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Original Article

RP-HPLC method development and validation for the simultaneous determination of lamivudine, abacavir and dolutegravir in pharmaceutical dosage forms

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ABSTRACT

Abacavir is an antiretroviral drug used to treat HIV/AIDS. Lamivudine (2′,3′-dideoxy-3′-thiacytidine, commonly called 3TC) is a potent nucleoside analog reverse transcriptase inhibitor (nRTI). Dolutegravir (DTG) is an FDA-approved drug for the treatment of HIV infection. Dolutegravir is an integrase inhibitor. A combination product of the above three drugs is being marketed under the brand name of Triumeq in India. In the present study focus is laid on this triple combination drug, The retention times of Lamivudine, Abacavir and Dolutegravir were found to be 2.2 min, 2.9 min & 7.4 min respectively. Regression coefficient r^2 value was 0.999 for Lamivudine, Abacavir and Dolutegravir and the response was linear. The percentage mean recovery of Lamivudine, Abacavir and Dolutegravir were found to be 100.04, 99.73 and 100.29% respectively. %RSD values of repeatability and intermediate precision were ≤2 and the method is precise. The solution stability studies of method indicate that the Lamivudine, Abacavir and Dolutegravir drugs were stable up to 24 hours. Hence, the developed method can be successfully employed for routine quality control of Lamivudine, Abacavir and Dolutegravir in drug testing laboratories and pharmaceutical industries.

Keywords: Lamivudine, Abacavir, Dolutegravir, RP-HPLC, HIV.

INTRODUCTION

Abacavir is an antiretroviral drug used to treat HIV/AIDS. It is of the nucleoside analog reverse transcriptase inhibitor (NRTI) type. Viral strains that are resistant to Zidovudine or Lamivudine are generally sensitive to Abacavir. It is well tolerated the main side effect is hypersensitivity, which can be severe, and in rare cases, fatal. Genetic testing can indicate whether an individual will be hypersensitive; over 90% of people can safely take Abacavir.

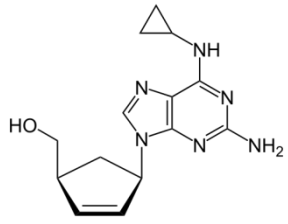

Fig1. Chemical Structure of Abacavir

Lamivudine (2′,3′-dideoxy-3′-thiacytidine, commonly called 3TC) is a potent nucleoside analog reverse transcriptase inhibitor (nRTI). It is marketed in the United States by GlaxoSmithKline under the tradenames Epivir and Epivir-HBV. Lamivudine has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV/AIDS. It improves the seroconversion of e-antigen positive hepatitis B and also improves histology staging of the liver. Long term use of Lamivudine leads to emergence of a resistant hepatitis B virus (YMDD) mutant. Despite this, Lamivudine is still used widely as it is well tolerated. It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.

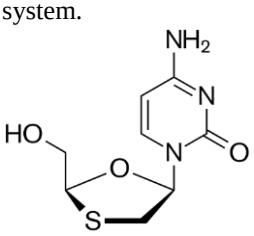

Fig 2 Chemical Structure of Lamivudine

Dolutegravir (DTG) is an FDA-approved drug for the treatment of HIV infection. Dolutegravir is an

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integrase inhibitor. Known as S/GSK1349572 or just "572" the drug is marketed as Dolutegravir by GlaxoSmithKline (GSK). In February, 2013 the Food and Drug Administration announced that it would fast track Dolutegravir's approval process. On August 13, 2013, Dolutegravir was approved by the FDA. On November 4, 2013, Dolutegravir was approved by Health Canada. On January 16, 2014, Dolutegravir was approved by the European Commission for use throughout the European Union.

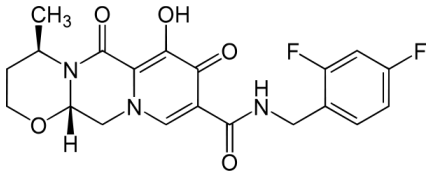

Fig 3 Chemical Structure of Dolutegravir

A combination product of the above three drugs is being marketed under the brand name of Triumeq in India. Since there were no methods available for the simultaneous estimation of the above three drugs in the combination product when we started our work. The analytical methods reported so far which are either in single or combination with other drugs are reviewed in the following literature survey.

Raja et al [1] reported a spectrophtometric method for the estimation of Abacavir sulfate in pharmaceutical formulations. Anil et al [2] reported a method for the simultaneous determination of antiretroviral drugs, Abacavir sulfate and Lamivudine in tablet dosage forms. Mandloi et al [3] reported a RP-HPLC method for the determination of Lamivudine in the bulk drug and tablet dosage forms. HariPrasad et al [4] reported a reverse phase-high performance liquid chromatographic method for the simultaneous determination of Lamivudine and Stavudine in tablet dosage forms. Lavanya et al [5] reported a RP-HPLC method for the estimation of Abacavir sulfate in bulk and pharmaceutical dosage forms. Sarat et al [6] reported a stability-indicating Ultra high-performance liquid chromatography (UPLC) method for the simultaneous estimation of Abacavir sulfate and Lamivudine in the capsule dosage forms. Palavan et al [7] reported a high performance liquid chromatographic method for the quantitative estimation of Abacavir, Lamivudine and Zidovudine simultaneously in tablet dosage forms. Pradeep Kumar et al [8] reported a RP-HPLC method for the estimation of Abacavir in bulk and in tablet dosage forms. Pradeep et al [9] reported development and validation of RP-HPLC chromatographic method for the estimation of Abacavir sulfate. Mohideen et al [10] reported a reverse phase HPLC method for the simultaneous analysis of Abacavir and Lamivudine in combined dosage forms. Raja et al [11] reported a method for the estimation of Abacavir, Lamivudine and Zidovudine by high performance liquid chromatography (HPLC) on a C18 column with UV detection at 270 nm. Sudha et al [12] reported a rapid high performance liquid chromatographic method for the estimation of Lamivudine and Abacavir simultaneously in combined dosage forms. Vaishali et al [13] reported an analytical method for the simultaneous estimation of Abacavir and Lamivudine in pure bulk drug and in combined tablet dosage form by UV spectrophotometric Vierodt's method. Vaishali et al [14] reported a RP-HPLC method for the simultaneous estimation of Abacavir (ABA) and Lamivudine (LAM) in pure bulk drug and in tablet dosage forms. Bennetto-Hood C et al [15] reported a sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) assay.

