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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DOLUTEGRAVIR AND LAMIVUDINE IN DRUG PRODUCT

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ABSTRACT

The aim of the method was to develop and validate a rapid, sensitive and accurate method for simultaneous estimation of Lamivudine and Dolutegravir in drug product by liquid chromatography. The chromatographic separation was achieved on the C18 column (Inertsil ODS 3V 250*4.6, 5um) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Trifluoroacetic acid in water: Acetonitrile (35:65). The flow rate was 0.8ml/ minute and ultraviolet detector at 260nm. The average retention time for Lamivudine and Dolutegravir found to be 2.984 min and 4.340 min. The proposed method was validated for selectivity, precision, linearity, and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 300.0 – 900.0µg/mL for Lamivudine and 50.0 - 150.0µg/mL of Dolutegravir.

KEYWORDS: Lamivudine, Dolutegravir, Isocratic, HPLC, Phenyl, Trifluoroacetic acid, Acetonitrile, Methanol, and validation.

1. INTRODUCTION

Lamivudine

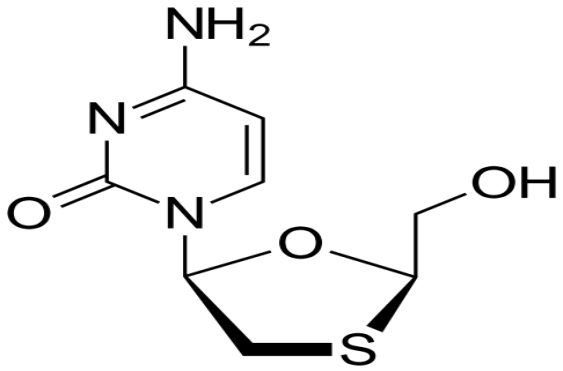


Fig. 1: Chemical structure: Lamivudine.

Lamivudine, commonly called **3TC**, is an antiretroviral medication used to prevent and treat HIV/AIDS. It is also used to treat chronic hepatitis B when other options are not possible. It is effective against both HIV-1 and HIV-2. It is typically used in combination with other antiretrovirals such as zidovudine and abacavir. Lamivudine may be included as part of post-exposure prevention in those who have been potentially exposed to HIV. Lamivudine is taken by mouth as a liquid or tablet.

Lamivudine is chemically designated as 4-Amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. Its molecular formula is $C_8H_{11}N_3O_3S$, and its molecular weight is 229.26 g/mol.

Dolutegravir

Dolutegravir (**DTG**) is a medication used for the treatment of HIV infection. Dolutegravir is an integrase inhibitor. The drug is marketed as **Tivicay** by Glaxo Smith Kline (GSK). Dolutegravir is approved for use in a broad population of HIV-infected patients. It can be used to treat HIV-infected adults who have never taken HIV therapy (treatment-naïve) and HIV-infected adults who have previously taken HIV therapy (treatment-experienced), including those who have been treated with other integrase strand transfer inhibitors. Tivicay is also approved for children ages 12 years and older weighing at least 40 kilograms (kg) who are treatment-naïve or treatment-experienced but have not previously taken other integrase strand transfer inhibitors.

Dolutegravir is chemically designated as Isopropyl (4*R*,12a*S*)-*N*-(2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2':4,5] pyrazino[2,1-*b*][1,3] oxazine-9-carboxamide. Its molecular formula is $C_{20}H_{19}F_2N_3O_5$, and its molecular weight is 419.38 g/mol.

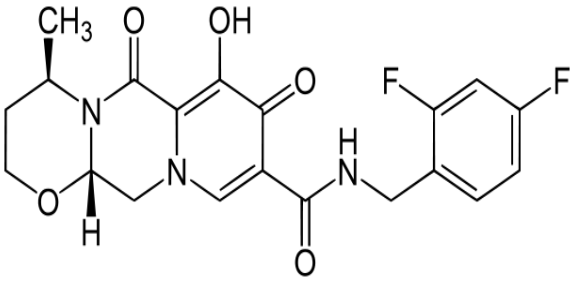


Fig.2. Chemical structure: Dolutegravir.

