WJPMR, 2026, 12(4), 183-201 Research Article Impact Factor: 7.555

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

ISSN (O): 2455-3301
ISSN (P): 3051-2557
Coden USA: WJPMBB

QBD BASED STABILITY INDICATING RP-HPLC METHOD FOR COMBINED ANTIRETROVIRAL TABLETS

Sridevi Pulakanam[1]*, Kumbha Prathyusha[2], Mule Venkatachennakesavareddy[3], Mangalapudi Rami Reddy[4], Maruprolu Anil Kumar Reddy[5]

[1,2]Department of Pharmaceutical Chemistry and Analysis, Bapatla College of Pharmacy, Bapatla -522101, Andhra Pradesh, India.

[3,4,5]Final Year Student of Bapatla College of Pharmacy, Bapatla -522101, Andhra Pradesh, India.

***Corresponding Author: Sridevi Pulakanam**

Department of Pharmaceutical Chemistry and Analysis, Bapatla College of Pharmacy, Bapatla -522101, Andhra Pradesh, India. **DOI:** https://doi.org/10.5281/zenodo.19330136

How to cite this Article: Sridevi Pulakanam[1]*, Kumbha Prathyusha[2], Mule Venkatachennakesavareddy[3], Mangalapudi Rami Reddy[4], Maruprolu Anil Kumar Reddy[5]. (2026). Qbd Based Stability Indicating rp-Hplc Method for Combined Antiretroviral Tablets. World Journal of Pharmaceutical and Medical Research, 12(4), 183–201.

Article Received on 21/02/2026 **Article Revised on 12/03/2026** **Article Published on 01/04/2026**

ABSTRACT

A tablet containing Dolutegravir, Lamivudine, and Tenofovir disoproxil fumarate is a combined drug of antiretroviral medication used to treat HIV/AIDS. WHO recognised this drug as the first-line treatment for adults. The primary purpose of this study is to develop and validate a simple, accurate, precise, and reproducible reverse-phase high-performance liquid chromatography (RP-HPLC) method for estimating dolutegravir, Lamivudine, and Tenofovir disoproxil fumarate in bulk and tablet dosage forms by optimising chromatographic parameters using a two-level full factorial design. A 2-level full factorial design was utilised to optimise the type of column and percentage of organic phase to maximise the theoretical plates and minimise the retention time of all three drugs. The optimised strength of acetonitrile and 1% ortho phosphoric acid buffer (70:30), flow rate and the volume of injection were found to be 1ml/min and 20 µl, respectively. The retention times were found to be 3.785min, 1.529min and 3.140 min for Dolutegravir, Lamivudine and Tenofovir, respectively. The R^2 values for Dolutegravir, Lamivudine and Tenofovir were found to be 0.99985, 0.99994 and 0.99923, respectively. The theoretical plate number and tailing factor for Dolutegravir, Lamivudine and Tenofovir were found to be NLT 2000 and should not be more than 2, respectively. The per cent RSD of peak areas of all measurements was found to be less than 2.0. The QBD approach was found to be an effective technique for optimising chromatographic conditions of the proposed method, which is useful for routine analysis of Dolutegravir, Lamivudine, and Tenofovir disoproxil fumarate in pharmaceutical dosage form.

KEYWORDS: RP-HPLC, Dolutegravir, Lamivudine, Tenofovir disoproxil fumarate, QbD, ICH guidelines.

1. INTRODUCTION

Antiretroviral therapy (ART)[1] has significantly evolved over the last few decades since the development of the first nucleoside analogues NRTIS[2] (nucleoside reverse transcriptase inhibitors). With the advent of triple therapy, majority of the challenges has been resolved. The latest class of the antiretroviral drugs developed was integrase inhibitors (INI).[3] Dolutegravir[4], (3S,7R)-N-[(2,4-difluorophenyl)methyl]-11-hydroxy-7-methyl-9,12-dioxo-4-oxa-1,8- diazatricyclo[8.4.0.0^{3,8}]tetradeca-10,13-diene-13- carboxamide[5] is an integrase inhibitor. It bocks HIV integrase enzyme by binding to the active site and obstructing the strand transfer step which is important in the HIV replication cycle and leads to the inhibition of viral activity.

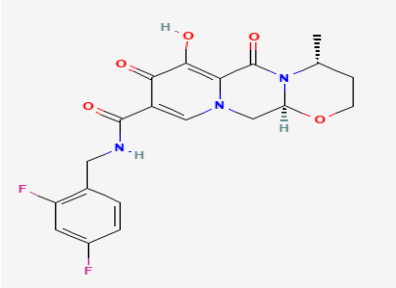


Fig. 1: Dolutegavir.

Lamivudine[6] b,4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5- yl]-1,2-dihydropyrimidin-2-one.[7] It has a structural similarity with cytosine. It can inhibit both HIV -1 &2 reverse transcriptase and the reverse transcriptase of hepatitis B virus. the phosphorylated active metabolite competes for incorporation into viral DNA.

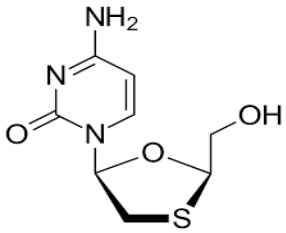


Fig. 2: Lamivudine.

Tenofovir[8-9] disoproxil Fumarate, 2E)-but-2-enedioic acid; bis({[(propan-2- yloxy)carbonyl]oxy}methyl) {[(2R)-1-(6-amino-9Hpurin-9-yl)propan-2-yl]oxy}methanephosphonate.[10] Tenofovir disoproxil fumarate is hydrolyzed to Tenofovir, which is then phosphorylated to Tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate, deoxy adenosine 5'-triphosphate, and by DNA chain termination.

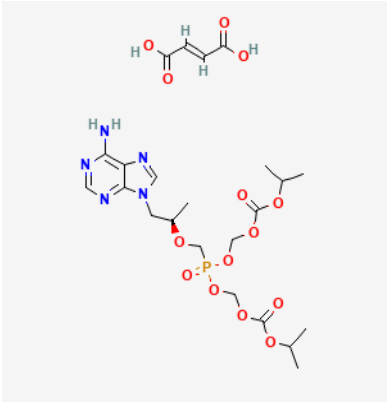


Fig. 3: Tenofovir.

Upon the literature survey there wre many analytical methods have been reported individually or in combination with other drugs.[11-19] However, there is no method reported for the simultaneous estimation of these three drugs by the HPLC method. Hence, the authors attempted to develop a stability indicating RP-HPLC method[20-25] for simultaneous estimation of drugs using QBD approach[26-36] to reduce no of trials, cost and time.

2. MATERIALS AND METHODS

2.1. Apparatus and Equipment

- HPLC studies were carried out on ALLIANCE Waters e 2695, Empower software 2.0 version, with a PDA detector set at 250nm for UV detection
- Waters Symmetry C18 (150 x 4.6mm) 3.5µ, X-Bridge Phenyl (150 x 4.6mm) 3.5 µ, columns were utilised in the study.
- Statistical software tool like Minitab version16.2.3 tab for DOE,
- pH meter – Eutech was used to check pH of all solutions.
- Other equipment like weighing balance – Sartouris,
- UV/VIS spectrophotometer SHIMADZU UV-1700 5, Ultra sonicator UCA 701 Unichrome.

2.2. Chemicals and Reagents

- Acetonitrile (HPLC grade)
- Ortho phosphoric acid (HPLC grade)
- Methanol (HPLC grade)
- HPLC grade water (Milli Q or equivalent)

Dolutegravir, lamivudine and tenofovir were gift samples obtained from Aurobindo Pharmaceuticals Ltd., Hyderabad. Tablets of Viropil (dolutegravir-50 mg, lamivudine-300 mg and tenofovir disoproxil fumarate-300mg) were purchased from a local pharmacy store.

2.3. Method development

2.3.1. Determination of Working Wavelength (λ_{max})

The λ_{max} of the solution of the drugs in a mixture of acetonitrile and 0.1% v/v OPA (50:50) was determined within the wavelength region of 200–400 nm against the same mixture as a blank. Thus, the isobestic point of two drugs at 250 nm was selected as the wavelength for further development of the method.

2.3.2. Initial Chromatographic Conditions

Different reverse-phase columns, as well as various organic solvents, such as OPA buffer and acetonitrile, were used to test the procedure at first. All the trials, observations and remarks were shown in Table **1.** Ultimately, a sufficient separation was carried out on X-Bridge Phenyl (150X4.6mm) 3.5µ using a combination of 0.1% OPA and acetonitrile.

From the trials, as the type of column and percentage of organic phase brought about a large change in retention time and theoretical plates they were selected as two factors to create 2^2 full factorial design[37 -49] and for method development.

2.3.3. Preparation Of Standard Stock Solution

Weighed accurately and transferred 10 mg of Dolutegravir, 60 mg of Lamivudine and 60mg of Tenofovir Disoproxil Fumarate working standard into a 100 ml clean dried volumetric flask and 20 ml diluent was added, sonicated, and the final volume was made with the diluent. From the above stock solution, 5 ml was pippeted into a 50 ml volumetric flask and diluted up to the mark with diluent (10ppm of Dolutegravir, 60ppm of Lamivudine and 60ppm of Tenofovir Disoproxil Fumarate).

2.3.4. Preparation of Sample Stock Solution

A total of 20 tablets were accurately weighed, and the average weight tablet was taken. 20 tablets were powdered, and an amount equivalent to 150 mg of tablet powder was accurately weighed into a clean, dried 100 ml volumetric flask. Diluent of 40 ml was added and sonicated for 20 mins, and volume was made up to the mark with the diluent. The solution was centrifuged at 5000 rpm for 10 mins, and 5ml of supernatant solution was pipeted into a 50 ml cleaned, dried volumetric flask and the final volume was made up with the diluent. Solution was passed through a 0.45-micron Millipore PVDF filter to get (10ppm of Dolutegravir, 60ppm of Lamivudine and 60ppm of Tenofovir Disoproxil Fumarate).

2.4. Optimisation of method

The QbD approach acknowledges dependent variables, independent variables and their interaction effects by a proper set of experiments on the responses to be analysed. Full factorial design (FFD), is one of the QbD approaches, an effective tool and commonly used for statistical optimization and analysis in scientific research and industrial applications.

2.4.1. Selection of factors

In this study, two independent variables were identified, such as columns and % of organic phase, which needed to be controlled to achieve the acceptable response range of dependent variables, such as theoretical plates NMT 5000 and retention time less than 4 min, were taken to create a factorial design.