Various analytical methods have been reported in literature to detect and quantify the individual drugs Abacavir, Lamivudine and Dolutegravir. But there is no official method reported for the simultaneous estimation of Abacavir, Lamivudine and Dolutegravir. Hence, a new analytical method development which is simple, accurate and precise. The main aim and objective of the present study is to develop and validate a new Reverse Phase High Performance Liquid Chromatographic method for the simultaneous determination of Abacavir, Lamivudine and Dolutegravir in pharmaceutical dosage form.

EXPERIMENTAL PROCEDURE

Instrumentation: Chromatography was performed with Alliance Waters 2695 HPLC provided with high speed auto sampler, column oven, degasser and & 2996 PDA detector to provide a compact and with class Empower-2 software.

Reagents and chemicals: The reference samples of of Abacavir, Lamivudine and Dolutegravir were provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade acetonitrile, HPLC grade methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial formulations (Brand Name: Triumeq Tablets; Lable Claim: Lamivudine 300mg, Abacavir 600mg and Dolutegravir 50mg) were purchased from the local pharmacy.

Preparation of buffer Solution: Accurately weighed 1.36gm of Potassium dihydrogen orthophosphate in a 1000ml of volumetric flask,

about 900ml of Milli-Q water added, sonicated and degassed, finally made up to the volume with water and pH was adjusted to 3.0 with dilute orthophosphoric acid.

Preparation of Standard Stock Solution: Accurately weighed and transferred 6mg of Lamivudine, 12mg of Abacavir and 5mg of Dolutegravir working standards into 10ml, 10ml and 50ml clean dry volumetric flasks separately, added 3/4th volume of diluents, sonicated for 30 minutes and made up to the final volume with the diluents to get stock solutions with concentration of 0.6mg/ml of Lamivudine, 1.2mg/ml of Abacavir and 0.1mg/ml of Dolutegravir respectively.

Preparation of Working Standard Solutions: Aliquots of 0.25, 0.5, 0.75, 1, 1.25 & 1.5 mL were pipette out from the above three stock solutions and transferred into a 10 ml volumetric flask and volume was made up to 10 ml with diluents. This gives solutions of 15,30,45,60,75 and 90 µg/ml of Lamivudine, 30,60,90,120, 150 and 180 µg/ml of Abacavir and 2.5,5,7.5,10,12.5 and 15 µg/ml of Dolutegravir respectively.

Sample preparation: 20 tablets were weighed, powdered and the average weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 70ml of diluent added and sonicated for 30 min, further the volume made up with the diluent and filtered. From the filtered solution 0.2ml was pipette out into a 10 ml volumetric flask and made up to 10ml with the diluent gives 60µg/ml Lamivudine, 120µg/ml Abacavir and 10 µg/ml of Dolutegravir.

Chromatographic condition: The chromatographic separation was carried out under the isocratic conditions. Chromatographic separation was achieved by injecting a volume of 10µl of standard solution into Inertsil ODS (250 x 4.6 mm, 5µm) column. The mobile phase of composition buffer: acetonitrile: methanol 50:20:30%v/v were allowed to flow through the column at a flow rate of 1.0 ml/min for a period of 11 min at 30^{0}C column temperature. Detection of the component was carried out at a wavelength of 225 nm.

Method Validation:

System Suitability Tests: Data from six injections of 10 µl of the working standard solutions of Lamivudine (60µg/ml) Abacavir (120µg/ml) and Dolutegravir (10µg/ml) were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates, retention time and resolution factor.

Specificity: The specificity of the method was performed by injecting blank solution, placebo solution and standard solutions of Lamivudine, Abacavir and Dolutegravir separately.

Linearity: By taking appropriate aliquots of the standard Lamivudine, Abacavir and Dolutegravir solutions with the mobile phase, six working solutions ranging between 15-90 µg/ml Lamivudine, 30-180 µg/ml Abacavir and 2.5-15 µg/ml Dolutegravir were prepared. Each experiment linearity point was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of Lamivudine, Abacavir and Dolutegravir to obtain the calibration curve.

Accuracy: Previously analyzed samples of Lamivudine, Abacavir and Dolutegravir to which known amounts of standard Lamivudine (60µg/ml) Abacavir (120µg/ml) and Dolutegravir (10µg/ml) corresponding to 50%, 100% and 150% of target concentration were added. The accuracy was expressed as the percentage of analyte recovered by the proposed method.

Precision: The repeatability and intermediate precision were determined by analyzing the samples of Lamivudine (60µg/ml) Abacavir (120µg/ml) and Dolutegravir (10µg/ml).

Limit of detection and the limit of quantification: Limit of detection (LOD) and limit of quantification (LOQ) of Lamivudine, Abacavir and Dolutegravir were determined by calibration curve method. Solutions of Lamivudine, Abacavir and Dolutegravir were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations.

$LOD = (3.3 \times Syx)/b$, $LOQ = (10.0 \times Syx)/b$

Where Syx is residual variance due to regression; b is slope.

Robustness: The robustness of themethod was performed by deliberately changing the chromatographic conditions. The parameters included slight variation in organic phase percentage in the mobile phase (45, 55%), flow rate (0.9, 1.1 ml/min) and column temperature (25, 35°C).

Stability: The sample solutions were injected at 0hr (comparison sample) and after 24hr (stability sample) by keeping at ambient room temperature. Stability was determined by determining %RSD for sample and standard solutions.

