Method development and Validation towards simultaneous estimation of Lamivudine and Dolutegravir in the bulk and tablet dosage forms under the influence of stress degradation conditions by using RP-HPLC method.

PAVAN KUMAR KASU*, B. SATYNARAYANA

*Department of chemistry, Osmania University, Hyderabad-500007, Telangana, India

ABSTRACT:

For the precise, simultaneous estimation of combined dolutegravir & lamivudine drugs in the tablet as well as bulk dosage forms, a simple accurate, and exact approach has been established. An Inertsil ODS (4.6 x 250mm, 5 µm) portable stage with an 80% buffer and 100% acetonitrile was used to run the chromatogram. The flow rate of 1 ml per min and the temperature was being maintained at 30 degree Celsius with improved wavelength was fixed at 260 nm. The retention time (RT) for Lamivudine & Dolutegravir were observed at 3.60 and 5.14 minutes, respectively. The percentage purity of Lamivudine and dolutegravir was detected to be 99.97% and 100.64%, respectively. The theoretical plates and tailing factor for Lamivudine and Dolutegravir were found to be 3381.91, 4959.43, 1.14, and 1.13, respectively, as system suitability characteristics. The percentage mean recovery was 99.64% and 100.01%, respectively, for Lamivudine and Dolutegravir, RSD was 0.4% and 0.8% towards repeatability, and 0.1 & 0.7 for the intermediate precision, respectively. %RSD for repeatability remained at 0.4 and 0.8 percent. The precision of the academic work remained exact, strong, and repeatable. The results for LOD and LOQ were 3.00 and 3.02, and 9.98 and 10.00. The results of the study revealed that the recommended Reverse Phase HPLC technique is exact, simple, powerful, quick, and reproducible. This could be used to regularly assess themass and tablet measurements of lamivudine and dolutegravir.

Keywords: Dolutegravir, Lamivudine, RP-HPLC, and simultaneous estimation

INTRODUCTION:

Lamivudine, a reverse transcriptase inhibitor which is used for the treatment of hepatitis B and HIV related infections. a reverse transcriptase inhibitor and zalcitabine analogue in which the pentose ring's 3' carbon is swapped out for a Sulphur atom. 1 It is used to treat Hepatitis B and type 1 human immunodeficiency virus (HIV)

1) Lamivudine is actually a synthetic nucleoside analogue which is phosphorylated intracellularly in order to obtain its relatively more active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This analogue is then incorporated into the viral DNA using HIV reverse transcriptase and HBV polymerase, which results in the termination of DNA chain.[2] IUPAC name of lamivudine is: 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3- oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. Molecular formula is $C_8H_{11}N_3O_3S$ with a average molecular weight is 229.25. This drug substance is highly soluble in the polar protic and aprotic organic solvents such as ethanol, dimethyl sulfoxide (DMSO), and dimethyl formamide (DMF). The solubility of

lamivudine in ethanol is nearly 0.5 mg per ml and approximately, 20mg per ml in DMSO and Dimethyl formamide. Dolutegravir is indicated in mixture with other antiretroviral agents for the treatment of HIV-1 infection that fulfil with the characteristics of adults or children aged from 12 years and older and present at least a weight of 40 kg^{-3} . The FDA has approved the use of dolutegravir and rilpivirine as a combination therapy for adults with HIV-1 infections whose virus is currently suppressed (50 copies/ml) on a stable regimen for at least six months, without a history of treatment failure, and who do not have any known substitutions linked to resistance to either of the two therapy components.. Dolutegravir is an HIV-1 antiviral agent and it inhibits HIV integrase by binding to the active site and blocking the strand transfer step of retroviral DNA integration in host cell.[4]

2) The strand transfer step is essential in the HIV replication cycle and results in inhibition of viral action.

Dolutegravir has a mean EC50 value of 0.5 µM (0.21 µg/mL) to 2.1 µM (0.85 µg/mL) in peripheral blood mononuclear cells (PBMCs) and MT-4 cells.[5] IUPAC name of dolutegravir is (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. Molecular Formula is $C_{20}H_{19}F_2N_3O_5$. Molecular weight is 419.3. Dolutegravir is highly soluble in mostly polar aprotic organic solvents such as DMSO, dimethyl formamide (DMF). Dolutegravir has a solubility of 5 mg per ml and 2.5 in DMF & DMSO, respectively. Additionally, dolutegravir is dissolved in ethanol very slightly.

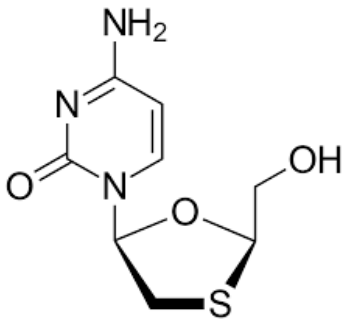


Figure 1: Assembly of Lamivudine

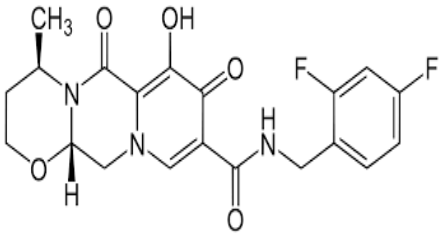


Figure 2: Assembly of Dolutegravir

Study of literature states, there are very few approaches reported towards the evaluation of Lamivudine and Dolutegravir either single or in combination with various other drugs in the pure form as well as drugs formulations by using HPLC, [5-14] LC/MS/MS,[15-19,] UV [22-24] HPTLC,[20-21] and UPLC [25]. In view of the requirement for an appropriate, economical RP-HPLC method for the regular analysis of Lamivudine and Dolutegravir synchronized evaluation of pharmaceutical dose. Attempts were made to establish simple, precise, accurate as well as economical process for the estimate of Lamivudine & Dolutegravir. The recommended approach will be validated as pr the standard ICH guidelines. The objective of the recommended work is to establish a new, precise, simple, exact and economical method as well as recognition for the Synchronized evaluation of Lamivudine& Dolutegravir in the pharmaceutical dose kind by utilizing RP-HPLC method.

LITERATURE REVIEW:

1. Anantha Kumar D, Srinivasa Rao G, JVLN SR (2010) towards Simultaneous determination of lamivudine, Zidovudine and Abacavir in the tablet dosage form by RP-HPLC method. E J of Chem7(1):180–184. https://doi.org/10. 1155/2010/473798 : a simple, accurate and reproducible RP-HPLC method was developed for the simultaneous determination of lamivudine, zidovudine and abacavir in tablet dosage form. Chromatography was carried out on HiQ Sil C18 V column using a mobile phase containing 0.01 M KH_2PO_4 (potassium dihydrogen orthophosphate) with pH is equal 3.0 and methanol (55:45 vol/vol) with a flow rate of 0.8 ml/min. The detection was made at 272 nm and stavudine used as the internal standard for this study. The retention time of lamivudine, abacavir and zidovudine were found to be 3.8, 6.3, 8.1 respectively. The calibration curves were found to be linear over the range 5-250 migro gram per ml for both zidovudine and abacavir and 5-140 microgram per ml for lamivudine. Therefore, the proposed method was validated as per ICH and USP guidelines and it was found suitable for the regular quality control analysis of the drugs in tablet dosage forms.

