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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LAMIVUDINE AND DOLUTEGRAVIR IN BULK AND FORMULATION BY UV SPECTROSCOPY

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ABSTRACT

A simple, rapid, precise and accurate spectrophotometric method has been developed for quantitative analysis of Dolutegravir and Lamivudine in tablet formulations. Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80 mL methanol and the flask was sonicated for about 10 min to solubilize the drug. The maximum absorbance of LAM and DLT was observed at 244.0 nm and 262.0 nm, respectively. LAM and DLT showed linearity in the concentration range of 10-50 µg/ml and 5-25 µg/ml at their respective maxima. The recovery of added standards (80 %, 100 % and 120 %) was found at three replicates and three concentrations level. The value of % means just close to 100, SD and % RSD are less then 2 indicate the accuracy of method. Parameters like linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported within the ICH guidelines. The assay value in tablet formulation was close to 100, SD and % RSD are less than 2 indicates no interference of excipients in the estimation of drugs. Correlation coefficient of Dolutegravir and Lamivudine was found to be 0.999 and 0.998 respectively. Developed method was found to be novel, accurate, precise, selective and rapid for simultaneous estimation of DLT and LAM. The method was validated as per the ICH guidelines. The developed method can be adopted in routine analysis of Dolutegravir in bulk or tablet dosage form and it involves relatively low cost solvents and no complex extraction techniques.

Keywords: Dolutegravir, Validation, Linearity, Precision, Accuracy.

1. INTRODUCTION

Dolutegravir (DTG) may be a medication used for the treatment of HIV infection. Dolutegravir is chemically designated as 4-{[(2S, 4R)-1-(4- Biphenylyl)-5-ethoxy-4-methyl-5-oxo-2-pentanyl]amino}-4-oxobutanoic acid [1-7]. Dolutegravir is an HIV integrase inhibitor, a replacement class of drug with a high barrier to drug resistance and few side effects. Dolutegravir is a HIV-1 antiviral agent. It binds to the active site and blocks the strand transfer step of retroviral DNA integration in the host cell and also inhibits the HIV integrase. The most essential step in the HIV replication cycle is stand transfer step which results in the inhibition of viral activity. Lamivudine, (2R-cis)-4-amino-1-[2-(hydro-xymethyl)-1, 3-oxathiolan-5-yl]-2(1H) pyrimidinone, [1] is a synthetic nucleoside analogue with activity against the human immunodeficiency virus (HIV) and hepatitis B virus (HBV). Lamivudine is a nucleotide reverse transcriptase inhibitor and works by blocking the HIV reverse transcriptase and hepatitis B virus polymerase. It is effective against both HIV-1 and HIV-2 [8]. Lamivudine is either formulated alone as a tablet/oral formulation or in combination with dolutegravir. HPLC is the most widely used technique for the estimation of lamivudine in human plasma, saliva, cerebrospinal fluid, and human blood cells, as well as for studying the drug metabolites in the urine. The present research work describes UV method for estimation of Lamivudine and Dolutegravir in bulk and pharmaceutical dosage form [9-14].

2. MATERIAL AND METHOD

2.1. Linearity range and calibration graph

2.1.1. Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80 mL methanol and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with methanol to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

2.1.2. Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml was withdrawn with help of pipette from standard stock solution A of LAM and DLT and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with pH 6.8 phosphate buffers that gave concentration of 100µg/ml (Stock-B).

2.1.3. Preparation of Working Standard Solution

Aliquots of 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml were withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with 6.8 phosphate buffer. This gave the solutions of 10 µg/ml, 20µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml respectively for LAM. 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with 6.8 phosphate buffer. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20 µg/ml and 25µg/ml respectively for DLT.

2.1.4. Selection of wavelength for linearity

Solutions of 10 µg/ml of LAM and 10 µg/ml DLT were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of LAM and DLT was observed at 244.0 nm and 262.0 nm, respectively. LAM and DLT showed linearity in the concentration range of 10-50 µg/ml and 5-25 µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration.

To study the linearity of LAM and DLT the selected wavelength were: λ_{max} of LAM 244.0 nm and λ_{max} of DLT 262.0 nm.

