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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND DOLUTEGRAVIR SODIUM IN BULK AND PHARMACEUTICAL DOSAGE FORMULATION

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Keywords:

HPTLC, Lamivudine, Dolutegravir sodium, Stability indicating method development, Validation, HIV

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ABSTRACT: A sensitive, accurate, and precise stability-indicating HPTLC method has been developed for the simultaneous estimation of lamivudine (LMV) and dolutegravir sodium (DOL) in bulk and pharmaceutical dosage formulation. The method employed chloroform: methanol: toluene formic acid (8:2:2:0.2 v/v/v/v) as a mobile phase and silica gel G 60 F254 TLC plates as stationary phase. Chromatographic detection was performed at 271 nm. The R_f Value of LMV and DOL was found to be 0.38 ± 0.02 and 0.62 ± 0.02 respectively. The method was validated in compliance with ICH Guideline for linearity, limit of detection (LOD), limit of quantification (LOQ), precision, specificity, accuracy and robustness. The linear regression analysis shown good linear relationship over the concentration range of 120 - 720 ng/spot for LMV (R^2 = 0.9994) and 20 - 120 ng /spot for DOL sodium (r^2 = 0.999). The LOD of LMV and DOL was found to be 8.86 ng/spot and 2.92 ng/spot respectively. The LOQ of LMV and DOL was found to be 26.5 ng/spot and 8.74 ng/spot respectively. The % recovery was calculated and found to be 98.83-101.27% for LMV and 98.95 - 100.97% for DOL respectively. LMV and DOL were subjected to acidic, alkaline, oxidative, neutral and thermal degradation conditions. The degradation products obtained were well resolved from the pure drugs with significantly different R_f values.

INTRODUCTION: DOL and LMV are indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection. HIV is a virus that attacks the immune system, which is our body's natural defence against illness. HIV infects vital cells in the human immune system such as helper T cells (specifically CD^{4+} T cells), macro-phages and dendritic cells [1, 2, 3].

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A two-drug treatment of dolutegravir and the well-tolerated lamivudine led to sustained viral suppression for most people starting antiretroviral therapy for the first line agent.

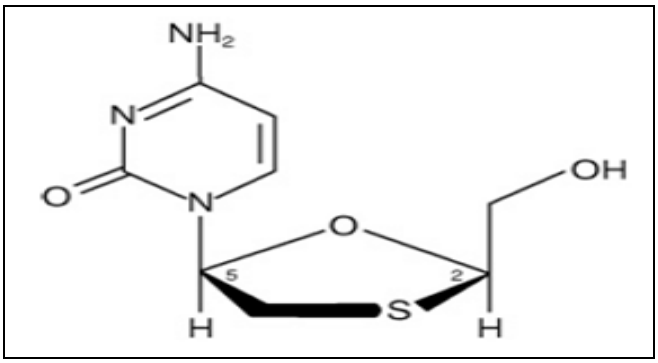

FIG. 1: CHEMICAL STRUCTURE OF LAMIVUDINE

The Dolutegravir and Lamivudine in a combination with once-daily are attractive, both drugs being safe, highly efficient and convenient for administration. Dual regimen of lamivudine and dolutegravir maintains virological efficacy up to 24 weeks and is associated to slight improvements of the immunologic and metabolic status [4, 5].

Lamivudine, chemically, 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydro-pyrimidin-2-one is a cytosine analogue with potent activity against human immunodeficiency (HIV) and hepatitis B viruses (HBV) through inhibition of reversed transcriptase activity **Fig. 1** [6].

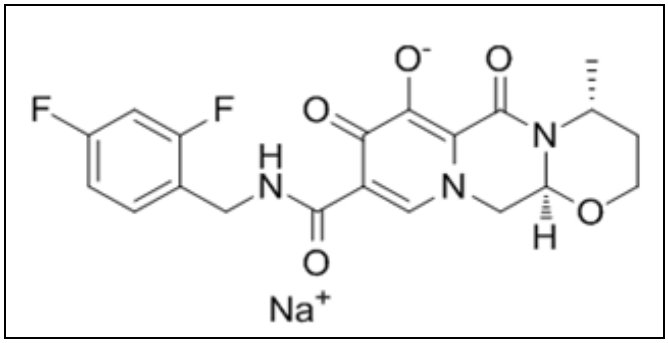

FIG. 2: CHEMICAL STRUCTURE OF DOLUTEGRAVIR SODIUM

Dolutegravir sodium, chemically, sodium (4R, 12aS)-9-[(2,4-difluorobenzyl)carbamoyl]-4methyl-6, 8- dioxo-3, 4, 6, 8, 12, 12a- hexahydro- 2H-pyrido[1', 2':4, 5]pyrazino[2,1-b][1,3]oxazin7-olate, is a novel transfer inhibitor active against human immunodeficiency virus. The drug is active against HIV type 1 (HIV-1) and also has some *in-vitro* activity against HIV type 2 (HIV-2) **Fig. 2** [7, 8, 9].

