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A novel rapid combined RP-HPLC stability method development and validation for antiviral HIV combinations lamivudine, tenofovir, doravirine in dosage form and its application to in vitro dissolution

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***Abstract*---**In the analysis of the pharmaceutical agents new sophisticated chromatographic methods have been utilized for the quality control purpose. In the current scenario ample amount of new drugs and newer pharmaceutical formulations are available intended in the cure of diseases. Diseases like HIV, AIDs, Hepatitis, and other viral diseases requires newer drugs and their combinations. As a result of this there is a need for analyse the drugs for quality control purposes. Here the api-drugs Lamivudine LAM, tenofovir TEN, Doravirine DOR, has been analysed by the RP-HPLC method in the tablet dosage-forms. This method is developed for the analysis, of these three drugs in combined forms for rapid analysis with very less amount of analytes drugs utilized for analysis purposes. The concentration range for the linearity selected was 7.5 to 45 µg/ml for Lamivudine LAM & Tenofovir TEN, whereas for Doravirine DOR it is 2.5 to 15 µg/ml. Wavelength selected for estimation was 269nm and chromatographic column used was Acclaim 120 C-18 column (250 mm x 4.6 mm, 5 µm id). The Retention time obtained were 2.16min for LAM, 2.65min for TEN and 3.25min for DOR. The correlation coefficient was found to be 0.9999 and this method is utilized for, the chemical analysis of drugs in synthetic mixtures and in formulation. The Stability & forced-degradations studies are carried out in the different stress conditions and the impurities as well as pure drug substances are efficiently detected by the developed HPLC method.

The application of this HPLC method in the in vitro dissolution for the tablet dosage forms of these three drugs can be analysed, rapidly in this single HPLC method.

***Keywords*---**RP-HPLC, stability, antivirals, lamivudine, tenofovir, doravirine, in vitro dissolution.

Introduction

In the analysis of the pharmaceutical agents there are many newer sophisticated chromatographic methods have been utilized for the quality control purpose. In the current scenario ample amount of new drugs and newer pharmaceutical formulations are available for the treatment of diseases. Even currently the use of the antiviral agents has been extensively used for the management of newer diseases like AIDS, Hepatitis, COVID-19, and many other respiratory diseases. [1]. The newly developed antimicrobial agents like Lamivudine LAM [2], Tenofovir TEN [3] and Doravirine DOR [4] are been widely applied for the treatment of diseases like HIV infections [5] and also in hepatitis. In pharmaceutical industries there are different individual methods of analysis for these drugs.

The literature reviews also suggests the individual and combinational HPLC methods [6] [7] of these drugs, but the methods are for single drugs estimations as well as for the other combinations. Hence there is a need for the rapid testing of these drugs by one single HPLC method as other methods are for individual estimations as well as in combinations only. The single HPLC method is developed in which all the three drugs LAM, TEN and DOR and their tablet dosage forms are analysed and assayed. Thereby this new quick & rapid HPLC method is developed for their analysis in the synthetic mixtures and in formulations as well as for the application of this single HPLC-method for the, in-vitro dissolution study of these three drugs in individual tablet dosage forms.

The antiviral drugs fit in to the class of non nucleoside reverse transcriptase inhibitor. [8] And they act by blocking the viral DNA replication in the hosts. Lamivudine , Tenofovir and Doravirine are highly potent drugs of the antiviral class [9]. Lamivudine is tricyclo derivative – 1 -[(2 - *R*, 5 - *S*) - 2 - (- hydroxyl – methyl) - 1,3—oxa-thio- lan – 5 – yl] - cytosine is the iupac name, is one of the efficient analogue, and Tenofovir is a purine analogue has iupac name [(2-*R*) - 1- (- 6 - amino - purin - 9 – yl -) - propan-2-yl] oxymethyl - phosphonic acid and Doravirine is a Di Aryl pyrimidine compound has iupac name:- 3- chloro - 5 - [[- 1 - [(-4,5 - dihydro - 4 - methyl – 5 - oxo – 1- *H* - 1,2,4 – tri - azol - 3 – yl) methyl] - 1,2 – di-hydro - 2 - oxo - 4 - (-tri- fluoro-methyl) - 3- pyridinyl] oxy] - -- benzonitrile[11] compound used in antiviral therapy.

Chemical structures of drugs

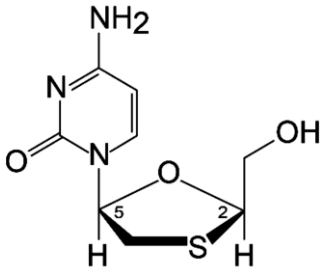

Figure 1. Lamivudine

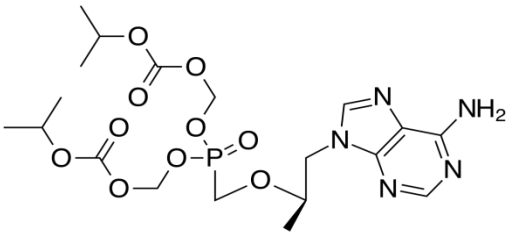

Figure 2. Tenofovir

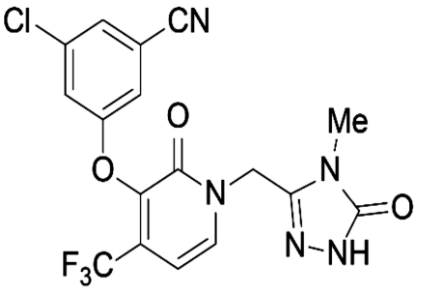

Figure 3. Doravirine

Materials and Methods

Materials

The Working Reference standards Lamivudine LAM, Tenofovir TEN, Doravirine DOR have been obtained from Sava pharma, Bizotech life sciences & Solesom pharma. The chemicals and reagents Methanol, Acetonitrile, orthophosphoric acid, potassium dihydrogen ortho phosphate, Azobisisobutyronitrile AIBN, has been used analytical grade Merck graded. HCl, NaOH analytical grade of Rankem were used. Milli-Q pure water has been used for sample and mobile phase preparations.