After defining the variables, the 2^2full factorial design, one of the powerful software techniques of DoE [50 -56] was utilised to create a design by the input data of two factors as type of column (Waters & X-bridge) and % Organic phase at limits of (low-30 & high-70) as shown in Table 2.

Table 2: Chromatographic conditions set for factorial design.

Factor	Name	Type	Low	High
A	Column	text	Waters	X-bridge
B	% organic phase	Numeric	30	70

2.4.2 Screening

In this step, the summary of the factorial design was generated by the response analyser, used for screening of method variables and process parameters at their respective low and high levels and shown in Table **3.**

Table 3: Summary of factorial design.

Std Order	Run Order	Center pt	Blocks	Column	%organic phase
1	1	1	1	Waters	30
4	2	1	1	X-bridge	70
8	3	1	1	X-bridge	70
6	4	1	1	X-bridge	30
2	5	1	1	X-bridge	30
7	6	1	1	Waters	70
5	7	1	1	Waters	30
3	8	1	1	Waters	70

The above table gives the summary of the design matrix as per the Full Factorial Design, showing Two-Factor Eight-Experimental runs along with duplicate studies of centre point (1, 1) runs.

With a small number of trials, the DOE of Minitab (version 16.2.3) was used to explore the effect of a variety of conditions on a specific response as well as their interactions. The generated data was analysed and found that the type of column and % organic phase had shown significant p values computed in ANOVA [57 – 63] and shown in Table **4 –9.**

A desirability function was applied to the optimised conditions by setting a target ranging from 4100-5400 for theoretical plates and 1.4-3.7 minutes for retention time. This desirability was studied on a scale that ranges from d=0 to d=1 for undesirable and fully desirable responses, respectively, for theoretical plates and retention time. The optimum conditions with desired target points as shown in the opti plot. (Fig. **5**). To finalise this optimum set of conditions, three replicate injections of 50 µg/ml of dolutegravir, 300µg/ml of lamivudine and 300µg/ml of tenofovir disoproxil fumarate were analysed to determine their observed retention time of all three drugs, resolution, asymmetry and theoretical plates were within the predicted ranges. It was calculated using formula of Relative error % and observed that the differences between the observed and predicted peak responses were less than 5%.

Relative error percentage = Predicted value – Experimental value/ Predicted value *100

2.4.3. Optimised chromatographic conditions

The optimised chromatographic conditions are shown in Table **10**. The chromatogram is shown in Fig. **6.**

Table 10: Optimised chromatographic conditions.

PARAMETERS	OBSERVATION
Instrument used	Waters HPLC with auto sampler and PDA detector.
Injection volume	10µl
Mobile Phase	Acetonitrile and 0.1% OPA(70:30)
Column	X-Bridge Phenyl (150x4.6mm) 3.5µ
Detection Wave Length	250 nm
Flow Rate	1 ml/min
Runtime	10min

2.5. Assay Procedure

The standard, a sample of 10 µl were injected into the chromatographic system and the areas for Lamivudine, Tenofovir Disoproxil Fumarate and Dolutegravir peaks were measured, and % assay was calculated by using the formula, and the results were given in Table **11**. and peaks were shown in (Fig. **7).**

$$\% \text{ ASSAY} = \frac{\text{sample area response}}{\text{standard area respnse}} \times \frac{\text{standard weight}}{\text{standard dilution}} \times \frac{\text{sample dilution}}{\text{sample weight}} \times \frac{\text{potency}}{(100 - \text{water or LOD})} \times 100$$

2.6. Method validation

The validation was performed for the optimised chromatographic analytical method as per ICH Q2(R1)[64 - 68] guidelines.

2.6.1. Linearity

The linearity was performed at six concentration levels, 1-15 µg/ml for dolutegravir, 6-90 µg/ml for lamivudine and 6-90µg/ml for tenofovir, respectively, and plots were constructed for concentration against peak area. The results obtained were given in Table.**12,** and plots were shown in (Fig. **9-10**).

2.6.2. Accuracy

Accuracy was carried out by adding a known amount of standard to the tablet solution for each drug at 50, 100, and 150 % levels in triplicate, and samples were analysed by the optimised method. The mean percentage recoveries were determined and given in Table **13-15**. and chromatograms were shown in (Fig **11 - 13**).

2.6.3. Precision

The samples of analytes were subjected to interday and intraday QC analyses for the determination of precision.

In method precision, each homogenous sample was analysed for 6 times.

The precision of the instrument was checked by injecting (n=6) solutions of 10ppm of

Dolutegravir, 60ppm of Lamivudine and 60ppm of Tenofovir Disoproxil Fumarate. In this % RSD was calculated for the peak areas obtained. The results were given in Table **16-17**. and chromatograms were shown in (Fig. **14-15).**

2.6.4. Robustness

The robustness of the method refers to its ability to remain unaffected by small and deliberate variations in method parameters. The robustness of the optimised method was investigated by injecting a standard solution of 10ppm of Dolutegravir 60ppm of Lamivudine, and 60ppm of Tenofovir Disoproxil Fumarate with minute slight changes in the chromatographic parameters, flow rate (0.8 ml/min to 1.2ml/min), proportion of solvent in mobile phase (45:55) and results were shown in Table **18-20**. Chromatograms were shown in (Fig. **16-19)**.

2.6.5. Limit of detection and Limit of quantification

LOD and LOQ for three drugs were evaluated using the standard deviation method. LOD was defined as 3.3 σ/S and LOQ as 10 σ/S based on the standard deviation of the response (σ) and slope of the calibration curve (S), and chromatograms were shown in (Fig. **20-21),** and results were shown in Table **21**.

2.6.6. System suitability

System suitability was checked by taking six replicates of three drugs at concentrations of 100 µg/ml. the retention time, theoretical plates, peak symmetry and %RSD of six injections were calculated, and the results are shown in Table **22.**

Apart from applying the QbD approach to dosage forms, we aimed to investigate the stability of the drugs under the stress conditions with optimised conditions.

2.7. Forced degradation studies[69-70]

Different stress conditions were applied to the samples, viz., acid, base, oxidative and thermal stress conditions separately and the solutions were analysed using HPLC.

2.7.1. Preparation of sample stock solution

20 tablets were weighed accurately, and the average weight was calculated. 20 tablets were powdered and 150 mg of equivalent tablet powder was accurately weighed into a clean, In a dried 100 ml volumetric flask, 40 ml of diluent was introduced, and the mixture was sonicated for 20 minutes with occasional shaking to ensure the contents dissolved. The solution was then topped up to the mark with the diluent. The solution was centrifuged at 5000 rpm for 10 mins. 5ml of supernatant solution was pipetted into a 50 ml cleaned, dried volumetric flask and

the final volume was made up to the mark with the diluent. The solution was filtered through a 0.45-micron Millipore PVDF filter.

2.7.2. Hydrolytic degradation under acidic conditions

5 ml of sample stock solution was pipetted into a 50 ml volumetric flask, and 3 ml of 1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and neutralised with 1 N NaOH and made up to 50 ml with diluent. The solution was filtered through a 0.45 micron Millipore PVDF filter. The chromatograms are shown in Fig. **22**.

2.7.3. Hydrolytic degradation under alkaline conditions

Five millilitres of the aforementioned solution were transferred into a 50 ml volumetric flask, followed by the addition of 3 ml of 1N NaOH. The flask was then maintained at a temperature of 60ºC for six hours. Afterwards, the solution was neutralised using 1N HCl and diluted to a total volume of 10 ml with a diluent. The resulting solution was filtered through 0.22-micron syringe filters and subsequently transferred into vials. The chromatograms were shown in (Fig. **23**).

2.7.4. Thermal-induced degradation

A Lamivudine sample was taken in petridish and kept in a hot air oven at 1100 C for 24 hours. Then the sample was taken, diluted with diluents and injected into the HPLC and analysed. The chromatograms are shown in (Fig. **24**).

2.7.5. Oxidative degradation

5ml of the stock solution was pipetted into a 50 ml volumetric flask. Then, 1 ml of a 3% w/v hydrogen peroxide solution was added to the flask, and the volume was adjusted to the mark using a diluent. The flask was left at room temperature for 15 minutes. Afterwards, the solution was filtered through 0.45-micron syringe filters and transferred into vials. The chromatograms were shown in (Fig. **25).**

3. RESULTS AND DISCUSSION

3.1. Determination of Working Wavelength (λ_{max})

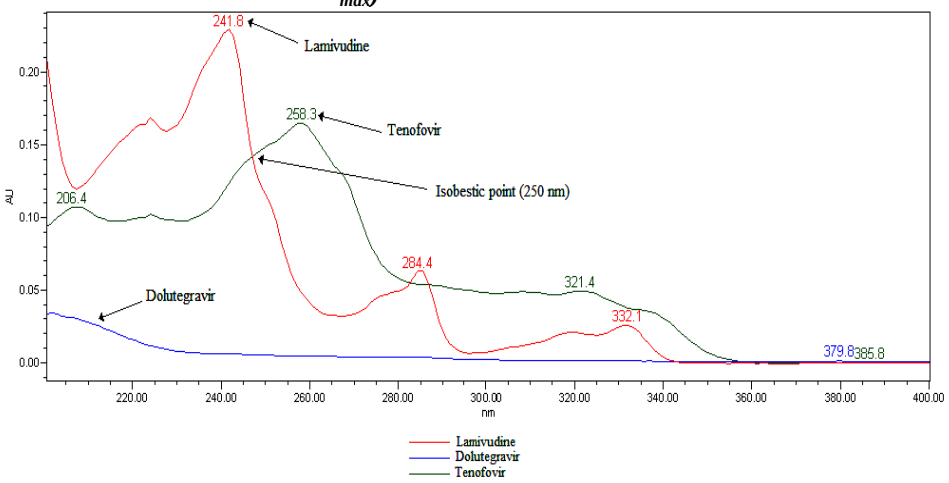


Fig. 4: Isobestic point of two drugs at 250 nm.

3.2. Method development by DOE

Table 1: Different trials with observations and remarks for method development.

