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DEVELOPMENT AND METHOD VALIDATION OF SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE IN BULK AND TABLET DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

Objective: Development of an accurate, simple, precise and rapid method for estimating lamivudine and Tenofovir disoproxil fumarate, simultaneously, in a combined tablet form. Determination of lamivudine and tenofovir disoproxil fumarate were estimated by RP-HPLC using Methanol: Ammonium acetate buffer solution (50:50) as mobile phase at pH 3.5 adjusted ortho phosphoric acid (OPA) with flow rate 1.0ml/min. Column used Kromasil C18 (250mm X 4.6mm i.d.) 5µm as a stationary phase. **Result:** The retention time were found to be 22 minutes of lamivudine and tenofovir disoproxil fumarate and peak was observed at 260nm which selected wavelength for quantities estimation. The LOD of Lamivudine and Tenofovir disoproxil Fumarate was found to be 0.99µg/ml and 0.58µg/ml. The LOQ of Lamivudine and Tenofovir disoproxil Fumarate was found to be 3.01µg/ml and 1.76µg/ml. **Conclusion:** The developed RP-HPLC method was simple specific accurate precise and robust for detection of Lamivudine and Tenofovir disoproxil Fumarate in bulk and tablet dosage form.

KEYWORDS

Lamivudine, Tenofovir disoproxil fumarate, RP-HPLC, Assay method, Linearity, Accuracy, Precision and Robustness.

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INTRODUCTON

Lamivudine and tenofovir disoproxil fumarate is nucleoside and nucleotide reverse transcriptase inhibitor (NRTIS)[1]. The antiretroviral drugs are used in treatment of infection of retroviruses such as HIV which still kill 5000 people a day[2]. Lamivudine is used in treatment of chronic hepatitis B at lower dose than for treatment of HIV and improve histology staging of liver. This combination product is used with other HIV medications to help control HIV infection[3-5]. The

chemical name of lamivudine is 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl] pyrimidine-2-one. The chemical name of tenofovir disoproxil fumarate is 9[(R)-2-[[Bis[[(isopropoxycarbonyl)methoxy]phosponyl]methoxy]propyl]adeninefumarate[6-8].

Lamivudine was approved by FDA (Food and drug administration) in Nov. 1995[9-11]. Tenofovir readily across the placenta; however, its concentration in maternal blood is about 3 times higher than in cord blood[12-13].

The Antiretroviral Pregnancy Registry has data on more than 4360 and 7072 women who have been exposed to lamivudine during their first and second/third trimesters, respectively, with newborn defect proportions of 3.1% and 2.9%, which are comparable to that of the general population. From these data, lamivudine appears to be safe in pregnancy. Lamivudine diffuses freely across the placenta from the maternal circulation to the fetal circulation and is secreted in breast milk[14-20]. In a systematic review of 903 infants whose mothers had received TDF for >2 weeks and most of them for several months during pregnancy, there was no increased risk of birth abnormalities[21].

The literature survey suggests UV method and RP-HPLC method for simultaneous estimation of lamivudine and tenofovir disoproxil fumarate in pharmaceutical formulation previous to our work to the reported best knowledge as per ICH guidelines. Thus efforts were made to develop analytical method sensitive, selective and fast for estimation of lamivudine and tenofovir disoproxil fumarate in combined dosage form by using RP-HPC.

The aim of the study was to develop simple, accurate, rapid specific and precise method for simultaneous estimation of lamivudine and tenofovir disoproxil fumarate in bulk and tablet dosage form.

MATERIAL AND METHODS

Material

Lamivudine 300mg and tenofovir disoproxil fumarate 300mg as pure drug were obtained as gift sample form torrent pharmaceuticals Gujarat, India. Methanol, Acetonitrile, Orthophosphoric acid, Ammonium acetate, HPLC Water was used throughout the experiment. Freshly prepared solution was employed.

Instruments

Instruments was used in Weighing balance (CY224), Digital PH Meter (LAMPH-10), Ultra Sonicator (2L300H), HPLC (1260 Infinity II), UV(V-550) in that HPLC binary gradient system is used and model no of HPLC is 1200series. Pump was used in 1260 infinity II Quaternary, pressure 600 bar Isocratic .The analysis was perform by using HPLC column Kromasil (250mm X 4.6 mm i.d.) 5µm with flow rate 1.0 ml/min and at Column oven temperature 40°C. The mobile phase composition was. The mobile phase Methanol: 25 mM ammonium acetate buffer solution (50:50v/v) the injection volume was 20µl and The HPLC system UV detector was used for analysis at 260nm with run time 22 min. Mobile phase filtered through 0.45µm nylon filter(Millipore) using filtration assembly with vacuum pump and ultrasonic water bath. The retention time 2.4 and 14.4min respectively. The proposed method was validated according to ICH guideline.

Chromatographic Conditions

Mode: Isocratic
Column: Kromasil C18
Column Dimension: (250 mm X 4.6 mm i.d.) 5µm
Column oven temp: 40°C
Detector: U.V. Detector
Wavelength: 260 nm
Flow Rate: 1.0 ml/min
Mobile phase: Methanol: 25mM ammonium acetate buffer solution (50:50)
Injection Volume: 20µl
Run time: 22 Minutes.

EXPERIMENTAL WORK

Preliminary characterization of drug

Lamivudine and tenofovir disoproxil fumarate

Lamivudine 300mg and tenofovir disoproxil fumarate 300mg is evaluated for various Preformulation parameters like color, odour and appearance and confirmed that they complied with official standards.