After a thorough literature survey it is clear that, there were no any article related to the simultaneous estimation and stability indicating method for combination of lamivudine and dolutegravir sodium by using HPTLC [10, 11, 12, 13, 14, 15, 16, 17, 18, 19].

MATERIALS AND METHODS:

Material: Lamivudine and dolutegravir sodium reference standard was procured from Lupin Pharmaceuticals Pvt., Ltd., Pune as gift sample. Tablet containing LMV (300mg) and DOL (50 mg) was purchased from local market, Pune. Silica Gel plates were purchased from E Merck India Pvt. Ltd. Mumbai. AR grade of solvents used for this study were purchased from Merck Pvt., Ltd., Mumbai.

Equipment: Camag HPTLC System (with TLC Scanner, Win CATS Software Version 4.0 and Linomat 5 application device) used for the analysis. The sample application was done by using Hamilton microlitre syringe.

Method Development:

Preparation of the Standard Stock Solution I: 10mg of Lamivudine and 10 mg of Dolutegravir sodium were separately weighed and transferred in to different 10 ml volumetric flasks and dissolved with methanol. The volume was made up to the mark, to get standard stock solution of Lamivudine (1000 µg/ml) and Dolutegravir (1000 µg/ml) respectively.

Preparation of the Working Standard Solution: 3 ml of standard stock solution of lamivudine was diluted with 10 ml methanol to get working standard solution of lamivudine 300 µg/ml and 0.5 ml of standard solution of dolutegravir sodium 1000 µg/ml was diluted with 10 ml methanol to get 50 µg/ml solution of dolutegravir sodium.

Preparation of Mixed Standard Solution (M): 1ml solution of lamivudine 300µg/ml and 1ml solution of dolutegravir sodium 50 µg/ml were mixed and diluted with 10 ml methanol to get mixed standard solution. This mixed standard solution (M) of containing lamivudine 30µg/ml and dolutegravir sodium 5 µg/ml respectively was used for stability indicating HPTLC analysis for simultaneous estimation of lamivudine and dolutegravir sodium.

Optimisation of Mobile Phase: Different mobile phase containing various ratios of ethyl acetate, chloroform, toluene and methanol were tried to get good and sharp resolution for lamivudine and dolutegravir sodium at 271 nm. The mobile phase containing chloroform: methanol: Toluene: Formic acid (8:2:2:0.2 v/v/v/v) with saturation time up to 45 min shows well defined and resolved peak. The optimum wavelength used for detection was 271nm.

Validation of Analytical Method: The developed HPTLC method was validated as per the ICH guidelines Q2 (R1) for linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness and specificity.

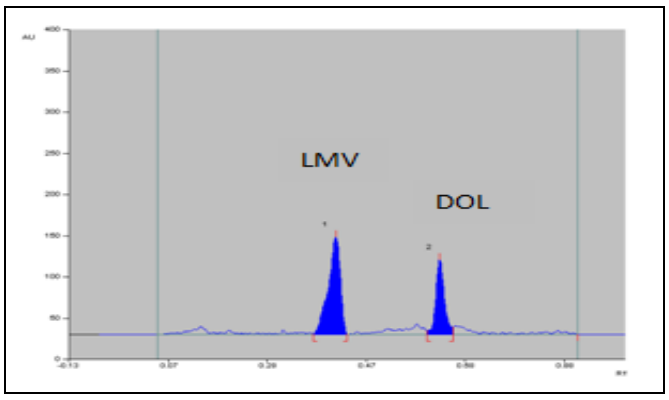

FIG. 3: REPRESENTATIVE DENSITOGRAM OF LAMIVUDINE (300 ng/SPOT) AND DOLUTEGRAVIR (50 ng/SPOT) BY HPTLC METHOD
LMV: lamivudine, DOL: Dolutegravir sodium

