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RP-HPLC Method Development and Validation of Dolutegravir and Lamivudine in Bulk and Its Formulation

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ABSTRACT
Back ground: *High Performance Liquid Chromatography (HPLC) is at present one of the most sophisticated tools of the analysis.*
Aim: *Dolutegravir and Lamivudine both are anti-viral agent. It is used for treatment of human immunodeficiency virus. The aim of the present research work deals with the analytical method and development and validation of lamivudine and dolutegravir by RP-HPLC as per International Conference on Harmonisation (ICH) guidelines.*
Method: *A novel developed method was established and precise simple economic and less time consuming. The chromatographic separation was achieved on Sun fire C_8 (150X4.6mm) 5µm to select the ideal mobile phase. Among that acetonitrile: potassium dihydrogen orthophosphate (55:45 v/v) was found to be ideal since it gave good resolution and peak shapes with perfect symmetry. The linearity was found to be concentration range of 2-10µg/ml, for dolutegravir and 12-60µg/ml for lamivudine.*
Results: *The correlation coefficient (r^2) was found to be 0.999, 0.999 and 0.999. The retention time of lamivudine and dolutegravir was found to be 1.6 min and 2.6 min respectively. The flow rate was found to be optimized at 1.0ml/min. detection was carried out at 260nm by UV detection. The develop method validate as per ICH guidelines.*
Conclusion: *The developed method was validated for linearity, accuracy, precision, the limit of detection and quantification, specificity. The method was applied successfully for the determination of dolutegravir and lamivudine in combined dosage form.*
Key words: *Lamivudine, dolutegravir, RP-HPLC, ICH guidelines, validation.*

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I. INTRODUCTION

Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter. Lamivudine is chemically 1-[(2R,5S)-2-(hydroxy methyl)-1-3 oxathiolan-5-yl] cytosine and used as an antiretroviral activity. Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B. The molecular formula of lamivudine is $C_8H_{11}N_3O_3S$ and molecular weight is 229.26 g/mol [1,2].

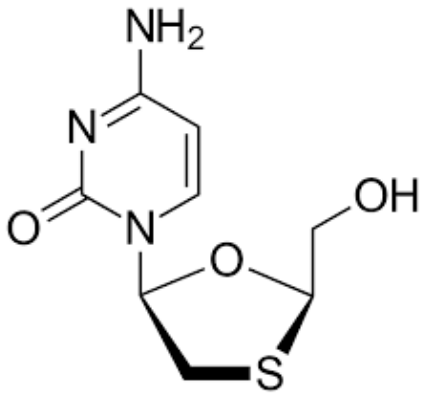

Figure 1: Chemical structure of lamivudine

Dolutegravir is an orally bio-available integrase strand transfer inhibitor (INSTI). It inhibits human immunodeficiency virus (HIV) integrase by binding to the active site and blocking the strand transfer step of retroviral deoxyribonucleic acid (DNA) integration in the host cell. The strand transfer step is essential in the HIV replication cycle and results in viral activity inhibition. If administered orally, it has half-life of approximately 15 h. The IUPAC name of dolutegravir is (3S,7R)-13-[(2,4-difluorophenyl) methyl carbamoyl]-7-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclo [8.4.0.03,8] tetradeca-10,13-dien-11-olate [3-5].

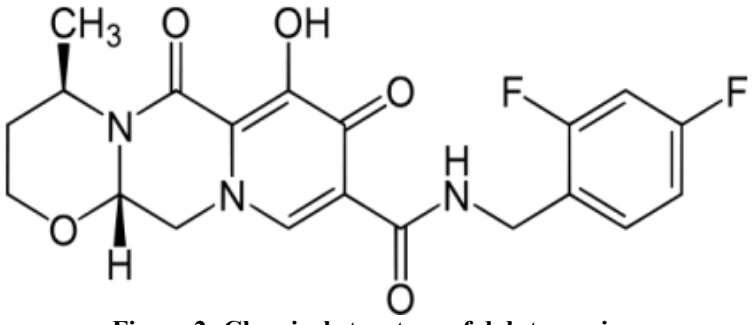

Figure 2: Chemical structure of dolutegravir

USFDA approved three drugs combination therapy (abacavir 600 mg, lamivudine 300 mg, dolutegravir 50 mg) as single pill regimen (combined dosage form) for people living with HIV. Both of these pharmaceuticals are available on the market alone and in conjunction with other medications. The USFDA authorised Lamivudine in conjunction with Dolutegravir Sodium on April 18, 2019. The combo medicine used to manage HIV infection and improve the function of your immune system [6,7].