Selection of analytical wavelength

Selection of solvent

Weighed approx 20mg of Lamivudine and Tenofovir disoproxil fumarate API and dissolved in methanol by means of sanitation. No particle seen after sonication.

Conclusion: Both drugs found freely soluble in Methanol, hence methanol will be used as a diluents for preparing stock solution. Further dilution will be prepared in mobile phase.

Selection of wavelength

Both drugs show significant absorption at 260nm wavelength. Hence 260nm wavelength will used for chromatography development

Selection of mobile phase

The pure drug of lamivudine and tenofovir disoproxil fumarate was injected into the HPLC system and run in different solvent into the systems. Mixture of different solvents were injected in order to determine optimum chromatographic conditions for effective elution of relative drug. After several permutation and combination, it was found that the Methanol: ammonium acetate buffer with pH 3.0 (50:50 v/v) give acceptable results as compared to other mobile phases. The pH was adjusted to pH 3 by the addition ammonium acetate .Finally, the optimal composition of the mobile phase selected as per design, which gives acceptable peak shape and symmetry of lamivudine and tenofovir disoproxil fumarate.

Selection of column

For RP-HPLC, various columns are available, but as the main aim of the method is to obtain a good peak of drug, a C18 column was preferred over other columns. Kromasil C18 (250mm X 4.6mm i.d.) 5µm was chosen to give good peak shape, good lifetime, and high resolution on compared to other C18 columns.

Method development by Rp-hplc

Stock solution preparation

Lamivudine stock solution

Weighed 10mg of lamivudine and dissolved in 10mL of methanol (1000PPM of Lamivudine).

Tenofovir disoproxil fumarate stock solution

Weighed 10mg of Tenofovir disoproxil fumarate and dissolved in 10mL of methanol (1000PPM of Tenofovir disoproxil fumarate).

Solution for UV scan

Lamivudine solution

Pipette out 0.4mL of Lamivudine stock solution and diluted up to 20mL with methanol. (20PPM of Lamivudine).

Tenofovir disoproxil fumarate solution

Pipette out 0.4mL of Tenofovir disoproxil fumarate stock solution and diluted up to 20mL with methanol. (20PPM of Tenofovir disoproxil fumarate) Methanol as a blank and both drug solution were scanned from 400nm to 200nm.

STANDARD STOCK SOLUTION

Lamivudine stock

Weigh accurately 20mg of Lamivudine and transfer to 20mL volumetric flask. Add 15mL of methanol, sonicate to dissolve it completely, make the volume up to the mark with methanol. (1000 PPM of Lamivudine)

Tenofovir disoproxil fumarate stock

Weigh accurately 20mg of Tenofovir disoproxil fumarate and transfer to 20mL volumetric flask. Add 15mL of methanol, sonicate to dissolve it completely, make the Volume up to the mark with methanol. (1000PPM of Tenofovir disoproxil fumarate).

Standard preparation

Pipette out 1mL of Lamivudine stock solution and 1mL of Tenofovir disoprexil fumarate stock solution and transfer in 20mL volumetric flask, make the volume up to the mark with Mobile phase. (50PPM of Lamivudine and 50PPM of Tenofovir disoproxil fumarate).

Observation: Blank spectra: (Methanol)

Observation

Absorption maxima of Lamivudine: 272nm, 236nm

Absorption maxima of Tenofovir disoproxil fumarate: 260nm.

Overlay Q point: 260nm

Conclusion

Both drugs show significant absorption at 260nm wavelength. Hence 260nm wavelength will used for chromatography development.

Mixture

Observation: Both drugs eluted and good chromatography observed

PREPARATION OF SOLUTIONS

Buffer solution

Dissolve 1.927gm of ammonium acetate buffer in a 1000mL of water.

Preparation of mobile phase

Preparation of mobile phase mixture of 50ml of methanol and 50 ml of 25Mm ammonium acetate and degassed it by sonication.

Standard Stock Solution:

Lamivudine stock

Weigh accurately 20mg of Lamivudine and transfer to 20mL volumetric flask. Add 15mL of methanol, sonicate to dissolve it completely, add methanol up to the mark. (1000PPM of Lamivudine).

Tenofovir disoproxil fumarate stock

Weigh accurately 20mg of Tenofovir disoproxil fumarate and transfer to 20mL volumetric flask. Add 15mL of methanol, sonicate to dissolve it completely, make the volume up to the mark with methanol. (1000PPM of Tenofovir disoproxil fumarate)

Standard preparation

Pipette out 1mL of Lamivudine stock solution and 1mL of Tenofovir disoprexil fumarate stock solution and transfer in 20 mL volumetric flask, the volume make the mark with Mobile phase. (50PPM of Lamivudine and 50PPM of Tenofovir disoproxil fumarate).

Tablet Sample preparation for assay

Weigh the 20 tablets and calculate the average weight of Tenofovir L tablet. Crush the same 20 tablets in mortar pestle and mix the contents uniformly with butter paper. Weigh the powder material equivalent to 50mg of lamivudine and 50mg of Tenofovir disoprexil fumarate. Transfer it in a clean and dry 50mL of volumetric flask, add 30-35ml of methanol sonicate it for 15 minutes with intermittent shaking after every 5 minutes. Make the volume up to the mark with methanol. Filter the solution through suitable 0.45 µ syringe filter discarding 3-5mL of filtrate. Further dilute 1ml of filtrate to 20 ml with mobile phase. (50PPM of Lamivudine and 50PPM of Tenofovir disoproxil fumarate)

API Sample preparation for assay

Weighed accurately 20mg of Lamivudine and 20mg of Tenofovir disoproxil fumarate and transfer to 20mL volumetric flask. Add 15mL of methanol, sonicate to dissolve incompletely, make the volume up to the mark with methanol. Further dilute 1 ml of stock solution to 20ml with mobile phase.