Type of Column	Mobile phase composition	Observation	Remarks
Waters Symmetry C_{18} (150 x 4.6mm, 3.5µ)	Acetonitrile and 0.1% OPA 60:40	Plate Count was not within the limit	Not satisfactory
	Acetonitrile and 0.1% OPA 30:70	The third peak was not eluted.	Not satisfactory
X-Bridge C_8 (150 x 4.6mm, 3.5µ)	Acetonitrile and 0.1% OPA 55:45	Only two peaks were eluted	Not satisfactory
	Acetonitrile and 0.1% OPA 60:40	Low plate count was observed	Not satisfactory
X-Bridge Phenyl C_{18}(150 x 4.6mm, 3.5 µ)	Acetonitrile and 0.1% OPA 50:50	Peak shapes were symmetrical with more no of theoretical plates.	Not Satisfactory
	Acetonitrile and 0.1% OPA 70:30	An unknown peak was formed at a 3.2 retention time	satisfactory

Table 4: Analysis of Variance for Theoretical plates of Lamivudine (coded units).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	2	475033	475033	237516	8.20	0.038
column	1	431520	431520	431520	14.90	0.018
% organic phase	1	43513	43512	43512	1.50	0.287
2-Way Interactions	1	219122	219122	219122	7.57	0.051
column*solvent	1	219122	219122	219122	7.57	0.051
Residual Error	4	115829	115829	28957		
Pure Error	4	115829	115829	28957		
Total	7	809984				

The p-values for column and % organic phase obtained were 0.018, 0.287, not significant values predicts theoretical plates of lamivudine are independent of % organic phase and column.

Table 5: Analysis of Variance for Theoretical plates of tenofovir disoproxil fumarate (coded units).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	2	784265	784265	392133	10.18	0.027
column	1	586445	586445	586445	15.23	0.018
% organic phase	1	197821	197821	197821	5.14	0.086
2-Way Interactions	1	140981	140981	140981	3.66	0.128
column*solvent	1	140981	140981	140981	3.66	0.128
Residual Error	4	154006	154006	38501		
Pure Error	4	154006	154006	38502		
Total	7	1079252				

The table showed that theoretical plates of tenofivir disoproxil fumarate are dependent on the column, as it shows p value<0.05.

Table 6: Analysis of Variance for Theoretical plates of dolutegravir (coded units).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	2	5396841	5396841	2698421	112.22	0.000
column	1	3199185	3199185	3199185	133.04	0.000
% organic phase	1	2197656	2197656	2197656	91.39	0.001
2-Way Interactions	1	430128	430128	430128	17.89	0.013
column*solvent	1	430128	430128	430128	17.89	0.013
Residual Error	4	96188	96188	24047		
Pure Error	4	96188	96188	24047		
Total	7	5923157				

From the table, significant p-values were found. The response of theoretical plates is dependent on the % organic phase and the column.

Table 7: Analysis of Variance for Retention time of lamivudine (coded units).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	2	15.2125	15.2125	7.6063	320.26	0.000
column	1	14.8513	14.8513	14.8513	625.32	0.000
% organic phase	1	0.3613	0.3613	0.3613	15.21	0.018
2-Way Interactions	1	0.0312	0.0312	0.0312	1.32	0.315
column*solvent	1	0.0312	0.0312	0.0312	1.32	0.315
Residual Error	4	0.0950	0.0950	0.0238		
Pure Error	4	0.0950	0.0950	0.0237		
Total	7	15.3388				

P= 0.000 for column and p=0.018 for % organic phase were obtained, and the retention time of lamivudine was found to be influenced by both factors.

Table 8: Analysis of Variance for Retention time of tenofovir disoproxil fumarate (coded units).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	2	11.0900	11.0900	5.5450	201.64	0.000
column	1	11.0450	11.0450	11.0450	401.64	0.000
% organic phase	1	0.0450	0.0450	0.0450	1.64	0.270
2-Way Interactions	1	0.0800	0.0800	0.0800	2.91	0.163

column*solvent	1	0.0800	0.0800	0.0800	2.91	0.163
Residual Error	4	0.1100	0.1100	0.0275		
Pure Error	4	0.1100	0.1100	0.0275		
Total	7	11.2800				

The significant p values were obtained for column and % organic phase. The response of retention time for tenofovir disoproxil fumarate was effected by column and % organic phase.

Table 9: Analysis of Variance for Retention time of dolutegravir (coded units).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	2	14.4625	14.4625	7.2312	175.30	0.000
column	1	13.2612	13.2612	13.2612	321.48	0.000
% organic phase	1	1.2013	1.2013	1.2013	29.12	0.006
2-Way Interactions	1	3.0012	3.0012	3.0012	72.76	0.001
column*solvent	1	3.0012	3.0012	3.0012	72.76	0.001
Residual Error	4	0.1650	0.1650	0.0413		
Pure Error	4	0.1650	0.1650	0.0413		
Total	7	17.6287				
P values obtained were less than 0.05 for the column and % Organic phase. Hence retention time response was dependent on both factors.						

3.3. Optimisation of method

From the opti plot column(x- bridge) and % organic phase ratio (70:30) were found to be optimal, based on desirability.

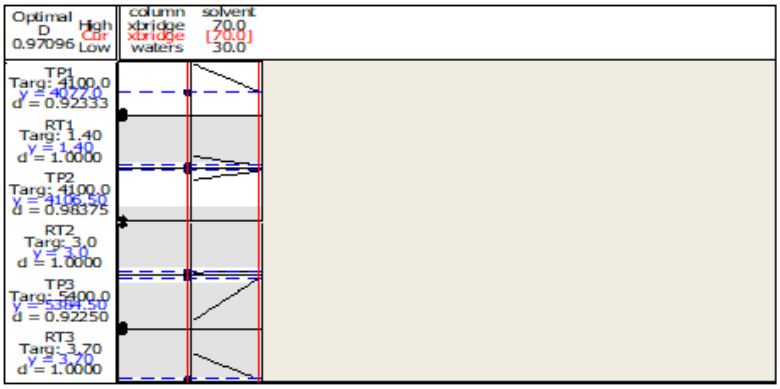


Fig. 5: Opti plot.

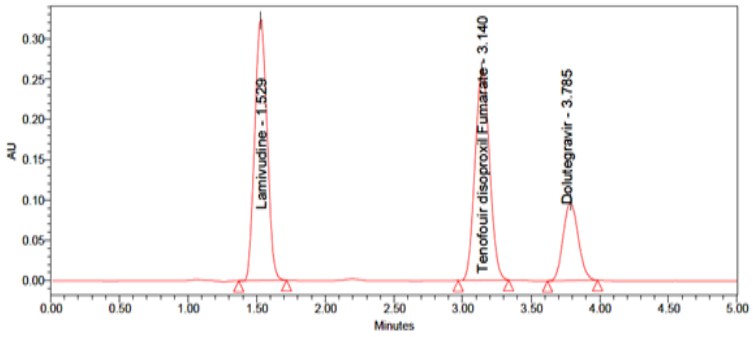


Fig. 6: Optimised chromatogram.

3.4. Assay

Table 11: Assay of Lamivudine and Tenofovir Disoproxil Fumarate and Dolutegravir.

Brand	Drug	Avg sample area (n=5)	Std (µg/ml)	Sample (µg/ml)	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
-	Dolutegravir	742368	10	10	50	99.9	10.2	99.75
	Lamivudine	2044223	60	60	300	99.9	60.1	99.72
	Tenofovir Disoproxil Fumarate	1946942	60	60	300	99.9	60.1	99.54

The % assay results were found to be 99.75, 99.72, 99.54 for dolutegravir, lamivudine and tenofovir disoproxil fumarate, respectively. The analysed tablets complies percentage purity within the acceptable limits as 100% ± 10 as per the ICH guidelines.

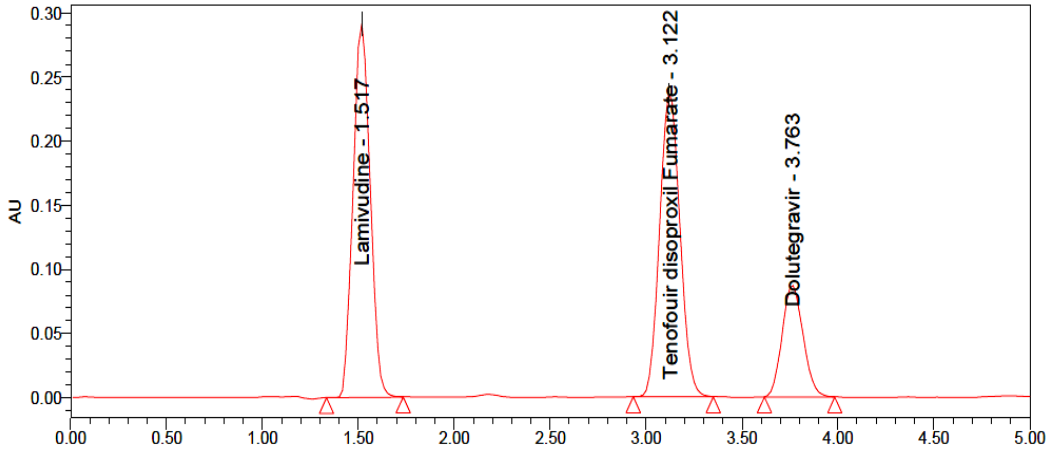


Fig. (7): Chromatogram of Assay.

3.5. Method validation

3.5.1 Linearity plots

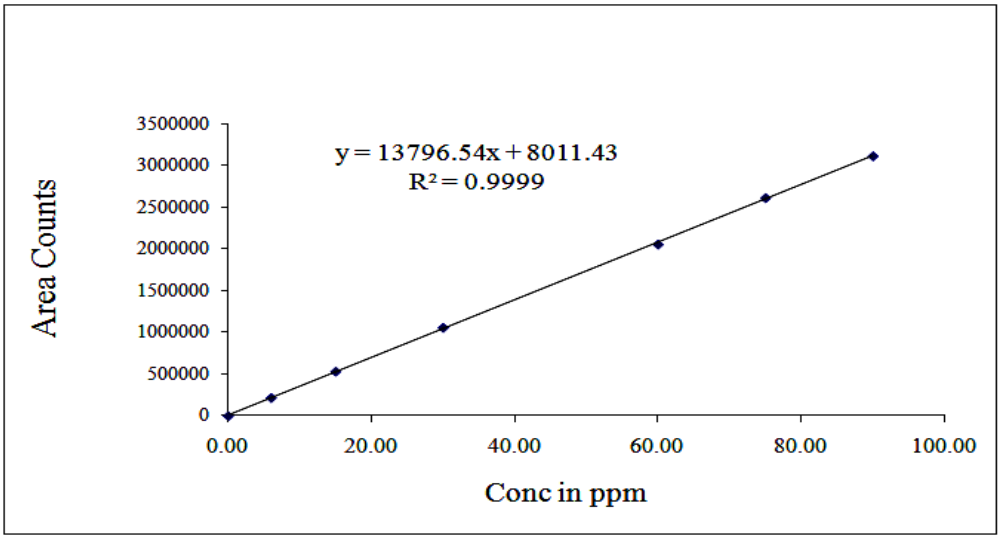


Fig. 8: Calibration curve for Lamivudine at 250 nm.