The present work deals with the analytical method and development and validation of lamivudine and dolutegravir by RP-HPLC as per International Conference on Harmonisation (ICH) guidelines.

MATERIALS AND METHODS [8-14]

The reference standard of dolutegravir and lamivudine were received as gift samples. HPLC grade acetonitrile, and methanol procured from Sigma Aldrich chemicals Pvt. Ltd., Hyderabad. The HPLC Waters instrument was used which consisted of Waters 515 solvent delivery system using a Sun fire C_8 (150×4.6mm) 3.5 µm and Waters 2489 UV detector. The software used was to Empower 2.

Preparation of mobile phase: The isocratic mobile phase was Acetonitrile: 0.05M Potassium Dihydrogen Orthophosphate buffer (55:45).The mobile phase was filtered through a 0.45 µm Millipore filter and degassed by sonication for 15 min.

Buffer preparation: 0.05 M Potassium Dihydrogen Orthophosphate buffer was prepared by dissolving 6.8 g of Potassium Dihydrogen Orthophosphate in 1000 ml of water.

Preparation of standard stock solution: Weighed about 10mg of dolutegravir and lamivudine reference standard and transferred into 10ml volumetric flask and make up the volume with the mobile phase and kept for sonication for 5 min. The concentration was approximately 1000µg/ml. Then Above stock solution was taken and further diluted and make up with mobile phase for 2-10µg/ml of dolutegravir and 12-60 µg/ml in lamivudine.

II. RESULTS AND DISCUSSION

Selection of wavelength

The selection of wavelengths for the analysis of dolutegravir and lamivudine was selected from the UV spectrum of drugs by scanning in the range of 200-400 nm. A UV Spectrum of 2-10µg/ml of dolutegravir and lamivudine both are diluted with acetonitrile and water was recorded. From this, the wavelength of 257 nm for dolutegravir and 271nm for lamivudine. The Isobestic contains 260nm. From the UV spectra, the wavelength was selected as 260nm as it shows good absorbance. Hence, from the spectrum, it was concluded that 260nm is the detection wavelength for the study.

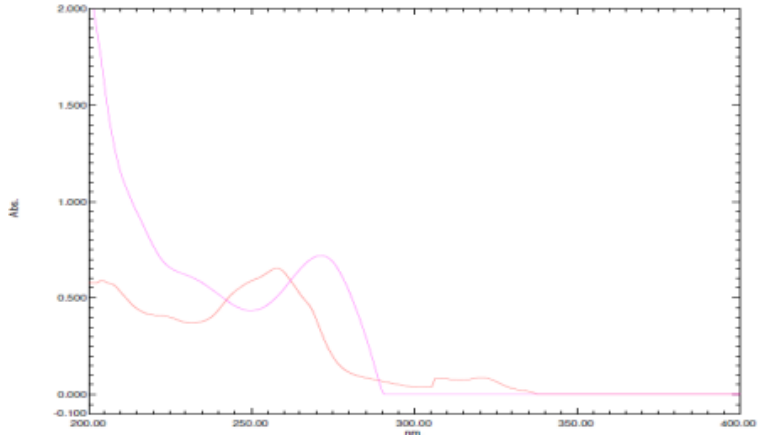

Figure 3: Overlay spectrum of dolutegravir and lamivudine

Optimization of chromatographic condition

Different mobile phases were tried, to select the ideal mobile phase. Among that acetonitrile: potassium dihydrogen orthophosphate (55:45 v/v) was found to be ideal since it gave good resolution and peak shapes with perfect symmetry. The retention time of lamivudine and dolutegravir was found to be 1.6 min and 2.6 min respectively. Stationary phase used was the Sun fire C_8 (150X4.6)5µm column. There was no change in pH done because better results were obtained in mobile phase pH itself. The flow rate was found to be optimized at 1.0ml/min. detection was carried out at 260nm by UV detection.