2. Ashok G, Mondal DS (2018) Development and validation of stability indicating method for the simultaneous estimation of batcaver sulphate, lamivudine and dolutegravir sodium in pharmaceutical dosage forms by RPHPLC Saudi. J Med Pharm Sci 4:289–296: A simple, rapid, specific, stability indicating method was developed and validated for the simultaneous estimation of Abacavir sulphate, lamivudine and dolutegravir sodium in pharmaceutical dosage form using RP-HPLC. The chromatographic separation was done using BDS column of dimensions 250nm x 4.6 mm, 5 micro particle size with mobile phase consisting of potassium dihydrogen phosphate buffer and acetonitrile in the ratio 45:55 v/v run in isocratic mode of flow rate 1.0 ml/ min. The column over temperature was maintained at 30 degree Celsius. The detection wavelength was 240 nm. The developed method was validated in accordance with ICH guidelines, evaluating accuracy, precision, ruggedness, robustness, LOD,LOQ, stability parameters and found to be within limits.

3. Khaleel N, Sk AR (2015) A validated stability indicating RP-HPLC method for simultaneous determination of abacavir, lamivudine and dolutegravir in bulk and pharmaceutical dosage form. W J of Pharm. Res 4(7):1453–1476: A fresh selective, rapid, accurate, precise and RP- HPLC stability indicating method was developed and validated for the quantitative simultaneous determination of dolutegravir and lamivudine in the bulk and pharmaceutical dosage form. A chromatographic separation ws done by using Inertsil ODS (250X4.6, 5 micrometer) column and mobile phase composed of phosphate buffer, pH 3.0, acetonitrile, methanol with 50:20:30 v/v/v with a flow rate of 1.0 ml/min and the detection limit of 257 nm using the PDA detector. These drugs were subjected to varied conditions like hydrolysis, oxidation, thermal, photochemical and UV. The suggested method was analyzed statistically and validated as per ICH guidelines a d the validation covered accuracy, precision, linearity, LOD, LOQ, robustness and specificity were within the limits.

4. Raja T, Lakshmana Rao A (2011) Development and validation of RP-HPLC method for estimation of abacavir, lamivudine and zidovudine in pharmaceutical dosage form. Int. J of Pharm

Tech Res. 3(2):852–857: A method has been developed and validated for the estimation of abacavir, lamivudine and zidovudine by HPLC on C18 column with UV detection of 270 nm. The mobile phase composition that provides an optimal resolution of components in an acceptable elution time in water:methanod (70:30 v/v) with 0.1 %

potassium dihydrogen phosphate buffer with pH 3.2. The powdered tablet was extracted with methanol and water (50:50 v/v) mixture and after addition of stavudine, an internal standard subjected to HPLC analysis and assayed by comparison of analyte to internal standard peak areas to concentration ratios. The method was successfully applied to pharmaceutical formulations because no chromatographic interferences from the tablet excipients were found. The method retained its accuracy and precision with standard addition technique was applied.

5. EXPERIMENTAL METHODOLOGY

INSTRUMENTS

Sl. No	Instrument	Model
1	HPLC	WATERS 2695 separation module Detector & software: Empower3
2	UV/VIS spectrophotometer	LABINDIA UV 12.500^{+}
3	pH meter	Adwa – AD 10100
4	Weighing machine	Afcoset ER-1000A
5	Pipettes & Burettes	Borosil
6	Beaker	Borosil

CHEMICALS:

SL. No	Chemical	Company Name
1	LAMIVUDINE	HETERO LABS
2	DOLUTEGRAVIR	HETERO LABS
3	KH_2PO_4	Finer chem LTD
4	Water & Methanol	LICHROSOLV (MERCK)
5	Acetonitrile	MOLYCHEM
6	Ortho phosphoric Acid	MERCK

HPLC METHOD DEVELOPMENT:

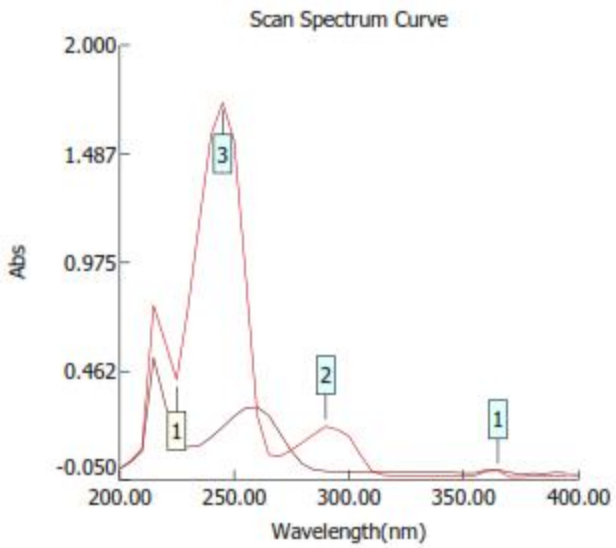
Mobile Phase Optimization:

During the procedure, the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer, Acetonitrile: methanol in different combinations of pH as well as varying amounts. Finally, the mobile phase was optimized to Phosphate buffer (pH 3.0), Acetonitrile in a fixed proportion 80 : 20 v/v respectively.

Wave length selection:

The UV spectrum of solution of 10 µg/ml Lamivudine and 10 µg per ml Dolutegravir in diluents with a mobile phase composition was recorded within the range of 400nm - 1000nm. From the UV spectrum wavelength is selected to be 260 nm. At this particular wavelength both drugs showed very good absorbance.

UVGraph



Optimization of the Column:

The method was carried out using the different columns, however best suited was C18 Phenomenex column, YMC, and state of art Inertsil-ODS column. This column (4.6 x 250mm, 5µm) was found to be ideal as it gave perfect peak shape & resolution at 1.0 ml/minute flow rate.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

HPLC Instrument	:	Waters HPLC with auto sampler and UV detector.
Temperature	:	Ambient
Column	:	Inertsil-ODS (4.6 x 250mm, 5 µm)
Buffer	:	3.4g - KH_2PO_4 in 1L of HPLC water P^H level was adjusted with OPA up to 3.0.

pH value	:	3.0
Mobile phase	:	80% of buffer 100% Acetonitrile
Flow rate	:	1 ml/minute
Wavelength	:	260 nm
Injection volume	:	20 µl
Run time	:	12 min.

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of standard phosphate buffer:

3.4g of KH_2PO_4 in a 1 Ltr of HPLC water and the pH is adjusted using OPA up to 3.0. Final solution was then filtered through 0.44 µm membrane filter and sonicated for 10 minutes.

Preparation of mobile phase:

Precisely measured 800 ml (80%) above buffer & 1L of Acetonitrile HPLC (100%) were added together and removed gases using an ultrasonic water bath for 10 min, then filtered through 0.45 µ filter using vacuum filtration method.

Diluent Preparation:

Mobile phase was prepared as the diluent.