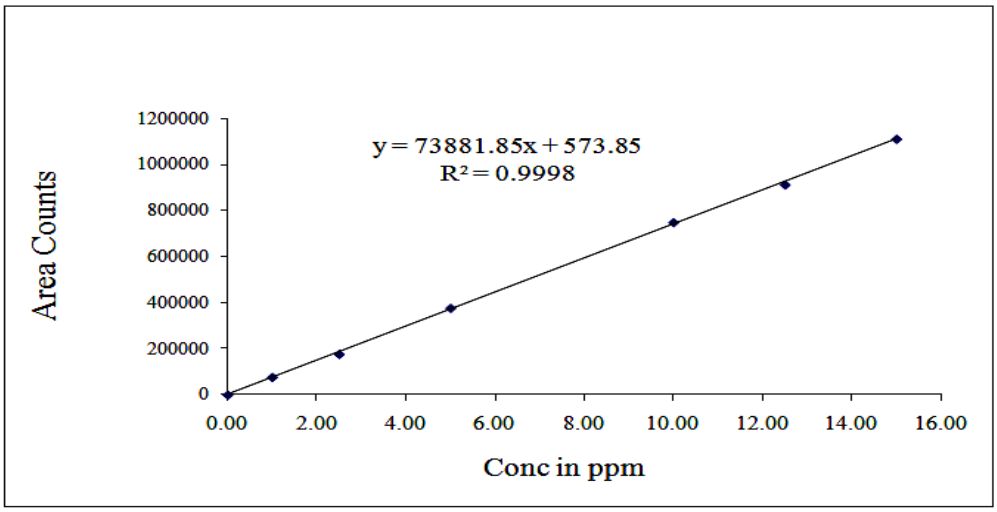


Fig. 9: Calibration curve for Dolutegravir at 250 nm.

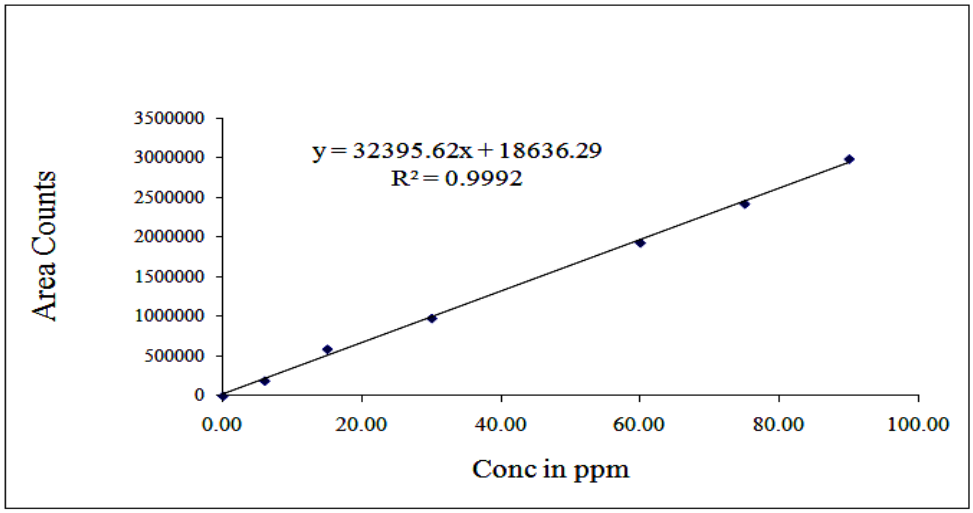


Fig. 10: Calibration curve for Tenofovir Disoproxil Fumarate at 250 nm.

Table 12. Results of linearity for Lamivudine, Tenofovir disoproxil Fumarate and Dolutegravir.

S.NO	Dolutegravir		Lamivudine		Tenofovir Disoproxil Fumarate	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	1.00	76689	6.00	214642	6.00	190489
2	2.50	176734	15.00	532381	15.00	586475
3	5.00	376658	30.00	1054869	30.00	974292
4	10.00	748573	60.00	2053049	60.00	1925405
5	12.50	912695	75.00	2610129	75.00	2415511
6	15.00	1111233	90.00	3110621	90.00	2979474
Regression equation	y = 73881.85x +573.85		y = 34491.34x +8011.43		y = 32395.62x +18636.29	
Slope	73881.85		34491.34		32395.62	
Intercept	573.85		8011.43		18636.29	
R^2	0.99985		0.99994		0.99923	

The developed analytical method was found linear over the given range of concentration and was examined using linear regression equation showing regression coefficients, 0.9998,0.9999, 0.9992 and slopes, 73881.85, 34491.34, 32395.62 for dolutegravir, lamivudine and tenofovir disoproxil fumarate respectively.

3.5.2. Accuracy

Table 13: Accuracy results of Dolutegravir by RP-HPLC method.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	370863	5	5.3	100.47	100.52
100%	744651	10	10.1	99.44	
150%	1122864	15	15.7	101.75	

Table 14: The Accuracy results for Lamivudine by RP-HPLC method.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1012605	30	30.2	100.16	100.57
100%	2041755	60	60.1	99.78	
150%	3065542	90	90.2	100.98	

Table 15: The Accuracy results for Tenofovir Disoproxil Fumarate by RP-HPLC Method.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	954918	30	30.2	100.77	100.92
100%	1952424	60	60.1	99.68	
150%	2863737	90	90.2	100.16	

The mean percentage recoveries for dolutegravir, lamivudine and tenofovir disoproxil fumarate were determined as 100.52, 100.57, 100.92 and depicts those were with in the acceptance limits as 100%±2 as per the Q2 specifications of ICH guidelines.

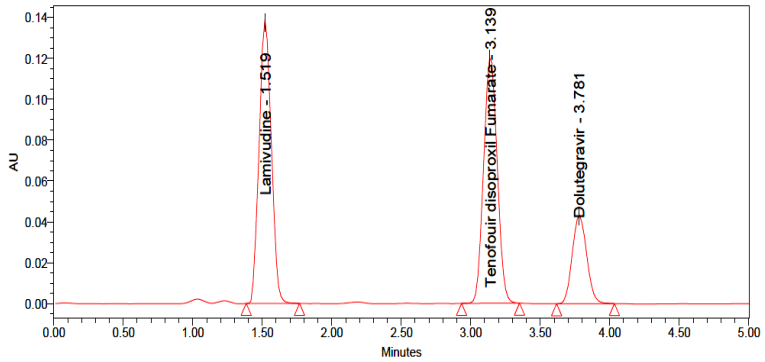


Fig. 11: Chromatogram for Accuracy 50%.

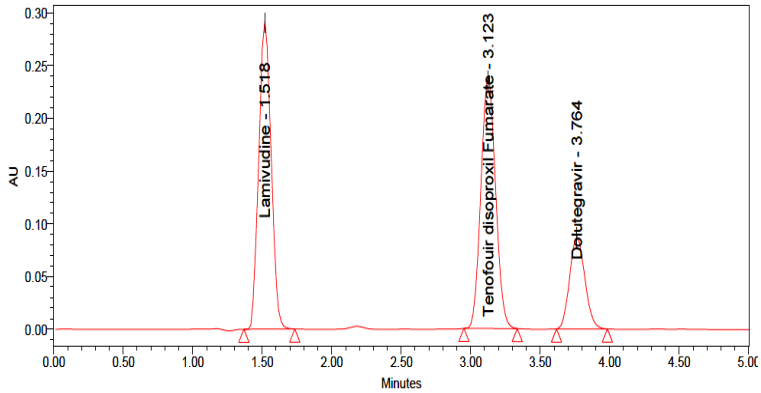


Fig. 12: Chromatogram for Accuracy 100%.

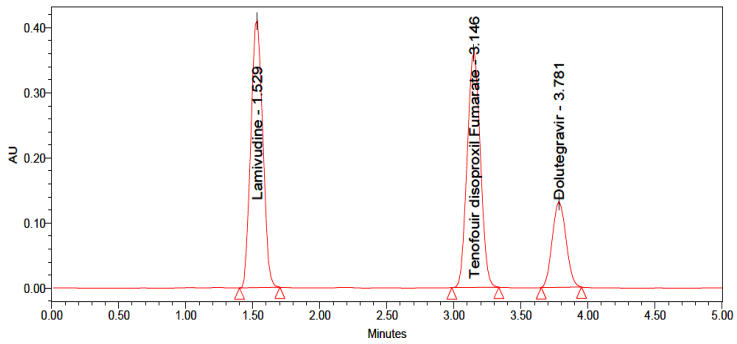


Fig. 13: Chromatogram for Accuracy 150%.

3.5.3. Precision

Table 16: Method Precision for Lamivudine and Tenofovir Disoproxil Fumarate and Dolutegravir by RP-HPLC method.

S. No.	Area for Dolutegravir	Area for Lamivudine	Area for Tenofovir Disoproxil Fumarate
1	742735	2043598	1949640
2	743895	2011696	1927598
3	748878	2032323	1952467
4	748481	2022357	1957796
5	741365	2021758	1939246
6	743815	2021665	1919763
Average	744862	2024066	1941085
Standard Deviation	3098.705	12173.12	14979.34
%RSD	0.42	0.60	0.77

The % RSD was computed as 0.42, 0.60,0.77 for dolutegravir, lamivudine and tenofovir disoproxil fumarate respectively & illustrates that they were within the acceptance criteria (≤2).

