: Results for linearity (n=3)

S.No	Parameters	Lamivudine	Dolutegravir
1	Slope	782071	396499
2	y intercept	387706	593776
3	Correlation coefficient r2	0.9995	0.9999
4	Regression Equation	y = 782071x + 387706	y =396499x –593776
5	Linearity range	50-250µg/ml	50-250µ g/ml
6	LOD	0.060	0.0142
7	LOQ	0.60	0.142

Table No.2: System suitability parameters

S.No	Parameters	Dolutegravir	Lamivudine
1	Retention time (min)	2.430	4.370
2	Theoretical plates (N)	22.16	22.16
3	Tailing factor (T)	1.69	1
4	Resolution (Rs)		1.090

Table No.3: Results for Accuracy (n=3)

S.No	Recovery level	Dolutegravir				Lamivudine			
		Amount Added (µg/ml)		Amount Found (µg/ml)	% Recovery	Amount Added (µg/ml)		Amount Found (µg/ml)	% Recovery
		Std	Test			Std	Test		
1	50%	100	100	197.5	98.75	100	100	199.2	99.6
2	100%	100	150	245.3	98.13	100	150	257.7	103
3	150%	100	200	296.6	98.89	100	200	299.3	99.76
4	Mean recovery	98.59				100.7			

Table No.4: Specificity parameters (n=5)

S.No	Parameters	Dolutegravir	Lamivudine
1	Retention time (min)	4.350	2.430
2	Theoretical plates (N)	1861	10512
3	Tailing factor (T)	1.30	1.05
4	Resolution (Rs)	1.7	

TableNo.5: Results for Robustness

S.No	Parameters (n=3)	%RSD	
		Dolutegravir	Lamivudine
1	Detection wavelength at 258nm	0.68	0.67
2	Detection wavelength at 262nm	0.16	0.86
3	Flow rate 1.4ml/min	0.28	0.97
4	Flow rate 1ml/min	0.51	0.39
5	Analyst I	0.69	0.72
6	Analyst II	0.95	0.85

