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DEVELOPMENT AND VALIDATION OF RP-UPLC METHOD FOR SIMULTANEOUS DETERMINATION OF LAMIVUDINE AND DOLUTEGRAVIR IN COMBINED DOSAGE FORM

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ABSTRACT:

A simple, accurate, precise method was developed for the simultaneous estimation of the Lamivudine and Dolutegravir in pharmaceutical dosage form by RP-UPLC. Chromatogram was run through HSS C18 (2.8 x 50 mm x 1.6 □m). Mobile phase containing Buffer Na2HPO4: Methanol taken in the ratio 70:30 was pumped through column at a flow rate of 0.3 mL/min. Buffer used in this method was Potassium dihydrogen phosphate. Temperature was maintained at 30°C. Optimized wavelength selected was 260 nm. Retention time of Lamivudine and Dolutegravir were found to be 1.408 min and 1.739 min. The percentage RSD of the Lamivudine and Dolutegravir were and found to be 0.8 and 0.8 respectively. The percentage recovery was obtained as 100.39% and 100.37% for Lamivudine and Dolutegravir respectively. LOD, LOQ values obtained from regression equations of Lamivudine and Dolutegravir were 0.41, 1.25 and 0.09, 0.26 respectively. Retention times were decreased and the run time was decreased, so the developed method was simple, economical and effective for the routine quality control test in industries.

Key Words: Lamivudine, Dolutegravir, RP-UPLC.

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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.

Dolutegravir IUPAC name have (4R,12aS)-7-h-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-carboxylic acid . 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

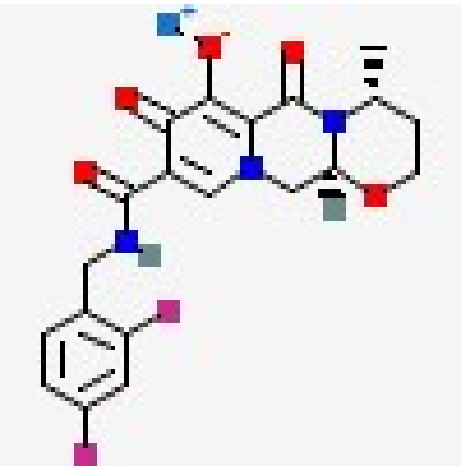


Fig. 1. Structure of Dolutegravir

Lamivudine IUPAC name have 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one.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

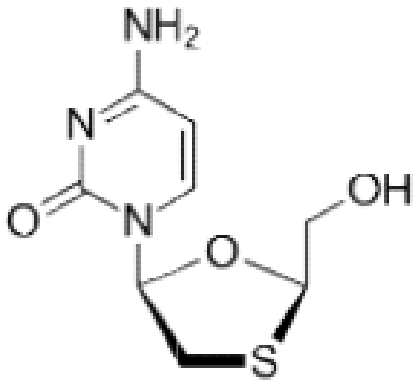


Fig. 2. Structure of Lamivudine

MATERIALS AND METHODS

- Lamivudine and Dolutegravir pure drugs (API), Combination Lamivudine and Dolutegravir oral tablets (**Dovato**), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer,Ortho-phosphoricacid.Alltheabovechemicalsandsolvents arefrom Rankem.

Methods:

Diluent: Based up on the solubility of the drugs, diluent was selected, Methanol and Water taken in the ratio of 50:50 as diluent.

Preparation of Standard stock solutions: Accurately weighed 75 mg of Lamivudine, 2.5 mg of Dolutegravir and transferred to individual 50 mL volumetric flasks separately. 3/4th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (1500 µg/mL of Lamivudine and 250 µg/mL of Dolutegravir)

Preparation of Standard working solutions (100% solution): 1mL from each stock solution was pipetted out and taken into a 10mL volumetric flask and made up with diluent. (150 µg/mL Lamivudine of and 25 µg/mL of Dolutegravir)

Preparation of Sample stock solutions: 10 tablets were weighed and was transferred into a 100 mL volumetric flask, 50 mL of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (3000 µg/mL of Lamivudine and 500 µg/mL of Dolutegravir)

Preparation of Sample working solutions (100% solution): 0.5 mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up with diluent. (150 µg/mL of Lamivudine and 25 µg/mL of Dolutegravir)

Preparation of buffer:0.01N Na2HPO4 Buffer: Accurately weighed 1.41 gm of sodium dihyrogen Ortho phosphate in a 1000 mL of Volumetric flask add about 900 mL of milli-Q water added and degas to sonicate and finally make up the volume with water.