2. MATERIALS AND METHODS

2.1 Equipments: The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase Phenyl column (Eclipse XDB-Phenyl 250*4.6, 5µm) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance and Vacuum microfiltration unit with a 0.45µ membrane filter.

2.2 Materials: Pharmaceutically pure sample of Lamivudine/Dolutegravir were obtained as gift samples from Fortune pharma training institute, Sri Sai Nagar Colony, KPHB, Hyderabad, India.

HPLC-grade Methanol and Acetonitrile were obtained from qualigens reagents Pvt Ltd. Trifluoroacetic acid (AR grade) was from sd fine chem.

2.3 Chromatographic conditions The sample separation was achieved on an (Eclipse XDB-Phenyl 250*4.6, 5µm) Phenyl column, aided by mobile phase mixture of 0.1%v/v Trifluoroacetic acid in water: Acetonitrile (35:65). The flow rate was 0.8 ml/ minute and ultraviolet detector at 260nm that was filtered and degassed prior to use, Injection volume is 10 µl and ambient temperatures.

Preparation of mobile phase

Buffer Preparation: Taken accurately 1ml of Trifluoroacetic acid in 1000mL of water.

Mobile phase: Then added 35 volumes of buffer and 65 volumes of Acetonitrile mixed well and sonicated for 5 min.

Diluents: Water: Acetonitrile: 50:50 v\v

2.4 Preparation of solutions

2.4.1 Standard solution: 150mg of pure Lamivudine and 25mg of Dolutegravir were weighed and transferred to 25 ml of volumetric flask and dissolved in the diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 1ml of solution is pipetted out into a 10 ml volumetric flask and volume was made up to mark with water to give a solution containing 600.0µg/ml of Lamivudine and 100.0µg/ml Dolutegravir.

2.4.2 Preparation of sample solution: Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 150mg of Lamivudine and 25mg of Dolutegravir sample and transferred to 25 ml of volumetric flask and dissolved in the diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 1ml of solution is pipetted out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 600.0µg/ml of Lamivudine and 100.0µg/ml Dolutegravir.

2.5 Method validation

2.5.1. System suitability

The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for system

2.5.2. Linearity

Linearity was studied by analyzing five standard solutions covering the range of 300.0 -900.0µg/ml for Lamivudine and 50.0 -150.0µg/ml Dolutegravir. From the primary stock solution 0.5ml, 0.75ml, 0.1ml, 1.25ml, 1.50 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 300.0 µg /mL, 450.0µg/mL, 600.0µg/mL, 750.0µg/mL and 900.0µg/mL of Lamivudine and 50.0g/mL, 75.0µg/mL, 100.0µg/mL, 125.0µg/mL and 150.0µg/mL of Dolutegravir.

A calibration curve with concentration verses peak areas was plotted by injecting the above-prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

2.5.3. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve.

$LOD = 3.3\ \delta/S$

$LOQ = 10\ \delta/S$

Where,

δ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

2.5.4. Method precision

The precision of the method was checked by repeated preparation (n=6) of 600.0µg/ml of Lamivudine and 100µg/ml Dolutegravir without changing the parameter of the proposed chromatographic method. And measured the peak areas and retention times.

2.5.5. Accuracy
The accuracy of the method was determined by calculating the recoveries of Lamivudine and Dolutegravir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Lamivudine and Dolutegravir.

2.5.6. Robustness
Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min.

3. RESULTS AND DISCUSSIONS
Determination of Working Wavelength (λ max): 10 mg of the Lamivudine and Dolutegravir standard drug is taken in a 10 ml volumetric flask and dissolved in diluent and volume made up to the mark, from this solution 0.1ml is pipette into 10 ml volumetric flask and made up to the mark with the Water to give a concentration of 10 µg/ml. The above-prepared solution is scanned in UV between 200-400 nm using Water as blank. The λmax was found to be 260nm.