RESULTS AND DISCUSSION

Method development: Initially reverse phase liquid chromatography separation was attempted by using various ratios of methanol and water, acetonitrile and water as mobile phases, in which both the drugs did not responded properly and also the peak shapes and separations were not achieved to the best of requirement .Hence, the organic content of mobile phase was further investigated to optimize the separation of both drugs.To improve the tailing factor, the pH of mobile phase was adjusted. Thereafter, buffer: acetonitrile and methanol were taken in ratio of 50:20:30%v/v/v and with a flow rate of 1.0 ml/min was employed which is ideal for the successful elution of the analytes. Preliminary development trials were performed with different analytical columns of different types from different manufacturers with different configurations. Among the analytical columns tried, Inertsil ODS column (250mmx, 4.6, 5µm particle size) was selected as the stationary phase to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 205nm to 280nm. Lamivudine, Abacavir and Dolutegravir showed maximum absorption at 225nm of wavelength and the same was selected as the detection wavelength for PDA detector. The retention times were found to about 2.2min, 2.9min and 7.4min for Lamivudine, Abacavir and Dolutegravir respectively. The chromatograms obtained for blank injection, placebo injection and optimized method were shown in the Fig.4, 5 and 6 respectively and optimized chromatographic conditions were shown in Table 1.

Table 1: Optimized chromatographic conditions

S. No.	Parameter	Condition
1	Mobile phase	Buffer: Acetonitrile:Methanol 50:20:30%v/v
2	pH	3
3	Diluents	Initially methanol and further with buffer
4	Column, make	Inertsil ODS 250 x 4.6 mm, 5µm
5	Column temperature	30^0C
6	Wave length	225nm
7	Injection volume	10µl
8	Flow rate	1.0ml/min
9	Run time	11min
10	Retention time (Lamivudine)	2.2 min
11	Retention time (Abacavir)	2.9 min
12	Retention time (Dolutegravir)	7.4 min

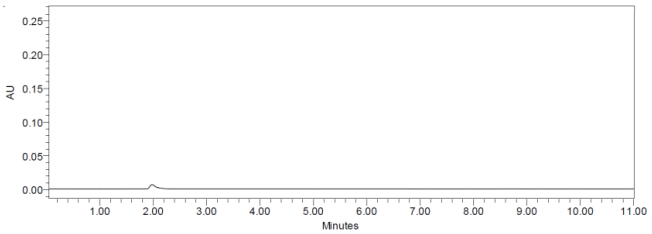

Fig 4 Chromatogram of Blank

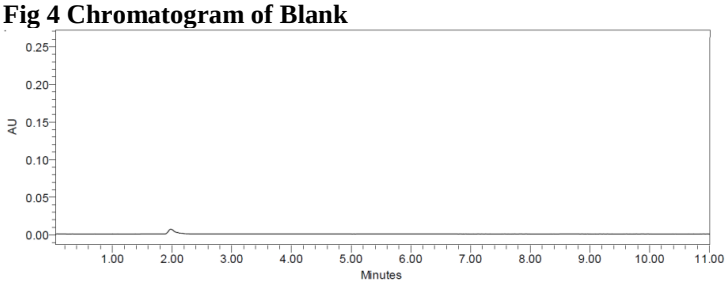

Fig 5 Chromatogram of Placebo

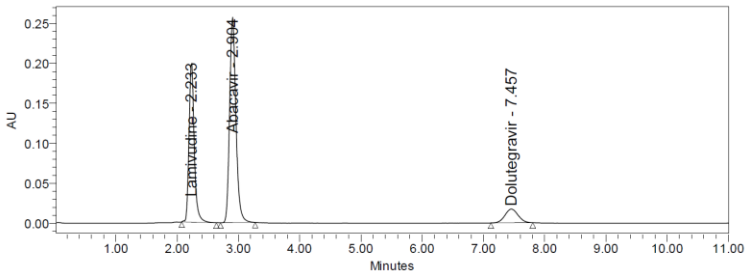

Fig 6. Chromatogram of Lamivudine, Abacavir and Dolutegravir standards

Method Validation:

System Suitability Test: Various system suitability parameters such as number of theoretical plates, peak tailing, retention time and resolution factor were determined. The total run time required for the method is only 11 minutes for eluting Lamivudine, Abacavir and Dolutegravir. The results obtained were shown in Table No.9.2 and 9.3. The number of theoretical plates was found to be > 2000, USP tailing was < 2 and USP resolution is above 2. The % RSD of areas for Lamivudine, Abacavir and Dolutegravir were 0.5%, 0.3% and 1.1% respectively.

Table 2. System Suitability of Lamivudine, Abacavir and Dolutegravir

S. No	Area of Lamivudine	Area of Abacavir	Area of Dolutegravir
1.	1173330	1846023	262501
2.	1178547	1837742	257149
3.	1183093	1832936	262798
4.	1190091	1841223	261374
5.	1182532	1837656	264736
6.	1186096	1846883	264958
Mean	1182282	1840411	262253
S.D	5836.71	5378.29	2851.82
%RSD	0.5	0.3	1.1

Table 3. System Suitability parameters for Lamivudine, Abacavir and Dolutegravir

Property	Lamivudine	Abacavir	Dolutegravir
Retention time (Rt)	2.2±0.3min	2.9± 0.3 min	7.4±0.3min
Theoretical plates (N)	3360± 163.48	3690± 163.48	5200± 163.48
Tailing factor (T)	1.25± 0.117	1.20 ± 0.117	1.08± 0.117

Specificity: The specificity of the method was performed by injecting blank solution, placebo solution and standard solutions separately. The chromatogram of the drug was compared with blank and placebo chromatogram to verify the interference. No interfering peak was observed at the retention time of Lamivudine, Abacavir and Dolutegravir. Hence, the method is specific for the determination of Lamivudine, Abacavir and Dolutegravir.

Linearity: Lamivudine showed a linearity of response between 15-90 µg/ml, Abacavir showed a linearity of response between 30-180 µg/ml and for Dolutegravir linearity response was between 2.5-15 µg/ml. These were represented by a linear regression equation as follows: y (Lamivudine) = 19325x + 717.24 (r^2=0.9999), y(Abacavir)= 15299x + 1679.5 (r^2=0.9998) and Dolutegravir was represented was regression equation was y(Dolutegravir) = 25702x + 762.94(r^2=0.9992) regression line was established by least squares method and correlation coefficient (r^2) for Lamivudine, Abacavir and Dolutegravir is found to be greater than 0.98. Hence, the curves established were linear. The results were shown in the Table 4 and Fig 7-14.