RESULTS AND DISCUSSION

Optimized method:

Chromatographic conditions:

Mobile phase: 70% 0.01N Na2HPO4: 30% Methanol

Flow rate : 0.3 mL/min

Column : HSS C18 (2.6 x 50 mm, 1.6 µm)

Detector wave length : 260 nm

Column temperature : 10° C

Injection volume : 1.0 mL

Run time : 3.0 min

Diluent : Water and Acetonitrile in the ratio 50:50

Results : In this trail by changing the buffer both peaks eluted.

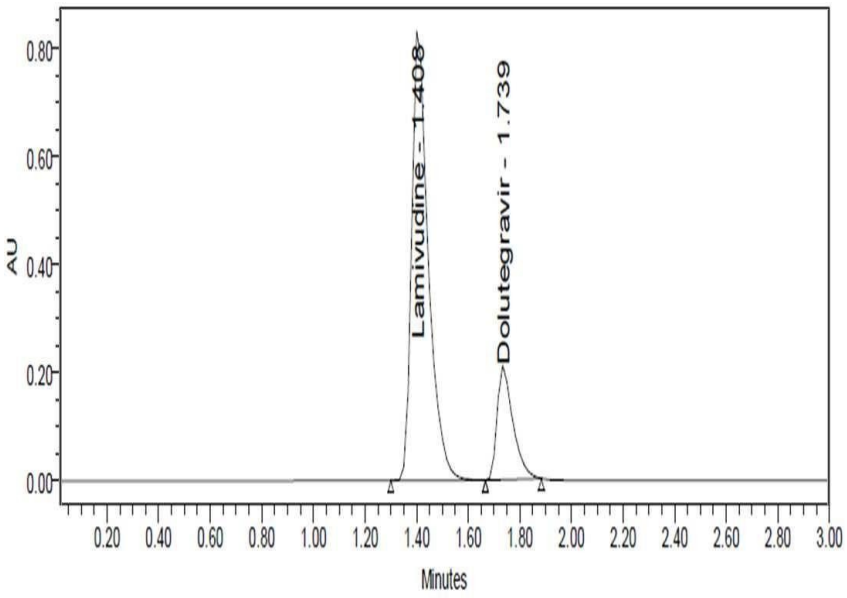


Fig 3: Optimized Chromatogram

Observation:

Lamivudine and Dolutegravir were eluted at 1.408 min and 1.739 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability:

All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Table: 1 System suitability parameters for Lamivudine and Dolutegravir

S.No	Lamivudine			Dolutegravir			Resolution
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	
1	1.376	2685	1.39	1.702	4512	1.37	3.1
2	1.385	2903	1.39	1.708	4172	1.42	3.0
3	1.390	3047	1.39	1.717	4553	1.38	3.1
4	1.391	2685	1.42	1.72	4483	1.39	3.0
5	1.400	2933	1.34	1.722	4448	1.39	3.0
6	1.408	2147	1.46	1.739	3764	1.46	2.8

