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Research Article

STABILITY INDICATING DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND DOLUTEGRAVIR RP-HPLC METHOD

[1]Addagetala Sowjanya, [2]Dr.B.Rajkamal

[1]M. Pharmacy, Department of Pharmaceutical Analysis, Malla Reddy Institute of Pharmaceutical science, Maisammaguda, Secunderabad, Hyderabad, 500100, Medchal District.

[2]Professor and Principal, Department of Pharmaceutical Analysis, Malla Reddy Institute of Pharmaceutical science, Maisammaguda, Secunderabad, Hyderabad, 500100, Medchal District.

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ABSTRACT:

Lamivudine and Dolutegravir were developed with Std Discovery 250 x 4.6 mm, 5m. Buffer-containing MP OPA :MeCN in the 55:45 ratio was poured across the column at 1 ml/min. This procedure employed 0.1% Perchloric acid buffer. The temperature was 30°C. Optimised wavelength was 230 nm. Lamivudine and Dolutegravir had 2.146 and 2.770 min retention times. Lamivudine and Dolutegravir had 0.4 and 0.5 RSD. %Recovery was 100.09% for Lamivudine and 100.62% for Dolutegravir. Lamivudine and Dolutegravir regression equations yielded LOD, LOQ values of 0.24, 0.73, and 0.15, 0.45. The regression equations for Lamivudine and Dolutegravir are y = 91520.x + 1773.9 and y = 179637x + 22360, respectively. Reduced retention and run time for better method development.

Key Words: Lamivudine and Dolutegravir, RP – HPLC.

INTRODUCTION

Worldwide the human immunodeficiency virus (HIV-1) affects more than 35 million people. Over the past decades, antiretroviral (ARV) therapy has made remarkable strides improving the quality-of-life of infected individuals. However, viral resistance to therapy continues to develop, highlighting the need for novel classes of ARVs directed at alternative targets. From the currently 28 FDA-approved drugs used in ARV therapy, none of them directly inhibits viral capsid stability [1]. Given that the proper capsid assembly and disassembly so finely regulates HIV-1 replication, it represents an emerging and very attractive target for drug development.

HIV-1 capsid plays a critical role in both early and late stages of the viral replication cycle. Early on, soon after viral entry and fusion of the viral and target cell membranes, the capsid core is released into the cytoplasm and disassembles by a process referred to as uncoating. Although uncoating is not completely understood, it is tightly connected with reverse transcription of the viral genome and subsequently with the nuclear import of viral DNA.[2–4]

Background

Lamivudine: Lamivudine is a reverse transcriptase inhibitor used to treat HIV and hepatitis B infections. It is chemically known as 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one.[5]

Address for Correspondence: Addagetala Sowjanya**,** M. Pharmacy, Department of Pharmaceutical Analysis, Malla Reddy Institute of Pharmaceutical science, Maisammaguda, Secunderabad, Hyderabad, 500100, Medchal District.; **E-Mail:** addagetalasowjanya1@gmail.com.

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Dolutegravir: It is an HIV-1 integrase inhibitor that blocks the strand transfer step of the integration of the viral genome into the host cell (INSTI)6. It is chemically known as (3S,7R)-N-[(2,4-difluorophenyl)methyl]-11-hydroxy-7-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclo[8.4.0.0^{3,8}]tetradeca-10,13-diene-13-carboxamide.7

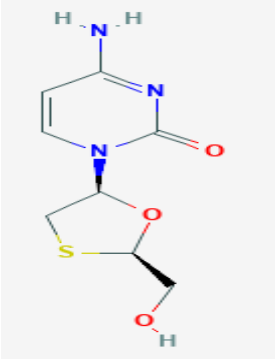

Figure 1: structure of Lamivudine

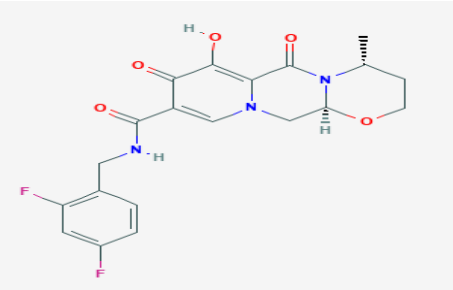

Figure 2: Structure of Dolutegravir

An extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Lamivudine, Dolutegravir, and their medicinal dose form using RP-HPLC.[8-13] must be validated and developed as per ICH guidelines.