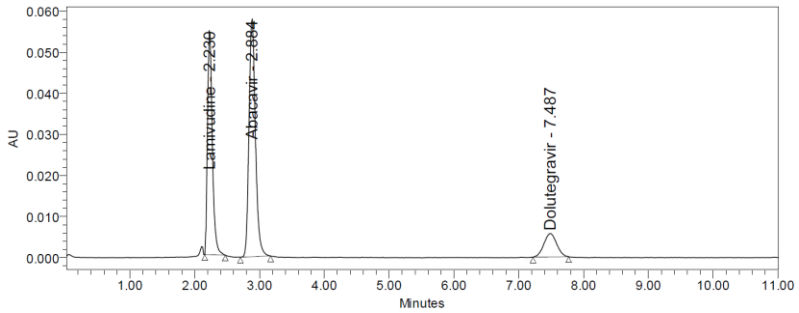

Fig 7 Linearity 25% chromatogram of Lamivudine, Abacavir and Dolutegravir

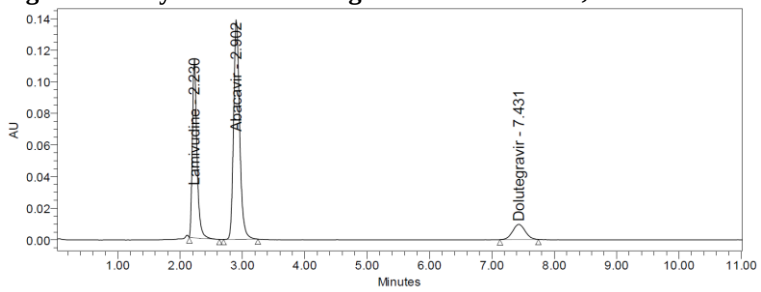

Fig 8 Linearity 50% chromatogram of Lamivudine, Abacavir and Dolutegravir

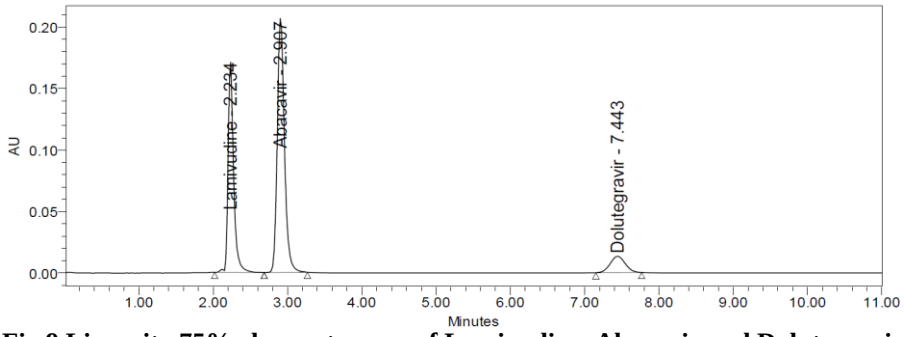

Fig 9 Linearity 75% chromatogram of Lamivudine, Abacavir and Dolutegravir

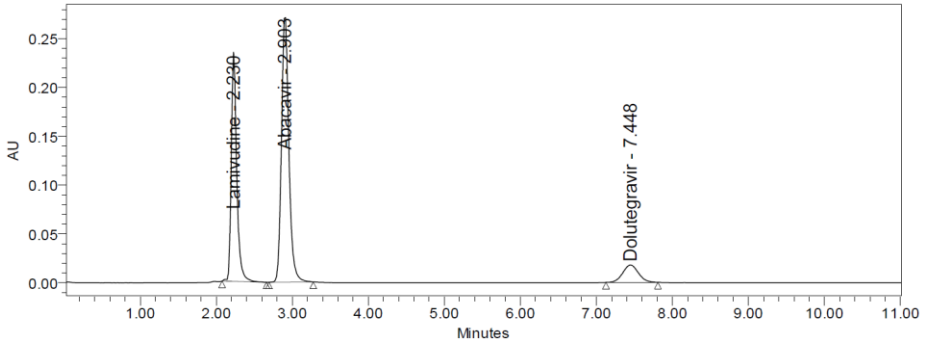

Fig 10 Linearity 100% chromatogram of Lamivudine, Abacavir and Dolutegravir

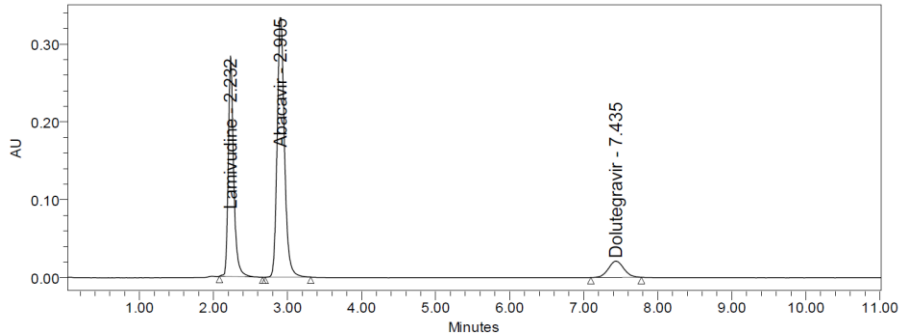

Fig 11 Linearity 125% chromatogram of Lamivudine, Abacavir and Dolutegravir

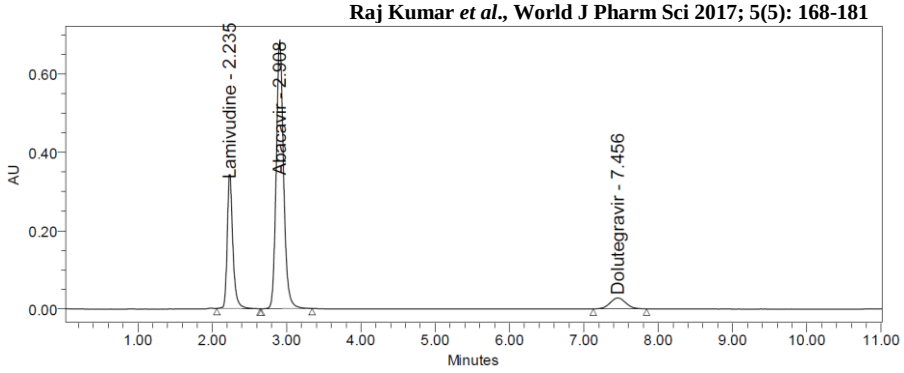

Fig 12 Linearity 150% chromatogram of Lamivudine, Abacavir and Dolutegravir

Table 4. Linearity data for Lamivudine, Abacavir and Dolutegravir

Lamivudine		Abacavir		Dolutegravir	
Conc (µg/ml)	**Peak area Average (n=3)**	**Conc (µg/ml)**	**Average peak area (n=3)**	**Conc (µg/ml)**	**Peak area Average (n=3)**
15	287355	30	442689	2.5	67362