RESULTS AND DISCUSSION

Final optimized method: Mixture

Observation: Both drugs eluted and good chromatography observed

System Suitability

HPLC system was optimized as per the chromatographic conditions. 20µl of Standard solutions of drugs were injected in triplicate into the chromatographic System. The chromatograms were recorded and measure the response for the major peak. System suitability parameter such as retention time, theoretical plate and Asymmetry factor

System suitability for filter study, solution stability, precision and accuracy

Observation summary

Acceptance criteria

% RSD for the area of 5 replicates of standard solution : NMT 2.0

Theoretical plate : NLT 2000

Asymmetry : NMT2.0

Conclusion

System suitability pass the crate

Routine sample analysis

API Sample

Observation summery and result

Acceptance criteria

API: NLT 98.0 and NMT102.0 of Lamivudine and Tenofovir disoproxil Fumarate.

Tablet: NLT 90.0 and NMT110.0 of Lamivudine and Tenofovir disoproxil Fumarate.

Validation of RP-HPLC method

Filter study

Filter study performed by using Centrifuged sample (Unfiltered), Sample passed through 0.45µ PVDF filter and 0.45µ Nylon filters, by discarding 5mL of solution. (Tablet mixture sample used for filter study).

Observation summery and result

Acceptance criteria

%Absolute difference NMT 2.0

Conclusion

Both filter PVDF and Nylon passes the criteria for filter study, hence both filters can be used.

SOLUTION STABILITY

Standard solution and sample solution injected at initial (0Hrs), after 12Hrs and 24Hrs percentage absolute difference calculated with respect to initial area.

Observation and Results of Solution stability

Acceptance criteria

% Absolute difference NMT 2.0

Conclusion

Standard solution and sample solution were found stable for 24 hrs, hence prepared solution can be used up to 24hours. User can check stability even after 24hrs depend on requirement.

SPECIFICITY

Injected blank, placebo, Standard solution and sample solution to check peak purity.

Results of Specificity

Acceptance criteria

Blank

There should be no Interference at R.T. of Lamivudine

Placebo

There should be no Interference at R.T. of Lamivudine Standard and sample solution: Peak purity: NLT 0.95 Sample solution Sample solution should exhibit at same R.T. as that of standard solution

Conclusion

Blank and Placebo were not having interference at R.T. of Level Lamivudine. Peak purity for both standard as well as sample were within limits. Sample solution exhibit same R.T. as that of standard solution. Hence developed chromatographic method passed the criteria for specificity

Linearity

5 Levels prepared from 10% to 150 % of working concentration. Each level injected in triplicate. Linearity graph plotted by Conc. vs. Mean Area. Calculated intercept, slope and regression coefficient.

Observation summary and Result

Acceptance criteria

Correlation coefficient: ≥

Conclusion

Regression coefficient was found well within acceptance limit for proposed range.

Accuracy

Recovery performed at three levels. 50% 100% and 150% level prepared. Each Level prepared in triplicate. The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added.

Observation summery and Result

Tenofovir disoproxil fumarate

Acceptance criteria

% Recovery: 98.0% to 102.0%.

Conclusion

% Recovery was found well within acceptance range at all three levels.

Precision

Precision performed by preparing 6 test samples

Observation summery and Result

Acceptance criteria

% Assay value for individual sample must be within 90 % to 110% of Lamivudine.

Conclusion

Precision pass the criteria, no variation found by preparing six different samples. Results are good reproducible.

Intermediate precision

Intermediate Precision performed by preparing 6 test sample by different analyst on different day.

Intermediate Precision sample preparation for Assay

Acceptance criteria

% Assay value for individual sample must be within 90% to 110% of Lamivudine % RSD for 6 intermediate precision samples NMT 2.0%.

% RSD for 12 sample (Precision and Intermediate Precision samples) NMT 2.0%.

Robustness

Standard of Intermediate precision and intermediate precision sample 1 for assay injected in robustness.

Observation and Result of Robustness

Theoretical plates: NLT 2000

Asymmetry: NMT 2.0

Limit of Detection (LOD) and Limit of Quantization (LOQ)

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope. The limit of detection (LOD) may be expressed as: LOD = 3.3 (SD)/S

Limit of Quantization (LOQ) is LOQ = 10 (SD)/S.

Acceptance criteria

0.99µg/ml The LOD of Lamivudine and Tenofovir disoproxil Fumarate was found to be 0.99µg/ml and 0.58µg/ml.

The LOQ of Lamivudine and Tenofovir disoproxil Fumarate was found to be 3.01µg/ml and 1.76µg/ml.