Linearity: Mixed standard solution containing lamivudine 30 µg/ml and dolutegravir sodium 5µg/ml were spotted on TLC plate with injection volume 4, 8, 12, 16, 20 and 24 µl to get concentration in the range of 120 to 720 ng/spot of lamivudine and 20 to 120 ng/spot of dolutegravir sodium. The plate was run with mobile phase chloroform: methanol: toluene: formic acid (8:2:2:0.2 v/v/v/v). The plate was dried and scanned at 271 nm using camagwin software version 1.4.4.6337 and densitograms were recorded and areas were reported. The procedure was repeated 3 times (n = 3) and the calibration curves were developed by plotting peak areas *vs.* Concentrations shown in **Table 1** and **Fig. 9 - 10**.

TABLE 1: REGRESSION DETAILS OF LAMIVUDINE AND DOLUTEGRAVIR

Parameter	Lamivudine	Dolutegravir
Linearity Range (ng/spot)	120-720	20-120
R^2	0.9994	0.999
Slope	8.4455	12.92
Intercept	236.89	117.74

R^2: Correlation coefficient, (n = 3)

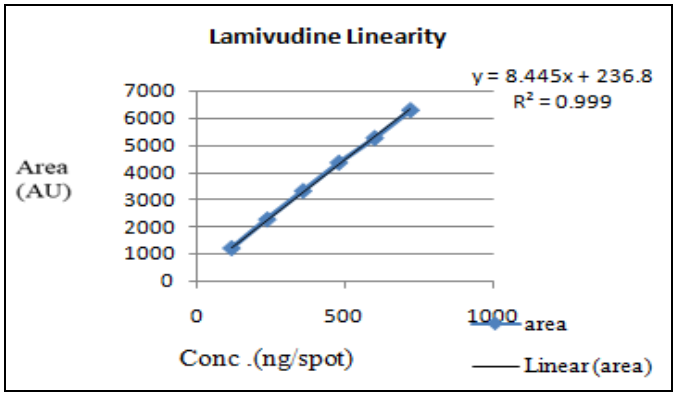

FIG. 4: LINEARITY GRAPH OF LAMIVUDINE

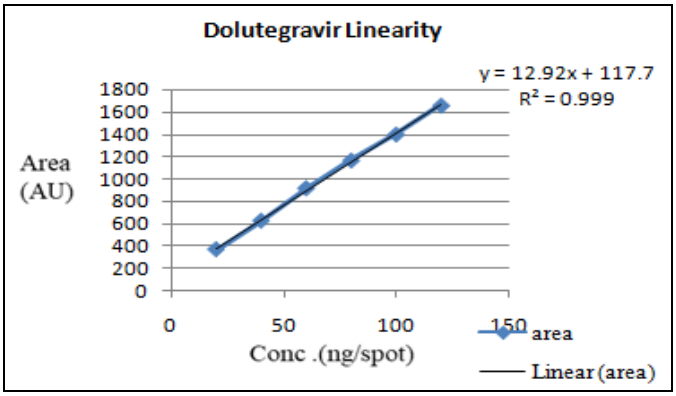

FIG. 5: LINEARITY GRAPH OF DOLUTEGRAVIR SODIUM

Precision: The precision of the method was verified by intraday precision and inter day precision studies. To evaluate intraday precision three spots of 8 µl, 12 µl, and 16 µl of mixed standard solution containing lamivudine 30 µg/ml and dolutegravir sodium 5µg/ml) were spotted on TLC plate for three times with different time intervals on same day. To evaluate interday precision three spots of 8 µl, 12 µl, and 16 µl of mixed standard solution containing lamivudine 30µg/ml and dolutegravir sodium 5 µg/ml were spotted on TLC plate within three consecutive days. The precision of method was evaluated by % RSD given in **Table 2** and **3**.

Recovery Study: Accuracy of the method was carried out by applying the method to the drug sample as lamivudine and dolutegravir sodium combination tablet to which a known amount of lamivudine and dolutegravir sodium standard powder corresponding to 80, 100, and 120% of label claim. Percentage recoveries were calculated given in **Table 4** and **5**.