30	577105	60	946792	5	133714
45	876113	90	1381750	7.5	188177
60	1171972	120	1833868	10	254075
75	1443174	150	2285640	12.5	320605
90	1736655	180	2759400	15	390766

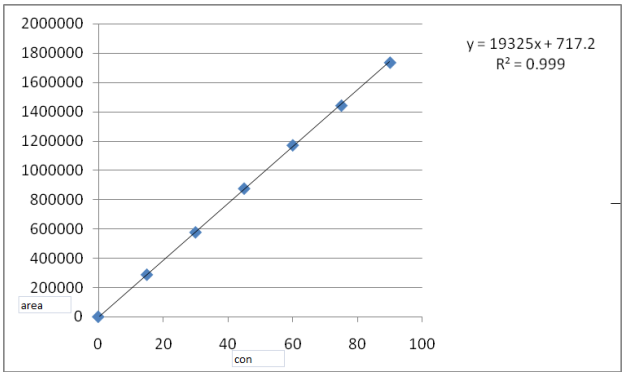

Fig 13. Calibration curve of Lamivudine

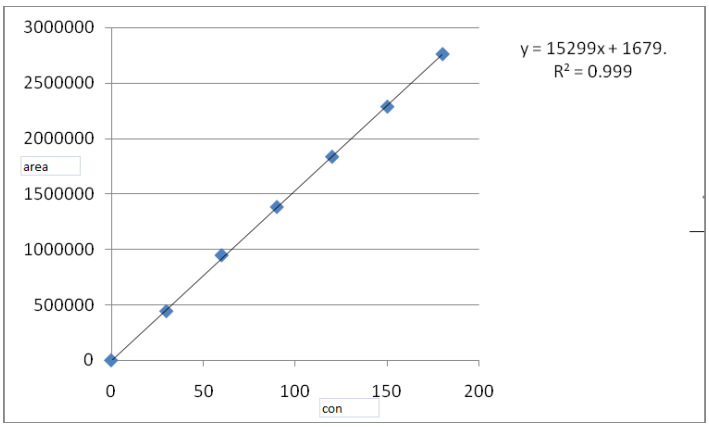

Fig 14. Calibration curve of Abacavir

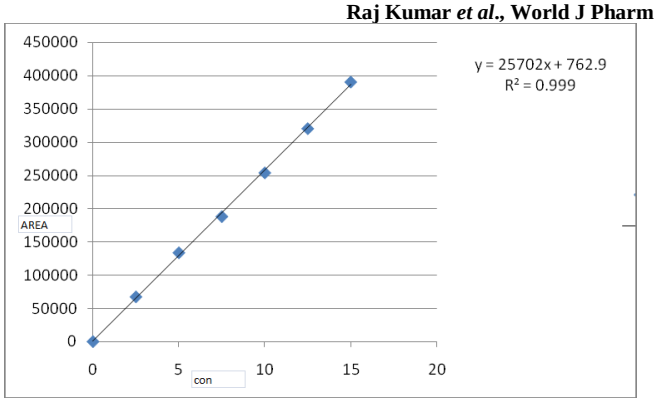

Fig 15. Calibration curve of Dolutegravir

Accuracy: To pre analyzed sample solution, a definite concentration of standard drug (50%, 100% & 150 % level) was added and recovery was studied. The % Mean recovery for Lamivudine, Abacavir and Dolutegravir are 100.04%, 99.73% and 100.29% respectively and these results are within acceptable limit of 98-102. The % RSD for Lamivudine, Abacavir and Dolutegravir are 0.8, 0.7 and 0.5 respectively and % RSD for Lamivudine, Abacavir and Dolutegravir are within limit of ≤2. Hence, the proposed method is accurate and the results are summarized in Table-5 and figure 16-18.