Table 1: Chromatographic conditions

Stationary phase	Sun fire C_8, (150X4.6mm) 5µm
Mobile phase	Acetonitrile: Potassium dihydrogen orthophosphate
Solvent ratio	55:45
Detection wavelength	260nm
Flow rate	1.0ml/min
Injection volume	20µl
Column temperature	25 ^{0}C
UV-Detector	Waters 2489

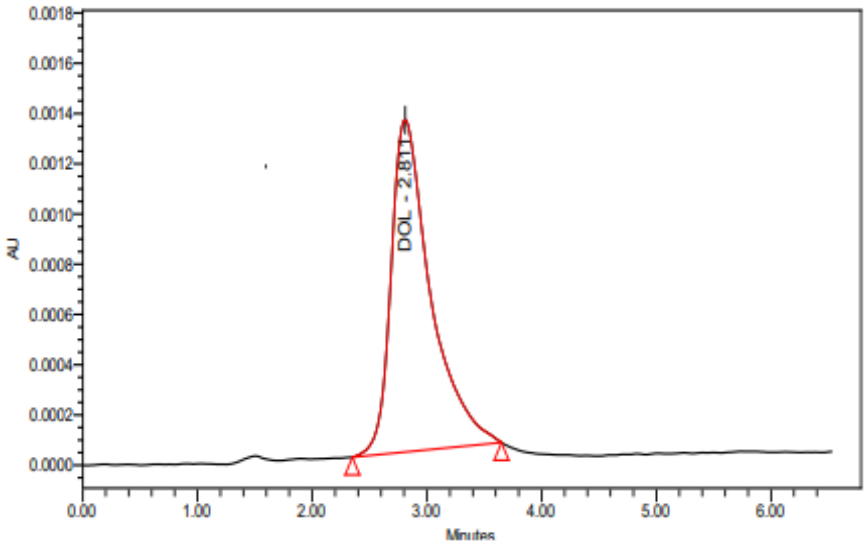

Figure 4: Standard chromatogram of dolutegravir

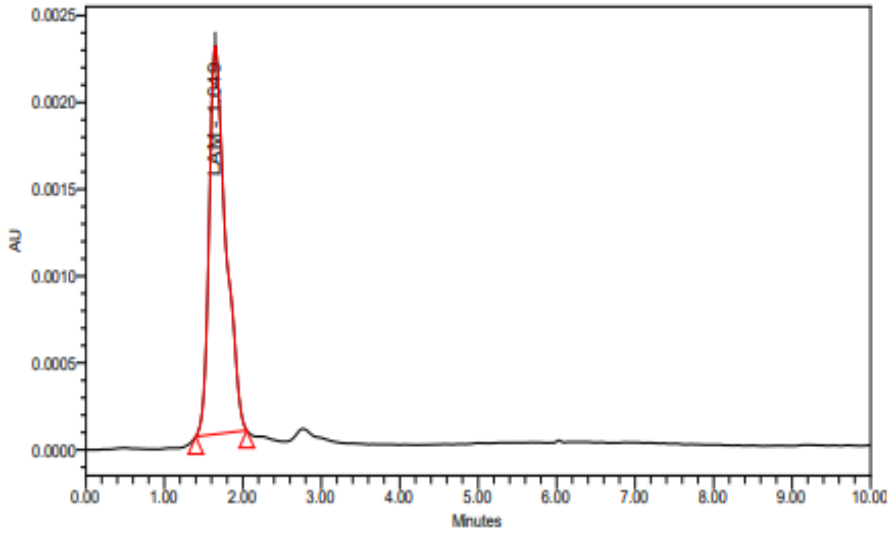

Figure 5: Standard chromatogram of lamivudine

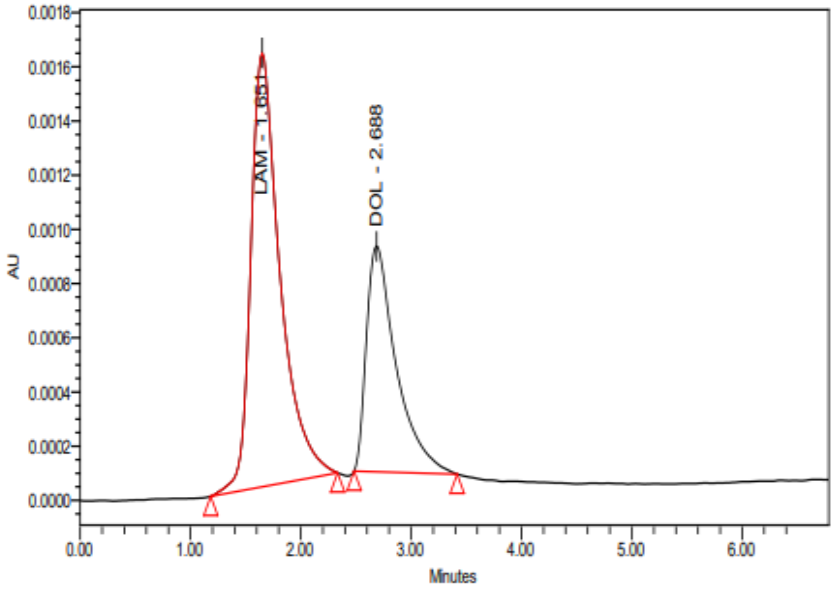

Figure 6: Chromatogram of dolutegravir and lamivudine

Method validation

Linearity

A Calibration curve is a relationship between the instrument response and a known concentration of the analyte. It was observed that the optimized methods were linear within a specific concentration range for individual drugs. The Calibration curve was constructed by plotting the Peak area Vs Concentration of calibration standard. The linear concentration ranges from 2-10µg/ml of dolutegravir and 12-60µg/ml of lamivudine. The correlation coefficient for both the drugs was found to be 0.999 and 0.999.

