S. Tamilarasan et al /J. Pharm. Sci. & Res. Vol. 13(4), 2021, 227-231

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Analytical Method Development and Validation for the Estimation of Dolutegravir and Lamivudine in Bulk Form by RP- HPLC

S. Tamilarasan, S. Malathi*

**Department of Pharmaceutical Analysis, PSG College of Pharmacy*
Coimbatore -641004, Tamil Nadu, India.
malathisanju@gmail.com

Abstract:

Aim: Dolutegravir and Lamivudine both are antiviral agent. It is used for treatment of human immunodeficiency virus. FDA approved for these two regimens. Dolutegravir is an integrase strand transfer inhibitor (INSTI) and Lamivudine is nucleoside analogue reverse transcriptase inhibitor (NRTI).

Method: A newly 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. 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.

Keywords: Dolutegravir, Lamivudine, RP-HPLC, ICH

INTRODUCTION

Chromatography, a separation technique, is mostly used in chemical analysis. High-performance liquid chromatography (HPLC) is an extremely versatile technique. Where analytes are separated by passage through a column packed with micrometer-sized particles. The reversed-phase chromatography is commonly used separation technique in HPLC [1-5].

Dolutegravir

IUPAC name have (4R,12aS)-N-(2,4-Difluorobenzyl)-7-hydroxy-4- methyl-6,8-dioxo-3, 4, 6, 8, 12,12a-hexahydro- 2H-pyrido (1:,2':4,5) pyrazino (2,1-b) (1,3) oxazine-9-carboxamide. The molecular formula of $C_{20}H_{19}F_2N_3O_5$ and molecular weight is 419.4gm/mol. Generally soluble in methanol, dimethylformamide (DMF), Dimethylsulphoxide (DMSO).The chemical structure shown in Fig.1

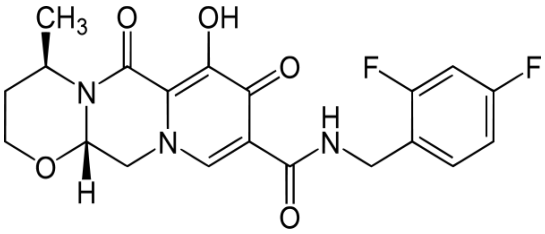

Fig. 1. Structure of Dolutegravir

Lamivudine

IUPAC name have 4-amino-1- [(2R, 5S)-2-(hydroxymethyl) - 1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2one.The molecular formula of $C_8H_{11}N_3O_3S$ and molecular weight is 229.26gm/mol. Generally soluble in Water, ethanol, methanol, dimethylformamide (DMF), Acetonitrile. The chemical structure shown in Fig.2

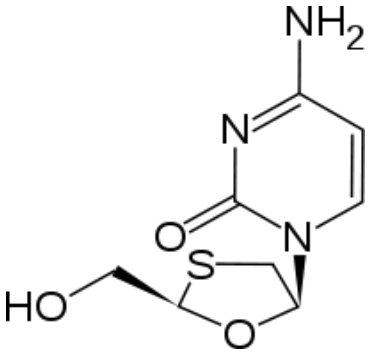

Fig. 2. Structure of Lamivudine

MATERIALS AND METHODS:

The reference standard of Dolutegravir and Lamivudine were received from Macleod's Pharmaceuticals Ltd, Himachal Pradesh respectively. HPLC grade acetonitrile, Methanol and methanol procured from Sigma Aldrich chemicals Pvt Ltd, Bangalore. Potassium dihydrogen orthophosphate received from Himedia laboratory Pvt. Ltd, Mumbai. The HPLC Waters instrument was used which consisted of Waters 515 solvent delivery system using a Sun fire C8 (150×4.6mm) 3.5 µm and Waters 2489 UV detector. The software used was to Empower 2. The mobile phase was sonicated using the Ultra Sonic Sonicator. The wavelength was fixed utilizing UV1650PC, Shimadzu.

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 of Dolutegravir:
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 minutes. The concentration was approximately 1000µg/ml. Then Above stock solution was taken and further diluted and make up with mobile phase for2-10µg/mL of Dolutegravir and 12-60 µg/mL in Lamivudine.

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 Dolutegrvir and 271nm for Lamivudine. The Isobestic contains 260nm. From the UV spectra, the wavelength was selected as 260nm (Fig 3) as it shows good absorbance. Hence, from the spectrum, it was concluded that 260nm is the detection wavelength for the study.