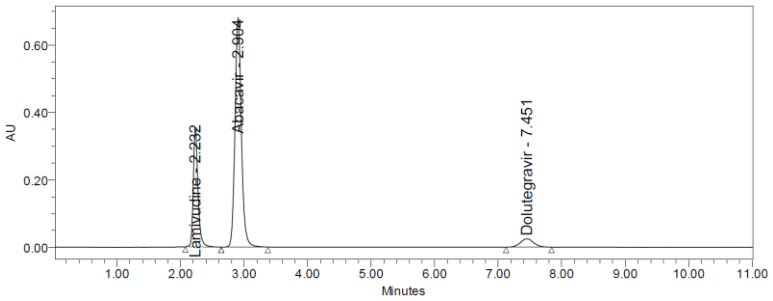

Fig 16 Accuracy 50% chromatogram of Lamivudine, Abacavir and Dolutegravir

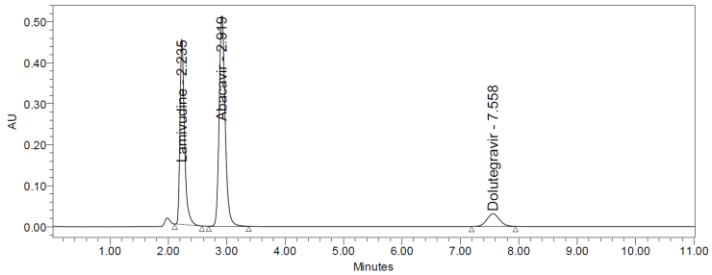

Fig 17 Accuracy 100% chromatogram of Lamivudine, Abacavir and Dolutegravir

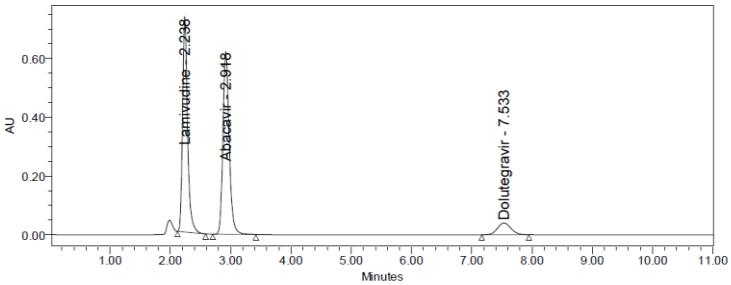

Fig 18 Accuracy 150% chromatogram of of Lamivudine, Abacavir and Dolutegravir

Table 5 Results of Recovery Experiments of Lamivudine, Abacavir and Dolutegravir

Preanalysed amount (µg/ml)			Spiked Amount (µg/ml)			% Recovered		
LMD	ABV	DTR	LMD	ABV	DTR	LMD	ABV	DTR
60	120	10	30	60	5	99.83	99.66	100.54
60	120	10	30	60	5	100.61	98.39	99.88
60	120	10	30	60	5	99.87	100.33	99.38
60	120	10	60	120	10	101.17	100.20	99.97
60	120	10	60	120	10	98.71	98.96	100.80
60	120	10	60	120	10	99.37	99.18	100.40
60	120	10	90	180	15	99.80	100.23	99.95
60	120	10	90	180	15	100.17	100.52	100.67
60	120	10	90	180	15	100.80	100.06	101.01
					MEAN	100.04	99.73	100.29
					SD	0.75	0.73	0.53
					%RSD	0.8	0.7	0.5

Precision: The repeatability and Intermediate precision data were summarized in Table 6 and 7, respectively and were assessed by the use of standard solutions of Lamivudine, Abacavir and Dolutegravir.

Repeatability: Six replicates injections in same concentration of Lamivudine, Abacavir and Dolutegravir were analyzed in the same day for repeatability and the % RSD for Lamivudine, Abacavir and Dolutegravir found to be 1.1, 0.3 and 1.2 respectively and % RSD for Lamivudine, Abacavir and Dolutegravir found to be within acceptable limit of ≤2 and hence, method is reproducible. The results were shown in the Table 6.

Intermediate precision (Day_ Day Precision): Six replicates injections in same concentration were analyzed on two different days with different analyst and column for verifying the variation in the precision and the % RSD for Lamivudine, Abacavir and Dolutegravir were found to be 0.4, 0.3 and 1.1 respectively and it is within acceptable limit of ≤2. Hence, the method is reproducible on different days with different analyst and column. This indicates that the method is precise. The results were shown in the Table7.

Robustness: **F**ew chromatographic conditions were deliberately altered to evaluate the robustness of the developed HPLC method. The robustness was established by changing the flow rate, column temperature and composition of the mobile phase within allowable limits from actual chromatographic conditions. It was observed that there were no marked change in mean R_t and RSD is within limit of ≤2 .The tailing factor, resolution factor and number of theoretical plates were found to be acceptable limits for Lamivudine, Abacavir and Dolutegravir. Hence, the method is reliable with variations in the analytical conditions and the results were shown in the Table 8 and Fig 19-24.

Table 6. Results of Repeatability of Lamivudine, Abacavir and Dolutegravir

S. No	Area of Lamivudine	Area of Abacavir	Area of Dolutegravir
1.	1168516	1846023	257402
2.	1171494	1837742	265811
3.	1181204	1832936	260258
4.	1198307	1841223	262643
5.	1195566	1837656	261564
6.	1175080	1846883	265572
Mean	1181695	1840411	262208
S.D	12570.67	5378.29	3218.76
%RSD	1.1	0.3	1.2

Table 7. Results of Intermediate precision of Lamivudine, Abacavir and Dolutegravir

S. No	Area of Lamivudine	Area of Abacavir	Area of Dolutegravir
1.	1185178	1850420	266606
2.	1189517	1844088	260972
3.	1192379	1838584	265900
4.	1190091	1844446	265141
5.	1194139	1843777	269278
6.	1198528	1851554	267744
Mean	1191639	1845478	265940
S.D	4536.51	4790.80	2834.12
%RSD	0.4	0.3	1.1

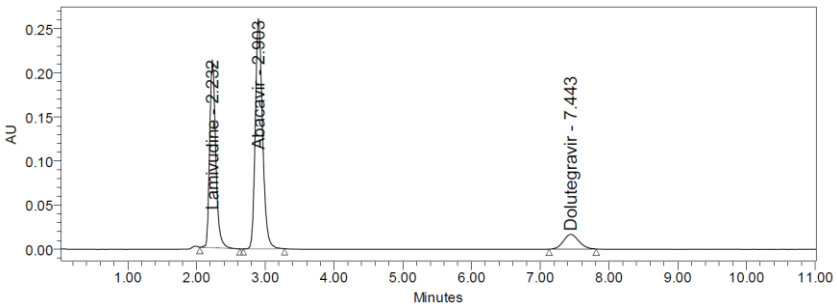

Fig 19. Robustness (Flow Minus: 0.9ml/min) chromatogram of Lamivudine, Abacavir and Dolutegravir