TABLE 2: INTER-DAY PRECISION DETAILS OF LAMIVUDINE

Concentration (ng/spot)	Avg. Area	Std. Deviation	% RSD
240	2272.15	2.38	0.10%
360	3319.77	2.84	0.08%
480	4351.01	2.06	0.04%

% RSD: Relative Standard Deviation, (n = 3)

TABLE 3: INTER-DAY PRECISION DETAILS OF DOLUTEGRAVIR SODIUM

Concentration (ng/spot)	Avg. Area	Std. Deviation	% RSD
40	656.49	5.95	0.9%
60	656.49	9.2	0.97%
80	1191.05	8.5	0.71%

% RSD: Relative Standard Deviation, (n = 3)

TABLE 4: RECOVERY STUDY OF LAMIVUDINE

Label Claim	% level	Initial amount (mg)	Amount added (mg)	% Recovery	% RSD
Lamivudine	80	300	240	98.83%	0.42%
(300 mg)	100	300	300	100.33%	0.59%
	120	300	360	101.27%	0.71%

% RSD: Relative Standard Deviation

TABLE 5: RECOVERY STUDY OF DOLUTEGRAVIR SODIUM

Label Claim	% level	Initial amount (mg)	Amount added (mg)	% Recovery	% RSD
Dolutegravir	80	50	40	98.95%	0.34%
sodium	100	50	50	100.15%	0.49%
(50mg)	120	50	60	100.97%	0.63%

% RSD: Relative Standard Deviation

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ of both drugs were calculated by using the signal-to-noise ratio following equations as per ICH guideline.

Values were given in **Table 6**.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response. S = slope of the calibration curve.

TABLE 6: LOD AND LOQ DETAILS OF LAMIVUDINE AND DOLUTEGRAVIR SODIUM

Parameters	LOD (ng/spot)	LOQ (ng/spot)
Lamivudine	8.86	26.5
Dolutegravir	2.92	8.74

LOD: Limit of Detection, LOQ: Limit of Quantitation

Robustness: Robustness studies were done by making small, deliberate changes in optimized condition like mobile phase composition ±0.5 ml; saturation time ±10 min and development distance ±5 mm. and percentage RSD were calculated given in **Table 7, 8**.

TABLE 7: ROBUSTNESS DETAILS OF LAMIVUDINE

Condition	R_f	Peak Area	%RSD
Mobile Phase Composition (±0.5 ml methanol)			
Chloroform: Methanol: Toluene: Formic acid (8:2.5:2:0.3 v/v/v/v)	0.40	3280.51	0.22%
Chloroform: Methanol: Toluene: Formic acid (8:2:2:0.2 v/v/v/v)	0.38	3289.24	
Chloroform: Methanol: Toluene: Formic acid (8:1.5:2:0.1 v/v/v/v)	0.35	3274.69	
Wavelength (±10 nm)			
261nm	0.39	3286.33	0.34%
271nm	0.38	3275.29	
281nm	0.37	3298.12	
Duration of Saturation (±10 min)			
55	0.39	3271.62	0.35%
45	0.38	3289.47	
35	0.37	3267.84	

R_f: Retention factor, % RSD: Relative Standard Deviation

TABLE 8: ROBUSTNESS DETAILS OF DOLUTEGRAVIR SODIUM

Condition	R_f	Peak Area	% RSD
Mobile Phase Composition (±0.5 ml methanol)			
Chloroform: Methanol: Toluene: Formic acid (8:2.5:2:0.3 v/v/v/v)	0.63	915.0	1.2%
Chloroform: Methanol: Toluene: Formic acid (8:2:2:0.2 v/v/v/v)	0.62	923.6	
Chloroform: Methanol: Toluene: Formic acid (8:1.5:2:0.1 v/v/v/v)	0.61	937.5	
Wavelength (±10 nm)			
261nm	0.63	926.89	1.21%
271nm	0.62	911.0	
281nm	0.60	903.6	
Duration of Saturation (±10 min)			
55	0.62	932.1	1.32%
45	0.62	908.2	
35	0.61	924.7	

R_f: Retention factor, % RSD: Relative Standard Deviation

Assay: To determine the content of lamivudine and dolutegravir sodium in tablets (containing 300 mg lamivudine and 50 mg dolutegravir sodium), 20 tablets were weighed accurately, crushed and average weight was calculated. The amount equivalent to 1 tablet was taken and dissolved and diluted up to the mark in with methanol to obtain a concentration equivalent to 30 µg/ml lamivudine and 5 µg/ml dolutegravir sodium.