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 C8 (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.

Fixed Chromatographic Condition

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 :	$25^{O}C$
UV-Detector	**:** Waters 2489

Method Validation
The developed method validation parameters were employed by ICH Guidelines

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.

Precision
Precision of the method was demonstrated by
1. Intraday precision
2. Interday precision
3. Repeatability

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.1**

Table No: 1. Intra - Day precision

Level	Concentration(µg/ml)		Peak area		%RSD	
	DOL	LAM	DOL	LAM	DOL	LAM
I	4	24	62804	161738	0.14	0.39
			62737	162841		
			62921	161742		
II	6	36	90987	242894	0.55	0.80
			91782	246165		
			90843	242682		
III	8	48	118592	329796	0.79	0.26
			117810	328093		
			119682	329381		

Table No: 2. Inter - Day precision

Level	Concentration(µg/ml)		Peak area		%RSD	
	DOL	LAM	DOL	LAM	DOL	LAM
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		

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.2.**

Repeatability

Standard solution of mixture of drug was injected five times and its %RSD was calculated and the result represented in **Table.3**.

Table No: 3. Repeatability of injection

Concentration(µg/ml)		Peak area		%RSD	
DOL	LAM	DOL	LAM	DOL	LAM
6	36	92857	242761	0.50	0.47
		92662	242961		
		93864	242901		
		92668	239989		
		92877	242700		
		92648	242789		

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 no: 4. LOD &LOQ

Drugs	Parameters	
	LOD	LOQ
DOL	0.05	0.15
LAM	0.10	0.30

System suitability studies

System suitability parameters like plate number, peak asymmetric factor, capacity factor, selectivity factor, resolution factor is calculated with the help of standard chromatogram. **Table.5**

Table no: 5. System suitability

Parameters	Drugs	
	DOL	LAM
Tailing factor	1.0	1.0
Resolution	1.03	
Retention time	2.6min	1.6min

