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Development and Validation for the Simultaneous Estimation of Lamivudine, Tenofovir Disproxil and Dolutegravir In Drug Product by RP-HPLC

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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, Tenofovir DF and Dolutegravir in drug product by liquid chromatography. The chromatographic separation was achieved on column (Luna C8 150*4.6mm) at ambient temperature .The separation was achieved employing a mobile phase consists of 0.1%v/v TFA in water and Acetonitrile with simple gradient programme. The flow rate was 1.0ml/ minute and ultra violet detector at 260nm. The average retention time for Lamivudine, Tenofovir DF and Dolutegravir found to be 2.023 min, 5.330 min and 7.673. 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 75.0 – 225.0µg/ml for Lamivudine, 75.0 – 225.0µg/ml of Tenofovir DF and 12.5 – 37.50µg/ml of Dolutegravir.

Key words: Lamivudine, Tenofovir DF and Dolutegravir, Isocratic, HPLC, LunaC8, TFA.

1. INTRODUCTION

Lamivudine

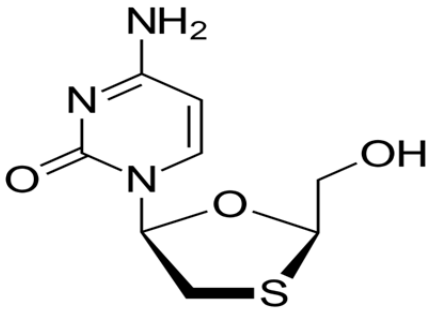


Fig. 1. Chemical structure: Lamivudine

Lamivudine 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 antiretroviral such as zidovudine and abacavir.

Common side effects include nausea, diarrhea, headaches, feeling tired, and cough. Serious side effects include liver disease, lactic acidosis, and worsening hepatitis B among those already infected. It is safe for people over three months of age and can be used during pregnancy. The medication can be taken with or without food. Lamivudine is a nucleoside reverse transcriptase inhibitor and works by blocking the HIV reverse transcriptase and hepatitis B virus polymerase.

Lamivudine is chemically designated as 4-Amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one

Its molecular formula is C8H11N3O3S, and its molecular weight is 229.26 g/mol.

Tenofovir DF

Tenofovir disoproxil is a medication used to treat chronic hepatitis B and to prevent and treat HIV/AIDS. It is generally recommended for use with other antiretroviral. It may be used for prevention of HIV/AIDS among those at high risk before exposure, and after a needlestick injury or other potential exposure.

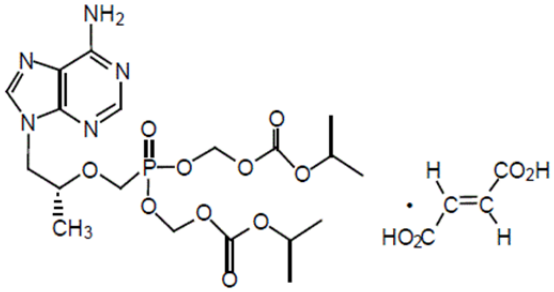


Fig. 2. Chemical structure: Tenofovir DF

Tenofovir DF is chemically designated as Bis{[(isopropoxycarbonyl)oxy]methyl} ({[(2*R*)-1-(6-amino-9*H*-purin-9-yl)-2-propanyl]oxy}methyl)phosphonate.

Its molecular formula is $C_{19}H_{30}N_5O_{10}P$, and its molecular weight is 519.443g/mol.

Dolutegravir

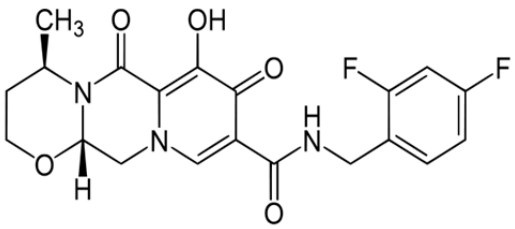


Fig. 3. Chemical structure: Dolutegravir

Dolutegravir is a medication used for the treatment of HIV infection. Dolutegravir is an integrase inhibitor. The drug is marketed as **Tivicay** by GlaxoSmithKline (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 (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 419.38g/mol

2. MATERIALS AND METHODS

2.1 Equipments: The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C8 column (Luna C8 150*4.6,3µ) as stationary phase ,Ultrasonic cleaner, Scaletech analytical balance ,Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

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

HPLC-grade Methanol and Acetonitrile was from qualigens reagents pvt ltd. Triflouro acetic acid (AR grade) was from sd fine chem.