PREPARATION OF THE LAMIVUDINE & DOLUTEGRAVIR STANDARD & SAMPLE SOLUTION:

Standard Solution Preparation:

In 10 ml clean and dry volumetric flask, weighed and transferred accurately 100 mg of lamivudine and 12.5 mg of dolutegravir as the working standard. Added approximately 7 mL of the diluent, and sonicate to completely dissolve it, and then added adequate liquid to make up the volume to the mark using the same solvent. (Stock answer)

Then pipetted 0.6 ml of aforementioned stock solutions into a 10 ml volumetric flask and added diluent to the mark.

Sample Solution Preparation:

Weigh 10 tablets precisely, crush them in a mortar and pestle, and transfer the sample, which is equal to 100 mg of lamivudine and 12.5 mg of dolutegravir, into a 10 mL clean, dry volumetric flask. Add about 7 mL of diluent, and sonicate the mixture for up to 15 minutes to completely dissolve it. Then, add enough liquid to the flask to make it the appropriate volume. It is subsequently filtered using a 0.45-micron Infusion channel. Pipette 0.6 ml of lamivudine and dolutegravir into a 10 ml volumetric flask from the aforementioned stock solution, diluted to the appropriate strength.

Use the formulas to determine the % Assay after injecting 20 µl of standard, processing the analyte through a chromatographic device, measuring the area of the purity peaks of the Lamivudine and Dolutegravir.

SYSTEM SUITABILITY:

Tailing factor of Lamivudine and Dolutegravir mixture analyte in their standard solution should not be more than 2.0. Moreover, Theoretical plates for the Lamivudine and Dolutegravir peaks in Standard solution should not be less than 2000. Also, to be noted that the Resolution for the Lamivudine and Dolutegravir peaks in standard solution should not be less than

2. **Calculation: (For Lamivudine)**

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{\text{Avg weight}}{\text{Label claim}} \times \frac{P}{100} \times 100$$

GIVEN:

AT = average area counts of sample preparation.

AS = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = Label Claim mg/ml.

RESULTS:

System Suitability Results:

1. Tailing factor obtained with standard injection = 1.14

2. Theoretical Plates observed using the standard injection = 3281.91

System suitability results for Lamivudine:

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.90	4361.01	1.24
2	1.00	3281.91	1.14
3	1.10	4137.68	1.18

System suitability results for Dolutegravir:

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	0.90	5749.13	1.09	6.44
2	1.00	4959.43	1.13	5.66
3	1.10	5286.06	1.04	6.11

***** Results for the actual flow rate i.e.,1.0ml/min have been considered from an assay standard.

***** Results for actual mobile phase composition (80:20) Buffer pH 3: Acetonitrile has been considered from Accuracy stand.

Assay Results: (Lamivudine)

$$\frac{855999}{854516.7} * \frac{100}{10} * \frac{0.6}{10} * \frac{10}{199} * \frac{10}{0.6} * \frac{398}{200} * \frac{99.8}{100} * 100 = 99.97\%$$

Calculation: (For Dolutegravir)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

RESULTS:

System Suitability Results:

1. Tailing factor Obtained using the standard injection = 1.13

2. Theoretical Plates Observed using the standard injection = 4959.43

3. Resolution Observed from the standard injection = 5.66

Assay Results: (For Dolutegravir)

$$\frac{115671}{114706} * \frac{12.5}{10} * \frac{0.6}{10} * \frac{10}{199} * \frac{10}{0.6} * \frac{398}{25} * \frac{99.8}{100} * 100 = 100.64\%$$

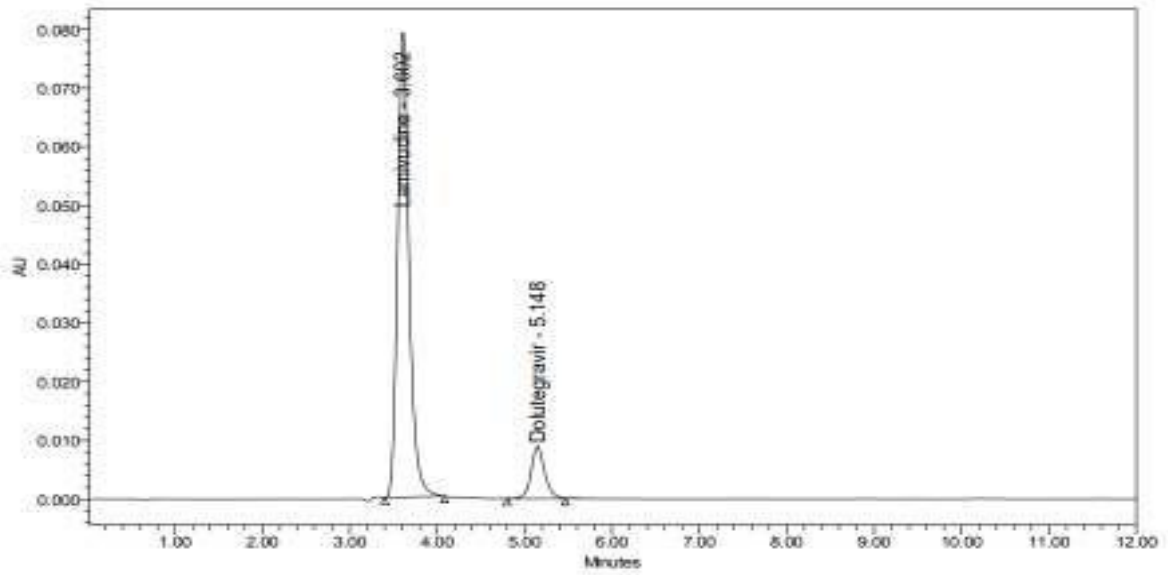


Figure 3: chromatogram of Standard

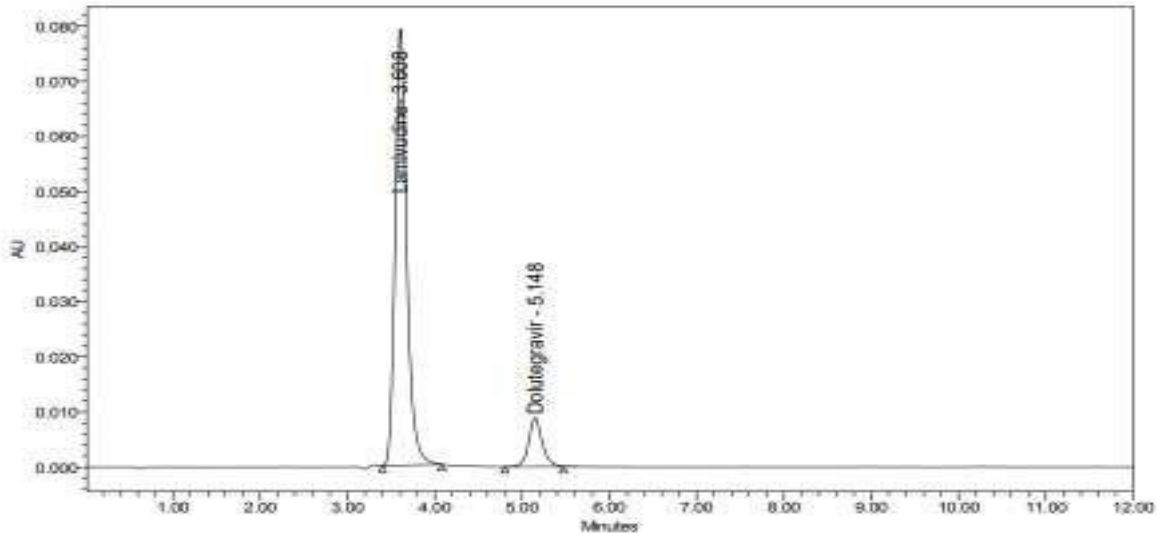


Figure 4: chromatogram of sample

SAMPLE AND STANDARD DETAILS

S. No.	Samples
1	Lamivudine & Dolutegravir Tablets 100 mg & 12.5 mg
2	Lamivudine & Dolutegravir

METHOD VALIDATION SUMMARY:

PRECISION:

Preparation of stock solution:

Weigh 10 tablets precisely, crush them in a mortar and pestle, and transfer the sample, which is equal to 100 mg of lamivudine and 12.5 mg of dolutegravir, into a 10 mL clean, dry volumetric flask. Add about 7 mL of diluent, and sonicate the mixture for up to 15 minutes to completely dissolve it. Then, add

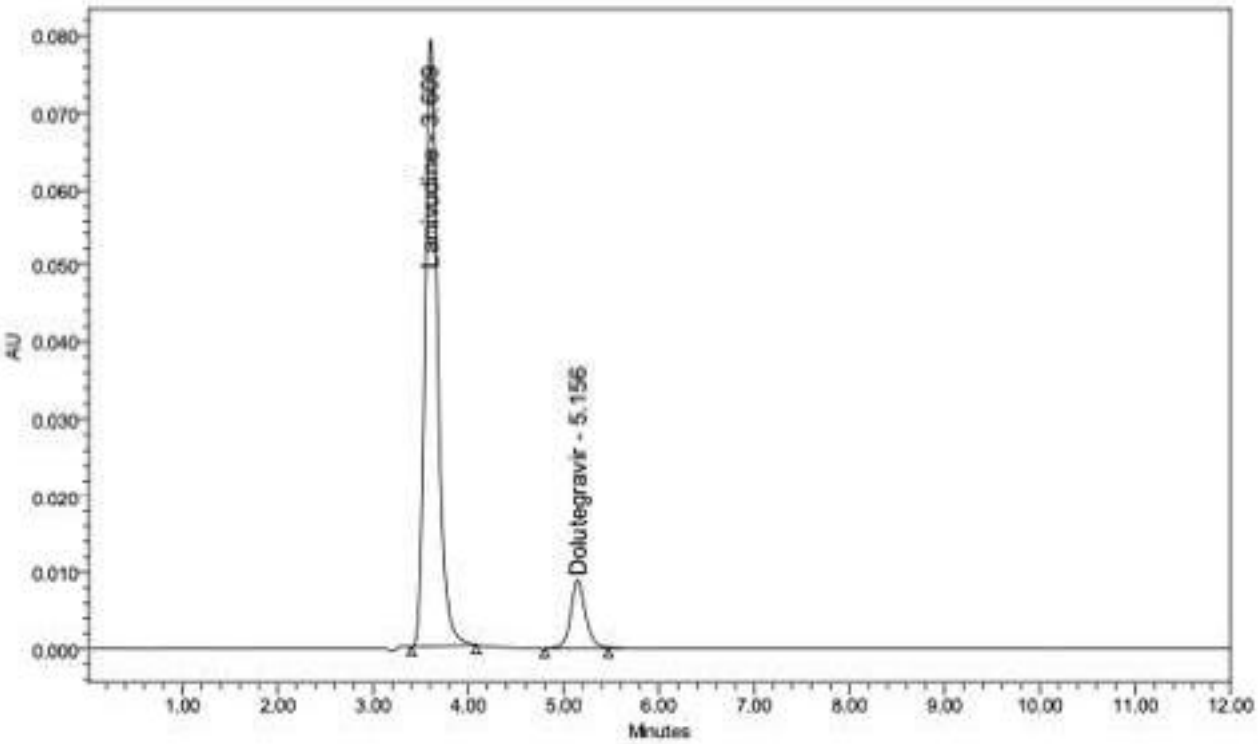
enough liquid to the flask to make it the appropriate volume. It is subsequently filtered using a 0.45-micron Infusion channel. Pipette 0.6 ml of lamivudine and dolutegravir into a 10 ml volumetric flask from the aforementioned stock solution, diluted to the appropriate strength.

Procedure:

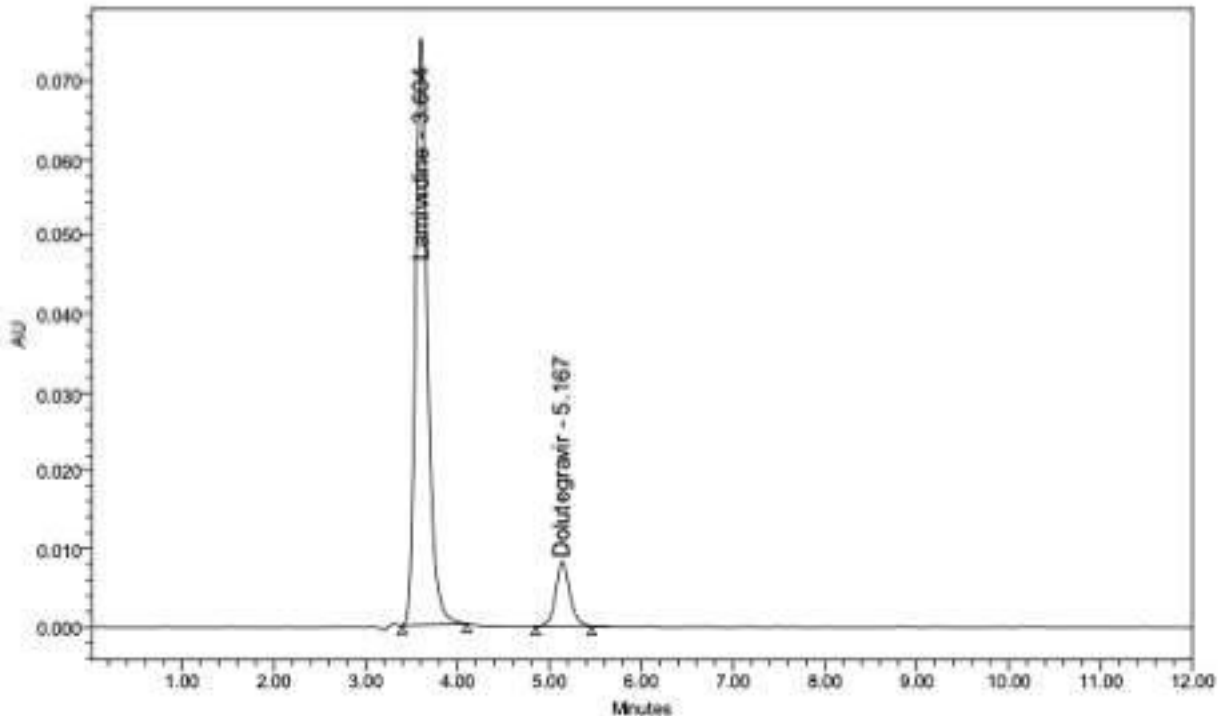
The area of each of the six injections of the reference solution into the HPLC was measured. The range of six duplicate injections'% RSD was found to be within the prescribed range.