After several initial trails with mixtures of methanol, water, Acetonitrile and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Trifluoroacetic acid in water: Acetonitrile (35:65). At flow rate was 0.8mL/ minute brought sharp peaks. The chromatogram was shown in Fig 3**.**

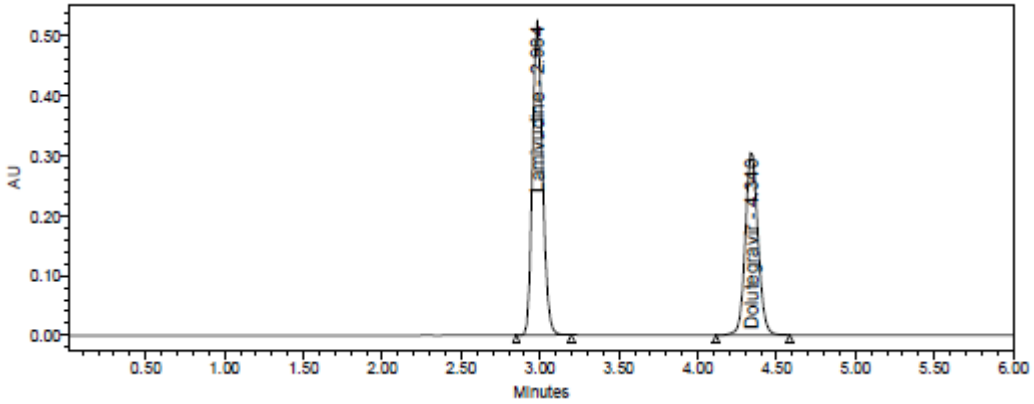


Fig. 3: Chromatogram of Lamivudine and Dolutegravir.

System suitability
The system suitability of the method was checked by repeated preparations for Dolutegravir and Lamivudine. The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for the system, System suitability data of Dolutegravir and Lamivudine are shown in Table 1.

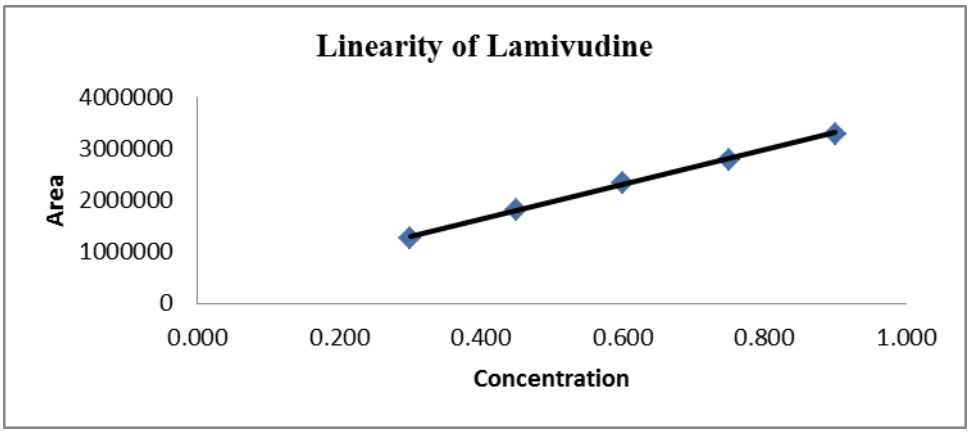
Table 1: System suitability data of Lamivudine and Dolutegravir.

Parameter	Lamivudine	Dolutegravir	Acceptance criteria
Retention time	2.984	4.342	+-10
Theoretical plates	10234	14485	>3000
Tailing factor	1.13	1.07	<1.50
% RSD	0.24	0.82	<2.00

Linearity
Linearity was studied by analyzing five standard solutions covering the range of 300.0 -900.0µg/ml for Lamivudine and 50.0 -150.0µg/ml Dolutegravir. From the primary stock solution 0.5ml, 0.75ml, 0.1ml, 1.25ml, 1.50 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 300.0 µg /mL, 450.0µg/mL, 600.0µg/mL, 750.0µg/mL and 900.0µg/mL of Lamivudine and 50.0g/mL, 75.0µg/mL, 100.0µg/mL, 125.0µg/mL and 150.0µg/mL of Dolutegravir in Table 2 and Table 3.