Materials and Methods

Spectrum pharma Research Solution provide with Lamivudine and Dolutegravir pure drugs (API) gift samples and Combination Lamivudine and Dolutegravir tablets (Dovato) received from local market. The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation

The development and method validation were conducted using a WATERS HPLC, specificaly the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective:

Developing a reliable analytical approach for the simultaneous measurement of Lamivudine and Dolutegravir in their pure state and during tablet manufacture is the main focus of this work. The method must be precise, accurate, sensitive, specific, consistent, and efficient.

Table 1: Chromatographic Conditions:

Mobile phase	0.1% TEA: Acetonitrile (60:40 v/v)
Flow rate	1 ml/min
Column	Agilent C18 (4.6 x 150mm, 5µm)
Wave length	265 nm
Column temperature	26°C
Injection volume	10µL
Run time	5.0 min
Buffer	Na2hpo4

Preparation

Preparation of Standard stock solutions: Accurately Weighed and transferred 37.5mg & 6.25mg of Lamivudine and Dolutegravir working Standards into a 50ml clean dry volumetric flask, add 25ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents .From the above stock solution. (750µg/ml Lamivudine,125 µg/ml of dolutegravir)

Preparation of Standard working solutions (100% solution): 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (75µg/ml lamivudine and 12.5 µg/ml of dolutegravir.

Preparation of Sample solutions: 20 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 500ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (600µg/ml Lamivudine,100 µg/ml of dolutegravir

Preparation of Sample working solutions (100% solution): 1.25ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (75µg/ml lamivudine,12.5 µg/ml of dolutegravir

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Lamivudine (75ppm) and Dolutegravir (12.5ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should be not more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Table 2: System suitability results

S.no.	Lamivudine			Dolutegravir			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resoluton
1	2.173	6367	1.54	2.727	7427	1.63	4.3
2	2.174	6386	1.58	2.727	7486	1.62	4.3
3	2.174	6326	1.53	2.728	7476	1.60	4.5
4	2.174	6396	1.54	2.73	7438	1.61	4.3
5	2.177	6377	1.54	2.73	7427	1.62	4.5
6	2.177	6299	1.55	2.731	7495	1.60	4.4

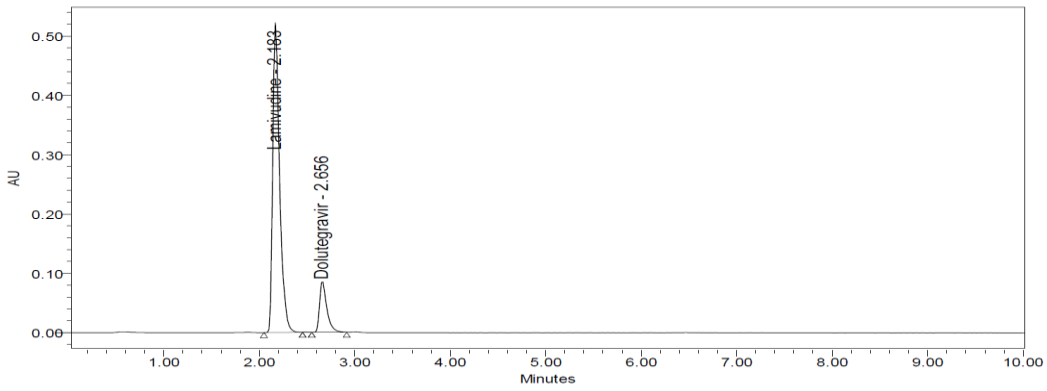

Figure 3: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time(mins)	Area
Lamivudine	2.171	2682148
Dolutegravir	2.662	451291