Instrumentation

Shimadzu HPLC system Class VP 2010 auto sampler has been used for the analysis equipped with PDA & UV detector. Shimadzu UV 1800 spectrophotometer had been utilized for the wavelength maxima estimation.
Veego VDA-8D Microprocessor Based Dissolution Test Apparatus has been utilized for the study of dissolution profiles of the drugs LAM, TEN, DOR in tablet dosage forms. Wist Temperature Chamber was used for thermal degradation study. Photostability Test Chamber Sanwood SM-LHH-GSD-UV Series was utilised.

Chromatographic conditions

The separation of the drugs Lamivudine LAM, Tenofovir TEN, Doravirine DOR has been made by using Acclaim 120 C-18 column (250 mm x 4.6 mm, 5 µm id). The mobile phase is been utilised consists of ratio of % Percentage by volume of Acetonitrile ACN (25): (75) Potassium Dihydrogen Phosphate buffer (0.02M) of pH 3.4. The flow rate adjusted 1ml/min and detection wavelength was 269nm. The temperature of column was 25 ^{0}C

Preparation of Solutions
Standard Solutions

The standard solutions of the three drugs working reference standards was prepared at concentrations range of 7.5 to 45 µg/ml for Lamivudine LAM & Tenofovir TEN, whereas for Doravirine DOR it is 2.5 to 15 µg/ml.

Sample Solution

The sample solutions were prepared from individual tablets the drugs LAM, TEN, DOR by weighing of the tablet powders. Twenty tablets had been wt and the avg wt was intended. The tablets were dissolved in methanol 50: buffer 50 ratio and sonicated and filtered off. The equivalent weight sufficient to prepare 100 µg/ml of Lamivudine LAM, Tenofovir TEN, Doravirine DOR in stock solution in single sample solution.

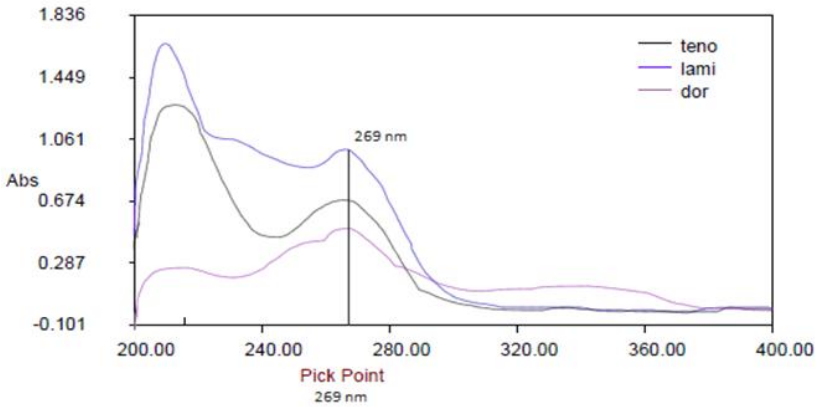

Figure 4. UV overlay Spectra of LAM, TEN DOR 269nm selected wavelength for estimation

Method validation

The Developed analytical method for Lamivudine LAM, Tenofovir TEN, Doravirine DOR is been validated ICH guideline in the terms of precision linearity accuracy LOD LOQ which are required to justify the purpose of the developed method. By employing the use of Acclaim 120 C-18 column the separation and resolution of the drugs was been efficiently made. The developed analytical method proves to be more quick rapid and efficient on the basis of the validation as per the ICH guideline protocols. It proves the practical application of the developed analytical method for the industries and other institutions it the future.

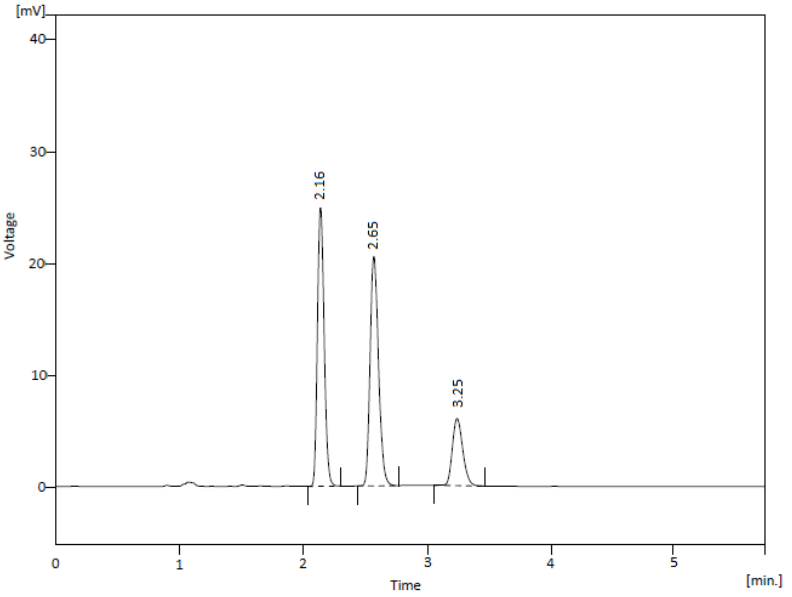

Figure 5. Chromatogram Standard LAM 30µg/ml, TEN 30µg/ml DOR 10µg/ml

System suitability

The developed RP-HPLC method is an accurate precise method following the system suitability parameters as per the USP and ICH protocols. The system suitability has been justified from the data of the retention time resolution of the drugs as well as on the basis of the tailing factors of the chromatograms and from the values of the theoretical plates can be treated as accurate and efficient for the analysis purposes.