10 µl spot of mixed standard solution, M (lamivudine 30 µg/ml and dolutegravir sodium 5µg/ml) and marketed formulation solution were spotted on TLC plate. The densitogram was recorded shown in **Fig. 6**

Forced Degradation Studies: In order to develop SIM for simultaneous estimation of lamivudine and dolutegravir sodium forced degradation studies were carried out according to ICH guidelines.

Preparation of Acid Induced Degradation Product: Initially degradation study performs by keeping mixed standard solution in 0.1N, 0.5N, 1N, 1.5N hydrochloric acid solution and kept at room temperature.

A mixture of 5ml mixed standard solution (lamivudine 30 µg/ml and dolutegravir sodium 5µg/ml) and 5 ml of 0.1N HCl was kept at room temperature for 3 h. The resultant solution spot was applied on TLC plates with concentration equivalent to 300 ng/spot of lamivudine and 50ng/spot of dolutegravir sodium and the plate was run with mobile phase, dried and scanned at 271 nm. Densitograms were recorded shown in **Fig. 7**.

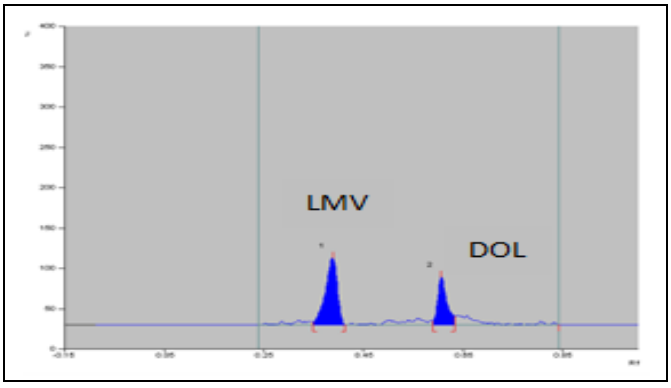

FIG. 6: HPTLC DENSITOGRAM OF LAMIVUDINE AND DOLUTEGRAVIR SODIUM IN PHARMACEUTICAL TABLET FORMULATION

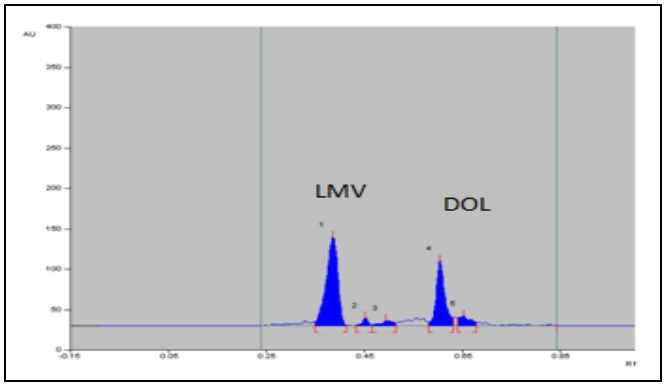

FIG. 7: ACID DEGRADATION OF LAMIVUDINE AND DOLUTEGRAVIR SODIUM

Preparation of Base Induced Degradation Product: Initially degradation study performs by keeping mixed standard solution in 0.1N, 0.5N, 1N, 1.5N NaOH solution and kept at room temperature. A mixture of 5ml mixed standard solution, M (lamivudine 30 µg/ml and dolutegravir sodium 5 µg/ml) and 5 ml of 1N NaOH, was kept at room temperature for 3 h. Spots of resultant degraded solution was applied on TLC plates with spot concentration equivalent to 300 ng/spot of lamivudine and 50 ng/spot of dolutegravir sodium and the plate was run with mobile phase, dried and scanned at 271 nm. Densitograms were recorded shown in **Fig. 8**.