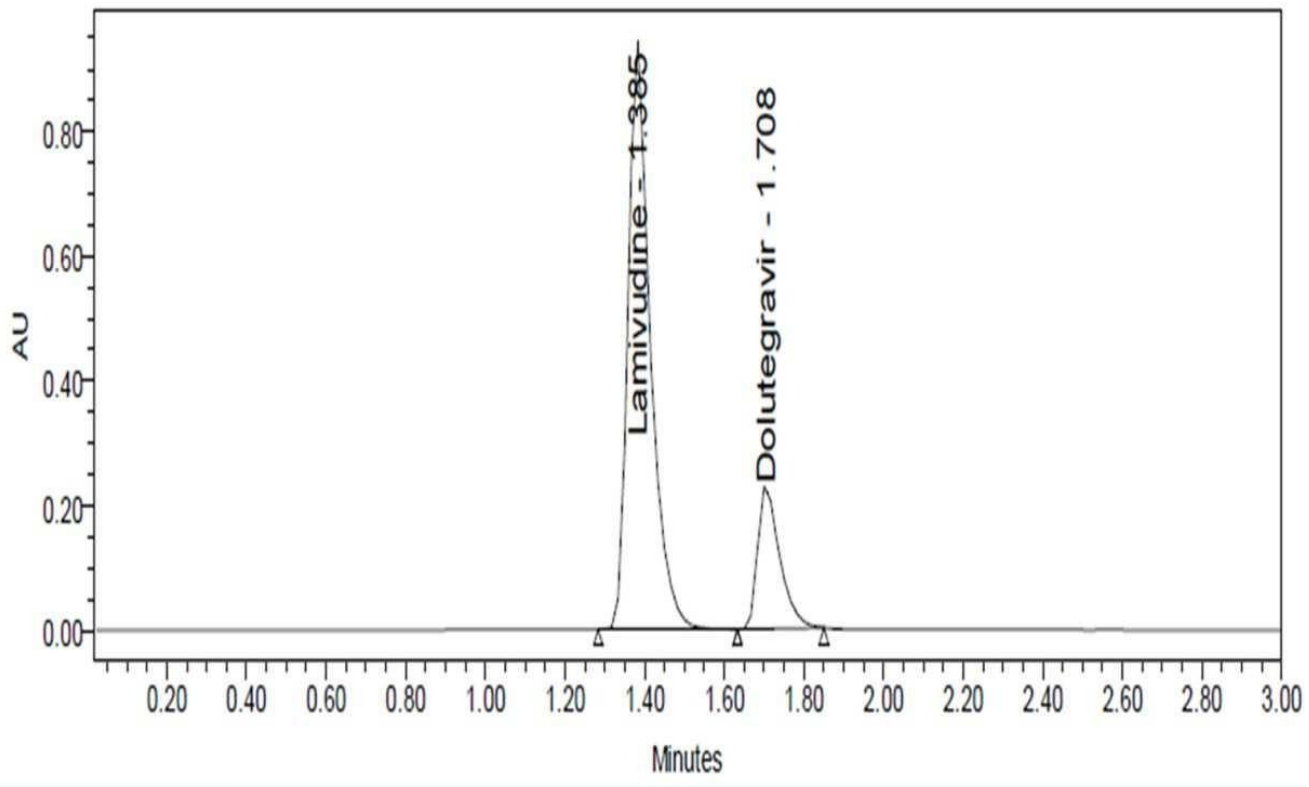


Fig 4: System suitability Chromatogram

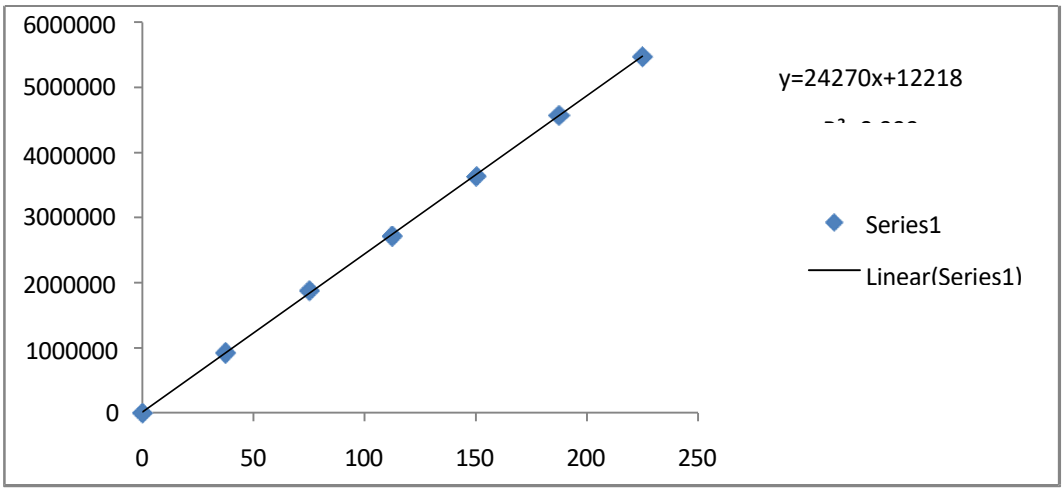
Discussion:

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

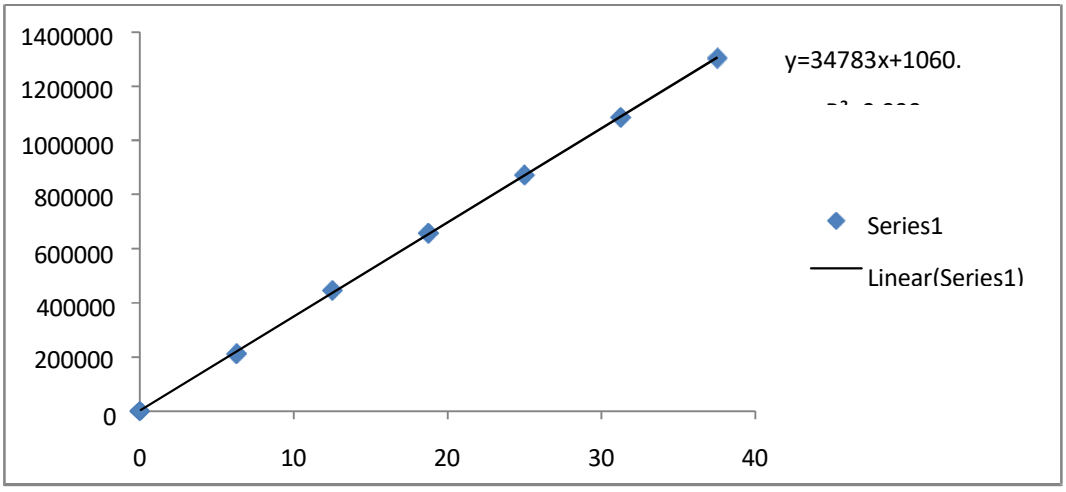
LINEARITY

Table2:LinearitytableforLamivudineandDolutegravir.

Lamivudine		Dolutegravir	
Conc(µg/mL)	**Peakarea**	**Conc(µg/mL)**	**Peakarea**
0	0	0	0
37.5	922806	6.25	210556
75	1878662	12.5	445258
112.5	2712498	18.75	655630
150	3638097	25	872691
187.5	4569129	31.25	1084994
225	5477284	37.5	1303577



FigNo.5Calibration curveofLamivudine



FigNo.6Calibration curveofDolutegravir

Discussion:

Six linear concentrations of Lamivudine (37.5-225 µg/mL) and Dolutegravir (6.25-37.5 µg/mL) were injected in a duplicate manner. Average areas were mentioned above and linearityequations obtained for Lamivudine was y= 24270x + 12218 and of Dolutegravir was y = 34783x + 1060. Correlation coefficient obtained was 0.999 for the two drugs.

ASSAY

Table 3: Assay Data of Lamivudine

S.No.	Standard Area	Sample area	%Assay
1	3640080	3639645	99.46
2	3662158	3653746	99.85
3	3623764	3640033	99.48
4	3646966	3601832	98.43
5	3650353	3616126	98.82
6	3710141	3612258	98.72
Avg	3655577	3627273	99.13
St dev	29577.7	20068.2	0.55
%RSD	0.8	0.6	0.6

Table 4: Assay Data of Dolutegravir

S.No.	Standard Area	Sample area	%Assay
1	882110	886576	100.74
2	882532	888945	101.00
3	870302	882568	100.28
4	872703	880422	100.04
5	878651	889162	101.03
6	889046	887665	100.86
Avg	882557	885890	100.66
St dev	6902.1	3594.3	0.4
%RSD	0.8	0.4	0.4