Table 1
System Suitability Parameters

Drug	Retention Time Min	Tailing Factor	Theoretical Plates
LAM	2.16	1.10	55641
TEN	2.65	1.11	62365
DOR	3.25	1.13	76231

Optimised chromatographic conditions

Table 2
Optimised Chromatographic Conditions

Parameters	Chromatographic Conditions
Mobile Phase Ratio	Acetonitrile (25) : (75) Phosphate buffer (0.02M) of pH 3.4
Coloumn	Acclaim 120 C-18 column (250 mm x 4.6 mm, 5 µm id)
Detector	PDA & UV
Coloumn Temp	25 ^{0}C
Wavelength	269nm
Flow Rate	1ml/min
Injection Volume	2µl

Specificity

The chromatogram of the standard reference drugs it was found to be accurate for the individual drug analysis as well as in the combination, and no other impurity or other analyte found to be overlapped in the chromatogram. It was confirmed by individual drug injecting multiple times to confirm that method is highly specific for analysis. Also the placebo was injected individually for the sample analysis and it does not interfere in the chromatogram.

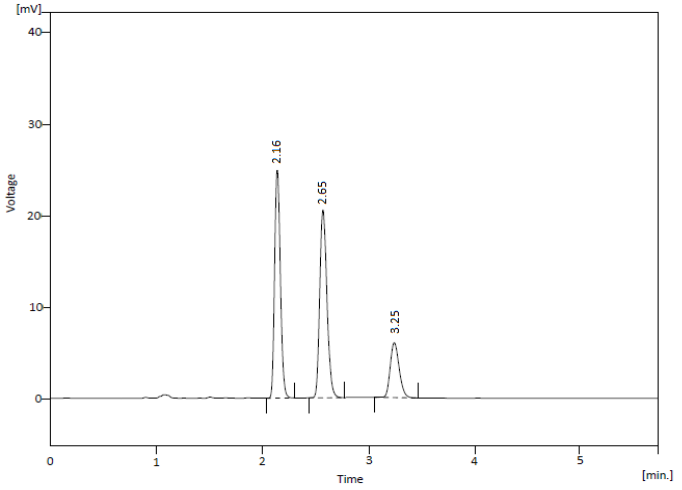

Figure 6. Chromatogram Standard LAM 30µg/ml, TEN 30µg/ml DOR 10µg/ml

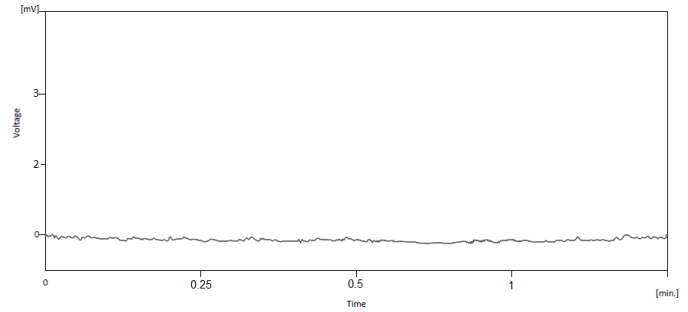

Figure 7. Chromatogram blank mobile phase with placebo

Table 3
Peak Purity Data

DRUG	PEAK PURITY ANGLE	PEAK PURITY THRESHOLD	PEAK PURITY
LAM	0.112	0.285	0.999
TEN	0.132	0.346	0.999
DOR	0.213	0.426	0.999

Linearity

The linearity of the drug response on the basis of variable concentration has been found to be within the range of 7.5 to 45 µg/ml for LAM & TEN that is 7.5, 15, 22.5, 30, 37.5 and 45µg/ml and for DOR is 2.5 to 15 µg/ml that is 2.5, 5, 7.5, 10, 12.5, 15 µg/ml. The graph of peak response vs concentration shows the linear graded response within the same range of concentrations. The correlation coefficient for the drug LAM, TEN, & DOR was found to be nearly 0.9997, 0.9997, & 0.9991 respectively. The dugs LAM , TEN and DOR were individually optimised for identical linearity with minimum conc, ranges 7.5 to 45 µg/ml and 2.5 to 15

µg/ml, so as to efficiently reduce the cost of the utilization of the working standard analyte drugs. It makes the method more efficient and cost effective as compared to other reported methods.

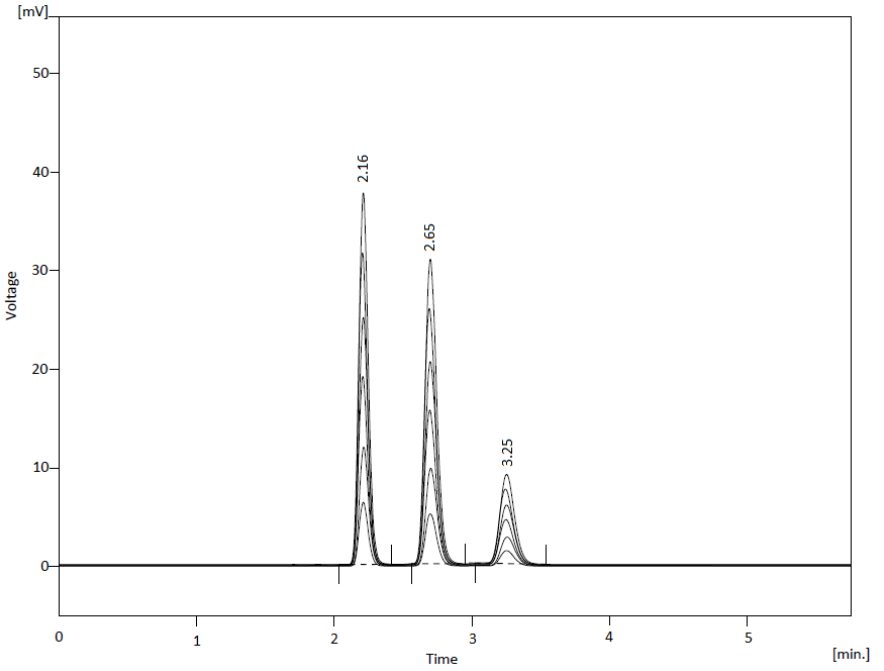

Figure 8. Overlay Chromatogram Linearity LAM, TEN 7.5 TO 45µg/ml, DOR 2.5 TO 15µg/ml