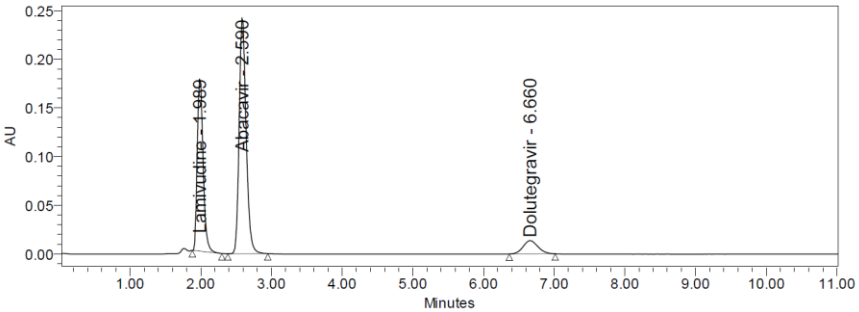

Fig 20. Robustness (Flow Plus: 1.1ml/min) chromatogram of Lamivudine, Abacavir and Dolutegravir

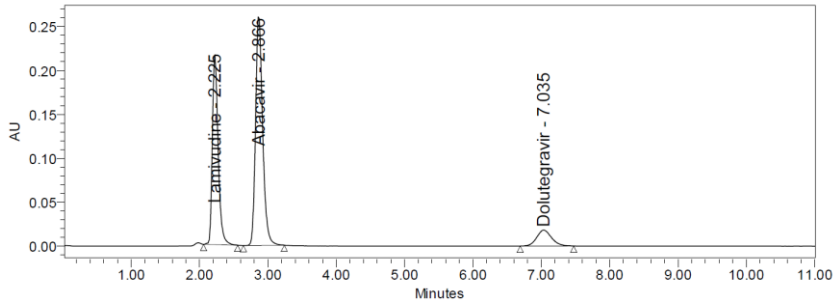

Fig 21. Robustness(Mobile phase minus:45%) chromatogram of Lamivudine, Abacavir and Dolutegravir

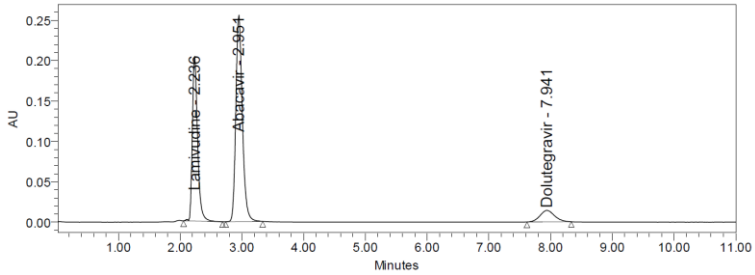

Fig 22. Robustness (Mobile Phase Plus: 55%) chromatogram of Lamivudine, Abacavir and Dolutegravir

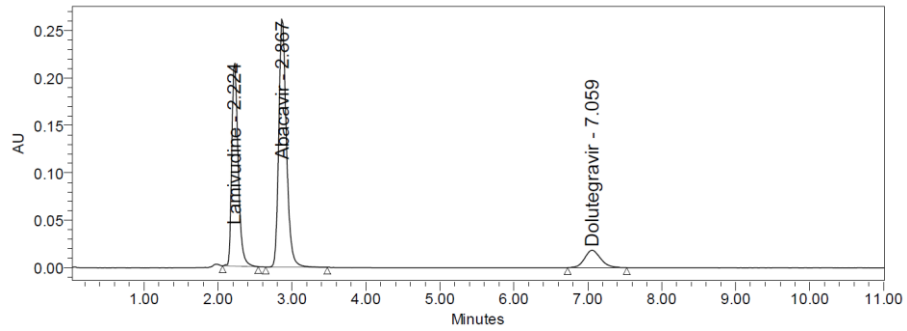

Fig 23. Robustness (Temperature Minus: 25 ^{o}C) chromatogram of Lamivudine, Abacavir and Dolutegravir

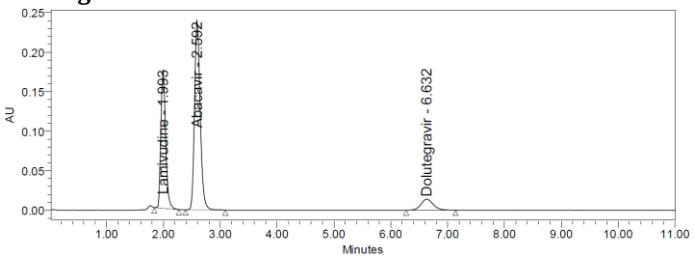

Fig 24. Robustness (Temperature Plus: 35 ^{o}C) chromatogram of Lamivudine, Abacavir and Dolutegravir

Table-8(a): Robustness – Flow Minus (n=6)

S.No.	Parameter	Abacavir	Lamivudine	Dolutegravir
1.	% RSD of area	0.3%	0.4%	1.1%
2.	Tailing Factor	1.22	1.32	1.12
3.	Plate count	3708	3207	5137

Table-8(b): Robustness- Flow Plus (n=6)

S.No.	Parameter	Abacavir	Lamivudine	Dolutegravir
1.	% RSD of area	0.6%	0.5%	1.3%
2.	Tailing Factor	1.30	1.21	1.10
3.	Plate count	3356	2880	4634

Table-8(c): Robustness - Mobile Phase Minus (n=6)

S.No.	Parameter	Abacavir	Lamivudine	Dolutegravir
1.	% RSD of area	0.5%	0.6%	1.1%
2.	Tailing Factor	1.24	1.34	1.12
3.	Plate count	3387	3756	5608

Table-8(d): Robustness – Mobile Phase Plus (n=6)

S.No.	Parameter	Abacavir	Lamivudine	Dolutegravir
1.	% RSD of area	0.4%	0.4%	1.7%
2.	Tailing Factor	1.26	1.35	1.12
3.	Plate count	3374	3687	5785

Table- 8(e): Robustness- Temperature Minus (n=6)

S.No.	Parameter	Abacavir	Lamivudine	Dolutegravir
1.	% RSD of area	0.4%	0.5%	0.5%
2.	Tailing Factor	1.24	1.32	1.14
3.	Plate count	3288	3865	5264

Table-8(f): Robustness – Temperature Plus (n=6)

S.No.	Parameter	Abacavir	Lamivudine	Dolutegravir
1.	% RSD of area	0.5%	0.6%	0.7%
2.	Tailing Factor	1.26	1.35	1.11
3.	Plate count	3214	3975	5457

Stability of sample solution: The sample solution injected after 24 hrs by keeping at ambient room temperature 30ºC did not show any appreciable change. The deviation in the assay is not more than 2 and the results are shown in Table 9.