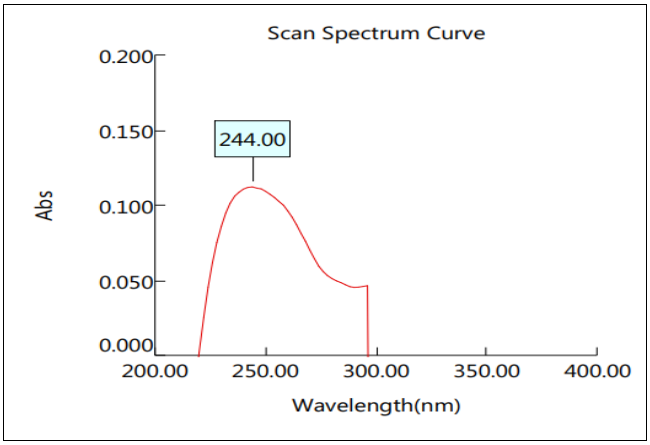

Fig. 1: Selection of λ_{max} of Lamivudine

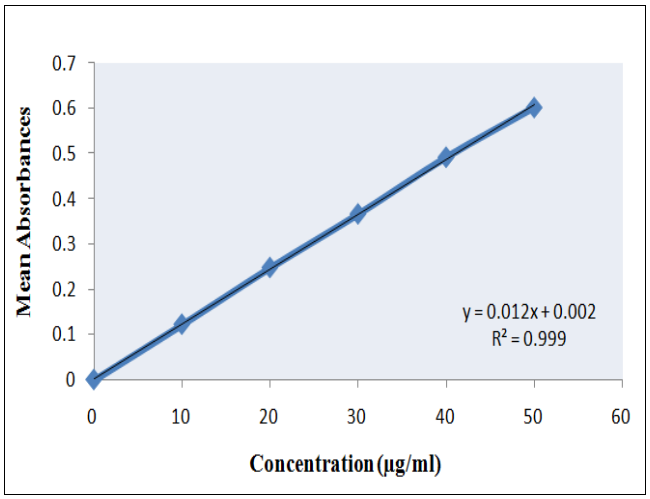

Fig. 2: Calibration Curve of LAM at λmax = 244.0 nm

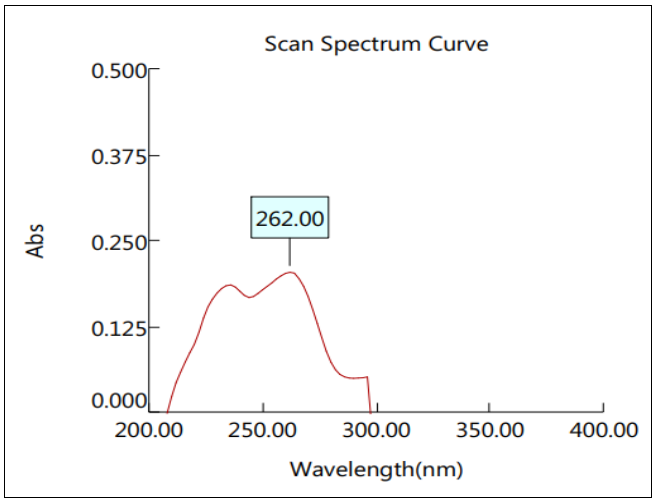

Fig. 3: Selection of λ_{max} of Dolutegravir

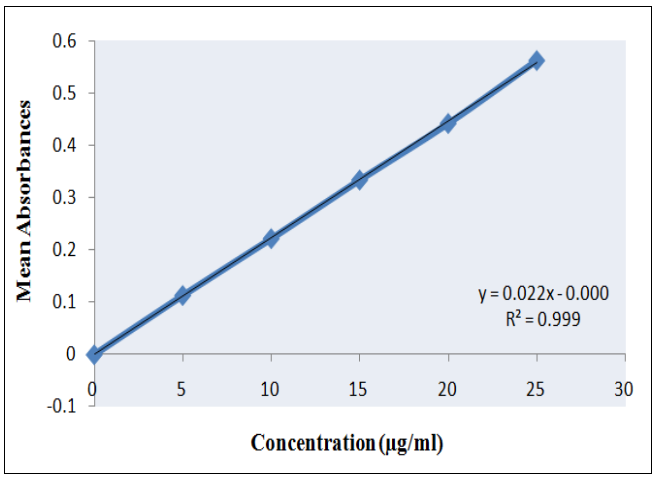

Fig. 4: Calibration Curve of DLT at λ_{max} = 262.0 nm

Table 1: Linearity of LAM At λmax = 244.0 nm

Standard Conc. (µg/ml)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean
0	0	0	0	0	0	0
10	0.122	0.123	0.121	0.122	0.124	0.122
20	0.248	0.249	0.248	0.247	0.248	0.248
30	0.365	0.366	0.367	0.368	0.367	0.367
40	0.492	0.493	0.493	0.491	0.492	0.492
50	0.602	0.601	0.602	0.603	0.603	0.602
Correlation Coefficient (r^2)						0.999
Slope (m)						0.021
Intercept (c)						0.002

Table 2: Linearity of DLT At λ_{max} = 262.0 nm

Standard Conc. (µg/ml)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean
0	0	0	0	0	0	0
5	0.114	0.113	0.112	0.114	0.113	0.113
10	0.221	0.222	0.223	0.221	0.222	0.222
15	0.334	0.333	0.334	0.335	0.334	0.334
20	0.443	0.442	0.443	0.441	0.442	0.442
25	0.565	0.564	0.555	0.564	0.564	0.562
Correlation Coefficient (r^2)						0.999
Slope (m)						0.022
Intercept (c)						0.000

2.2. Simultaneous equation method

2.2.1. Study of Overlay Spectra