2.3 Chromatographic conditions The sample separation was achieved on a (3µ, 150 cm X 4.6 mm i.d.) Luna C8 column, aided by mobile phase mixture of 0.1%v/v Trofluoro acetic acid in water and Methanol. The flow rate was 1.0 ml/ minute and ultra violet detector at 260nm that was filtered and degassed prior to use, Injection volume is 5 µL and ambient temperatures.

Gradient programme:

	Time	%A	%B
1	0.0	70	30
2	1.00	70	30
3	2.00	40	60
4	8.00	40	60
5	9.00	70	30
6	13.00	70	30

Preparation of mobile phase:

Buffer Preparation: Taken accurately 1.0ml of Trofluoro acetic acid in 1000mL of water

Mobile phase-A: Buffer

Mobile phase-B: Methanol

Diluent: Water: Acetonitrile (50:50 v\v)

2.4 Preparation of solutions

2.4.1 Standard solution: A 75 mg of Lamivudine, 75 mg of Tenofovir DF and 12.5mg of Dolutegravir were weighed and transferred to 50 ml of volumetric flask and dissolved in 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 pipette out into a 10 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 150µg/ml of Lamivudine, 150 µg/ml Tenofovir DF and 25 µg/ml Dolutegravir.

2.4.2 Preparation of sample solution: Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 75mg of Lamivudine, 75mg of Tenofovir DF and 12.5mg of Dolutegravir sample were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 1 ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 75 µg/ml of Lamivudine, 75 µg/ml Tenofovir DF and 12.5 µg/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 75.0 -225.0 µg/ml for Lamivudine, 75.0 -225.0 µg/ml Tenofovir DF and 12.5 -35.5 µg/ml for Dolutegravir. From the primary stock solution 0.5ml,0.75ml,1.0ml,1.25ml,1.5 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 75.0 µg /mL , 112.5µg/mL ,150.0µg/mL ,187.0µg/mL and 225.0 µg/mL of Tenofovir DF and 75µg/mL,112.5 µg/mL ,150.0µg/mL ,187.0µg/mL and 225.0 µg/mL Lamivudine and 12.5 µg/mL, 18.8 µg/mL, 25.0 µg/mL, 31.3 µg/mL 37.5 µg/mL Dolutegravir.

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,

σ = 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 75µg/ml of Lamivudine, 75µg/ml of Tenofovir DF and 12.5µg/ml of Dolutegravir without changing the parameter of the proposed chromatographic method.

2.5.5. Accuracy

The accuracy of the method was determined by calculating the recoveries of Lamivudine, Tenofovir DF and Dolutegravir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Lamivudine, Tenofovir DF 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, Tenofovir DF 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 upto 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, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v TFA in water: Methanol. The flow rate was 1.0 ml/ minute brought sharp peaks. The chromatogram was shown in Fig 4**.**