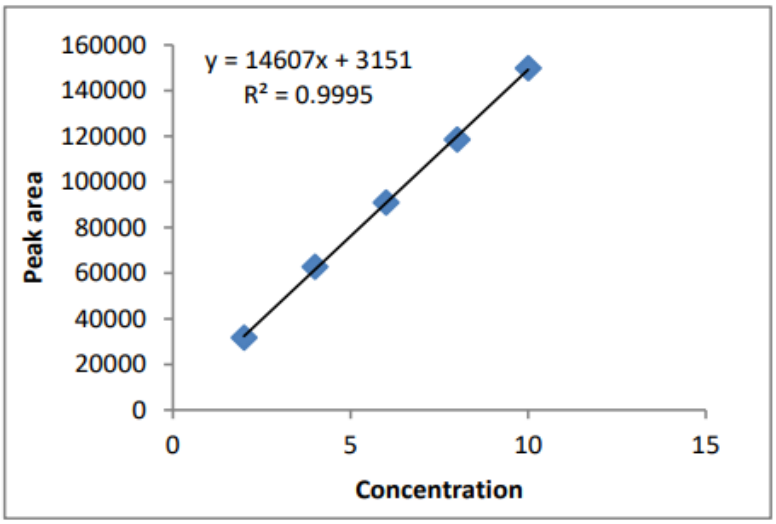

Figure 7: Calibration curve of dolutegravir

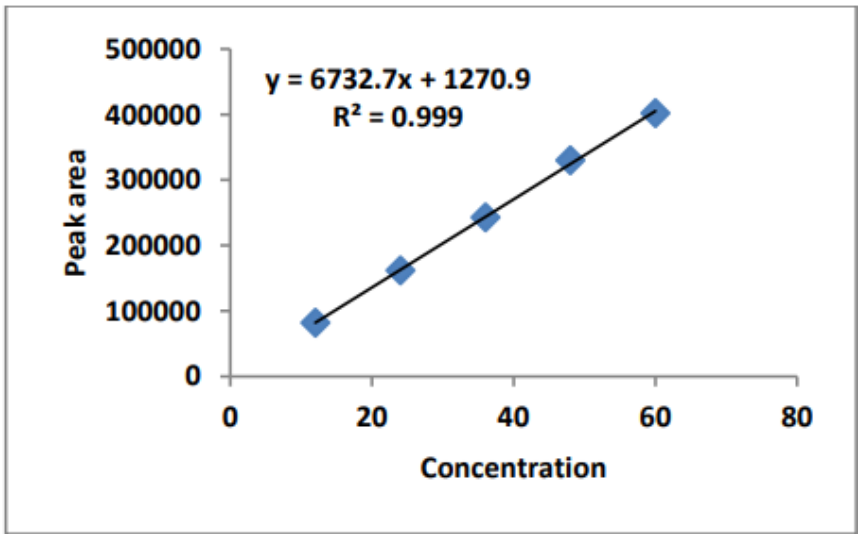

Figure 8: Calibration curve of lamivudine

Precision

Intraday precision: Intraday precision was found by carrying out the analysis at three different concentrations in the linearity range for three times on the same day. % RSD was calculated and the results are represented in Table 2.

Table 2: Intraday precision

Level	Concentration (µg/ml)		Peak area		% RSD	
	Dolutegravir	Lamivudine	Dolutegravir	Lamivudine	Dolutegravir	Lamivudine
I	4	24	62805	161739	0.14	0.39
			62736	162840		
			62922	161741		
II	6	36	90988	242895	0.55	0.80
			91781	246164		
			90843	242681		
III	8	48	118591	329796	0.79	0.26
			117811	328092		
			119682	329382		

Interday precision: Interday precision was found by carrying out the analysis at three different concentrations in the linearity range for three days over a period of one week. % RSD was calculated and the results are represented in Table 3.

Table 3: Interday precision

Level	Concentration (µg/ml)		Peak area		% RSD	
	Dolutegravir	Lamivudine	Dolutegravir	Lamivudine	Dolutegravir	Lamivudine
I	4	24	65712	164542	0.31	0.58
			65830	163841		
			66119	165735		
II	6	36	92853	275562	0.72	0.17
			92710	274653		
			91620	275432		
III	8	48	130771	349782	0.19	0.25
			131082	351380		
			130585	349876		

Limit of detection (LOD) and limit of quantification (LOQ)

The lowest amount of analyte in a sample that can be detected under stated experimental conditions and Limit of quantification is the lowest amount of analyte in the sample that is quantified and is usually established by injecting the lowest concentration of standard solution at which the peak was determined. Preparation of calibration curve from the serial dilution of standard was repeated for six times. The limit of detection and limit of quantification was calculated by using the average value of standard deviation and slope. The LOD and LOQ were determined from the linearity studies and the values were tabulated Table 4.