Table 4
Linearity Data of LAM, TEN ,DOR

Linearity	LAM		TEN		DOR	
	Conc. µg/ml	Area	Conc. µg/ml	Area	Conc. µg/ml	Area
1	7.5	628.03	7.5	609.38	2.5	239.24
2	15	1169.14	15	1135.28	5	423.38
3	22.5	1858.71	22.5	1805.05	7.5	673.10
4	30	2437.74	30	2367.36	10	882.74
5	37.5	3051.53	37.5	2976.69	12.5	1094.71
6	45	3656.61	45	3552.51	15	1324.11

LOD limit of detection & LOQ limit of quantification

The method developed is highly sensitive in very dilute solutions that can be observed from the LOD and LOQ values. LOD of the Lamivudine LAM, Tenofovir TEN, Doravirine DOR is found to be 1.426, 1.431 and 0.589 µg/ml correspondingly and the LOQ values of LAM, TEN and DOR are respectively 4.322, 4.336 and 1.785 µg/ml.

Forced degradation studies

The HPLC method is also applicable in the stability study during forced-degradation stress studies of the drug products and drug substances. It was carried out by ICH Q1 AR2 guidelines in which the degradation of these drug substances was been made out, - by different- stress conditions like Acid, Alkali, Hydrolytic, Thermal and Photo degradation for the drugs substances. In the forced degradation study the 0.1N HCl was utilised and the drug samples were subjected for degradation for at different time intervals of 30, 60, 120 minutes and also on a thermostat heat bath at 60^0C temperature to accelerate the degradation process. Further Degradation was also carried out at 1N HCl, 2N HCl to analyse higher rate of degradations. Identical process was carried out in alkaline degradation by using 0.1N NaOH at 30, 60, 120min time intervals and higher rate degraded by using 1N NaOH & 2N NaOH.

The control and the samples were neutralized and then analyzed by the developed HPLC method. The Thermal degradation was carried out in a controlled oven Wist Temperature Chamber at different ranges of 60, 80, 100 ^{0}C, and the samples were analysed at time intervals of 60mins and at longer durations 120mins, 180mins to 5hrs for analyzing higher degradation rates. Oxidative stress was applied by using 3% H_2O_2 and parallel with 0.01M Azobisisobutyronitrile AIBN for the degradation of the drug samples for 30,60,120 minutes has been done. Photo UV stress has been applied by putting the drug samples in Photostability Test Chamber Sanwood SM-LHH-GSD-UV Series , Photolytic (1.2million lux hrs and 200watt hrs)**,** the time interval was 6hrs, 12hrs, 24hrs and 48hrs to check the degradation pattern in longer duration of time.

The samples and standard of different degradation methods were filtered through nylon membrane 0.45um and injected individually as well as in the combined forms. The developed HPLC method efficiently detects the drug samples in the chromatograms and can be compared with the reference standard chromatograms. The major peaks of individual drugs are unaffected by impurity peaks, and can be efficiently resolved with peak purity analysis that justifies no interference, merging or overlapping of other peaks.