Working standard solution from the standard stock solution prepared as in concentration 10 µg/ml of LAM and 5µg/ml of DLT were scanned in the spectrum mode over the range of 200-400 nm against phosphate buffer pH 6.8 as blank and the overlain spectra of the two were recorded. LAM showed an absorbance peak at 244.0 nm, whereas DLT at 262.0 nm. The overlain spectra also showed isoabsorptive points at 248.0 nm.

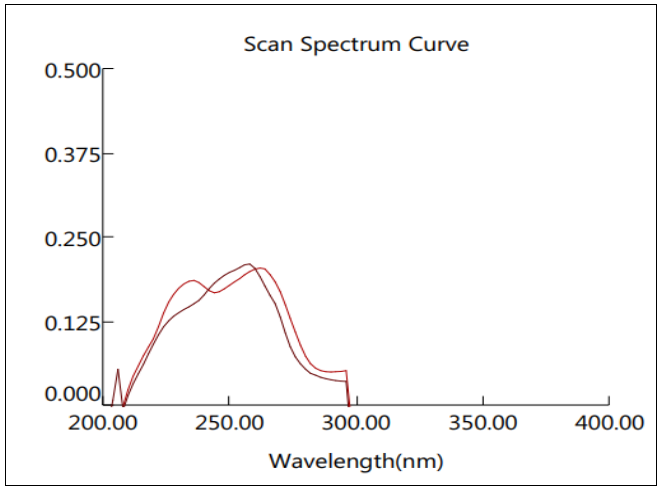

Fig. 5: Overlay Spectra of LAM and DLT

Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method.

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 244.0 nm and 262.0 nm that are λ_{max} of LAM and DLT respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%,\ 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C_{LAM} = \frac{A_1 ay_2 - A_2 ay_2}{ax_1 ay_2 - ax_2 ay_1} \quad \text{..... Eq (1)}$$

$$C_{DLT} = \frac{A_1 ax_2 - A_2 ax_1}{ax_1 ay_2 - ax_2 ay_1} \quad \text{..... Eq (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 244.0 nm and 262.0 nm respectively, ax_1 and ax_2 are absorptivities of LAM at λ_1 (244.0 i.e. λ_{max} of LAM) and λ_2 (262.0 i.e. λ_{max} of DLT) respectively and ay_1 and ay_2 are absorptivities of DLT at λ_1 and λ_2 respectively. C_{LAM}

and C_{DLT} are concentrations of LAM and DLT respectively. Fig. 5 represent the overlain spectra of both the drugs in 1:1 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the LAM and DLT.