Observation summary

Table No.1: System suitability parameters for lamivudine

S.No	Standard solution	Area	Asymmetry	Theoretical plates
1	Standard 1	27796584	1.19	6538
2	Standard 2	27868495	1.19	6544
3	Standard 3	27685896	1.19	6531
4	Standard 4	27794286	1.18	6559
5	Standard 5	27958474	1.19	6547
6	Mean	27820747	1.19	6544
7	STD	100892.79623	----------	----------
8	%RSD	0.36	----------	----------

Table No.2: System suitability parameters for tenofovir disoproxil fumarate

S.No	Standard solution	Area	Asymmetry	Theoretical plates
1	Standard 1	17125901	1.02	12999
2	Standard 2	17185403	1.02	12991
3	Standard 3	17248671	1.02	12964
4	Standard 4	17338462	1.03	13018
5	Standard 5	16985476	1.02	13014
6	Mean	17176783	1.02	12997
7	STD	132824.72864	----------	----------
8	%RSD	0.77	----------	----------

Observation summery and result

Table No.3: Routine sample analysis of sample lamivudine and tenofovir disoproxil fumarate

S.No		Sample	Area	%Assay
1	Lamivudine	Sample 1	27867489	100.57
		Sample 2	27956849	100.81
	Tenofovir disoproxil Fumarate	Sample 1	17186425	100.95
		Sample 2	17128763	100.54

Table No.4: Filter study of lamivudine

S.No	Sample	Area	% Absolute difference
1	Unfiltred	28076953	NA
2	0.45µ PVDF filter	27862864	0.76
3	0.45µ Nylon filter	27969832	0.38

Table No.5: Filter study of tenofovir disoproxil Fumarate

S.No	Sample	Area	% Absolute difference
1	Unfiltered	17298768	NA
2	0.45µ pvdf filter	17247524	0.40
3	0.45µ Nylon filter	17297658	0.11

Observation and Results of Solution stability

Table No.6: Solution stability study of lamivudine

S.No	Sample solution			Standard solution		
	Time point	Area	%Absolute difference	Time point	Area	% Absolute difference
1	Initial	27958476	NA	Initial	27958164	NA
2	12Hours	27898963	0.21	12Hours	27958643	0.00
3	24Hours	27846153	0.40	24Hours	27894784	0.23

Table No.7: Solution stability study tenofovir disoproxil fumarate

S.No	Sample solution			Standard solution		
	Time point	Area	% Absolute difference	Time point	Area	% Absolute difference
1	Initial	17298768	NA	Initial	17246840	NA
2	12Hours	17254804	0.25	12Hours	17176583	0.41
3	24Hours	17208476	0.52	24Hours	17149682	0.56

Results of Specificity

Table No.8: Specificity study of lamivudine

S.No	Description	Observation
1	Blank	No interference at R.T. of lamivudine in blank
2	Placebo	No interference at R.T. of lamivudine in placebo
3	Standard solution	Peak purity was 0.997
4	Test sample	Peak purity was 0.996

Table No.9: Specificity study of tenofovir disoproxil fumarate

S.No	Description	Observation
1	Blank	No interference at R.T. of lamivudine in blank
2	Placebo	No interference at R.T. of lamivudine in placebo
3	Standard solution	Peak purity was 0.998
4	Test sample	Peak purity was 0.998

Observation summary and Result

Table No.10: Result of linearity study lamivudine

S.No	Levels	Conc. (µg/mL)	Area	Mean	% RSD
1	10%	5.02	2759141 2746343 2748564	2751349	0.249
2	50%	25.1	13637671 13587694 13584621	13603329	0.219
3	100%	50.2	27800364 27982365 27842815	27875181	0.342
4	125%	62.75	34437981 34461751 34376853	34425528	0.127
5	150%	75.30	41645196 41317564 41432846	41465202	0.401

Table No.11: Result of linearity study tenofovir disoproxil

S.No	Levels	Conc. (µg/mL)	Area	Mean	% RSD
1	10%	5.02	1706620 1715768 1715768	1711640	0.271
2	50%	25.1	8585827 8546813 8546817	8559819	0.263
3	100%	50.2	17280362 17384581 17518642	17394528	0.687
4	125%	62.75	21326457 21418506 21551480	21832148	0.383
5	150%	75.30	26234401 26078236 26115842	26142826	0.312

Data for calibration curve of Lamivudine andTenofovir Disoproxil Fumarate

Table No.12: Result of calibration curve of Lamivudine and Tenofovir Disoproxil Fumarate

S.No	Parameter	Lamivudine	Tenofovir Disoproxil Fumarate
1	Detection Wavelength	260nm	260nm
2	Beer's law limit	5-75µg/ml	5-75µg/ml
3	Intercept	-74328.973	-98421.778
4	Correlation coefficient (R^2)	0.99994	0.99998

Lamivudine

Table No.13: Result of accuracy study of lamivudine

S.No	Level (%)	Area	Lamivudine Recovered conc.	lamivudine Added Conc. (µg/mL)	% Recovery	Mean % Recovery	% RSD
1	50	13775483	24.88	25.10	99.13	99.46	0.332
		13765866	24.86	25.00	99.46		
		13867852	25.05	25.10	99.79		
2	100	27884257	50.36	50.10	100.53	100.65	0.125
		27968547	50.52	50.20	100.63		
		27952864	50.49	50.10	100.78		
3	150	41628576	75.19	75.00	100.25	99.90	0.317
		41507627	74.97	75.10	99.83		
		41370581	74.72	75.00	99.63		

Tenofovir disoproxil Fumarate

Table No.14: Result of accuracy study of Tenofovir disoproxil Fumarate

S.No	Level (%)	Area	Tenofovir Recovered conc.	Tenofovir disoproxil fumarate Added Conc. (µg/mL)	% Recovery	Mean % Recovery	% RSD
1	50	8579521	25.22	25.20	100.09	99.99	0.376
		8568143	25.19	25.30	99.57		
		8597125	25.28	25.20	100.30		
2	100	17184621	50.52	50.10	100.84	100.49	0.344
		17086243	50.23	50.00	100.47		
		17065482	50.17	50.10	100.15		
3	150	25418624	74.73	75.00	99.64	100.17	0.481
		25618624	75.32	75.10	100.29		
		25726981	75.64	75.20	100.58		