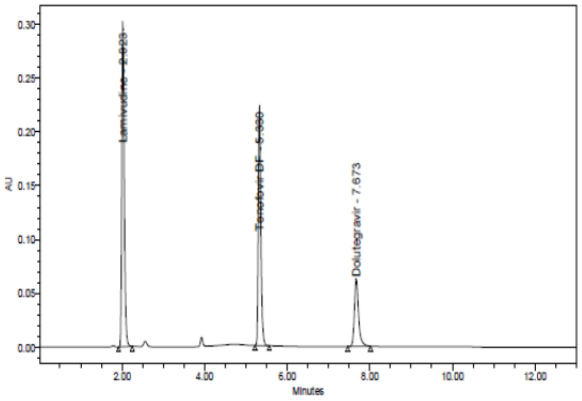


Fig 4 Chromatogram of Lamivudine/Tenofovir DF/Dolutegravir

System suitability

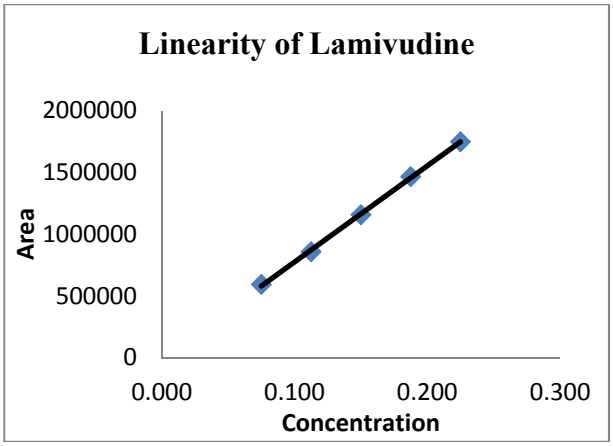
The system suitability of the method was checked by repeated preparations for Lamivudine,Tenofovir DF and Dolutegravir. The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >1500. The retention time, peak area, theoretical plates and tailing factor were evaluated for system , System suitability data of Lamivudine,Tenofovir DF and Dolutegravir are shown in Table 1

parameter	Lamivudine	Tenofovir DF	Doultegravir	Acceptance criteria
Retention time	2.027	5.327	7.682	+-10
Theoretical plates	5432	36561	31434	>3000
Tailing factor	1.23	1.26	1.29	<1.50
% RSD	0.22	0.24	0.27	<2.00

Table 1 System suitability data of Lamivudine,Tenofovir DF and Dolutegravir

Linearity:

Linearity was studied by analyzing five standard solutions covering the range of 75.0 -225.0 µg/ml for Lamivudine, 75.0 -225.0 µg/ml Tenofovir DF and 12.5 -37.5 µg/ml for Dolutegravir. From the primary stock solution 0.5ml,0.75ml,1.0ml,1.25ml,1.5 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 75.0 µg /mL , 112.5µg/mL ,150.0µg/mL ,187.0µg/mL and 225.0 µg/mL of Tenofovir DF and 75µg/mL,112.5 µg/mL ,150.0µg/mL ,187.0µg/mL and 225.0 µg/mL Lamivudine and 12.5 µg/mL, 18.8 µg/mL, 25.0 µg/mL, 31.3 µg/mL 37.5 µg/mL of Dolutegravir. Correlation coefficient values for Lamivudine, The linearity data for Lamivudine, Tenofovir DF and Dolutegravir are shown in Table 2 , Table 3 and Table 4



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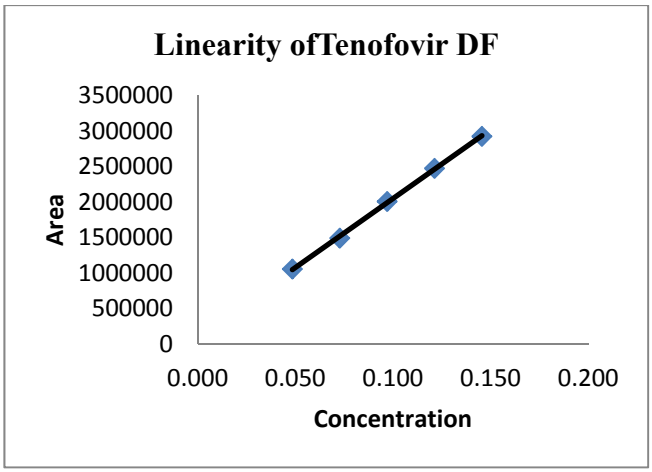

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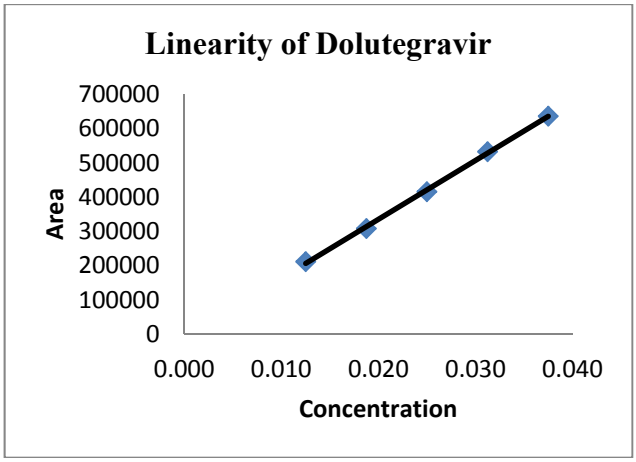