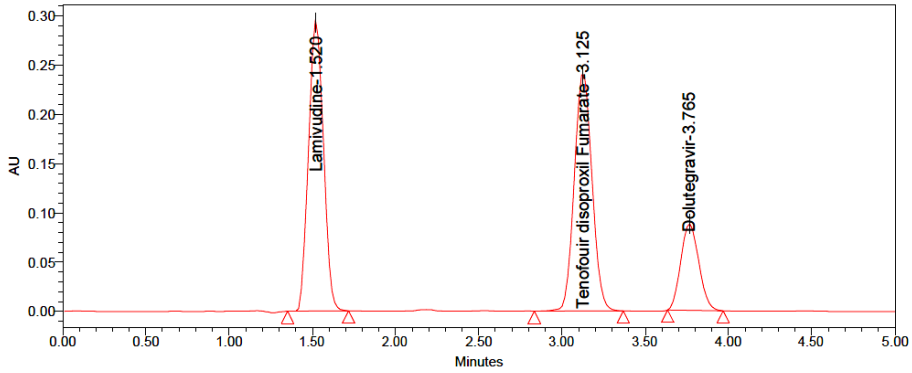


Fig. 14: Chromatogram for Method Precision.

Table 17: Intermediate Precision for Lamivudine and Tenofovir Disoproxil Fumarate and Dolutegravir by RP-HPLC method.

S. No.	Area for Dolutegravir	Area for Lamivudine	Area for Tenofovir Disoproxil Fumarate
1	744471	2057759	1927241
2	743722	2041695	1925517
3	745628	2040724	1967192
4	744699	2031596	1932355
5	744816	2041691	1918983
6	749074	2023213	1913152
Average	745402	2039446	1930740
Standard Deviation	1900.601	11595.086	19070.627
%RSD	0.25	0.57	0.99

The % RSD was calculated as 0.25, 0.57, 0.99 for all three drugs were found to be within the acceptance limits (≤3) and depicts precision of the method.

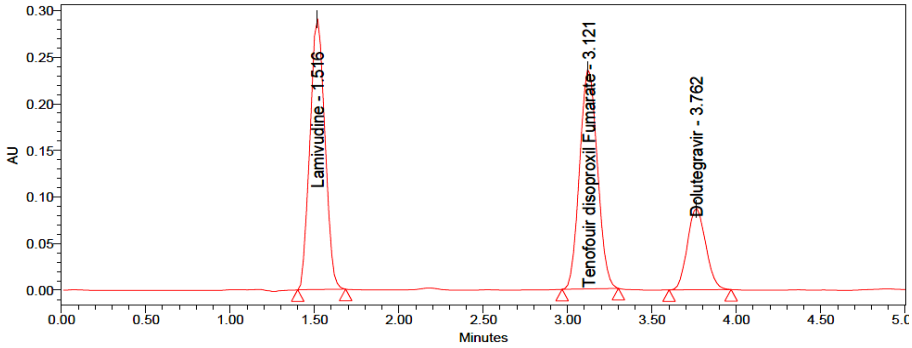


Fig. 15: Chromatogram for Intermediate Precision.

3.5.4. Robustness

Table 18: Robustness results of Dolutegravir by RP-HPLC.

Parameter	Dolutegravir					
	Condition	**Retention time(min)**	**Peak area**	**Resolution**	**Tailing**	**Plate count**
Flow rate Change(mL/min)	Less flow(0.8ml)	4.699	885281	2.26	1.04	4879
	Actual(1ml)	3.756	743647	3.21	1.01	4845
	More flow(1.2ml)	3.145	543615	2.28	1.05	4792
Organic Phase change	Less Org (45:55)	3.877	834887	2.24	1.05	4893
	Actual(50:50)	3.748	746592	2.28	1.04	4728
	More Org(55:45)	2.71	714246	2.39	1.04	4896

Table 8: Robustness results of Lamivudine by RP-HPLC.

Parameter	Lamivudine					
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count
Flow rate Change (mL/min)	Less flow(0.8ml)	1.887	2255736	12.26	1.05	8979
	Actual(1ml)	1.545	2041658	13.21	1.10	8942
	More flow(1.2ml)	1.286	1462681	12.28	1.05	8979
Organic Phase change	Less Org (45:55)	1.290	2575975	12.24	1.06	8916
	Actual(50:50)	1.532	2016975	14.28	1.03	8984
	More Org(55:45)	1.517	1815046	12.39	1.12	8995

Table 20: Robustness results of Tenofovir Disoproxil Fumarate by RP-HPLC.

Parameter	Tenofovir Disoproxil Fumarate					
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count
Flow rate Change(mL/min)	Less flow(0.8ml)	3.897	2176684	2.26	1.11	12579
	Actual(1ml)	3.164	1945782	3.21	1.14	12542
	More flow(1.2ml)	2.616	1412008	2.28	1.05	12579
Organic Phase change	Less Org (45:55)	3.239	2222648	2.24	1.06	12516
	Actual(50:50)	3.140	1903495	2.28	1.03	12584
	More Org(55:45)	2.475	1742291	2.39	1.12	12595

The retention time and plate count were found unaffected with slight deliberate changes in flow rate and mobile phase ratio that represents the robustness of the method.

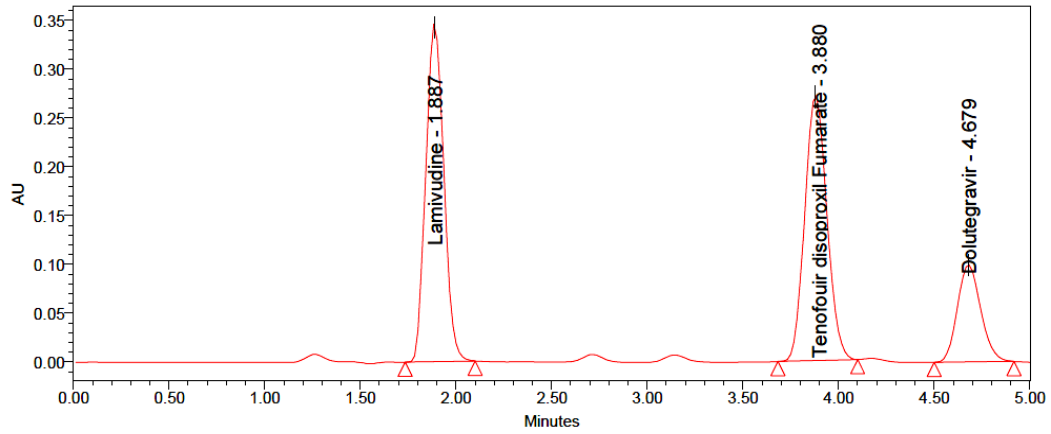


Fig. (16): Chromatogram for less flow rate (0.8 ml).

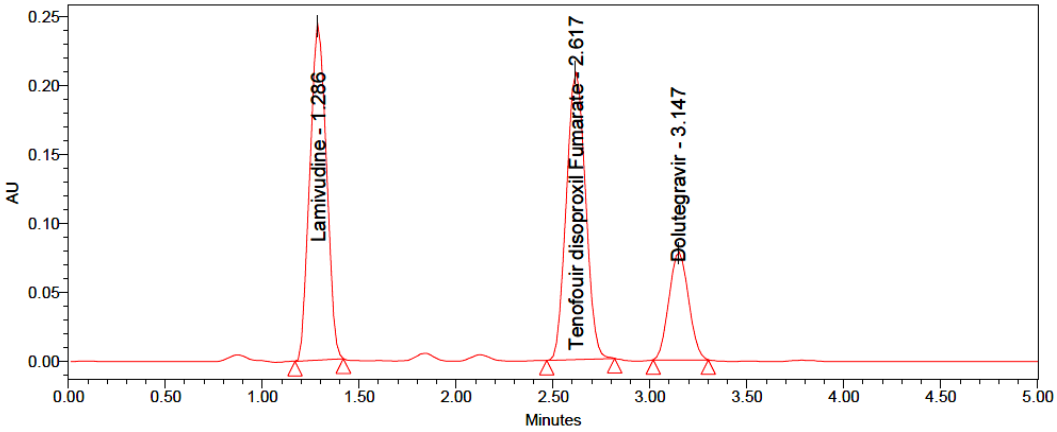


Fig. (17): Chromatogram for more flow rate (1.2ml).

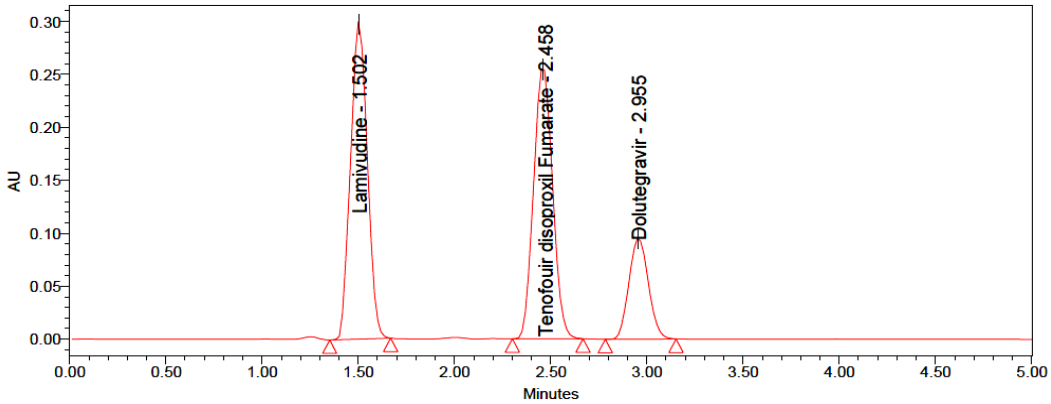


Fig. (18). Chromatogram for more Organic Phase (55:45).

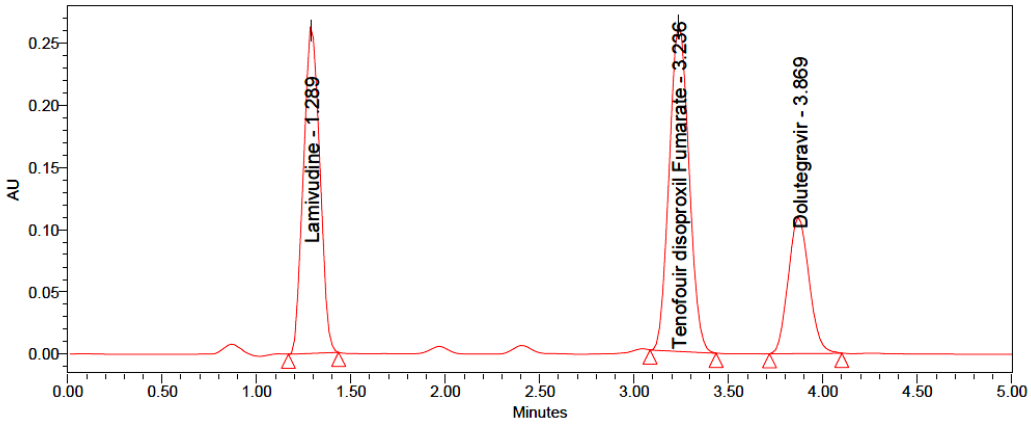


Fig. (19): Chromatogram for Less Organic (45:55).