Observation summery and Result

Table No.15: Precision result of lamivudine

S.No	Sample	Area	% Assay
1	Sample 1	27868214	100.96
2	Sample 2	27795261	100.62
3	Sample 3	27864752	100.71
4	Sample 4	27945712	100.93
5	Sample 5	27864024	100.79
6	Sample 6	27963081	101.15
7	Mean		100.86
8	STD DEV		0.190895
9	% RSD		0.189

Table No.16: Precision result of tenofovir disoproxil fumarate

S.No	Sample	Area	% Assay
1	Sample 1	17124852	100.99
2	Sample 2	17124862	100.91
3	Sample 3	16985247	99.93
4	Sample 4	17184962	101.02
5	Sample 5	17218451	101.38
6	Sample 6	17045812	100.36
7	Mean		100.76
8	STD DEV		0.524443
9	% RSD		0.520

Intermediate Precision sample preparation for Assay

Observation summary and Results

Table No.17: Result of intermediate precision of lamivudine

S.No	Sample	Area	% Assay
1	Sample 1	27864382	100.73
2	Sample 2	27914627	100.91
3	Sample 3	27862843	100.57
4	Sample 4	27942815	100.70
5	Sample 5	27904627	100.80
6	Sample 6	27904867	100.72
7	Mean		100.74
8	STD DEV		0.113598
9	% RSD		0.113
10	Precision plus intermediate precision	Mean	100.799
		STD DEV	0.16265
		% RSD	0.161

Table No.18: Result of Intermediate precision of tenofovir disoproxil Fumarate

S.No	Sample	Area	% Assay
1	Sample 1	17028545	100.68
2	Sample 2	17058462	100.85
3	Sample 3	16895247	99.73
4	Sample 4	16914286	99.69
5	Sample 5	16945268	100.11
6	Sample 6	16975243	100.20
7	Mean		100.21
8	STD DEV		0.477524
9	% RSD		0.477
10	Precision plus intermediate precision	Mean	100.487
		STD DEV	0.55909
		% RSD	0.556

Observation and Result of Robustness

Table No.19: Result of robustness of lamivudine

S.No	Change in Parameter	Standard area	Sample Area	% Assay	Abs Diff w. r. t. Precision
1	Wavelength by +3 NM	30027595	30127138	102.05	0.185
2	Wavelength by -3 NM	26028098	26075104	100.89	0.033
3	Flow rate by +10% (1.1mL/min)	25288071	25276844	100.67	0.193
4	Flow rate by -10% (0.9mL/min)	31013735	31075149	100.91	0.051
5	Column oven temp by +2ºC	27894183	27864171	100.60	0.257
6	Column oven temp by -2ºC	27961476	27985476	100.80	0.062

Table No.20: Result of robustness tenofovir disoproxil fumarate

S.No	Change in Parameter	Standard area	Sample Area	% Assay	Abs Diff w. r. t. Precision
1	Wavelength by +3 NM	16445644	16374518	100.77	0.015
2	Wavelength by -3 NM	16392726	16247698	100.32	0.443
3	Flow rate by +10% (1.1mL/min)	16247698	15101483	100.14	0.619
4	Flow rate by -10% (0.9mL/min)	18621453	18468124	100.38	0.381
5	Column oven temp by +2ºC	18621453	17084517	100.27	0.494
6	Column oven temp by -2ºC	17184628	17048142	100.41	0.351

Table No.21: Result of (LOD) and (LOD) of lamivudine and tenofovir disoproxil fumarate

S.No	-	Lamivudine	Tenofovir disoproxil Fumarate
1	Limit of Detection	0.99 µg/ml	0.58 µg/ml
2	Limit of Quantization	3.01µg/ml	1.76 µg/ml