2.2.2. Working Linearity

To get the working concentration range, linearity was observed at 244 nm and 262 nm for LAM and DLT respectively. Standard stock solution of LAM and DLT was prepared in water. The absorbtivity of both drugs were calculated by using equation

$$A = abc$$

Where A=Absorbance, a=Absorbtivity, b=Pathlength, c = Concentration.

2.3. Validation of Simultaneous Equation Method

A_1: Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug was calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

B_1: Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of LAM and DLT to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

C_1: Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week. The results are shown in table 5.

2.3.1. Analysis of tablet sample

Twenty marketed tablets of LAM and DLT were weighed and ground to a fine powder; amount equal to 30 mg of LAM was taken in 10 ml volumetric flask. The DLT present in this amount of tablet powder was 5 mg. 5 ml of methanol was then added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with methanol. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with phosphate buffer pH 6.8 to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from Simultaneous Equation Method. The procedure was repeated for five times.

3. RESULTS AND DISCUSSION

3.1. Method I: Simultaneous equation method

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 244.0 nm and 262.0 nm that are λ_{max} of Lamivudine (LAM) and Dolutegravir (DLT) respectively. Then developed method was validated by using various parameters.

3.1.1. Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the results was recorded. The results of linearity are reported in table 3.

Table 3: Linearity of Lamivudine and Dolutegravir

PARAMETER	LAM	DLT
Concentration (µg/ml)	10-50	5-25
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.012	0.022
Intercept (c)*	0.002	-0.000

**value of three replicates*

3.1.2. Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80 %, 100 % and 120 %) was found at three replicates and three concentrations level. The value of % means just close to 100, SD and % RSD are less than

2 indicate the accuracy of method. Result of recovery study shown in table 4.

Table 4: Recovery study

% LEVEL	% MEAN±SD*	
	DLT	LAM
80%	98.66±0.556	98.49±1.676
100%	98.54±0.687	99.69±0.118
120%	99.25±0.696	99.60±0.166

***** *Value of three replicates and five concentrations*

3.1.3. Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less then 2 indicate the precision of method. Result of precision is shown in table 5.

Table 5: Results of precision

PARAMETER	% MEAN±SD*	
	DLT	LAM
Repeatability	99.26±0.092	99.50±0.107
Intermediate precision		
Day to day precision	98.82±0.114	99.62±0.068
Analyst-to-Analyst	99.57±0.044	99.57±0.086
Reproducibility	99.04± 0.105	99.35±0.092

***** *Value of five replicates and five concentrations*

3.2. Assay of tablet formulation

The results of the analysis of tablet formulation were reported. The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipients in the estimation of drugs.

Table 6: Assay of tablet formulation

Conc. Present (µg/ml)		% Conc. Found	
DLT	LAM	DLT	LAM
10	5	97.00	99.80
20	10	99.80	99.25
30	15	97.67	99.93
40	20	99.80	99.95
50	25	99.12	99.70

*****Average of three replicates and five concentrations*

4. CONCLUSION

The proposed method for the determination of Lamivudine and Dolutegravir in solid dosage form was found to be precise, selective, rapid and economical. The maximum absorbance of LAM and DLT was observed at 244.0 nm and 262.0 nm, respectively. LAM and DLT showed linearity in the concentration range of 10-50 µg/ml and 5-25 µg/ml at their respective maxima. The recovery of added standards (80 %, 100 % and 120 %) was found at three replicates and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. The proposed method can be used for the drug analysis in routine quality control and method proves to be more economical. The developed method can be adopted in routine analysis of Dolutegravir in bulk or tablet dosage form and it involves relatively low cost solvents and no complex extraction techniques. Hence the proposed method can be effectively applied for the routine quality control analysis of Lamivudine and Dolutegravir in bulk and in tablet dosage form.

Conflict of interest

None declared

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