LOD = 3.3*(SD/slope)
LOQ = 10*(SD /slope)

Table 4: LOD and LOQ

Drugs	Parameters	
	LOD	LOQ
Dolutegravir	0.05	0.15
Lamivudine	0.10	0.30

Assay

20 tablets (each contains 300mg of lamivudine and 50mg of dolutegravir) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Weight equivalent to one tablet powder of lamivudine and dolutegravir was dissolved in sufficient mobile phase. After that filtered the solution using 0.45µm membrane filter under vacuum filtration and sonicated for 5min and dilute to 100ml with mobile phase. Peak area of both standard and test was determined. The percent of assay was calculated from the peak area of standard and sample. The percent of assay was calculated by using the following formula. The results were shown in the Table 5. Acceptance criteria: The % assay should be within 99.00-101.00%.

Table 5: Assay

Drug	Label claim (mg)	% Assay
Lamivudine	300	101.10
Dolutegravir	50	99.94

III. CONCLUSION

An isocratic novel method has been developed for the estimation of Dolutegravir and Lamivudine in bulk. By RP-HPLC coupled UV detector. The above data concluded that the developed method is simple, rapid, and time consuming. This method ability to separate components within 5 min. When compared with pre-reported methods, this method is easier and faster with high specificity and sensitivity and also economical. The developed method is fully validated as per ICH guidelines results show that the method is reliable and acceptable. The main advantage of the proposed methods is suitable for routine analysis.