C

Fig. 5 Calibration curve: (A), Lamivudine; (B), Tenofovir DF(C) Dolutegarvir

Level	Concentration (mg/mL)	Peak area
50%	0.075	591945
75%	0.113	857258
100%	0.150	1156190
125%	0.188	1464972
150%	0.225	1747388

Table 2 Linearity data for Lamivudine

Level	Concentration (mg/mL)	Peak area
50%	0.075	398197
75%	0.113	616981
100%	0.150	890618
125%	0.188	1132833
150%	0.225	1350967

Table 3 Linearity data for Tenofovir DF

Level	Concentration (mg/mL)	Peak area
50%	0.013	211867
75%	0.019	308682
100%	0.025	416213
125%	0.031	533065
150%	0.038	636856

Table 4 Linearity data for Dolutegravir

RESULT

A linear relationship between peak areas versus concentrations was observed for Lamivudine**,** Tenofovir DF and Dolutegravir and Dolutegravir in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.9997, 0.9993 and 0.9996 for Lamivudine**,** Tenofovir DF and Dolutegravir which prove that the method is linear in the range of 50% to 150%.

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.

	Lamivudine mg	Tenofovir DF mg	Dolutegravir mg
LOD	0.005	0.008	0.001
LOQ	0.017	0.026	0.003

Table 5 LOD and LOQ values Calculated from calibration curve

Method precision (repeatability)

The precision of the method was checked by repeated preparation(n=6) of 75µg/ml of Lamivudine, 75µg/ml of Tenofovir DF and 12.5µg/ml of Dolutegravir without changing the parameter of the proposed chromatographic method. And measure the peak areas and retention times. The precision of the method (% RSD) of was found to be <1% showing good repeatability. The values of percentage RSD for Lamivudine. Tenofovir DF and Dolutegravir are shown in Table 6, Table 7 and Table 8.

Sample No	Retention time	Peak area	% Assay
1	2.035	1186180	99.8
2	2.03	1168311	98.9
3	2.025	1284146	99.5
4	2.024	1212404	99.3
5	2.025	1193391	99.1
6	2.032	1197975	99.5

Table 6 Method precision data for Lamivudine

Sample No	Retention time	Peak area	% Assay
1	5.332	911891	99.5
2	5.328	897659	99.0
3	5.335	980781	99.2
4	5.317	928665	99.3
5	5.322	918331	99.2
6	5.331	920578	100.4

Table 7 Method precision data for Tenofovir DF

Sample No	Retention time	Peak area	% Assay
1	7.716	423392	99.5
2	7.71	413404	99.9
3	7.72	449736	100.1
4	7.698	441627	99.9
5	7.701	420715	98.5
6	7.716	432462	99.6

Table 8 Method precision data for Dolutegravir

Accuracy (recovery study):
The accuracy of the method was determined by calculating the recoveries of Lamivudine,Tenofovir DF and Dolutegravir by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Lamivudine, Tenofovir DF and Dolutegravir. The percentage recovery results obtained are listed in Table 9 ,10 &11.

LEVEL	S.No	%Recovery of Lamivudine	Average
50	1	99.7	99.8%
	2	99.2	
	3	100.4	
100	1	99.8	99.4%
	2	98.9	
	3	99.5	
150	1	99.2	98.9%
	2	98.6	
	3	98.9	

Table 9 Recovery data for Lamivudine

LEVEL	S.No	%Recovery of Tenofovir DF	Average
50	1	99.1	99.1%
	2	98.6	
	3	99.5	
100	1	99.5	99.2%
	2	99.0	
	3	99.2	
150	1	98.9	99.3%
	2	99.5	
	3	99.4	

Table 10 Recovery data for Tenofovir DF

LEVEL	S.No	%Recovery of Dolutegravir	Average
50	1	99.7	99.6%
	2	99.2	
	3	99.9	
100	1	99.5	99.8%
	2	99.9	
	3	100.1	
150	1	99.0	99.0%
	2	98.8	
	3	99.4	

Table 11 Recovery data for Dolutegravir

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 12, 13 and 14) The results of Robustness of the present method had shown that changes are not significant we can say that the method is Robust.