3.5.5. LOD and LOQ (µg/ml)

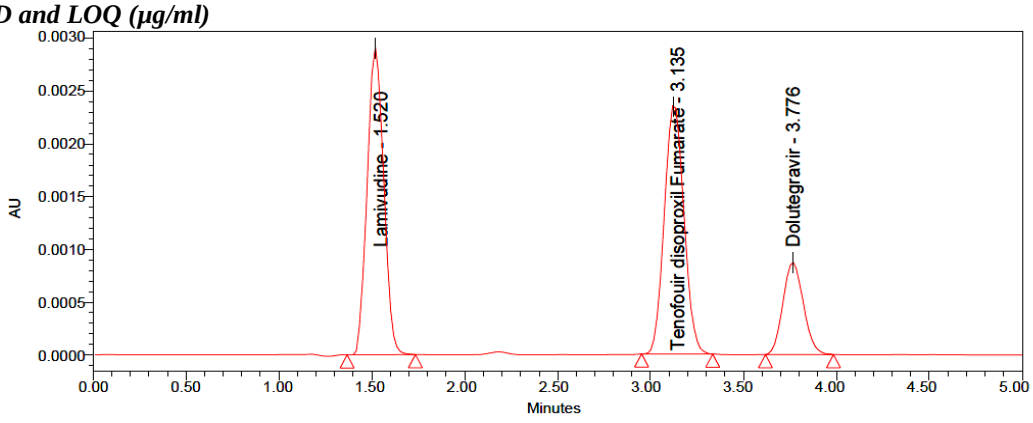


Fig. (20): Chromatogram for LOD.

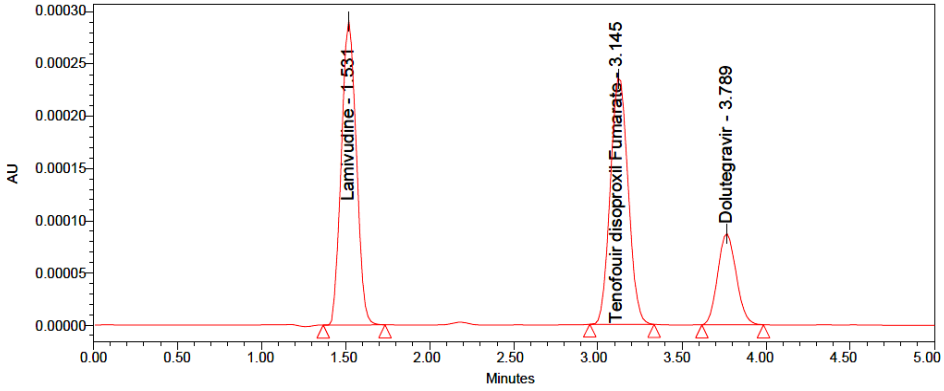


Fig. (21): Chromatogram for LOQ.

Table 21: Sensitivity parameters (LOD & LOQ) by RP-HPLC.

Name of drug	LOD(µg/ml)	LOQ(µg/ml)
Dolutegravir	**0.01**	**0.1**
Lamivudine	**0.06**	**0.6**
Tenofovir Disoproxil Fumarate	**0.06**	**0.6**

The LODs were obtained as 0.01, 0.06, 0.06 µg/ml, and the LOQs were obtained as 0.1, 0.6, 0.6 µg/ml, which indicates the method has good sensitivity.

3.5.6. System suitability

Table 22: System suitability parameters for optimised trial.

S.No	Name	Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
1	Dolutegravir	3.785	744791	3.18	1.08	5448
2	Lamivudine	1.529	2036345	43.22	1.05	4154
3	Tenofovir Disoproxil Fumarate	3.140	1942634	41.13	1.08	4098

The retention time was less than 4 minutes, and plate count NMT 10,000 was observed for all three drugs. The acceptance criteria were ± 2% for the per cent coefficient of variation for the peak area, retention time of the drug, USP plate count.

3.6. Degradation studies

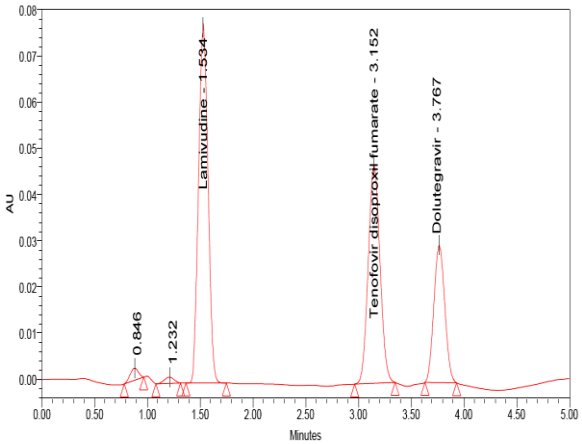


Fig. (22). Chromatogram of Acid degradation.

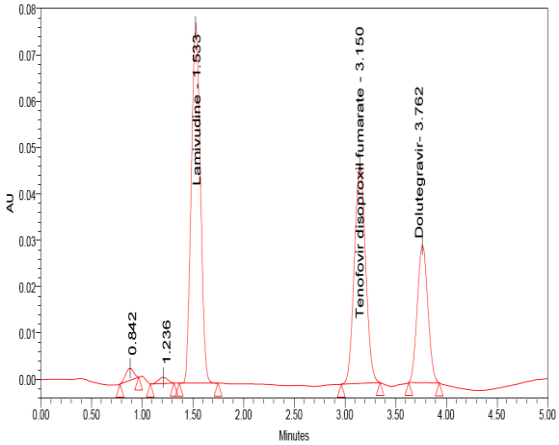
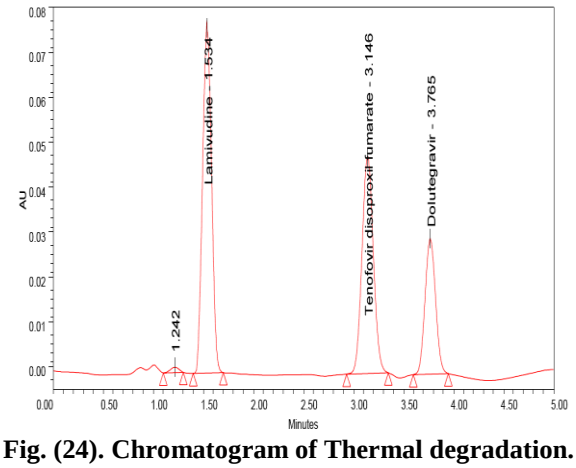


Fig. (23). Chromatogram of Alkali degradation.



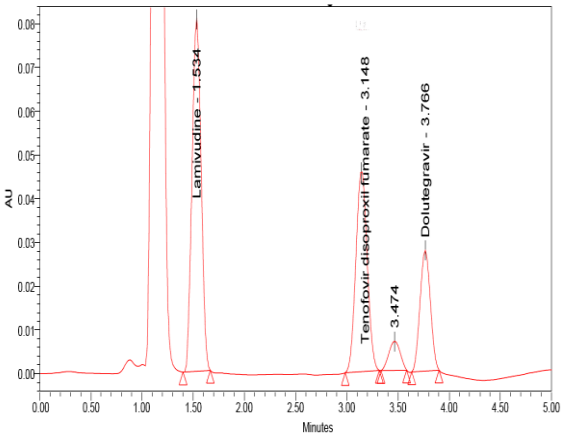


Fig. (24). Chromatogram of Thermal degradation. Fig. (25). Chromatogram of Peroxide degradation.

CONCLUSION

A simple, accurate, and reproducible HPLC method was developed to determine dolutegravir, lamivudine and tenofovir disoproxil fumarate based on a QbD approach using Design Expert® software, Minitab version(16.2.3). The optimised conditions wereanalyzed for thrice, and the developed method was validated as per ICH guidelines, and the results obtained were within the limits. The degradation studies were also performed for the drug by using various stress conditions, and no

degradation products were found in acidic, basic, and oxidative conditions.

ACKNOWLEDGMENTS

The authors are thankful to Aurobindo Laboratories, Hyderabad, for providing the active pharmaceutical ingredient to carry out the research work and expressing gratitude to the management of Bapatla College of Pharmacy for providing library facilities throughout the research.