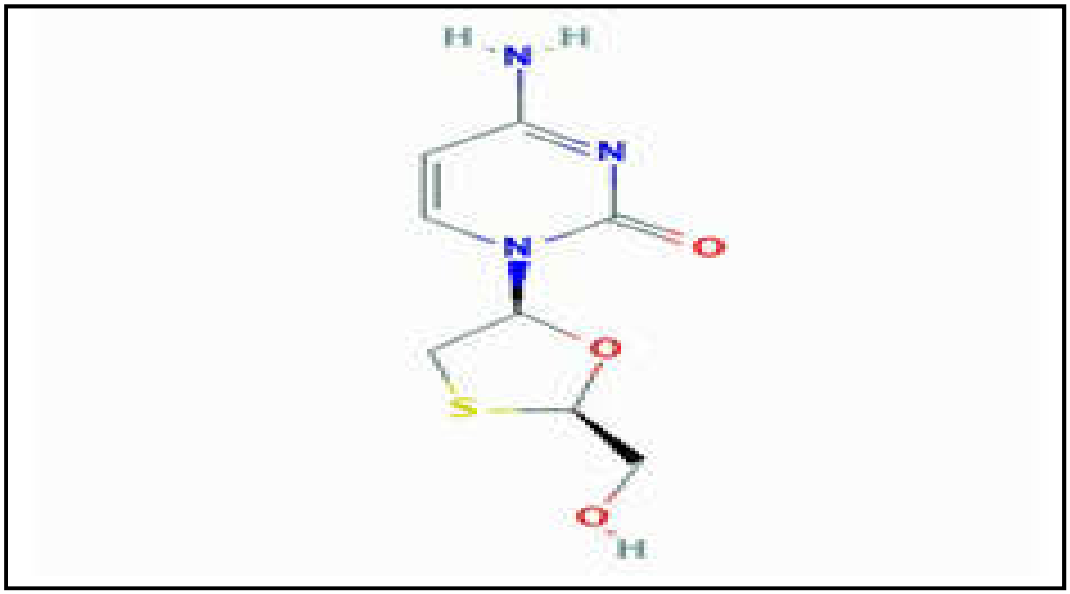

Figure No.1: Structure of Lamivudine

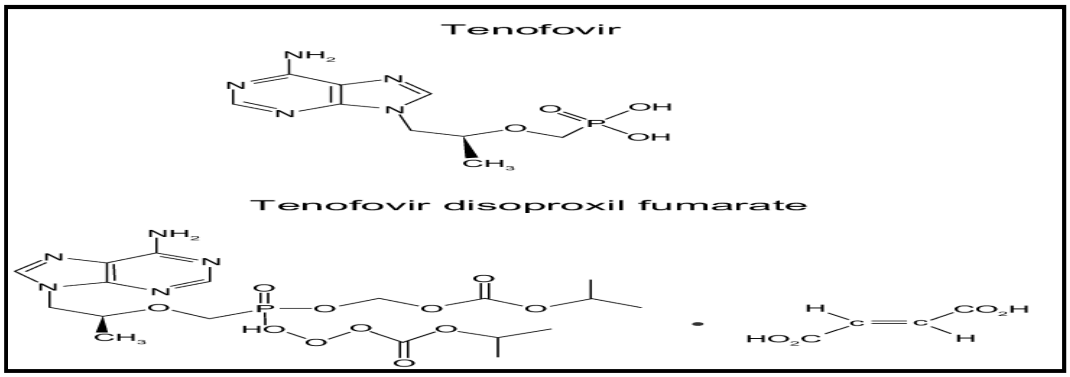

Figure No.2: Structure of tenofovir disoproxil fumarate

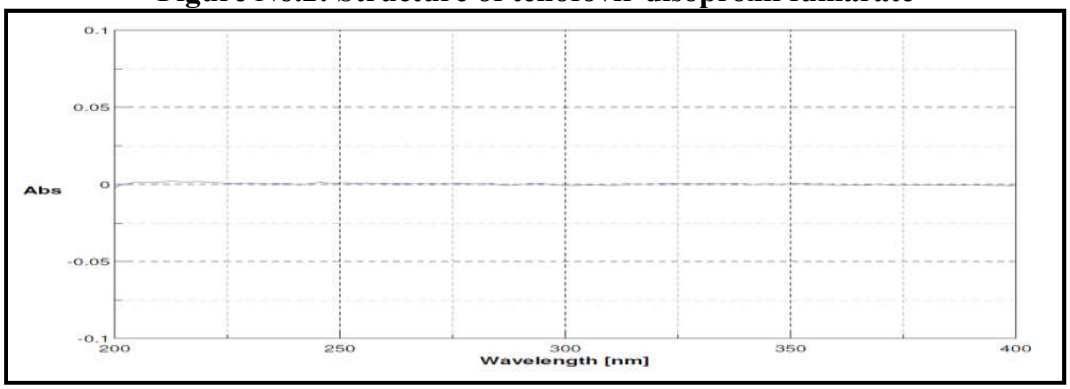

Figure No.3: Blank methanol spectra

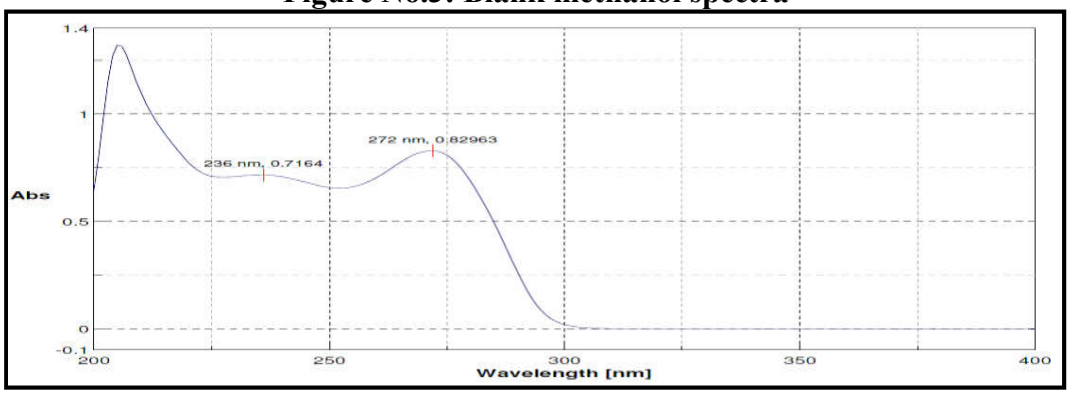

Figure No.4: Lamivudine spectra

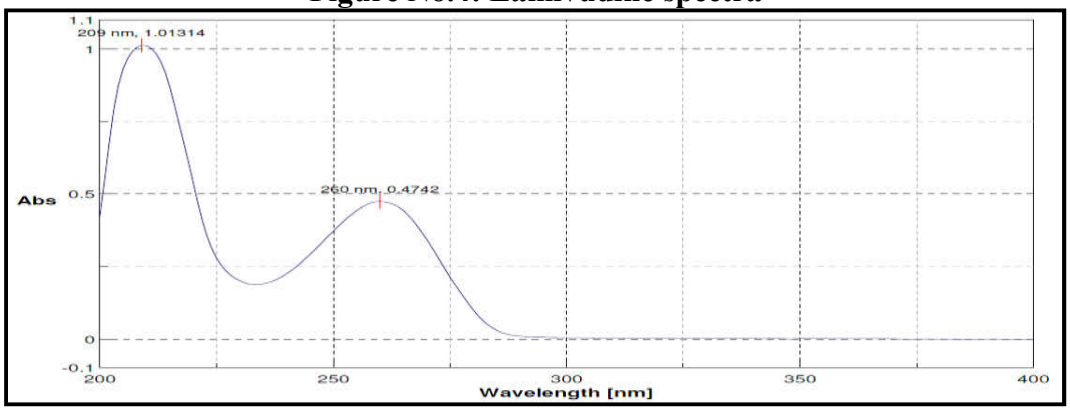

Figure No.5: Tenofovir disoproxil fumarate spectra

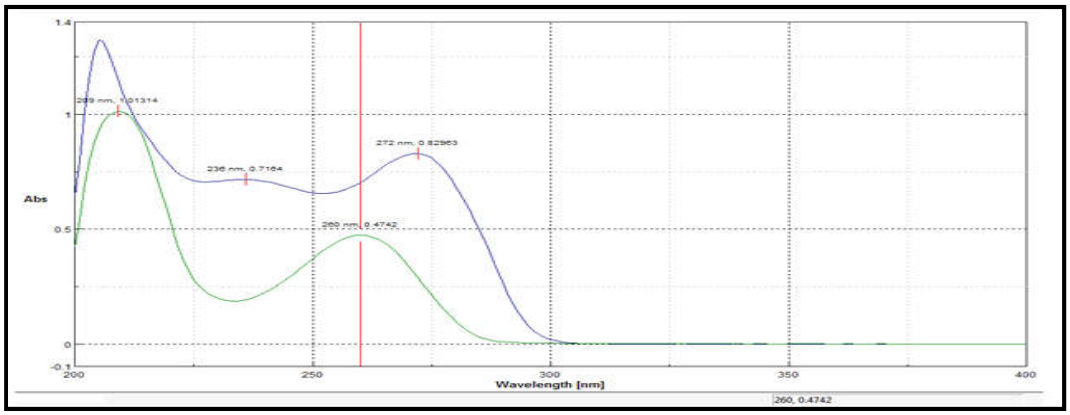

Figure No.6: Overlay Lamivudine and Tenofovir disoproxilfumarate spectra Mixture

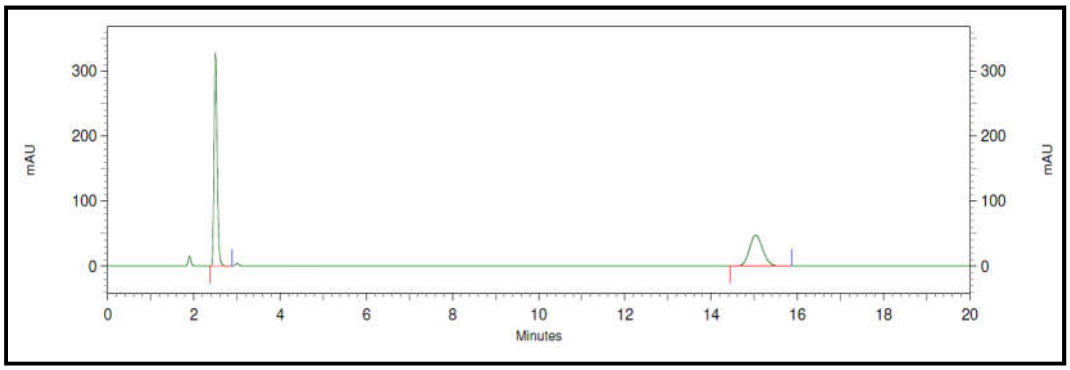

Figure No.7: Lamivudine and Tenofovir mixture

Final optimized method: Mixture

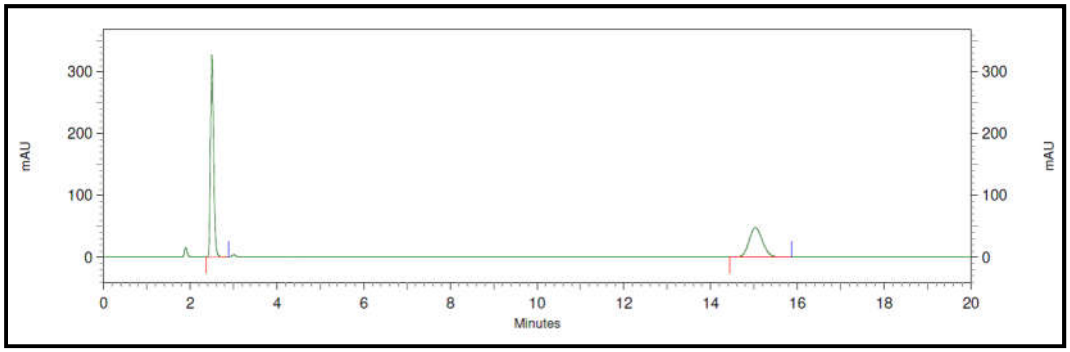

Figure No.8: Lamivudine and Tenofovir disoproxil Fumarate optimized

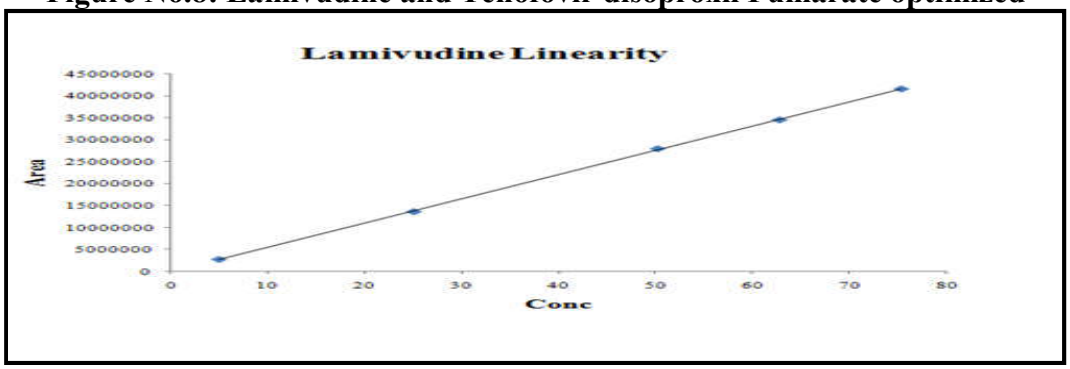

Figure No.9: Calibration chromatogram of lamivudine linearity

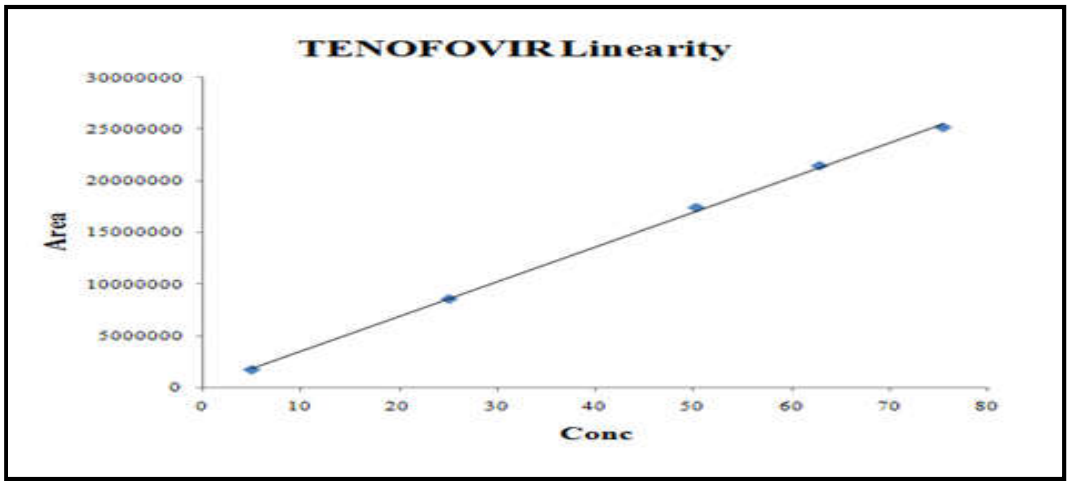

Figure No.10: Calibration Chromatogram of Tenofovir disoproxil fumarate linearity

CONCLUSION

Analytical method attempted to developed and validated for simultaneous estimation of lamivudine and tenofovir disoproxil fumarate in bulk and tablet dosage form by RP-HPLC method. Determination of lamivudine and tenofovir disoproxil fumarate were