Competing interest statement

All authors declare that there is no conflict of interests regarding publication of this paper.

Additional information

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Ethical approval

Not required.

REFERENCES

[1]. Priya DS, Sankar DG. Simultaneous stability-indicating method for the determination of abacavir, dolutegravir and lamivudine by RP-HPLC. International journal of pharmaceutical sciences and research. 2016; 7(7):2905.

[2]. Bennetto-Hood C, Tabolt G, Savina P, Acosta EP. A sensitive HPLC–MS/MS method for the determination of dolutegravir in human plasma. Journal of Chromatography B. 2014; 945:225-32.

[3]. Jayakar B, Kumar M, Saravanan C, Kumudhavalli MV. Method development and validation of RP-HPLC method for simultaneous determination of Lamivudine and Zidovudine. J Chem Pharm Res. 2010; 2(1):478-81.

[4]. Induri Madhusudhana Reddy, Raju B. Development and validation of UPLC method for simultaneous estimation of Efavirenz and Lamivudine in pharmaceutical formulations. Journal of Applied Pharmaceutical Science. 2016; 6(3):029-33.

[5]. Ganesh Akula, Srinu Naik Sapavatu, Rajendra Kumar Jadi, Jainendra Kumar Battineni, Narender Boggula. Analytical method development and validation for the estimation of tramadol in bulk and its formulations by UV-spectroscopy. Journal of Advanced Scientific Research. 2021; 12(2):77-83.

[6]. Yusuff I, Vara Prasad MV, Shaheedha SM, Habeeb M. A New Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Dolutegravir and Rilpivirine in Bulk and its Dosage Forms. Iranian Journal of Pharmaceutical Sciences. 2019; 15(4):53-72.

[7]. Thaidala Sriveni, Vanamala Naveen, Vemula Sai Rupa, Aeruva Renuka, Sunil Porika, M Akiful Haque, Vasudha Bakshi, Narender Boggula. Development and Validation of Dolutegravir in Bulk and Formulation: An Anti-Retroviral Drug Using UV-Spectroscopy. International Journal of Pharmaceutical Quality Assurance. 2021; 12(1):57-60.

[8]. Deepali G, Elvis M. UV Spectrophotometric Method for Assay of the Anti-Retroviral Agent Lamivudine in Active Pharmaceutical Ingredient in its Tablet Formulation. Journal of Young Pharmacists. 2010; 2(4):417-9.

[9]. Sharma R, Mehta K. Simultaneous spectrophotometric estimation of tenofovir disoproxil fumarate and lamivudine in three component tablet formulation containing efavirenz. Indian Journal of Pharmaceutical Sciences. 2010; 72(4):527.

[10]. Mirza Shahed PPS, MHG Dehghan, SN Mokale. Simultaneous Spectrophotometric Estimation of Abacavir sulfate and Lamivudine in Tablet Dosage Form. Asian J Research Chem. 2009; 2(4):461-463.

[11]. Devmurari. VP. Simultaneous Spectrophotometric Determination of Lamivudine and Abacavir in the Mixture. Asian J Research Chem. 2010; 3(3):707-709.

[12]. Chaitanya Kallu, Nikhil Raj Dachaboina, Mohammed Sayeed, Naveen Pathakala, Aravinda Nalla, Jithendar Reddy Mandhadi, Sandeep Bolla, Narender Boggula. Development and Validation for Determination of Apremilast in Bulk and in Tablets by UV Spectrophotometer. Asian Journal of Pharmaceutics. 2024; 18(3):841-847.

[13]. Srinivas Lella, Venkateswararao P, Srinivasrao N: A stability indicating RP-HPLC methods for simultaneous estimation of the abacavir, lamivudine and dolutegravir in bulk and pharmaceutical dosage form. International J of Research in Pharmacy and Chemistry 2019; 9(3):146-153.

[14]. Boggula N, Bhadru B, More K. RP-HPLC Method Validation for Levomilnacipran Estimation in Bulk and Formulation. International Journal of Pharmaceutical Quality Assurance. 2023; 14(4):900-903.