The results are summarized for Lamivudine and Dolutegravir

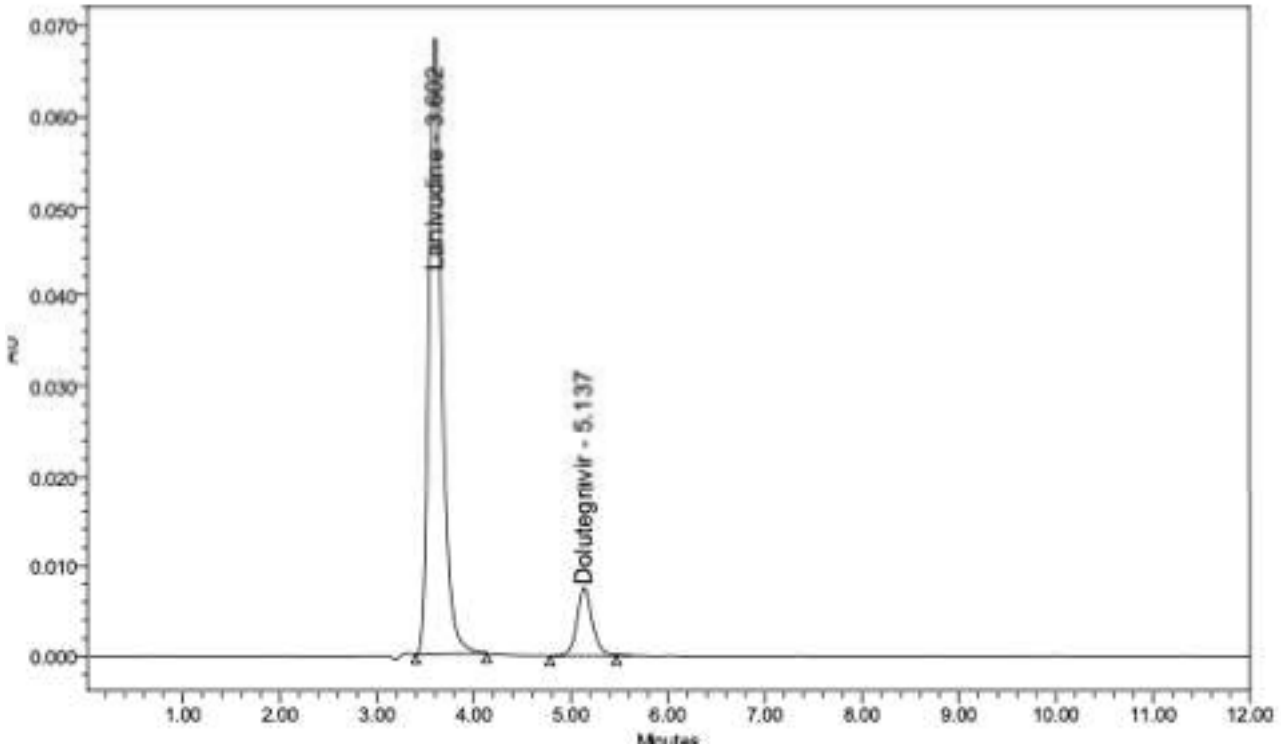
Injection	**Area for Dolutegravir**	**Area for Lamivudine**
1st Injection	111367	852827
2nd Injection	112718	852338
3rd Injection	112654	858354
4th Injection	113940	852840
5th Injection	112514	858514
6th Injection	112281	857581
Avg	112662.31	855409.01
Standard Deviation	846.70	12.523.51
%RSD	0.80	0.40