estimated by RP-HPLC using Methanol: Ammonium acetate buffer solution (50:50) as mobile phaseat pH 3.5 adjusted ortho phosphoric acid (OPA) with flow rate 1.0 ml/min. Column used Kromasil C18250 mm X 4.6 mm i.d.) 5µm as a stationary phase. The retention time were found to be 22 minutes of lamivudine and tenofovir disoproxil fumarate and peak was observed at 260 nm which selected wavelength for quantities estimation. After development of the method it was validated for linearity, precision, intermediate precision, accuracy, robustness, studies according to ICH guidelines. The system suitability parameter also reveals that the values within the specific limit for the proposed method.

Calibration curve was linear over the range of 5-75µg/mL for lamivudine and tenofovir disoproxil fumarate. The linearity was observed with correlation coefficient (R^2) found to be 0.99994 and 0.99998 lamivudine and tenofovir disoproxil fumarate respectively. The result of assay was found to be 100.89 lamivudine and 100.54 tenofovir disoproxil fumarate. The assay result found closed to 100%.

The result of accuracy shown in table it was be found value of pure drugs of % Recovery 98.0% to 102.0% which indicates that the method accurate % Recovery was found well within acceptance range at all three levels.

The relative standard derivative and intermediate precision% RSD for 12 sample (Precision and Intermediate Precision samples) NMT 2.0% and the % RSD was found 0.189 and 0.520% Assay value for individual sample must be within 90% to 110% lamivudine and tenofovir disoproxil fumarate.

The result of robustness was found to be satisfactory within range. The change in wavelength was found to be absolute difference between Assay of precision study and change in wavelength (+3NM and -3NM)) NMT 2.0. % RSD of change in flow rate Absolute difference between Assay of precision study and change in Flow rate (-10 and +10%) NMT 2.0 and change in column oven temperature Absolute difference between Assay of precision study and change in Column oven temp (-2°C and +2°C) NMT 2.0

The LOD of Lamivudine and Tenofovir disoproxil Fumarate was found to be 0.99 µg/ml and 0.58µg/ml. The LOQ of Lamivudine and Tenofovir disoproxil Fumarate was found to be 3.01µg/ml and 1.76µg/ml. The developed RP-HPLC method was simple specific accurate precise and robust for detection of Lamivudine and Tenofovir disoproxil Fumarate in bulk and tablet dosage form.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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