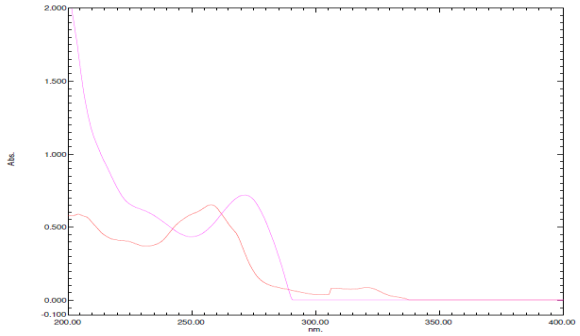

Fig.3. Overlay Spectrum of Dolutegravir and Lamivudine

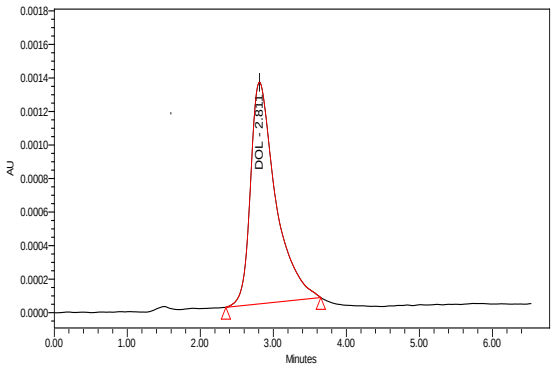

Fig.4. Standard Chromatogram of Dolutegravir

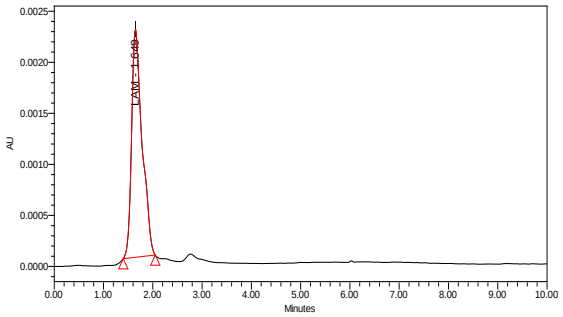

Fig.5. Standard Chromatogram of Lamivudine

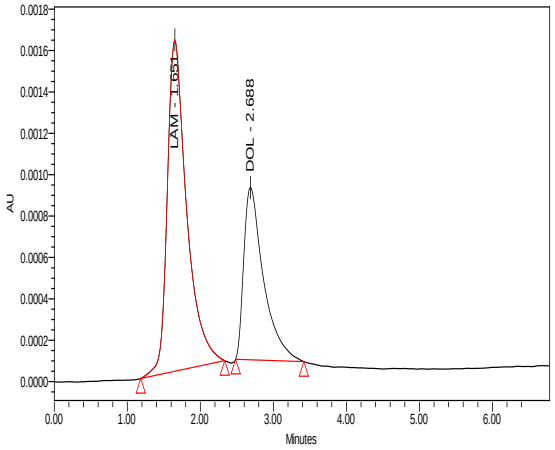

Fig.6. Chromatogram of Bulk (Dolutegravir, Lamivudine)

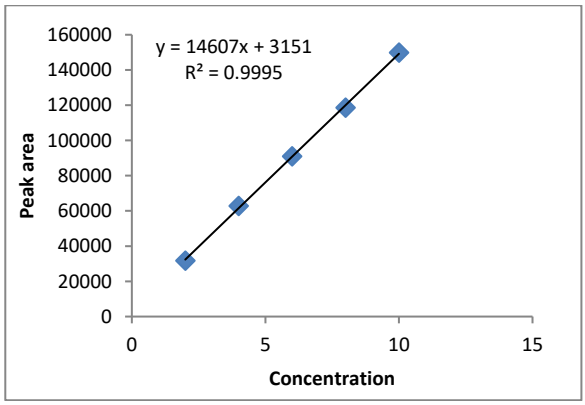

Fig.7. Calibration Curve of Dolutegravir

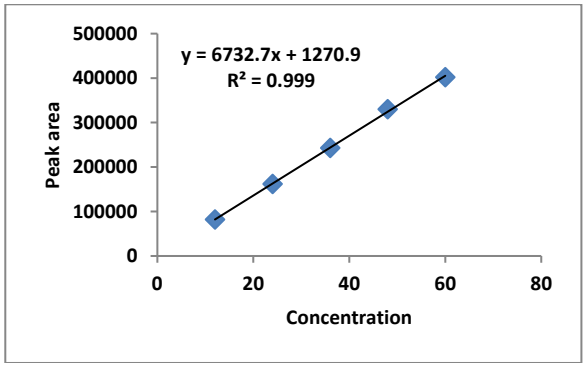

Fig.8. Calibration Curve of Lamivudine

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 minutes. 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.

REFERENCES

1. A.H Beckett and J.BStenlake, A practical pharmaceutical chemistry, CBS publishers &distributors, fourth edition-part two, Page no: 284,285.
2. L.R. Snyder, J.J. Kirkland and J.L. Glajch, Practical HPLC Method Development, Wiley, edn2 (1997).
3. Anonymous, Guide for Industry. Bioanalytical method validation, U.S Department of Health and Human services Food and Drug Administration (FDA), Vol 2,191-210, 2001,
4. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, *Geneva*, March 1996
5. Veeraswami B, Naveen V. Development and validation of RP-HPLC method for the estimation of dolutegravir and rilpivirine in bulk and pharmaceutical dosage form and its application to rat plasma development. *Asian Journal of Pharmaceutical and Clinical Research*, Vol. 12, no. 2, Jan. 2019, pp. 267-71.
6. Dubey SO, Duggirala MA. Simultaneous estimation of lamivudine, abacavir and dolutegravir by UPLC method. Int. J. App. Pharm. 2018; 10(1):46-52.
7. AlffenaarJW. Development and Validation of a Bioanalytical Method for the Simultaneous Determination of 14 Antiretroviral Drugs using Liquid Chromatography-Tandem Mass Spectrometry. Journal of Applied Bionalysis. 2018 Apr15; 4(2):37.
8. Saidulu P, Mastanamma SK. Stability indicating gradient RP-HPLC method for the simultaneous estimation of lamivudine, abacavir and dolutegravir in bulk and their combined dosage form. Int. J. Pharm. Sci. Re. 2016; 37(2).
9. Kalpana T, Rajeswari DT, Ganji RR. Development and validation of analytical method for determination of Dolutegravir sodium, Lamivudine and tenofovir disoproxil fumarate using reverse phase high performance liquid chromatography. Der Pharma Chemica. 2017; 9(8):117-27.
10. Singh VD, Sanjay JD. Optimization of an RP-HPLC method for simultaneous estimation of lamivudine and raltegravir in the binary mixture by using design of the experiment. Eurasian J Anal Chem. 2012; 12:179-95.
11. Devanna N, Dudekula B, Ramachandraiah C. Development and validation for the simultaneous estimation of dolutegravir and lamivudine in drug product by RPHPLC. International Journal of Research in Pharmaceutical and Nano Sciences. 2017; 6(4).
12. Nekkala K, Kumar VS, Ramachandran D. Development and Validation for the Simultaneous Estimation of Lamivudine, Tenofovir Disproxil and Dolutegravir in Drug Product by RP-HPLC. Journal of Pharmaceutical Sciences and Research. 2017 Sep 1; 9(9):1505.
13. Cozzi V, Charbe N, Baldelli S, Castoldi S, Atzori C, Cattaneo D, Clementi E. Development and validation of a chromatographic ultraviolet method for the simultaneous quantification of dolutegravir and rilpivirine in human plasma. Therapeutic Drug Monitoring. 2016 Jun 1; 38(3):407-13.
14. 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 Jul 1; 7(7):2905.
15. Rao NM, Sankar DG. Development and validation of stability-indicating HPLC method for simultaneous determination of Lamivudine, Tenofovir, and Dolutegravir in bulk and their tablet dosage form. Future Journal of Pharmaceutical Sciences. 2015 Dec