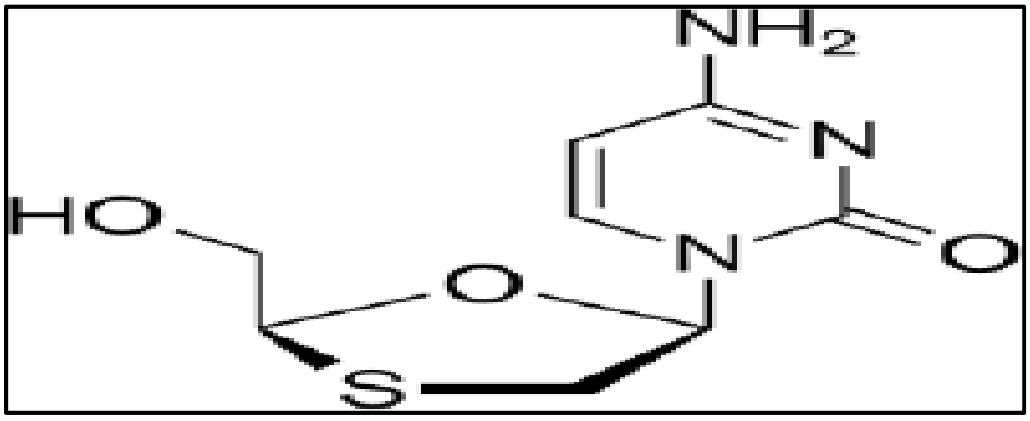

Figure No.1: Chemical structure of Lamivudine

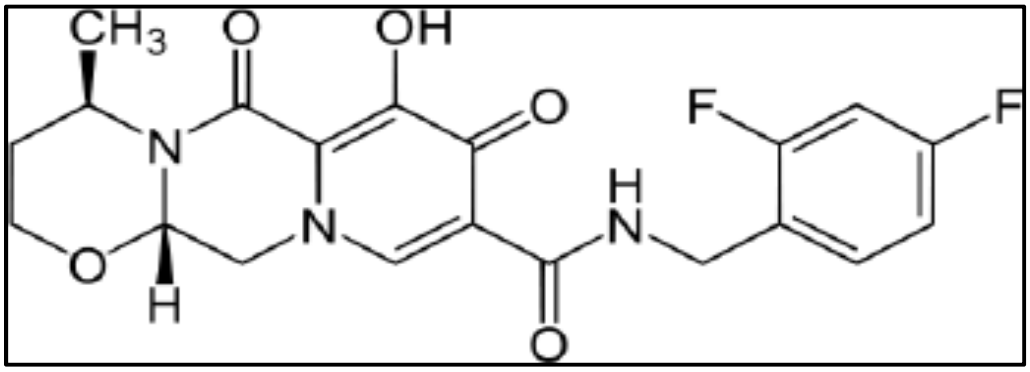

Figure No.2: Chemical structure of Dolutegravir

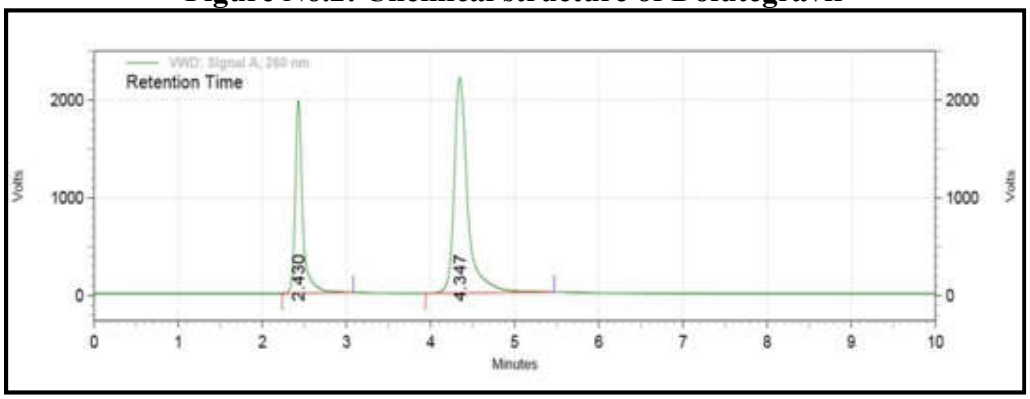

Figure No.3: Optimized chromatogram for the Lamivudine and Dolutegravir

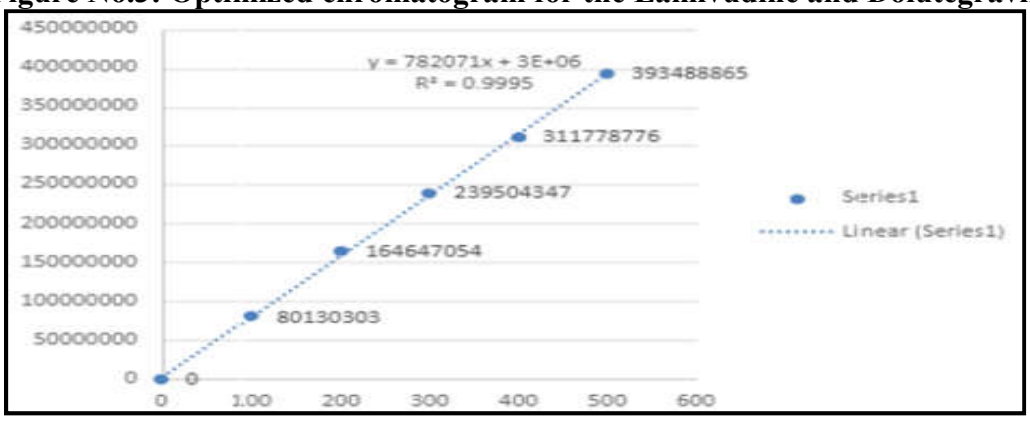

Figure No.4: Calibration plot of Dolutegravir

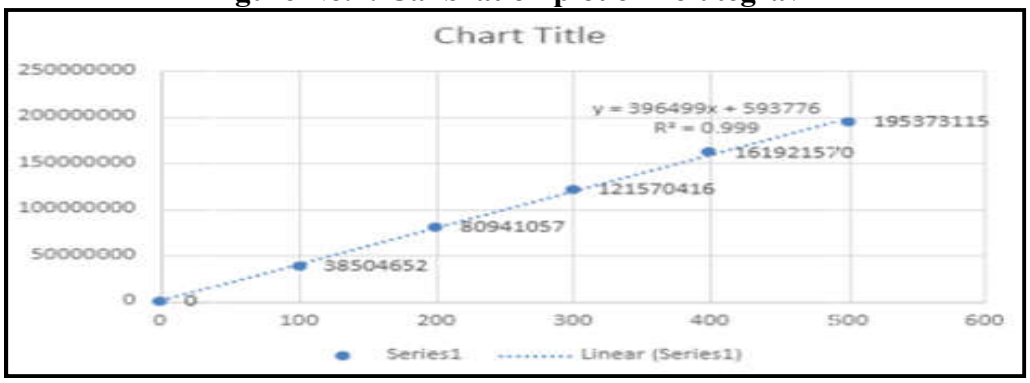

Figure No.5: Calibration plot of Lamivudine

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Dolutegravir and Lamivudine in bulk and tablet dosage form. Retention time of Dolutegravir and Lamivudine were found to be 2.4mins and 4.3mins. 0.87 and 0.40 %RSD of the Dolutegravir and Lamivudine were and found to be 0.4, 0.3 and 0.4 respectively. % Assay was obtained as 98.46%, 99.2% for, Dolutegravir and Lamivudine respectively. Regression Coefficient was 0.99 for all the two drugs. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Pharmaceutical Analysis, M.L. College of Pharmacy, Singarayakonda, Andhra Pradesh, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Neeli Sri Swapna and Karavadi Thejomoorthy. Analytical method development and validation for the estimation of dolutigravir and lamivudine by RP-HPLC, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 9(4), 2021, 187-194.