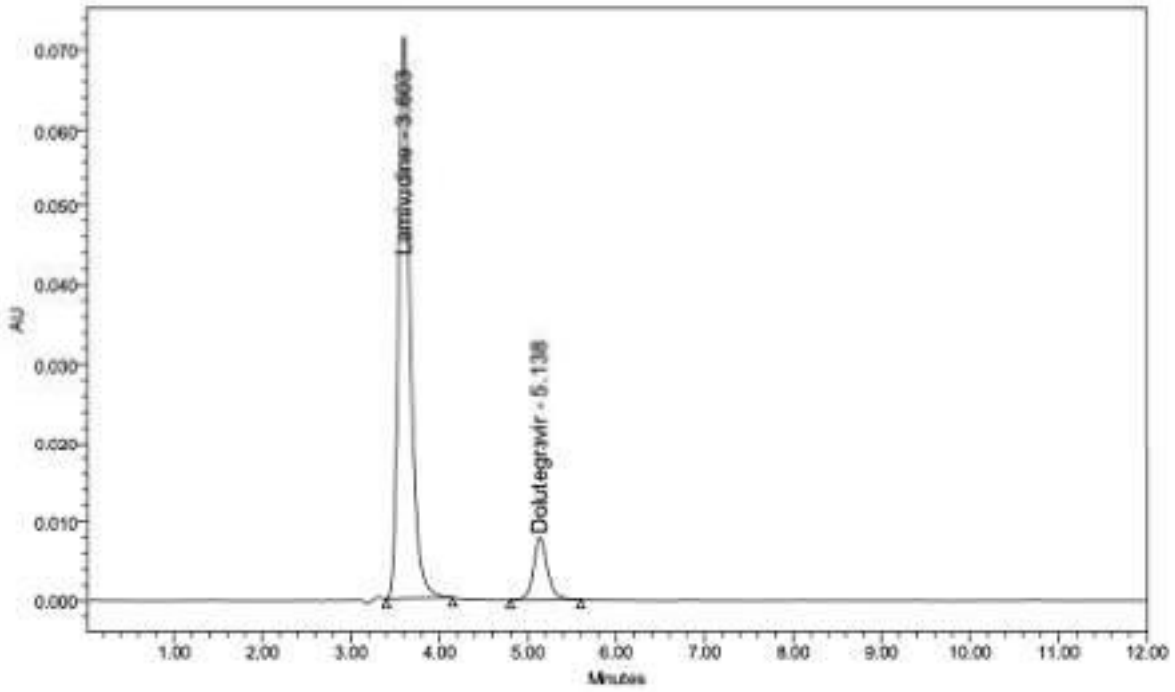
PRECISION CHROMATOGRAM OF INJECTION 1



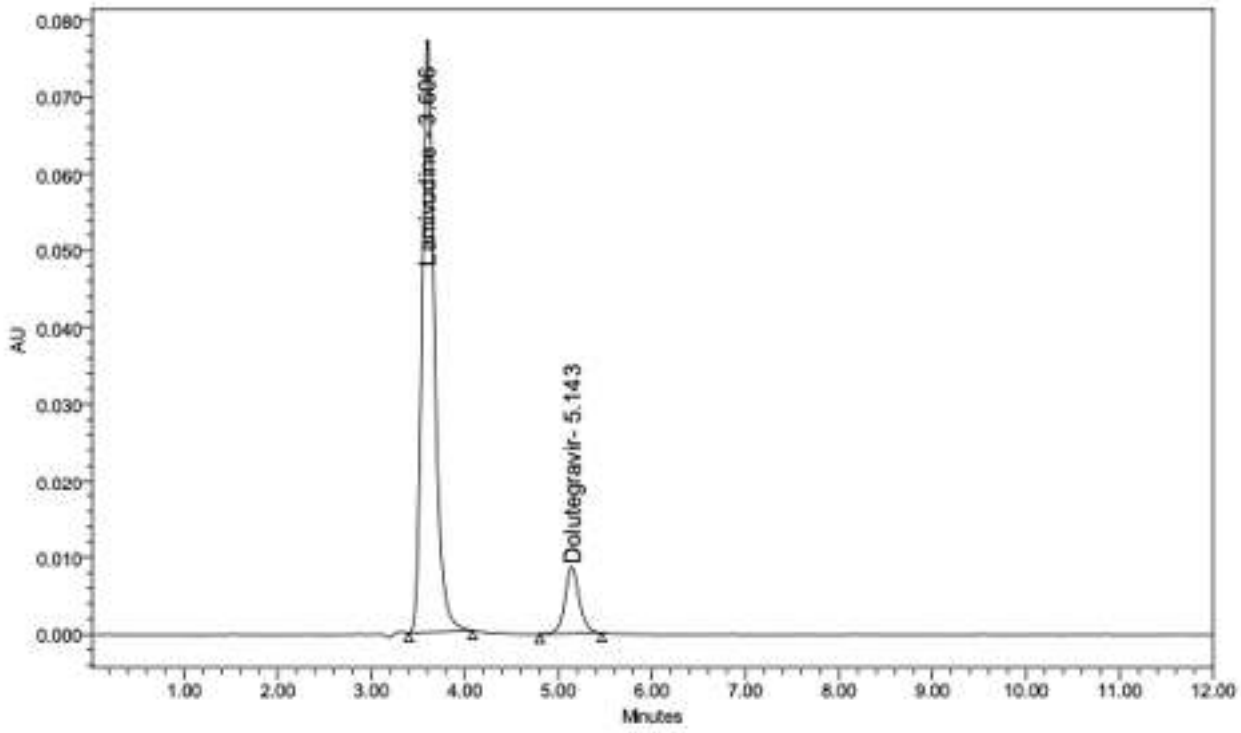
PRECISION CHROMATOGRAM OF INJECTION 2



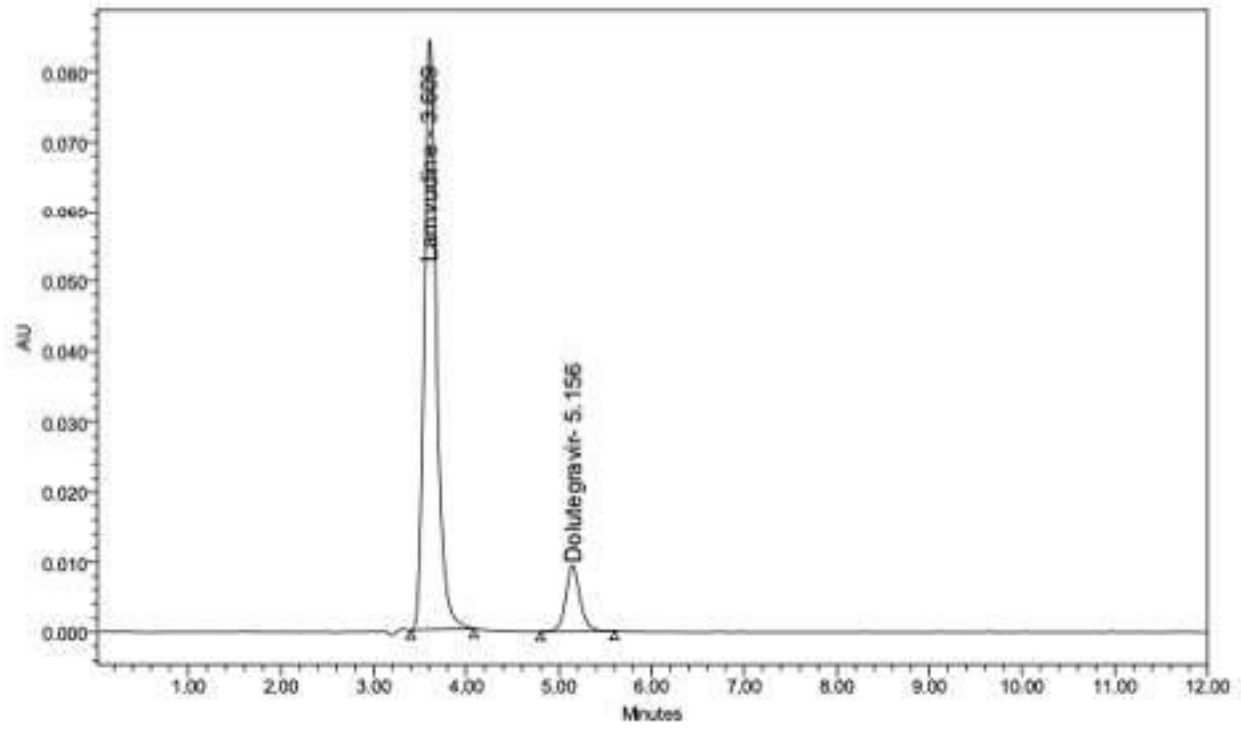
PRECISION CHROMATOGRAM OF INJECTION 3



PRECISION CHROMATOGRAM OF INJECTION 4



PRECISION CHROMATOGRAM OF INJECTION 5



PRECISION CHROMATOGRAM OF INJECTION 6

Acceptance Criteria: The area of results from six standard injections should have an RSD of no more than 2%.

INTERMEDIATE PRECISION/RUGGEDNESS:

To determine to be intermediate precision, which is also known as ruggedness of the method, Precision was performed on different days.