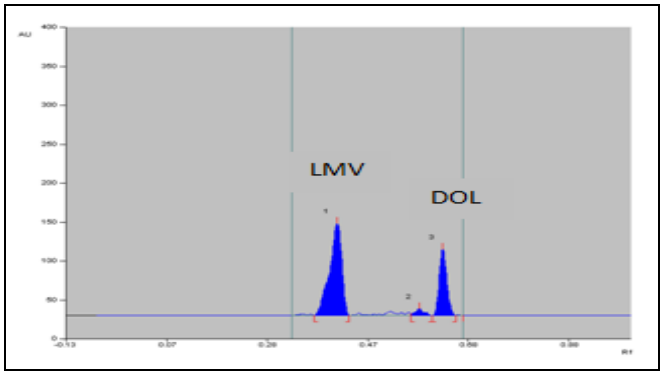

FIG. 8: BASE DEGRADATION OF LAMIVUDINE AND DOLUTEGRAVIR SODIUM

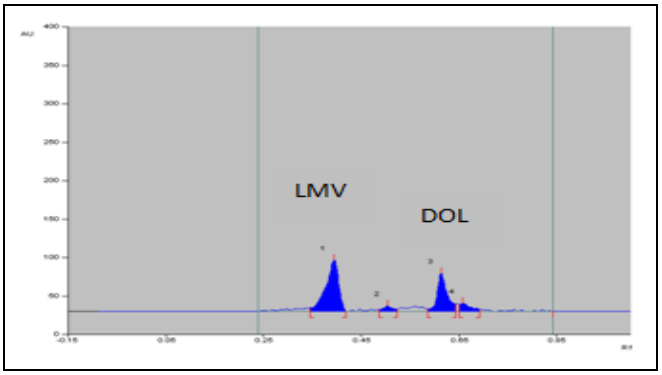

FIG. 9: OXIDATIVE DEGRADATION OF LAMIVUDINE AND DOLUTEGRAVIR SODIUM

Preparation of Hydrogen-Peroxide Induced Degradation Product: A mixture of 5 ml mixed standard solution, M (lamivudine 30 µg/ml and dolutegravir sodium 5 µg/ml) and 5 ml of 3% hydrogen peroxide was kept at room temperature for 3 h. Spots of resultant degraded solution was applied on TLC plates with spot concentration equivalent to 300 ng/spot of lamivudine and 50 ng/spot of and the plate was run with mobile phase, dried and scanned at 271nm. Densitograms were recorded shown in **Fig. 9**.

Photolytic Degradation Study: 300 mg of lamivudine and 50 mg of dolutegravir sodium powder was mixed and put in a sunlight for 3 h. Powdered sample of 10 mg were withdrawn after 30 min, 1 h, 2 h, 3 h and 4 h intervals. Spot of resultant degraded solution was applied on TLC plate with spot concentration equivalent to 300 ng/spot of lamivudine and 50 ng/spot the plate was run with mobile phase, dried and scanned at 271 nm. Densitograms were recorded shown in **Fig. 10**.

Preparation of Neutral Induced Degradation Product: A mixture of 5 ml mixed standard solution M (lamivudine 30 µg/ml and dolutegravir sodium 5 µg/ml) and 5ml of Distilled water kept for 24 h at room temperature. The resultant degraded solution spot was applied on TLC plates with concentration equivalent to 300 ng/spot of lamivudine and 50 ng/spot of dolutegravir sodium and the plate was run with mobile phase dried and scanned at 271 nm. Densitograms were recorded shown in **Fig. 11**.