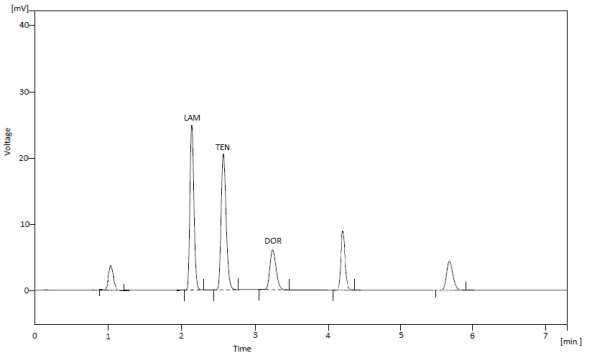

Figure 9. Acid Degradation LAM, TEN , DOR

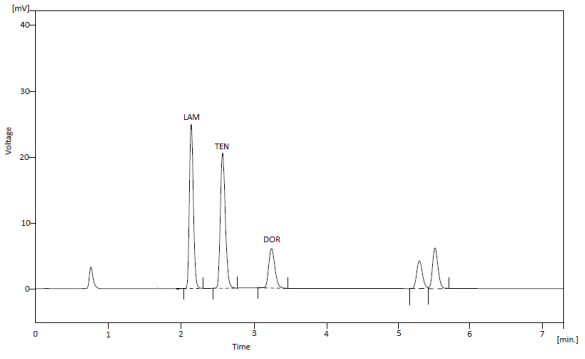

Figure 10. Base Degradation LAM, TEN, DOR

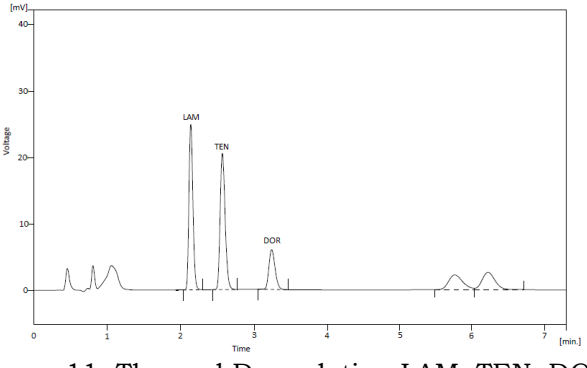

Figure 11. Thermal Degradation LAM, TEN, DOR

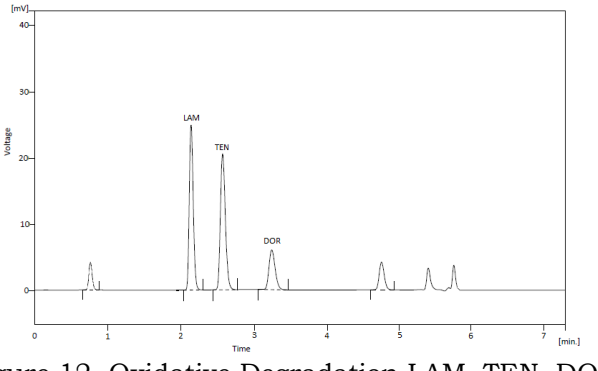

Figure 12. Oxidative Degradation LAM, TEN, DOR

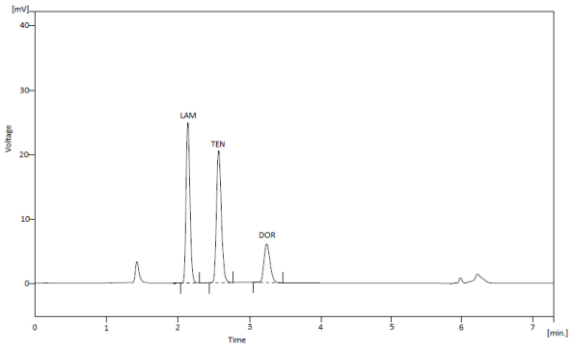

Figure 13. Hydrolytic Degradation LAM, TEN, DOR

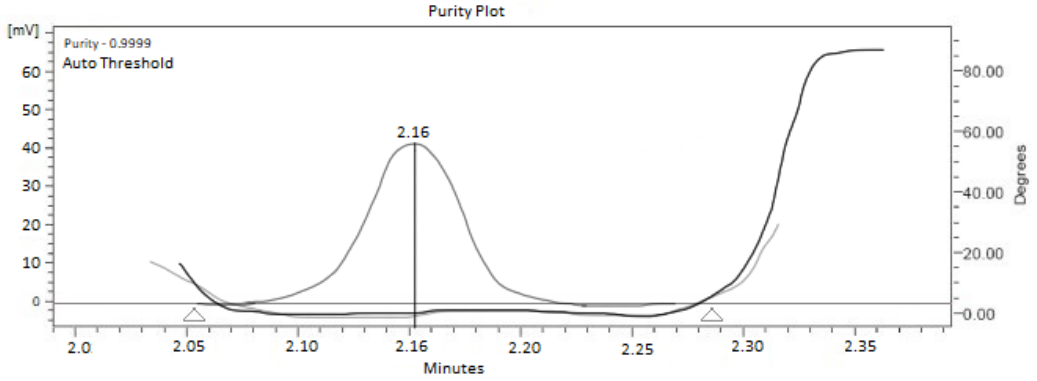

Figure 14. Peak Purity of LAM

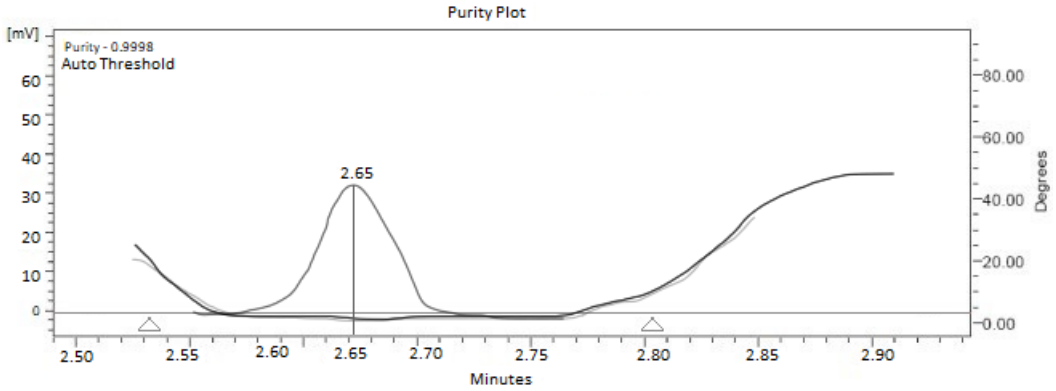

Figure 14. Peak Purity of LAM

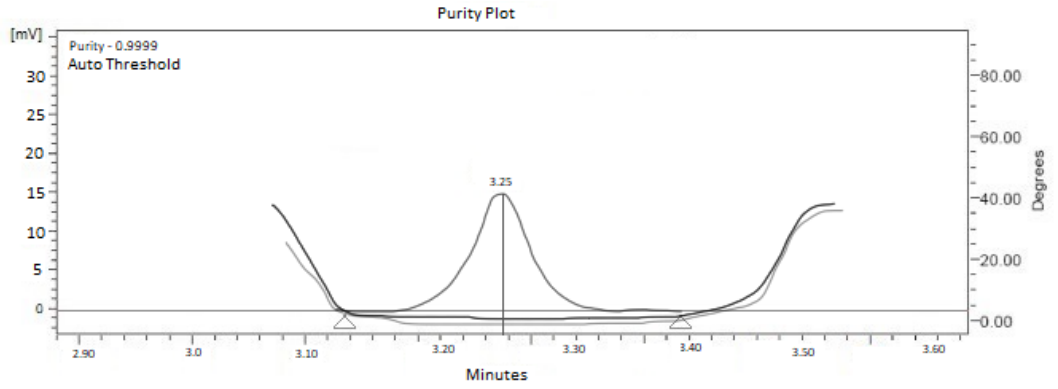

Figure 16. Peak Purity of DOR

Table 5
Stress Degradation Peak Purity Data

	PEAK PURITY ANGLE			PEAK PURITY THRESHOLD			PEAK PURITY		
	LAM	TEN	DOR	LAM	TEN	DOR	LAM	TEN	DOR
Acid	0.110	0.181	0.210	1.245	1.241	1.210	0.999	0.999	0.997
Base	0.112	0.211	0.167	1.256	1.256	1.294	0.998	0.996	0.998
Oxidative	0.218	0.310	0.116	1.236	1.264	1.267	0.999	0.997	0.999
Thermal	0.187	0.246	0.132	1.299	1.246	1.210	0.997	0.997	0.999
Photolytic	0.199	0.174	0.198	1.241	1.298	1.244	0.998	0.999	0.999
Hydrolytic	0.164	0.119	0.310	1.361	1.310	1.298	0.999	0.999	0.998