1; 1(2):73-7.
16. 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 Jan 15; 945:225-32.
17. 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.
18. Dhanwate SS, Pachauri AD, Ghode PD, Khandelwal KR. Development and validation of analytical method for the estimation of Lamivudine and Dolutegravir sodium in dosage form. Journal of Pharmaceutical Sciences and Research. 2019 Aug 1; 11(8):2886-90.
19. Pal N, Rao AS, Ravikumar P. Simultaneous HPLC method development and validation for estimation of Lamivudine, Abacavir and Dolutegravir in combined dosage form with their stability studies. Asian journal of chemistry. 2016 Jan 1; 28(2):273.
20. Fatima SS, Nagaraju P, Mounika V, Priyadarshini GI, Naik VV. Stability-indicating method development and validation of Rp-Hplc method for simultaneous estimation of lamivudine, abacavir, dolutegravir in pharmaceutical dosage forms. Indo American journal of pharmaceutical sciences. 2017 Feb 1; 4(2):359-67.
21. VeereshamCiddi, Quantitative Bio-Analysis of Tenofovir Disoproxil Fumarate, Lamivudine and Efavirenz Simultaneously in Human Plasma Using Reverse-Phase Liquid Chromatography". Acta Scientific Pharmaceutical Sciences 2.10 (2018): 17-27.
22. Mastanamma S, Jyothi JA, Saidulu P, Varalakshmi M. Development and validation of RP-HPLC method for the simultaneous estimation of Lamivudine, Tenofovir Alafenamide and Dolutegravir bulk and their combined dosage form. Pharm Methods. 2018 Jul 1; 9:49-55.
23. Wang X, Penchala SD, Amara A, Else L, McClure M, Boffito M. A validated method for quantification of dolutegravir using ultra performance liquid chromatography coupled with UV detection. Therapeutic Drug Monitoring. 2016 Jun 1;38(3):327-31
24. 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 Oct 1; 15(4):53-72.
25. Balasaheb BG, Balasaheb AK, Subhash TR, Jijabapu K, Sudhakar PS. Development and validation of UV spectrophotometric method for estimation of dolutegravir sodium in tablet dosage form. Malaysian Journal of Analytical Sciences. 2015; 19(6):1156-63.
26. Nalini CN, Nithya A, Afreen A, Banu AA, Hemalatha P, Gayathri EJ. Analytical Methods for Determination of Lamivudine and their Applicability in Biological Studies. Research Journal of Pharmacy and Technology. 2018; 11(11):5166-72.
27. Sarat M, Krishna PM, Rambabu C. Development and Validation of RP-UPLC Method for Simultaneous Estimation of Abacavir Sulphate and Lamivudine in Combined Tablet Dosage Form. International Journal of ChemTech Research. 2012 Jul; 3(4):939-44.
28. https://www.drugbank.ca/drugs/DB08930.
29. Indian Pharmacopeia 2018, Volume II, Published by the Indian Pharmacopoeia Commission, pg. 2376 - 2385.