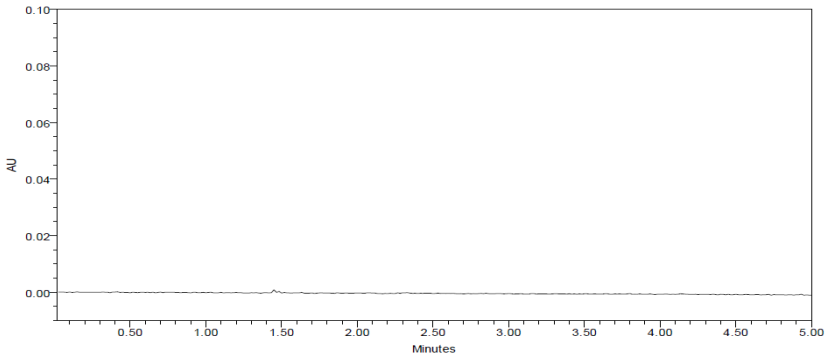

Figure 4: Blank

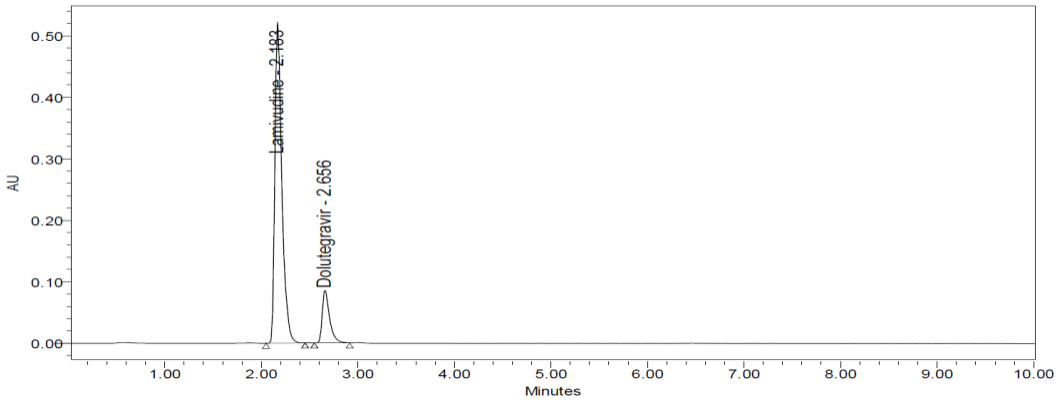

Figure 5: Specificity of Lamivudine and Dolutegravir

Linearity:

Calibration data is given in table 4 and regression data in table 4 and calibration curve in figure 6, 7

Table 4: Calibration data of Lamivudine and Dolutegravir

Lamivudine		Dolutegravir	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
30	917879	5	224154
60	1766414	10	413515
90	2676800	15	633501
120	3391138	20	794628
150	4389663	25	1017448
180	5179365	30	1222899

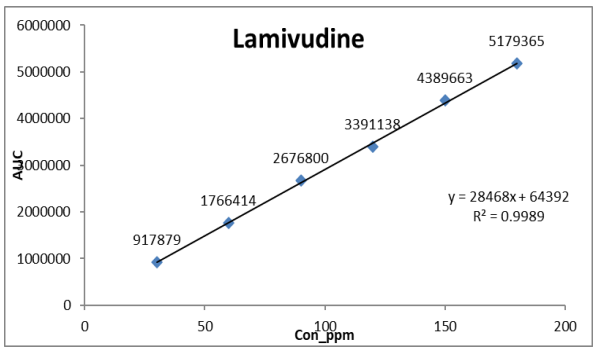

Figure 6 Calibration curve of Lamivudine

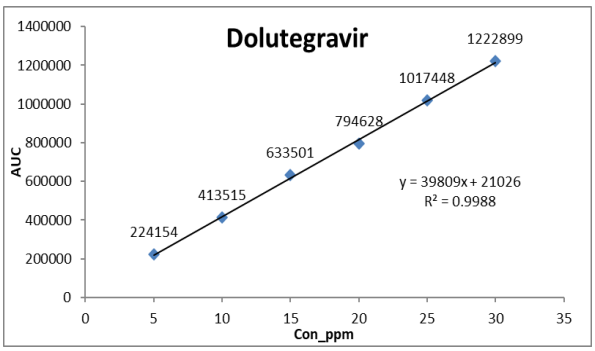

Figure 7 Calibration curve of Lamivudine

Table 5: regression data

Parameter	Lamivudine	Dolutegravir
Conc range (µg/mL)	30-180	5-30
Regression Equation	y = 28468x + 64392	y = 39809x + 21026
Co-relation	0.999	0.999

Accuracy:

Recovery data shown in table 6

Table 6: recovery data of Lamivudine and Dolutegravir

	Lamivudine			Dolutegravir		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	60	60.10	100.16	10	10.07	100.70
	60	59.77	99.62	10	10.02	100.17
	60	60.27	100.45	10	9.91	99.07
100%	120	121.29	101.08	20	20.03	100.16
	120	121.18	100.98	20	20.31	101.53
	120	120.79	100.66	20	19.99	99.96
150%	180	178.31	99.06	30	30.28	100.94
	180	178.59	99.22	30	30.32	101.07
	180	179.22	99.57	30	30.43	101.43
% recovery	100.09			100.56		

System precision was performed and the data was shown in table 7

Table 7: System precision of Lamivudine and Dolutegravir

S. No	Area of Lamivudine	Area of Dolutegravir
1.	3311401	790713
2.	3305718	795496
3.	3288610	794943
4.	3306880	796483
5.	3319219	796901
6.	3285581	797474
Mean	**3302902**	**795335**
S.D	**13164.4**	**2444.9**
%RSD	**0.4**	**0.3**

The % RSD for the peak areas of Lamivudine and Dolutegravir obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Lamivudine and Dolutegravir and shown in table 8.

Table 8: method Precision

S. No	Area of Lamivudine	Area of Dolutegravir
1.	3286339	792187
2.	3294580	797745
3.	3325437	796380
4.	3280334	795969
5.	3314953	791564
6.	3290337	792160
Mean	**3298663**	**794334**
S.D	**17641.1**	**2664.7**
%RSD	**0.5**	**0.3**

From the above results, the % RSD of method precision study was within the limit for Lamivudine and Dolutegravir.

Robustness: Robustness conditions like Flow minus (1.1ml/min), Flow plus (1.3ml/min), mobile phase minus (50B:50A), mobile phase plus (65B:40A), temperature minus (21°C) and temperature plus(31°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 9: Robustness data for Lamivudine and Dolutegravir.

Condition	%RSD of Lamivudine	%RSD of Dolutegravir
Flow rate (-) 0.7ml/min	0.1	0.5
Flow rate (+) 0.9ml/min	0.2	0.2
Mobile phase (-) 55B:45A	0.2	0.2
Mobile phase (+)65B:35A	0.1	0.5
Temperature (-) 27°C	0.9	0.2
Temperature (+) 33°C	0.8	0.2

Sensitivity:

Table 10: sensitivity of Lamivudine and Dolutegravir.

Molecule	LOD	LOQ
Lamivudine	0.68	2.07
Dolutegravir	0.53	1.62

Force Degradation Studies: table 11 shows degradation conditions and table 12 shows the obtained degraded data and purity plot chromatograms.

Table 11: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxdation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

Table 12: degradation data

Type of degradation	Lamivudine			Dolutegravir		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	3165014	95.44	4.56	748697	94.04	5.96
Base	3182117	95.96	4.04	749812	94.18	5.82
Peroxide	3200164	96.50	3.50	750625	94.28	5.72
Thermal	3253863	98.12	1.88	776356	97.52	2.48
Uv	3263971	98.43	1.57	782988	98.35	1.65
Water	3300623	99.53	0.47	789862	99.21	0.79

Assay: Dovato Tablet, bearing the label claim Lamivudine 400mg, Dolutegravir 100mg. Assay was performed with the above formulation. Average % Assay for Lamivudine and Dolutegravir obtained was 99.71% and 99.73%.respectively.

Table 11: assay data

	Lamivudine			Dolutegravir		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	3311401	3286339	99.10	790713	792187	99.50
2	3305718	3294580	99.35	795496	797745	100.20
3	3288610	3325437	100.28	794943	796380	100.03
4	3306880	3280334	98.92	796483	795969	99.98
5	3319219	3314953	99.96	796901	791564	99.43
6	3285581	3290337	99.22	797474	792160	99.50
Avg	**3302902**	**3298663**	**99.47**	**795335**	**794334**	**99.77**
Stdev	**13164.4**	**17641.1**	**0.53**	**2444.9**	**2664.7**	**0.33**
%RSD	**0.4**	**0.5**	**0.53**	**0.3**	**0.3**	**0.34**

Assay was calculated by the formula:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{10}{1} \times \frac{1}{10} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure 8 formula for assay

Conclusion:

The results showed that the new approach of estimating Lamivudine and Dolutegravir at the same time is simple, accurate, and exact. One reason it works so well is because of its excellent resolution, short retention time, and ability to separate degradants. The suggested method works well for pharmaceutical industry standardised evaluations and doesn't break the bank.

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