REFERENCES

1. Tseng A, Seet J, Phillips EJ. The evolution of three decades of antiretroviral therapy: challenges, triumphs and the promise of the future. British Journal of Clinical Pharmacology [Internet], Apr. 15, 2014; 79(2): 182–94. Available from: https://doi.org/10.1111/bcp.12403
2. Amblard F, Patel D, Michailidis E, Coats SJ, Kasthuri M, Biteau N, et al. HIV nucleoside reverse transcriptase inhibitors. European Journal of Medicinal Chemistry [Internet], Jun. 20, 2022; 240: 114554. Available from: https://doi.org/10.1016/j.ejmech.2022.114554
3. Shalbi F, Ali AR. A mini-review on integrase inhibitors: The cornerstone of next-generation HIV treatment. European Journal of Medicinal Chemistry [Internet], Sep. 27, 2024; 279: 116900. Available from: https://doi.org/10.1016/j.ejmech.2024.116900
4. Noorbasha K, Nurbhasha S. A new validated stability-indicating RP-HPLC method for simultaneous quantification of dolutegravir and lamivudine in bulk and pharmaceutical dosage form. Future Journal of Pharmaceutical Sciences [Internet], Jul. 28, 2020; 6(1). Available from: https://doi.org/10.1186/s43094-020-00060-y
5. Godela R, G S. An effective stability indicating RP-HPLC method for simultaneous estimation of Dolutegravir and Lamivudine in bulk and their tablet dosage form. Future Journal of Pharmaceutical Sciences [Internet], Apr. 15, 2020; 6(1). Available from: https://doi.org/10.1186/s43094-020-00026-0
6. Somkuwar K, Sabale P, Sawale V, Rahangdale P. Comparative study of UV spectroscopy, RP-HPLC and HPTLC methods for quantification of antiviral drug lamivudine in tablet formulation. Future Journal of Pharmaceutical Sciences [Internet], Jun. 24, 2024; 10(1). Available from: https://doi.org/10.1186/s43094-024-00651-z
7. Namegabe LM, Mahano AO, Sarr SO. Development, validation and application of a spectrofluorimetric method for the quantification of nevirapine in pharmaceutical formulations tablets and suspensions. American Journal of Analytical Chemistry [Internet], Jan. 1, 2022; 13(06): 206–27. Available from: https://doi.org/10.4236/ajac.2022.136015
8. Attaluri T, Seru G, Varanasi SNM. Development and validation of a stability- indicating RP-HPLC method for the simultaneous estimation of bictegravir, emtricitabine, and tenofovir alafenamide fumarate. Turkish Journal of Pharmaceutical Sciences [Internet], Sep. 15, 2020; 18(4): 410–9. Available from: https://doi.org/10.4274/tjps.galenos.2020.70962
9. Chhabra GS, Rajora A, Mishra DK. Stability indicating RP-HPLC method for the determination of Tenofovir in pharmaceutical formulation. Research Journal of Pharmacy and Technology [Internet], Dec. 28, 2021; 6335–9. Available from: https://doi.org/10.52711/0974-360x.2021.01095
10. Itigimatha N, Chadchan KS, Yallur BC, Hadagali MD. Simple and sensitive RP-HPLC and UV spectroscopic methods for the determination of remogliflozin etabonate in pure and pharmaceutical formulations. Turkish Journal of Pharmaceutical Sciences [Internet], Apr. 15, 2021; 19(2): 213–9. Available from: https://doi.org/10.4274/tjps.galenos.2021.55381
11. Maheshwari M, Soni P. RP-HPLC Method development and Validation for Rapid estimation of Diazepam in Bulk and Pharmaceutical Dosage Form. Research Journal of Pharmacy and Technology [Internet], May 30, 2022; 1938–42. Available from: https://doi.org/10.52711/0974-360x.2022.00322
12. Lal B, Kapoor D, Jaimini M. A review on analytical method validation and its regulatory perspectives. Journal of Drug Delivery and Therapeutics [Internet], Mar. 14, 2019; 9(2): 501–6. Available from: https://doi.org/10.22270/jddt.v9i2.2403
13. Patil BR, Bhusnure OG, Paul BN, Ghodke AY, Mulaje SS. ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DILTIAZEM HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC. International Journal of Drug Regulatory Affairs [Internet], Feb. 12, 2018; 2(2): 78–84. Available from: https://doi.org/10.22270/ijdra.v2i2.133
14. Rathod S, Chopade J, Mahaparale S, Jadhao YP. Analytical method development and validation for estimation of emtricitabine in tablet dosage form by reverse phase high performance liquid chromatography. Indian Journal of Pharmaceutical Sciences [Internet], Jan. 1, 2024; 86(1). Available from: http://dx.doi.org/10.36468/pharmaceutical-sciences.1277
15. Pakalapati S, Rumalla CS, Gudapati AR, Korupolu RB, Gajbhiye SB, Kaliyaperumal M. A novel RP-HPLC method development and validation for determination and estimation of eluxadoline drug with its impurities. SN Applied Sciences [Internet], May 8, 2020; 2(6). Available from: https://doi.org/10.1007/s42452-020-2834-9
16. Sharma A, Ankalgi AD, Devi A, Pandit V, Ashawat MS. Analytical Method Development and Validation for Simultaneous estimation of Methotrexate and Hydroxychloroquine sulfate in

bulk drug by using RP-HPLC. Asian Journal of Pharmaceutical Analysis [Internet], May 13, 2021; 73–8. Available from: https://doi.org/10.52711/2231-5675.2021.00014
17. Rathod SM, Patel PU. Development and validation of RP – HPLC method for estimation of lamivudine and dolutegravir sodium in synthetic mixture. Research Journal of Pharmacy and Technology [Internet], Jan. 1, 2020; 13(6): 2864. Available from: https://doi.org/10.5958/0974-360x.2020.00510.7
18. Kamal AH, Marie AA, Hammad SF. Stability indicating RP-HPLC method for simultaneous determination of omeprazole and aspirin in the presence of salicylic acid as degradation product. Microchemical Journal [Internet], Oct. 20, 2019; 152: 104350. Available from: https://doi.org/10.1016/j.microc.2019.104350
19. Shakoor A, Ahmed M, Ikram R, Hussain S, Tahir A, Jan BM, et al. Stability-indicating RP-HPLC method for simultaneous determination of metformin hydrochloride and vildagliptin in tablet and biological samples. Acta Chromatographica [Internet], Jan. 15, 2020; 32(1): 39–43. Available from: https://doi.org/10.1556/1326.2019.00555
20. Attimarad M, Venugopala KN, Islam MM, Shafi S, Altaysan AI. Rapid Simultaneous Quantitative Analysis of Hypoglycemic agents by RP HPLC: Development, Validation and Application to Medicine. Indian Journal of Pharmaceutical Education and Research [Internet], Mar. 16, 2022; 56(2): 564–72. Available from: https://doi.org/10.5530/ijper.56.2.79
21. Nihila K. METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE, DOLUTEGRAVIR AND TENOFOVIR DISOPROXIL FUMARATE IN BULK AND PHARMACEUTICAL DOSAGE FORM USING RP-HPLC AND ITS APPLICATION TO IN-VITRO DISSOLUTION STUDY. Journal of Medical Pharmaceutical and Allied Sciences [Internet], Sep. 15, 2021; 10(4): 3202–7. Available from: https://doi.org/10.22270/jmpas.v10i4.1165
22. Vetapalem R, Yejella RP, Atmakuri LR. Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of teneligliptin and metformin. Turkish Journal of Pharmaceutical Sciences [Internet], Apr. 1, 2020; 17(2): 141–7. Available from: https://doi.org/10.4274/tjps.galenos.2018.16768
23. Adin SN, Gupta I, Aqil M, Mujeeb M. Application of QbD based approach in development and validation of RP-HPLC method for simultaneous estimation of methotrexate and baicalin in dual-drug-loaded liposomes. Biomedical Chromatography [Internet], Jan. 7, 2023; 37(4): e5581. Available from: https://doi.org/10.1002/bmc.5581
24. Kumbhar PL, Kulkarni AS, Chakole RD, Charde MS. QBD Approach to Method Development, Validation and Degradation Profiling of Antifungal Drugs by RP-HPLC. Asian Journal of Pharmaceutical Analysis [Internet], May 28, 2024; 69–75. Available from: http://doi.org/10.52711/2231-5675.2024.00013
25. Bhaskaran NA, Kumar L, Reddy MS, Pai GK. An analytical "quality by design" approach in RP-HPLC method development and validation for reliable and rapid estimation of irinotecan in an injectable formulation. Acta Pharmaceutica [Internet], Jul 20, 2020; 71(1): 57–79. Available from: https://doi.org/10.2478/acph-2021-0008
26. Shah J, Kotadiya R, Patel R. Analytical quality by Design-Based robust RP-HPLC method for quantitative estimation of pregabalin and etoricoxib in Fixed-Dose combination tablet formulation. Journal of AOAC International [Internet], Jun. 29, 2022; 105(6): 1536–47. Available from: https://doi.org/10.1093/jaoacint/qsac082
27. Urmi KF, Nawaz MdS, Islam SMA. Analytical quality by design approach to RP-HPLC method development and validation for simultaneous estimation of esomeprazole and naproxen in modified-release dosage form. Future Journal of Pharmaceutical Sciences [Internet], Jan. 7, 2022; 8(1). Available from: https://doi.org/10.1186/s43094-021-00396-z
28. Puranik MP. ANALYTICAL QUALITY-BY-DESIGN (AQBD) APPROACH FOR THE DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF LAMOTRIGINE IN BULK AND TABLET FORMULATION. Journal of Medical Pharmaceutical and Allied Sciences [Internet], Oct. 15, 2021; 10(5): 3591–6. Available from: https://doi.org/10.22270/jmpas.v10i5.1506
29. Prajapati P, Patel A, Shah S. DOE-Based Analytical Quality Risk Management for Enhanced AQBD Approach to Economical and Eco-Friendly RP-HPLC Method for synchronous estimation of multiple FDC products of antihypertensive drugs. Journal of Chromatographic Science [Internet], Sep. 17, 2021; 60(8): 786–99. Available from: https://doi.org/10.1093/chromsci/bmab123
30. Gurumukhi VC, Bari SB. Quantification and Validation of Stability-Indicating RP-HPLC Method for Efavirenz in Bulk and Tablet Dosage Form using Quality by Design (QbD): A Shifting Paradigm. Journal of Chromatographic Science [Internet], May 3, 2021; 60(2): 143–56. Available from: https://doi.org/10.1093/chromsci/bmab061
31. Sha'at M, Spac AF, Stoleriu I, Bujor A, Cretan MS, Hartan M, et al. Implementation of QBD approach to the analytical method development and validation for the estimation of metformin hydrochloride in tablet dosage forms by HPLC. Pharmaceutics [Internet], May 31, 2022; 14(6): 1187. Available from: https://doi.org/10.3390/pharmaceutics14061187