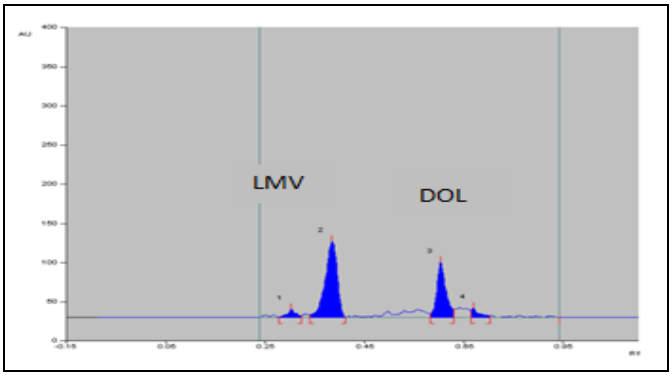

FIG. 10: PHOTOLYTIC DEGRADATION OF LAMIVUDINE AND DOLUTEGRAVIR SODIUM

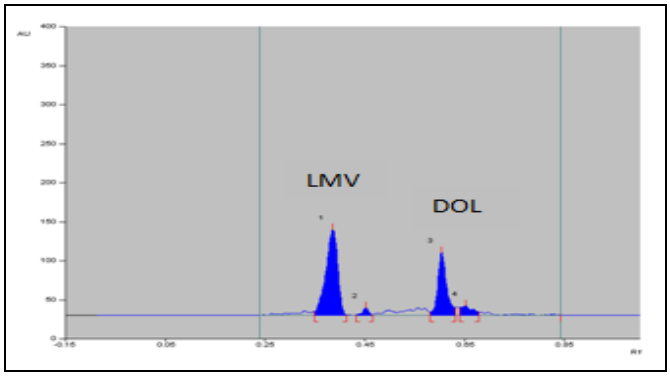

FIG. 11: NEUTRAL DEGRADATION OF LAMIVUDINE AND DOLUTEGRAVIR SODIUM

TABLE 9: SUMMARY OF VALIDATION PARAMETERS

S. no.	Validation parameter	Results	
		Lamivudine	Dolutegravir Sodium
1	Linearity Equation	y = 8.4455x + 236.89 R^2 = 0.9994	y = 12.92x + 117.74 R^2 = 0.999
2	Accuracy	98.83-101.27%	98.95-100.97%
3	LOD	8.86 ng/spot	2.92ng/spot
4	LOQ	26.5 ng/spot	8.74 ng/spot
5	Precision (%RSD)	0.42-0.71 %	0.34-0.64%
6	Robustness (%RSD)	0.22-0.35%	1.2-1.32%

TABLE 10: SUMMARY OF FORCE DEGRADATION PARAMETERS

S. no.	Degradation Parameter	% degradation		R_f of degraded Products	
		Lamivudine	Dolutegravir	Lamivudine	Dolutegravir
1	Acid	12.08 %	10.04%	0.42,0.49	0.65
2	Base	7.22%	6.66%	0.56	-
3	Oxidation	14.78%	16.54%	0.49	0.66
4	Thermal	6.12%	8.28%	0.28	0.66
5	Neutral	6.11%	6.37%	0.45	0.67

RESULTS AND DISCUSSION: The mobile phase containing chloroform: methanol: toluene: formic acid (8:2:2:0.2 v/v/v/v), with saturation time up to 45 min shows well defined and resolved peak. The optimum wavelength used for detection was 271 nm. The retention factor was found to be 0.38 ± 0.02 for lamivudine and 0.62 ± 0.02 for dolutegravir sodium respectively.

The developed method found to be linear within the range 120 to 720 ng/spot of lamivudine and 20 to 120 ng/spot of dolutegravir sodium. The linear regression data for the calibration curves (n=3) showed good linear relationship and R^2 were 0.9994 for lamivudine and 0.999 for dolutegravir respectively. The accuracy of method was determined at 80%, 100%, 120% level. The % recoveries were found to be 98.83 - 101.27% for lamivudine and 98.95 - 100.97% for dolutegravir sodium. The LOD of lamivudine and dolutegravir sodium was found to be 8.86 ng/spot and 2.92 ng/spot respectively. The LOQ of lamivudine and dolutegravir sodium was found to be 26.5 ng/spot and 8.74 ng/spot respectively.

The developed method was found to be precise as the % RSD values for intra-day and inter-day were found to be less than 2%. The method was also found to be robustness indicated by the % RSD values which are less than 2%. The summary of validation parameters of proposed HPTLC method is shown in **Table 9**. The stress degradation studies were carried out for the drug in acid, base, photolytic, oxidation and neutral conditions and results are given in **Table 10**.

CONCLUSION: A simple, specific, precise and accurate HPTLC method has been developed for quantitative determination of lamivudine and dolutegravir sodium in bulk drug and in tablet formulation. The separation was achieved using Silica gel pre-coated aluminium plate 60F254 (10X10cm) with 250µm thickness as a stationery phase and using chloroform: methanol: toluene: formic acid (8:2:2:0.2 v/v/v/v) as a mobile phase. The developed HPTLC method was validated based on ICH guidelines. Statistical analysis proves that the method is reproducible for the analysis lamivudine and dolutegravir sodium as bulk drug and in pharmaceutical formulations without any interference from the excipients.

In this study, intrinsic stability of lamivudine and dolutegravir sodium is observed under stress conditions. The drug was found to be degraded in acid, base, oxidative, neutral and thermal conditions. As the method could effectively separate principle drug peak from degradation product peaks. It can be employed as a stability indicating method.

The advantages of the proposed methods involve a simple procedure for sample preparation and relatively short time of analysis. The proposed HPTLC method is suitable for the analysis of lamivudine and dolutegravir sodium in commercial tablets.

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CONFLICT OF INTEREST: Nil

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