A linear relationship between peak areas versus concentrations was observed for Lamivudine and Dolutegravir in the range of 50% to 150% of nominal concentration. The correlation coefficient was 0.9993 and 0.9995 for Lamivudine and Dolutegravir.

A



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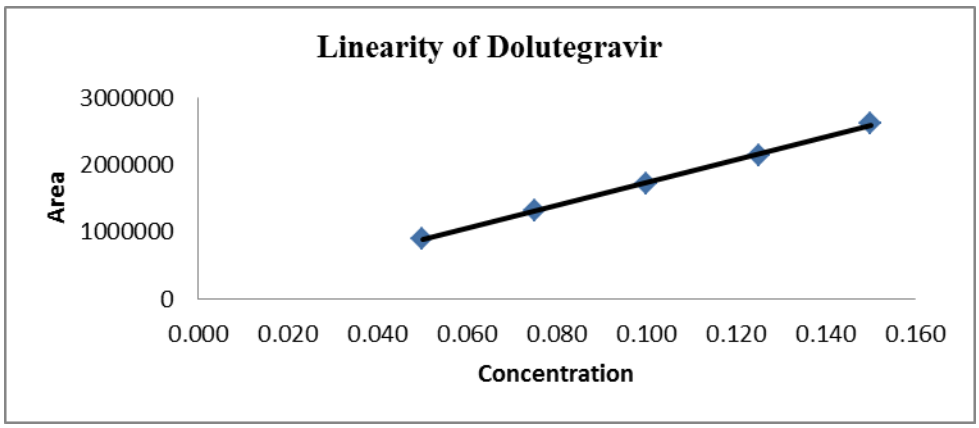

Fig. 4: Calibration curve: (A) Lamivudine: (B) Dolutegravir.

Table 2: Linearity data of Lamivudine.

Level	Concentration (mg/mL)	Peak area
50%	0.30	1265839
75%	0.45	1818420
100%	0.60	2345003
125%	0.75	2786044
150%	0.90	3293018
Correlation		**0.9993**

Table 3: Linearity data of Dolutegravir.

Level	Concentration (mg/mL)	Peak area
50%	0.05	906024
75%	0.75	1313084
100%	0.1	1724552
125%	0.125	2140755
150%	0.150	2619121
Correlation		**0.9995**

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

LOD = 3.3 σ /S ……. (1)

LOQ =10 σ /S …….. (2)

Where,

σ = the standard deviation of the response (STEYX)

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Table 4: LOD and LOQ values Calculated from the calibration curve.

	Lamivudine mg	Dolutegravir mg
LOD	0.035	0.005
LOQ	0.105	0.015

Method precision (repeatability)

The precision of the method was checked by repeated preparation (n=6) of 600.0µg/ml of Lamivudine and 100.0µg/ml Dolutegravir without changing the parameter of the proposed chromatographic method. And measure the peak areas and retention times. The precision of the method (% RSD) was found to be <1% showing good repeatability. The values of percentage RSD for Lamivudine and Dolutegravir are shown in Table 5 and Table 6.

Table 5: Summary of peak areas for method precision of Lamivudine.

Sample. NO	Retention time	Peak area	% Assay
1	2.984	2378719	98.8
2	2.983	2321042	99.6
3	2.984	2329586	100.2
4	2.983	2319944	99.6
5	2.984	2322890	98.9
6	2.984	2398834	99.6
Mean	2.984	2345169	99.5
%RSD	**0.02**	**1.47**	**0.53**

Table 6: Summary of peak areas for method precision of Dolutegravir.