32. Wong WH, Lee WX, Ramanan RN, Tee LH, Kong KW, Galanakis CM, et al. Two level half factorial design for the extraction of phenolics, flavonoids and antioxidants recovery from palm kernel by-product. Industrial Crops and Products [Internet], Oct. 28, 2014; 63: 238–48. Available from: https://doi.org/10.1016/j.indcrop.2014.09.049
33. Jaynes J, Ding X, Xu H, Wong WK, Ho C. Application of fractional factorial designs to study drug combinations. Statistics in Medicine [Internet], Aug. 1, 2012; 32(2): 307–18. Available from: https://doi.org/10.1002/sim.5526
34. Godbole MD, Sabale PM, Mathur VB. Development of lamivudine liposomes by three-level factorial design approach for optimum entrapment and enhancing tissue targeting. Journal of Microencapsulation [Internet], Jun. 16, 2020; 37(6): 431–44. Available from: https://doi.org/10.1080/02652048.2020.1778806
35. Kumar SRJ, Rao VK, Katari NK, Jyothi NS, Kowtharapu LP. Determination and quantification of related substances and degradation products in bictegravir by full factorial design evaluated HPLC and mass spectrometry. Analytical Methods [Internet], Jan. 1, 2023; 15(10): 1274–85. Available from: https://doi.org/10.1039/d2ay02106d
36. Jankovic A, Chaudhary G, Goia F. Designing the design of experiments (DOE) – An investigation on the influence of different factorial designs on the characterization of complex systems. Energy and Buildings [Internet], Jul. 22, 2021; 250: 111298. Available from: https://doi.org/10.1016/j.enbuild.2021.111298
37. Fukuda IM, Pinto CFF, Moreira CDS, Saviano AM, Lourenço FR. Design of Experiments (DoE) applied to Pharmaceutical and Analytical Quality by Design (QbD). Brazilian Journal of Pharmaceutical Sciences [Internet], Nov. 8, 2018; 54(spe). Available from: https://doi.org/10.1590/s2175-97902018000001006
38. Beg S, Swain S, Rahman M, Hasnain MS, Imam SS. Application of Design of Experiments (DOE) in pharmaceutical product and process optimization. In: Elsevier eBooks [Internet], 2019; 43–64. Available from: https://doi.org/10.1016/b978-0-12-815799-2.00003-4
39. Vogt FG, Kord AS. Development of Quality-By-Design Analytical Methods. Journal of Pharmaceutical Sciences [Internet], Sep. 9, 2010; 100(3): 797–812. Available from: https://doi.org/10.1002/jps.22325
40. Raman NVVSS, Mallu UR, Bapatu HR. Analytical quality by design approach to test method development and validation in drug substance manufacturing. Journal of Chemistry [Internet], Jan. 1, 2015; 2015(1): 1–8. Available from: https://doi.org/10.1155/2015/435129
41. Raman NVVSS, Mallu UR, Bapatu HR. Analytical quality by design approach to test method development and validation in drug substance manufacturing. Journal of Chemistry [Internet], Jan. 1, 2015; 2015(1): 1–8. Available from: https://doi.org/10.1155/2015/435129
42. Patel KY, Dedania ZR, Dedania RR, Patel U. QbD approach to HPLC method development and validation of ceftriaxone sodium. Future Journal of Pharmaceutical Sciences [Internet], Jul. 13, 2021; 7(1). Available from: https://doi.org/10.1186/s43094-021-00286-4
43. Karmarkar S, Garber R, Genchanok Y, George S, Yang X, Hammond R. Quality by design (QBD) based development of a stability indicating HPLC method for drug and impurities. Journal of Chromatographic Science [Internet], Jul. 1, 2011; 49(6): 439–46. Available from: https://doi.org/10.1093/chrsci/49.6.439
44. Megahed SM, Habib AA, Hammad SF, Kamal AH. Experimental design approach for development of spectrofluorimetric method for determination of favipiravir; a potential therapeutic agent against COVID-19 virus: Application to spiked human plasma. Spectrochimica Acta Part a Molecular and Biomolecular Spectroscopy [Internet], Nov. 28, 2020; 249: 119241. Available from: https://doi.org/10.1016/j.saa.2020.119241
45. Ganorkar SB, Shirkhedkar AA. Design of experiments in liquid chromatography (HPLC) analysis of pharmaceuticals: analytics, applications, implications and future prospects. Reviews in Analytical Chemistry [Internet], Jul. 27, 2017; 36(3). Available from: https://doi.org/10.1515/revac-2016-0025
46. Veni DK, Gupta NV. Development and evaluation of Eudragit coated environmental sensitive solid lipid nanoparticles using central composite design module for enhancement of oral bioavailability of linagliptin. International Journal of Polymeric Materials [Internet], Feb. 5, 2019; 69(7): 407–18. Available from: https://doi.org/10.1080/00914037.2019.1570513
47. Prajapati PB, Jayswal KV, Shah SA. Estimation of multiple Fixed-Dose combination products of Ramipril and Aspirin by GERV-Chromatography using DOE and Risk-Based Enhanced Analytical Quality by Design approach. Journal of AOAC International [Internet], May 15, 2021; 104(6): 1726–41. Available from: https://doi.org/10.1093/jaoacint/qsab073
48. Saha P, Pandey MM. Design of experiment (DOE)-Approach based RP-HPLC analytical method development and validation for estimation of efavirenz in bulk and formulations. Journal of Chromatographic Science [Internet], Feb. 22, 2021; 60(1): 35–44. Available from: https://doi.org/10.1093/chromsci/bmab029
49. Fouad MA, Elsabour SA, Elkady EF, Elshazly HM. Design of experiment (DOE), multiple response optimization and utilizing the desirability function in the simultaneous HPLC separation of five antihypertensive drugs. Journal of the Iranian Chemical Society [Internet], Jun. 23, 2021; 19(1):

269–82. Available from: https://doi.org/10.1007/s13738-021-02316-7
50. Stojanović J, Krmar J, Protić A, Svrkota B, Đajić N, Otašević B. Experimental design in HPLC separation of pharmaceuticals. Arhiv Za Farmaciju [Internet], Jan. 1, 2021; 71(4): 279–301. Available from: https://doi.org/10.5937/arhfarm71-32480
51. Lakens D, Caldwell AR. Simulation-Based power analysis for factorial analysis of variance designs. Advances in Methods and Practices in Psychological Science [Internet], Jan. 1, 2021; 4(1). Available from: https://doi.org/10.1177/2515245920951503
52. Veni DK, Gupta NV. Development and evaluation of Eudragit coated environmental sensitive solid lipid nanoparticles using central composite design module for enhancement of oral bioavailability of linagliptin. International Journal of Polymeric Materials [Internet], Feb. 5, 2019; 69(7): 407–18. Available from: https://doi.org/10.1080/00914037.2019.1570513
53. Olsen AA, McLaughlin JE, Harpe SE. Using multiple linear regression in pharmacy education scholarship. Currents in Pharmacy Teaching and Learning [Internet], Jun. 12, 2020; 12(10): 1258–68. Available from: https://doi.org/10.1016/j.cptl.2020.05.017
54. Kartechina NV, Bobrovich LV, Nikonorova LI, Pchelinceva NV, Abaluev RN. Practical application of variance analysis of four-factor experience data as a technology of scientific research. IOP Conference Series Materials Science and Engineering [Internet], Sep. 1, 2020; 919(5): 052030. Available from: https://doi.org/10.1088/1757-899x/919/5/052030
55. Connelly LM. Introduction to Analysis of Variance (ANOVA). Medsurg Nursing [Internet], Jan. 1, 2021; 30(3): 218. Available from: https://doi.org/10.62116/msj.2021.30.3.218
56. Deshmukh TB, Deo SS, Inam FS, Lambat TL. Development and validation of novel HPTLC method for the simultaneous estimation of Amlodipine Besylate and Telmisartan in tablet dosage form using ICH Q2 (R1) directions. Journal of the Chinese Advanced Materials Society [Internet], Oct. 2, 2018; 6(4): 329–40. Available from: https://doi.org/10.1080/22243682.2018.1425905
57. Radić I, Runje M, Babić S. Development of an analytical method for the determination of pimavanserin and its impurities applying analytical quality by design principles as a risk-based strategy. Journal of Pharmaceutical and Biomedical Analysis [Internet], Apr. 24, 2021; 201: 114091. Available from: https://doi.org/10.1016/j.jpba.2021.114091
58. Orlandini S, Hancu G, Szabó ZI, Modroiu A, Papp LA, Gotti R, et al. New Trends in the quality control of enantiomeric Drugs: Quality by Design-Compliant Development of Chiral Capillary Electrophoresis Methods. Molecules [Internet]. Oct. 19, 2022; 27(20): 7058. Available from: https://doi.org/10.3390/molecules27207058
59. Rajmane AD, Shinde KP. A review of HPLC method development and validation as per ICH guidelines. Asian Journal of Pharmaceutical Analysis [Internet], Jun. 3, 2023; 143–51. Available from: https://doi.org/10.52711/2231-5675.2023.00024
60. Palandurkar K, Bhandre R, Boddu SHS, Harde M, Lakade S, Kandekar U, et al. Quality risk assessment and DoE – Practiced validated stability-indicating chromatographic method for quantification of Rivaroxaban in bulk and tablet dosage form. Acta Chromatographica [Internet], Jan. 11, 2022; 35(1): 10–20. Available from: https://doi.org/10.1556/1326.2021.00978
61. Marzouk HM, Rezk MR, Gouda AS, Abdel-Megied AM. A novel stability-indicating HPLC-DAD method for determination of favipiravir, a potential antiviral drug for COVID-19 treatment; application to degradation kinetic studies and in-vitro dissolution profiling. Microchemical Journal [Internet], Oct. 18, 2021; 172: 106917. Available from: https://doi.org/10.1016/j.microc.2021.106917
62. Kumari MV, Reddy ChB. STABILITY INDICATING VALIDATED HPLC METHOD FOR THE DETERMINATION OF ZANUBRUTINIB IN BULK AND PHARMACEUTICAL DOSAGE FORM. Asian Journal of Pharmaceutical and Clinical Research [Internet], Aug. 15, 2020; 159–62. Available from: https://doi.org/10.22159/ajpcr.2020.v13i10.38621
63. Edrees FH, Draz ME, Saad AS, Hammad SF, Mohamed HM. A sustainable approach for the degradation kinetics study and stability assay of the SARS-CoV-2 oral antiviral drug Molnupiravir. Scientific Reports [Internet], May 31, 2023; 13(1): 8797. Available from: https://doi.org/10.1038/s41598-023-34537-6
64. Kashid SK, Tapkir A, Choudhari P. ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR STABILITY INDICATING HPTLC METHOD FOR ASSAY OF STIRIPENTOL IN BULK AND DOSAGE FORM. Journal of Applied Pharmaceutical Sciences and Research [Internet], Jan. 25, 2021; 3(4): 26–30. Available from: https://doi.org/10.31069/japsr.v3i4.5
65. Ali SNS, Mobina L, Mehfuza M, Seema P, Ahmed A, Khan GJ. Analytical Method Development and Validation and Forced Degradation Stability-Indicating studies of Favipiravir by RP-HPLC and UV in bulk and pharmaceutical dosage form. Journal of Pharmaceutical Research International [Internet], Nov. 10, 2021; 254–71. Available from: https://doi.org/10.9734/jpri/2021/v33i48b33283
66. Marzouk HM, Gouda AS, Rezk MR, Abdel-Megied AM. Innovative eco-friendly stability-indicating HPLC-PDA method for simultaneous determination of the emerging antiviral drugs against COVID-19 infection molnupiravir and favipiravir; degradation kinetic studies along with LC-MS based structure elucidation. Microchemical Journal [Internet], Jul

14, 2024; 205: 111197. Available from: https://doi.org/10.1016/j.microc.2024.111197