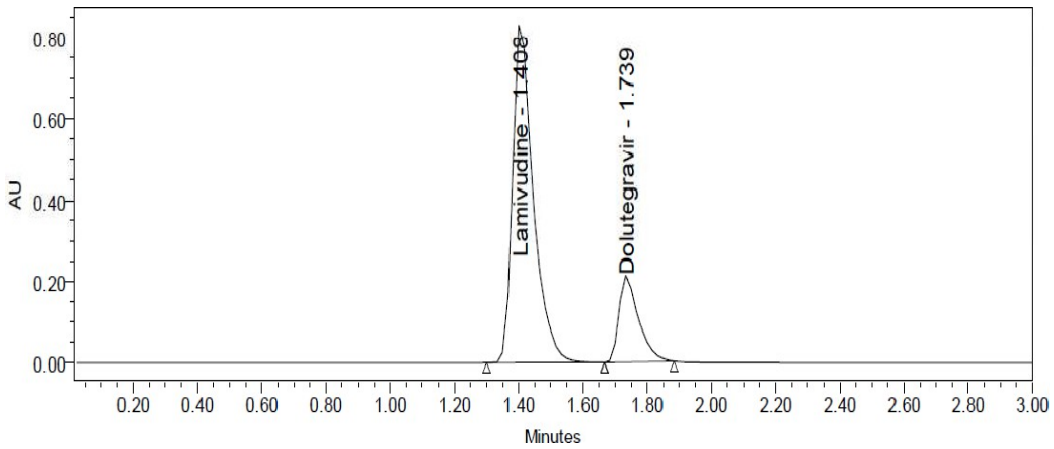
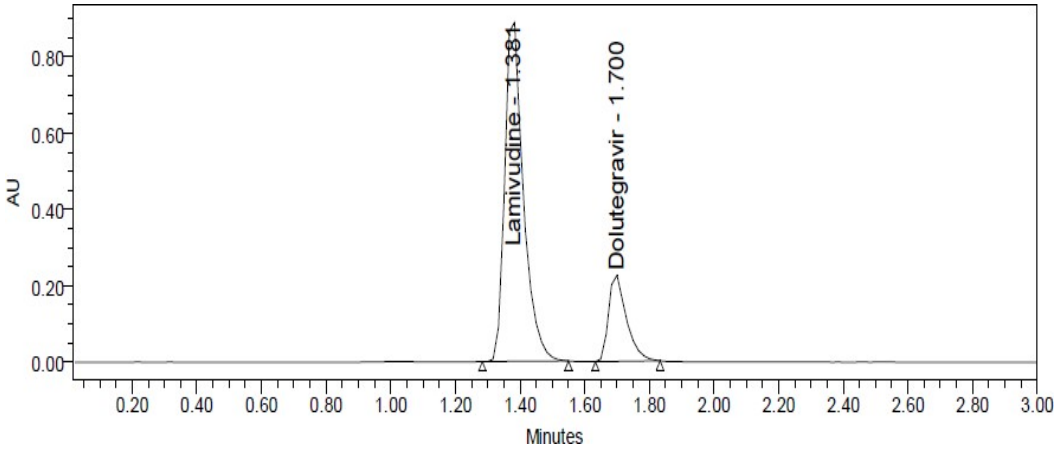


Fig7: Chromatogram of working standard solution



FigNo.8: Chromatogram of working sample solution

Discussion

(ROCKLATAN), bearing the label claim Lamivudine 0.2 mg, Dolutegravir 0.05 mg. Assay was performed with the above formulation. Average %Assay for Lamivudine and Dolutegravir obtained was 99.13% and 100.66% respectively.

SUMMARY& CONCLUSION:

Table5: SummaryTable

Parameters		Lamivudine	Dolutegravir	LIMIT
Linearity Range(µg/mL)		37.5-225µg/mL	6.25-37.5µg/mL	R<1
Regressioncoefficient		0.999	0.999	
Slope(m)		24270	34783	
Intercept(c)		12218	1060	
Regressionequation (Y=mx+c)		y=24270x+ 12218	y=34783x+1060.	
Assay(%mean assay)		99.13%	100.66%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision % RSD		0.8	0.8	NMT2.0%
Method precision % RSD		0.6	0.4	NMT2.0%
Accuracy%recovery		100.39%	100.37%	98-102%
LOD		0.41	0.09	NMT3
LOQ		1.25	0.26	NMT10
Robustness	**FM**	0.7	1.0	%RSD NMT 2.0
	FP	0.3	0.5	
	MM	0.2	0.4	
	MP	0.7	1.1	
	TM	0.1	0.1	
	TP	0.2	0.7	

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Lamivudine and Dolutegravir in Tablet dosage form. Retention time of Lamivudine and Dolutegravir were found to be 1.408 min and 1.739 min. The % RSD of the Lamivudine and Dolutegravir were and found to be 0.8 and 0.8 respectively. The %Recovery was obtained as 100.39% and 100.37%for Lamivudine and Dolutegravir respectively. LOD, LOQ values obtained from regression equations of Lamivudine and Dolutegravir were 0.41, 1.25 and 0.09, 0.26 respectively. Regression equation of Lamivudine is y = 24270 x + 12218 and y = 34783 x + 1060 of Dolutegravir. Retention times were decreased and that run time was decreased, so the method developed was simple and cost effective that can be adopted in regular Quality control test in Industries.

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