Parameter	Rt of Lamivudine	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	2.265	1.20	5880
Increased flow rate (1.2ml/min)	1.864	1.13	5135
Wave Length 258nm	2.023	1.27	5433
262	2.029	1.20	5438

Table 12 Robustness data for Lamivudine

Parameter	Rt of Tenofovir DF	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	5.766	1.28	38767
Increased flow rate (1.2ml/min)	5.021	1.25	36881
Wave Length 258nm	5.330	1.24	36901
262	5.331	1.24	37337

Table 13 Robustness data for Tenofovir DF

Parameter	Rt of Dolutegravir	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	8.439	1.24	29406
Increased flow rate (1.2ml/min)	7.210	1.23	28663
Wave Length 258nm	7.673	1.30	30331
262	7.684	1.29	31932

Table 14 Robustness data for Dolutegravir

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The results were shown in Table no.15, 16 & 17. The %RSD assay values between two analysts was calculated, this indicates the method was rugged.

		%Assay	%RSD
Analyst-1	LAMIVUDINE	99.8	0.64%
Analyst-2		98.9	

Table 15 Robustness data for Lamivudine

		%Assay	%RSD
Analyst-1	TENOFOVIR DF	99.5	0.36%
Analyst-2		99.0	

Table 16 Robustness data for Tenofovir DF

		%Assay	%RSD
Analyst-1	DOLUTEGRAVIR	99.5	0.28%
Analyst-2		99.9	

Table 17 Robustness data for Dolutegravir

CONCLUSION

From the above experimental results it was concluded that, this newly developed method for the simultaneous estimation of LAMIVUDINE, TENOFOVIR DF 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 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://www.drugbank.ca/drugs/DB08930

[5] https://www.drugbank.ca/drugs/DB00709

[6] https://www.drugbank.ca/drugs/DB08930

[7] Development and Validation of Analytical Method for Determination of Dolutegravir Sodium, Lamivudine and Tenofovir Disoproxil Fumarate Using Reverse Phase High Performance Liquid Chromatography **Author(s):** Talari Kalpana, Dr. Tiruveedula Raja Rajeswari, Ramana Reddy Ganji

[8] Development and validation of stability-indicating HPLC method for simultaneous determination of Lamivudine, Tenofovir, and Dolutegravir in bulk and their tablet dosage form **.Nagasarapu MallikarjunaRao[a].** Dannana Gowri Sankarb.

[9] Stability Indicating Gradient RP-HPLC Method for the Simultaneous Estimation of Lamivudine, Abacavir and Dolutegravir in Bulk and their Combined Dosage Form P. Saidulu*,Sk.Mastanamma

[10] Simultaneous Estimation of Tenofovir DF and Lamivudine in Synthetic Mixture by RP-HPLC Method Kena H. Patel1 , Shailesh V. Luhar2 , Sachin B. Narkhede3

[11] Development & Validation of HPLC Method for Analysis of Some Antihypertensive Agents in their Pharmaceutical Dosage Forms Shalini pachauria , Sarvesh paliwalb , Kona.S.Srinivasa , Yogendra Singha , Varun Jain

[12] DEVELOPMENT OF ASSAY METHOD AND FORCED DEGRADATION STUDY OF LAMIVUDINE AND TENOFOVIR DF BY RP-HPLC IN TABLET FORMULATIONS. *Naazneen, A. Sridevi*

[13] RP-HPLC method for the estimation of Lamivudine in pharmaceutical dosage form M.Akiful Haque*, S.Hasan Amrohi, Prashanth Kumar.K, Nivedita.G, Pradeep Kumar.T, Dibyalochan Mohanty and Prakash.V. Diwan

[14] Development and Validation of HPLC Method for Simultaneous Determination of Amlodipine, Lamivudine, Hydrochlorothiazide in Dosage Form and Spiked Human Plasma Samya M. El-Gizawy1 , Osama H. Abdelmageed2 , Mahmoud A. Omar3 , Sayed M. Deryea3 , Ahmed M. Abdel-Megied4

[15] New Method Development and Validation for the Simultaneous Estimation of Tenofovir DF and Lamivudine in A bulk and Pharmaceutical Dosage Forms Swathi Vaka

[16] http://www.irjponline.com/admin/php/uploads/1029_pdf.pdf

[17] http://www.ncbi.nlm.nih.gov/pubmed/20502563