Sample No	Retention time	Peak area	% Assay
1	4.343	1724831	99.9
2	4.34	1706302	98.9
3	4.34	1773083	100.3
4	4.340	1727906	99.5
5	4.342	1737634	100.1
6	4.342	1776207	99.9
Mean	4.341	1740994	99.8
%RSD	**0.03**	**1.61**	**0.52**

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Lamivudine and Dolutegravir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Lamivudine and Dolutegravir. The percentage recovery results obtained are listed in Table 7 & 8.

Table 7: Recovery data of Lamivudine.

LEVEL	S.NO	%Recovery of Lamivudine	Average
50	1	99.1	99.3%
	2	99.2	
	3	99.4	
100	1	98.8	99.5%
	2	99.6	
	3	100.2	
150	1	99.1	99.1%
	2	98.6	
	3	99.8	

Table 8: Recovery data of Dolutegravir.

LEVEL	S.NO	%Recovery of Dolutegravir	Average
50	1	99.0	99.3%
	2	99.2	
	3	99.6	
100	1	99.9	99.7%
	2	98.9	
	3	100.3	
150	1	99.4	99.5%
	2	99.1	
	3	99.9	

Robustness: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table 9&10) the results of Robustness of the present method had shown that changes are not significant was found to be the method is Robust.

Table 9: Results of Lamivudine.

parameter	Rt of Lamivudine	Theoretical plates	Asymmetry
Decreased flow rate (0.7ml/min)	3.397	10969	1.14
Increased flow rate (0.9ml/min)	2.655	9002	1.12
Wave Length 258nm	2.984	10221	1.13
262nm	2.985	10246	1.12

Table 10: Results of Dolutegravir.

parameter	Rt of Dolutegravir	Theoretical plates	Asymmetry
Decreased flow rate (0.7ml/min)	4.945	15107	1.02
Increased flow rate (0.9ml/min)	3.867	12644	1.02
Wave Length 258nm	4.340	14541	1.09
262nm	4.345	14353	1.04

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The results were shown in Table 11&12. The %RSD assay values between two analysts were calculated, this indicates the method was rugged.

Table 11: Ruggedness data for Lamivudine.

		%Assay	%RSD
Analyst-1	**LAMIVUDINE**	98.8	0.57%
Analyst-2		99.6	

Table 12: Ruggedness data for Dolutegravir.

		%Assay	%RSD
Analyst-1	**DOLUTEGRAVIR**	99.9	1.00%
Analyst-2		98.5	

CONCLUSION

From the above experimental results it was concluded that, newly developed method for the simultaneous estimation of LAMIVUDINE and DOLUTEGRAVIR was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost-effective and it can be effectively applied for routine analysis in research institutions, quality control department in pharmaceutical industries, approved testing laboratories.

REFERENCES

1. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, October 1994.
2. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March 1996.
3. http://www.ich.org/
4. https://en.wikipedia.org/wiki/Dolutegravir
5. https://www.drugbank.ca/drugs/DB08930
6. https://en.wikipedia.org/wiki/Lamivudine
7. https://www.drugbank.ca/drugs/DB00709
8. Development and validation of stability-indicating HPLC method for simeltaneous determination of Lamivudine, Tenofovir, and Dolutegravir in bulk and their tablet dosage form. Nagasarapu Mallikarjuna Raoa. Dannana Gowri Sankar.
9. Development and Validation of Analytical Method for Determination of Dolutegravir Sodium, Lamivudine and Tenofovir Disoproxil Fumarate Using Reverse Phase High Performance Liquid Chromatography Talari Kalpana, Dr. Tiruveedula Raja Rajeswari, Ramana Reddy Ganji.
10. Simultaneous HPLC Method Development and Validation for Estimation of Lamivudine, Abacavir and Dolutegravir in Combined Dosage Form with their Stability Studies, Narottam Pal.
11. Simultaneous stability-indicating method for the determination of abacavir, dolutegravir and lamivudine by rp-hplc Sindu Priya* and D. Gowri Sankar.