Table 6
Stress Degradation study summarized data

Degradation Condition	Peak Area			% Drug Recovered			% Degraded		
	LAM	TEN	DOR	LAM	TEN	DOR	LAM	TEN	DOR
Acid	2285.62	2291.33	845.39	93.79	97.04	95.87	6.20	2.95	4.12
Base	2350.44	2239.73	831.25	96.45	94.85	94.27	3.54	5.14	5.72
Oxidative	2286.33	2276.99	851.78	93.82	96.43	96.60	6.17	3.56	3.39
Thermal	2345.66	2217.23	829.32	96.26	93.90	94.05	3.73	6.09	5.94
Photolytic	2364.71	2268.38	856.79	97.04	96.06	97.17	2.95	3.93	2.82
Hydrolytic	2411.62	2334.77	872.13	98.96	98.87	98.91	1.03	1.12	1.08

Precision

The developed method is validated and has been marked precise as per the validation parameter performed in this method. Different samples and standards in six sample and standards were injected multiple times and the SD and RSD was determined. The assay results were also performed and checked for each drug. It shows the how the method is precise.

Table 7
Precision Repeatability Data of LAM & TEN 30 µg/ml DOR 10µg/ml

Number of Injections	LAM Area 30 µg/ml	TEN Area 30 µg/ml	DOR Area 10 µg/ml
1	2437.33	2367.33	882.64
2	2440.85	2361.33	885.64
3	2435.40	2371.44	890.21
4	2442.33	2375.54	889.44
5	2441.02	2362.45	885.31
6	2435.55	2369.33	888.81
AVG	2438.747	2367.90	887.00
SD	3.02	5.40	2.94
% RSD	0.12	0.22	0.33

Accuracy

The accuracy studies- has been carried out as per the guideline at three different levels. The level of 50, 100 and 150 % has been made,- for justify the accuracy of the developed method. For the standard and the test samples the accuracy study had been done for showing accurate performance of the developed method.

Table 8. Accuracy Data at Three different levels

DRUG	Sample Amount µg/ml	% Conc. Level	Total Area	Net Area	Amount STD Added µg/ml	Amount STD Recovered µg/ml	% Recovery	Mean Recovery
LAM	15	50	1752.01	582.86	7.5	7.47	99.70	99.37
		100	2329.41	1160.26	15	14.88	99.24	
		150	2908.32	1739.17	22.5	22.31	99.17	
TEN	15	50	1699.15	563.86	7.5	7.45	99.33	99.70
		100	2265.19	1129.90	15	14.92	99.52	
		150	2842.56	1707.27	22.5	22.55	100.25	
DOR	5	50	633.38	209.99	2.5	2.47	99.19	99.13
		100	839.23	415.84	5	4.91	98.21	
		150	1058.45	635.06	7.5	7.49	99.99	

Dissolution studies

The major application of the dissolution studies in the analysis is to study the drug releasing profile from the solid oral dosage forms in the simulated gastric and intestinal fluids. The amount of the drug substance liberated in a particular time interval is most important for the detection of the efficacy of the drug substances. The use of Veego VDA-8D Microprocessor Based Dissolution Test Apparatus USP apparatus –II (paddle type) at 50 RPM speed, has been done and dissolution bowl 900ml capacity were used. The phosphate buffer with 5% SLS has been used in weak acidic and neutral medium as well as in acidified pH was adjusted with orthophosphoric acid. The temperature was 37 ± 0.5 ^{0}C kept and

the n = 6 tablet samples were studied for sink conditions in the dissolution study. The sampling was done at diversified-, time intervals of the 5,10,15,30,45,60 mins and samples were filtered through nylon filters 0.45um, carried out in a automated sampler apparatus. The dissolution profile shows % Dissolution starting from 15 mins around 88% and reaches upto 99% at 45 mins for the tablet dosage forms, and it meets the USP criteria within Q= 88 to 100% within 15 to 45mins time interval. The dissolution profiles of drugs in the, tablet dosage forms have been studied as per below:

Table 9
Dissolution Profile of dugs

Medium	LAM % Dissolved			TEN % Dissolved			DOR % Dissolved		
	15 min	30 min	45 min	15 min	30 min	45 min	15 min	30 min	45 min
pH 1.2	88.42	94.75	98.23	84.73	92.31	97.15	88.21	92.78	98.24
pH 4.5	86.44	92.46	95.27	87.86	94.47	98.80	90.42	94.68	98.66
pH 6.8	88.12	97.99	98.37	89.15	96.73	97.06	90.23	97.40	99.13