LOD and LOQ: LOD and LOQ for Lamivudine were 0.10 and 0.32 µg/ml; for Abacavir were 0.11 and 0.33 µg/ml; for Dolutegravir were 0.06 and 0.18 µg/ml respectively. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive and the results were shown in Table-10.

Assay: The percentage assay of labeled claim of Lamivudine, Abacavir and Dolutegravir present in the Triumeq Tablets were 99.85%, 100.11% and 99.88% respectively. % RSD values for Lamivudine, Abacavir and Dolutegravir were within limit of ≤2 and the results were shown in Figure No. Table 11 and Fig 25.

Table 9: Stability data of Abacavir, Lamivudine and Dolutegravir

Drug	%Assay at 0 hr*	%Assay at 24hr*	Deviation
Lamivudine	99.85	99.12	0.52
Abacavir	100.11	99.47	0.45
Dolutegravir	99.88	98.65	0.87

* n=6 for each parameter.

Table 10 LOD and LOQ data of Lamivudine, Abacavir and Dolutegravir

Lamivudine			**Abacavir**			**Dolutegravir**		
S.NO	SLOPE	Y-INTERCEPT	S.NO	SLOPE	Y-INTERCEPT	S.NO	SLOPE	Y-INTERCEPT
1	19199	1421	1	15280	1165	1	25783	513.1
2	19310	357.6	2	15370	1698	2	25692	485.1
3	19466	372.2	3	15246	2174	3	25631	1290
AVG	19325	716.9	AVG	15299	1679	AVG	25702	762.7
SD		609.78	SD		504.77	SD		456.84
LOD		0.10	LOD		0.11	LOD		0.06
LOQ		0.32	LOQ		0.33	LOQ		0.18

Table 11 Assay Data of Lamivudine, Abacavir and Dolutegravir

S. No.	Drug Name	Amount injected (µg/mL)	Amount found (µg/mL)	% Assay ± SD*
1	Lamivudine	60	59.91	99.85±1.06
2	Abacavir	120	120.13	100.11±0.96
3	Dolutegravir	10	9.988	99.88±1.23

* n=6 for each parameter; Lable Claim: Triumeq Tablets Lamivudine 300mg, Abacavir 600mg and Dolutegravir 50mg.

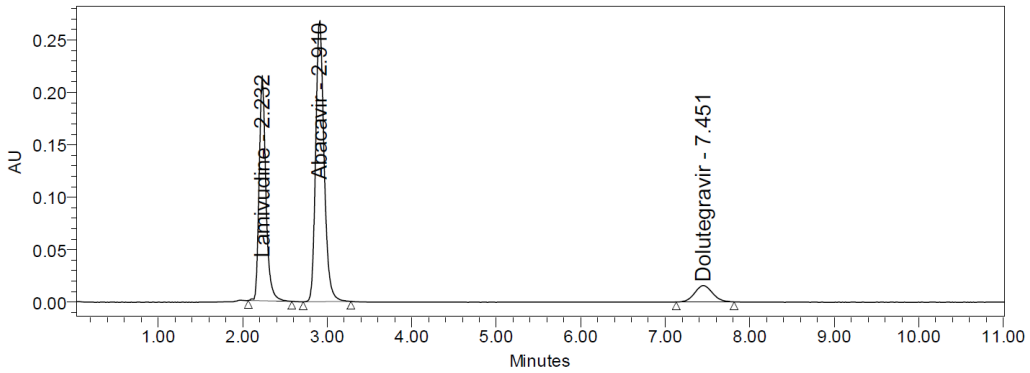

Fig 9.25 Assay chromatogram of Lamivudine, Abacavir and Dolutegravir

DISCUSSION

The present study involves Inertsil ODS C18 (250 x 4.6 mm, 5µm) column as the stationary phase. Phosphate buffer (pH 3.0), acetonitrile, methanol were taken in the ratio 50:20:30%v/v/v and used as mobile phase at a flow rate of 1.0 ml/min. In this method, the numbers of theoretical plates were above 2000. The retention times of Lamivudine, Abacavir and Dolutegravir were found to be 2.2 min, 2.9 min & 7.4 min respectively. Tailing factor is less than 2 and % RSD of peak area is less than 2, this indicates that the optimized method met the system suitability parameters. The regression coefficient r^2 value was 0.999 for Lamivudine, Abacavir and Dolutegravir and the response was linear. The percentage mean recovery of Lamivudine, Abacavir and Dolutegravir were found to be 100.04, 99.73 and 100.29% respectively and it showed that the proposed method is accurate. %RSD values of repeatability and intermediate precision were ≤2 and the method is precise. The lowest values of LOD and LOQ as obtained by the proposed HPLC method indicate that the method is sensitive. The solution stability studies of method indicate that the Lamivudine, Abacavir and Dolutegravir drugs were stable up to 24 hours. In robustness chromatographic conditions were changed as flow minus: 0.9 ml/min; flow plus: 1.1ml/min; temperature minus: 25ºC; temperature plus: 35ºC; mobile phase minus: organic phase 45%v/v; mobile phase plus: organic phase 55%v/v. These changes didn't show any variation in results and it showed the reliability of the method.

CONCLUSION

A new simple, precise and accurate HPLC method was developed and validated for the simultaneous estimation of Lamivudine, Abacavir and Dolutegravir in pharmaceutical dosage form.

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