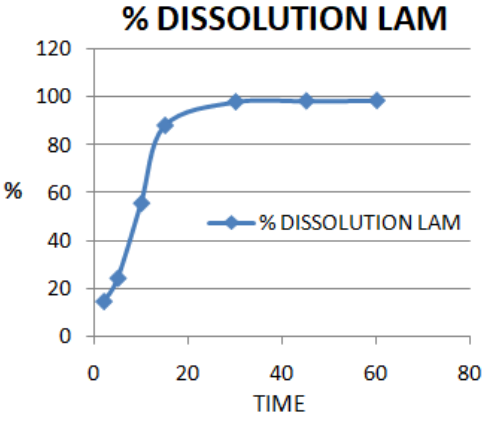

Figure 17. % Dissolution vs. Time mins LAM

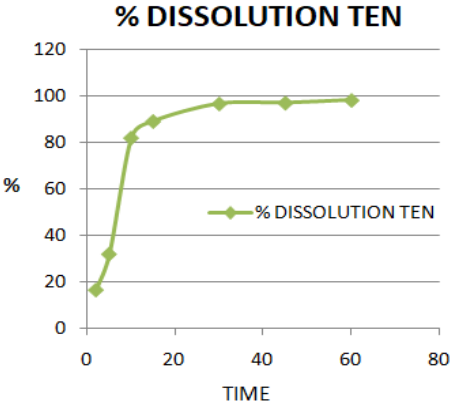

Figure 18. % Dissolution vs. Time mins TEN

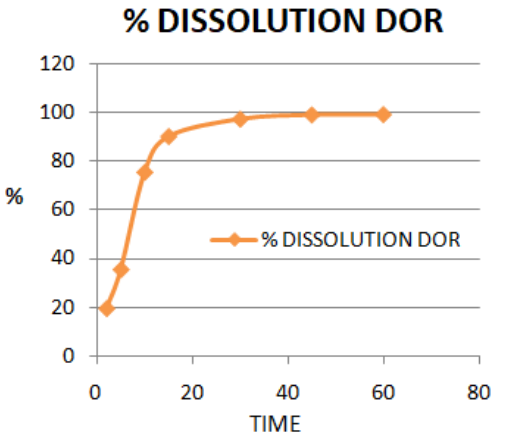

Figure 19. % Dissolution vs. Time mins DOR

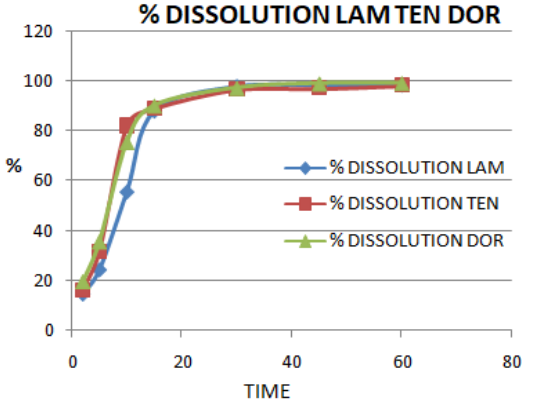

Figure 20. % Dissolution vs. Time mins LAM TEN DOR Combined

Assay of marketed formulations

The foremost purpose of this method is to develop a quick & specific single assay,- method for the tablet dosage forms. Each Tablet contains 300mg of Lamivudine, 300mg of Tenofovir and 100mg of Doravirine in the tablet dosage form DORLATVIR™ Tablets, similar to in house developed dosage forms in synthetic mixtures.

Table 10
Assay of Drugs in Tablet dosage form

	Sample No	Label Claim (mg)	Result (mg)	% Label Claim	% Avg Assay	SD	% RSD
LAM	1	300	298.33	99.44	99.41	0.32	0.32
	2	300	297.25	99.08			
	3	300	299.19	99.73			
TEN	1	300	297.12	99.04	99.27	0.47	0.47

	2	300	299.44	99.81			
	3	300	296.89	98.96			
DOR	1	100	98.77	98.77			
	2	100	99.65	99.65	98.95	0.62	0.63
	3	100	98.43	98.43			

Results and Discussions

The New Rapid Precise; Accurate, RP-HPLC method is been successfully developed for the three drugs Lamivudine LAM, Tenofovir TEN, Doravirine DOR within a very short run time within 4 minutes all the three drugs can be qualitatively and quantitatively analyses. The results are summarized in table given below:

Table 11
Summarized Results for the Developed HPLC Method

Serial No	Parameters	Criteria of Acceptance	Results
1	System Suitability	Theoretical Plates NLT 2000	LAM- 55641 TEN- 63365 DOR- 76231
		Tailing Factor NMT 2.0	LAM- 1.10 TEN- 1.11 DOR- 1.13
		Retention Time Min	LAM- 2.16 TEN- 2.65 DOR- 3.25
2	Precision	% RSD NMT 2.0	LAM- 0.12 TEN- 0.22 DOR- 0.33
3	Linearity	Correlation Coefficient NLT 0.999	LAM- 0.9997 TEN- 0.9997 DOR- 0.9991
4	Accuracy	% Recovery 98-102%	LAM- 99.37 TEN- 99.70 DOR- 99.13
5	LOD	1:3 (Conc. in µg/ml)	LAM- 1.426 TEN- 1.431 DOR- 0.589
6	LOQ	1:10 (Conc. in µg/ml)	LAM- 4.322 TEN- 4.336 DOR- 1.785
7	Assay	% Label Claim	LAM- 99.41 TEN- 99.27 DOR- 98.95
8	% Dissolution	% Drug Release at 45min	LAM- 98.37 TEN- 98.80 DOR- 99.13

The method is helpful in the assay analysis for the drugs three drugs Lamivudine LAM, Tenofovir TEN, Doravirine DOR, having % Assay results LAM 99.41% TEN 99.27% DOR 98.95% as compared with the label claimed. As well as this applicable on the dissolution profile study of these drugs in tablet dosage forms showing the % Drug release from 88 to 99 % within the USP criteria. The method has very accurate working response within very lower range of concentration of 7.5 to 45µg/ml for LAM & TEN and 2.5 to 15µg/ml for DOR in individual as well as in the dosage forms. This helps in the detection of the analyte drugs by using less amount of the working standards making the method very cost effective.

Conclusion

The developed analytical method is able to detect the drugs Lamivudine LAM, Tenofovir TEN, Doravirine DOR in individual as well as in the samples and , in the combined dosage forms with the accuracy and precision parameters as per the ICH guideline. The Stability method i.e. forced degradation study helps to understand the different impurities, degraded products, generated in stress conditions and the method effectively detects the drug analytes pure peaks, without any interference of other peaks. Also the analytical method is successfully validated as per the ICH guidelines and is useful for the assay and recovery study from the marketed formulations. The method effectively used for the in vitro dissolution profile study of the drugs in the solid oral tablet dosage forms in a single HLPC run time which is beneficial and time saving for the analysis of the drugs in different dosage forms as well as in the combinations.

Conflict of interest

The authors, have none of the conflicts of interest, regarding the investigation research works**.**

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References

1. Mercadel CJ, Skelley JW, Kyle JA, Elmore LK. Lamivudine. Journal of Pharmaceutical Technology. 2014;30(6):216-226. doi:10.1177/8755122514544126
2. Fantauzzi A, Mezzaroma I. Lamivudine: clinical efficacy and role in HIV therapy. Therapeutics Advance Chronic Disease. 2014;5(4):164-177. doi:10.1177/2040622314530461
3. Ray AS, Fordyce MW, Hitchcock MJM. Tenofovir alafenamide: A novel prodrug of tenofovir for the treatment of Human Immunodeficiency Virus. Antiviral Res. 2016;125:63-70. doi:10.1016/j.antiviral.2015.11.009

4. Ameen F, Mamidala E, Davella R, Vallala S. Doravirine inhibits SARS-CoV-2 protein targets: A potential multi-target drug. Journal of Infect Public Health. 2021;14(10):1454-1460. doi:10.1016/j.jiph.2021.07.012
5. Cihlar T, Fordyce M. Current status and prospects of HIV treatment. Current Opinion Virology. 2016;18:50-56. doi:10.1016/j.coviro.2016.03.004
6. Al-Zoman NZ, Maher HM, Al-Subaie A. Simultaneous determination of newly developed antiviral agents in pharmaceutical formulations by HPLC-DAD. Chem Cent J. 2017;11(1):1. doi:10.1186/s13065-016-0232-6
7. Mallikarjuna Rao N, Gowri Sankar D. Development and validation of stability-indicating HPLC method for simeltaneous determination of Lamivudine, Tenofovir, and Lamivudine in bulk and their tablet dosage form. Future Jorunal of Pharmaceutical Sciences. 2015;1(2):73-77. doi:10.1016/j.fjps.2015.11.002
8. Zhuang C, Pannecouque C, De Clercq E, Chen F. Development of non-nucleoside reverse transcriptase inhibitors (NNRTIs): our past twenty years. Acta Pharm Sinica B. 2020;10(6):961-978. doi:10.1016/j.apsb.2019.11.010
9. Sluis-Cremer N, Tachedjian G. Mechanisms of inhibition of HIV replication by non-nucleoside reverse transcriptase inhibitors. Virus Res. 2008;134(1-2):147-156. doi:10.1016/j.virusres.2008.01.002
10. Rao JN, Sudhakar C, Dubey SS. RP-HPLC Method Validation for the Assay of Tenofovir Disoproxil Orotate. Research Journal of Pharmaceutical Technology. Published online July 19, 2021:3855-3859. doi:10.52711/0974-360X.2021.00668
11. National Center for Biotechnology Information. PubChem Compound Summary for CID 584600047 Doravirine
12. ICH Q2 (R1), Validation of analytical procedures: text and methodology, 2005
13. ICH Stability Testing of New Drug Substances and Products Q1A (R2), International Conference on Harmonization (2003)
14. K. Nekkala, V. Shanmukha Kumar, and D. Ramachandran, Development and validation for the simultaneous estimation of lamivudine, tenofovir disproxil and Lamivudine in drug product by RP-HPLC, Journal of Pharmacutical. Sciences, Res., vol. 9, no. 9, 2017. pp. 1505–1510.
15. A. Vejendla, S. Talari, R. Moturu, S. N. M. Boddapati, and A. E. Kola, Method development and validation for Cabotegravir and Doravirine by using HPLC and its degradants are characterized by LCMS and FTIR, Futur. Journal of Pharmaceutical Sciences., vol. 7, no. 1, 2021, pp. 226, DOI: 10.1186/s43094-021-00355-8
16. I. K. Ramöller et al., HPLC-MS method for simultaneous quantification of the antiretroviral agents Doravirine and cabotegravir in rat plasma and tissues, Journal of Pharmacutical Biomedical Analalysis., vol. 213, 2022, pp. 114698, DOI: 10.1016/j.jpba.2022.114698.
17. Atul A. S., Charushila H. B., Sanjay J. S. Application of UV-spectrophotometric methods for estimation of tenofovir disoproxil fumarate in tablets. Pakistan Journal of Pharmaceutical Sciences.2009; 22(1): 27–29.
18. Kavitha K. Y., Geetha G., Hariprasad R., Venkatnarayanan R., Kaviarasu M. Development and validation of RP-UPLC analytical method for simultaneous estimation of the emtricitabine, tenofovir disoproxil fumerate and rilpivirine and its pharmaceutical dosage form. International Research Journal of Pharmacy. 2013;4(1):150–155

19. L. Kovač, Z. Časar, T. Trdan Lušin, and R. Roškar, Development of an Analytical Method for Determination of Related Substances and Degradation Products of Cabotegravir Using Analytical Quality by Design Principles, ACS Omega, vol. 7, no. 10, 2022, pp. 8896–8905, DOI: 10.1021/acsomega.1c07260.
20. Y. Zheng et al., HPLC-MS/MS method for the simultaneous quantification of Lamivudine, elvitegravir, Doravirine, darunavir, ritonavir, raltegravir and raltegravir-β-d-glucuronide in human plasma, Journal of Pharmacy Biomedical Analalysis., vol. 182, 2020, pp 113119, DOI: 10.1016/j.jpba.2020.113119.
21. I. Yusuff, M. V. Vara Prasad, S. M. Shaheedha, and M. Habeeb, A New Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Lamivudine and Doravirine in Bulk and its Dosage Forms, Iran. Journal of Pharmaceutical Sciences., vol. 15, no. 4, 2019, pp. 53–72.
22. International Conference on Harmonization, ICH Guidelines Q2 (R1), Validation of Analytical Procedures, Text and Methodology Ref. CPMP/ICH/381/95
23. J. Nageswara Rao, Sudhakar. RP-HPLC Method Validation for the Assay of Tenofovir Disoproxil Orotate, Research Journal of Pharmacy and Technology. 2021; 14(7):3855-9. Doi: 10.52711/0974-360X.2021.00668
24. Singh S., Bakshi M. Guidance on conduct of stress tests to determine inherent stability of drugs. Pharmatech. 2000;24:1–14
25. Q1AR2: stability testing of new drug substances, products IFPMA. Proceedings of the International Conference on Harmonization (ICH '03); 2003; Geneva, Switzerland.