Preparation of stock solution:

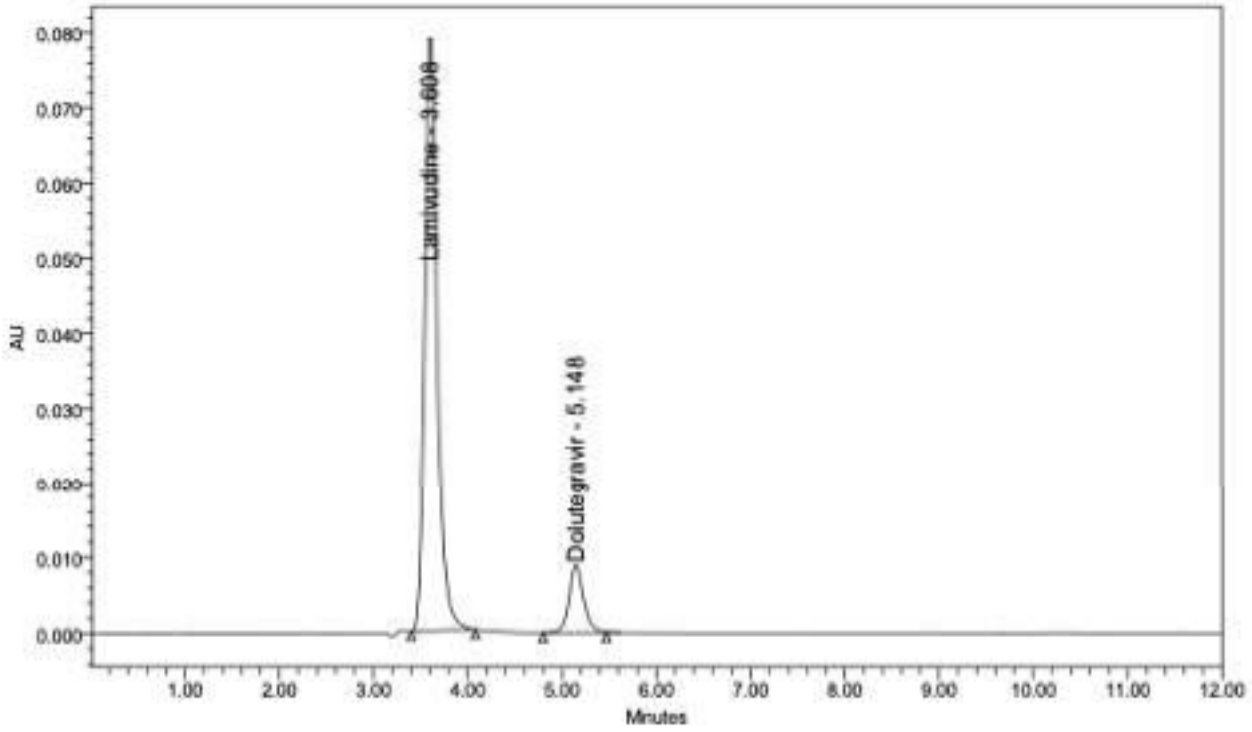
In 10 ml clean and dry volumetric flask, weighed and transferred accurately 100 mg of lamivudine and 12.5 mg of dolutegravir as the working standard. Added approximately 7 mL of the diluent, and sonicate to completely dissolve it, and then added adequate liquid to make up the volume to the mark using the same solvent. (Stock answer)

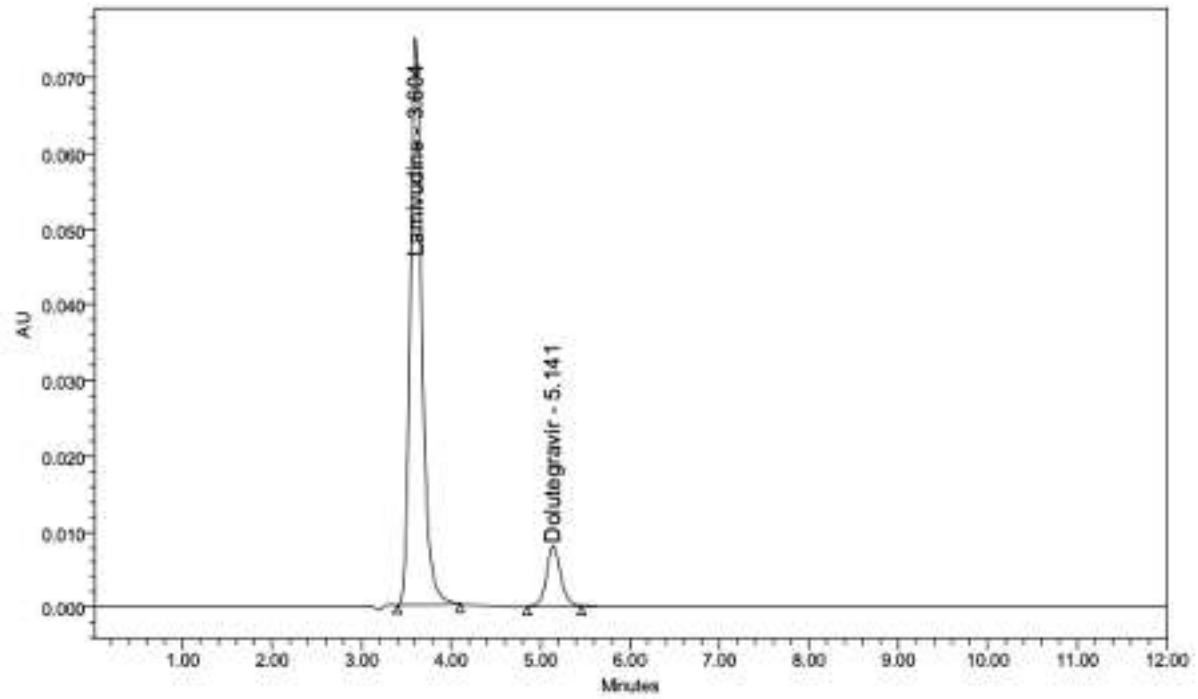
Then pipetted 0.6 ml of aforementioned stock solutions into a 10 ml volumetric flask and added diluent to the mark.

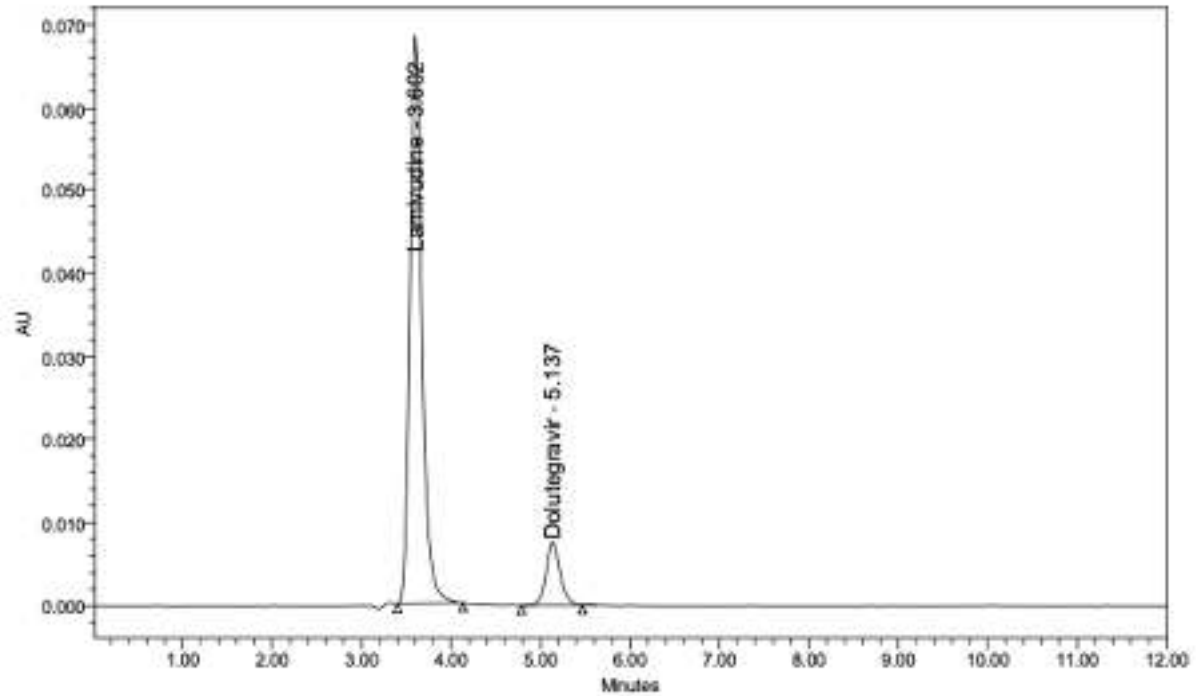
Procedure:

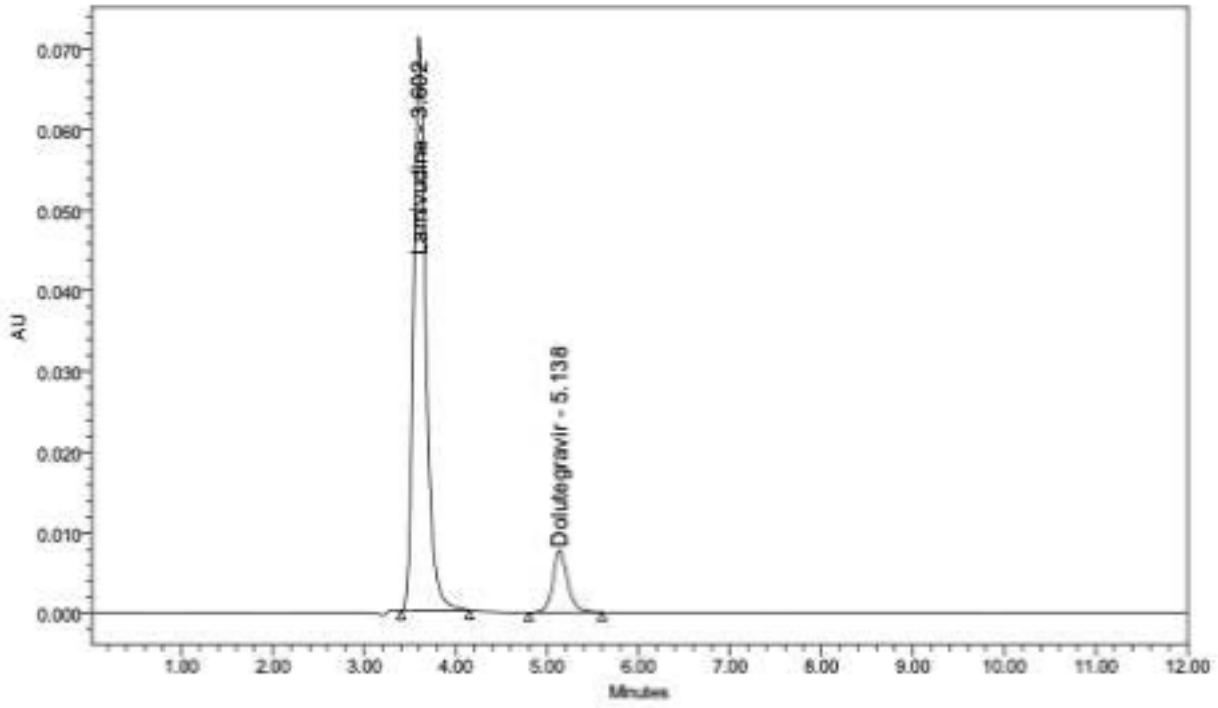
The area of each of the six injections of the reference solution into the HPLC was measured. The% RSD for the area of six replicate injections was found to be within the prescribed range. The results are summarized for Lamivudine and Dolutegravir

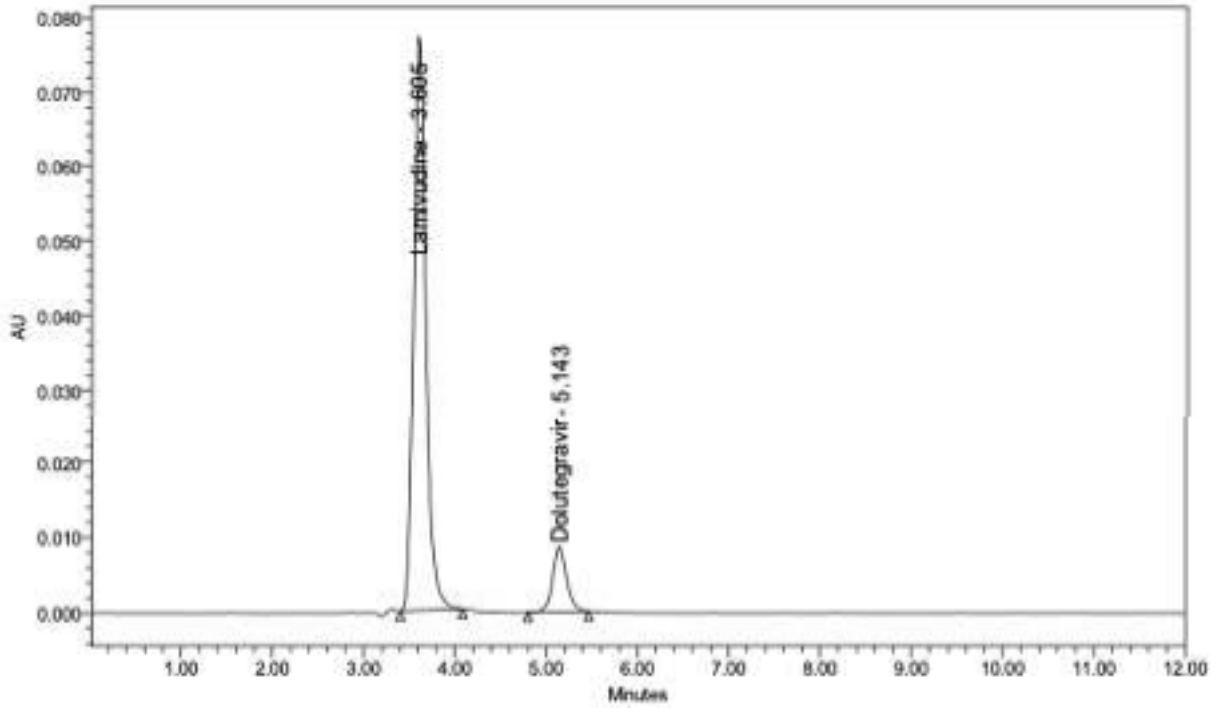
Injection	**Area for Lamivudine**	**Area for Dolutegravir**
1st Injection	859454	112534
2nd Injection	857161	111225
3rd Injection	859459	112914
4th Injection	858376	113392
5th Injection	858483	113107
6th Injection	859770	112960
Avg	858784.8	112688.8
Std. Deviation	976.1	769.6
% of RSD	0.10	0.70

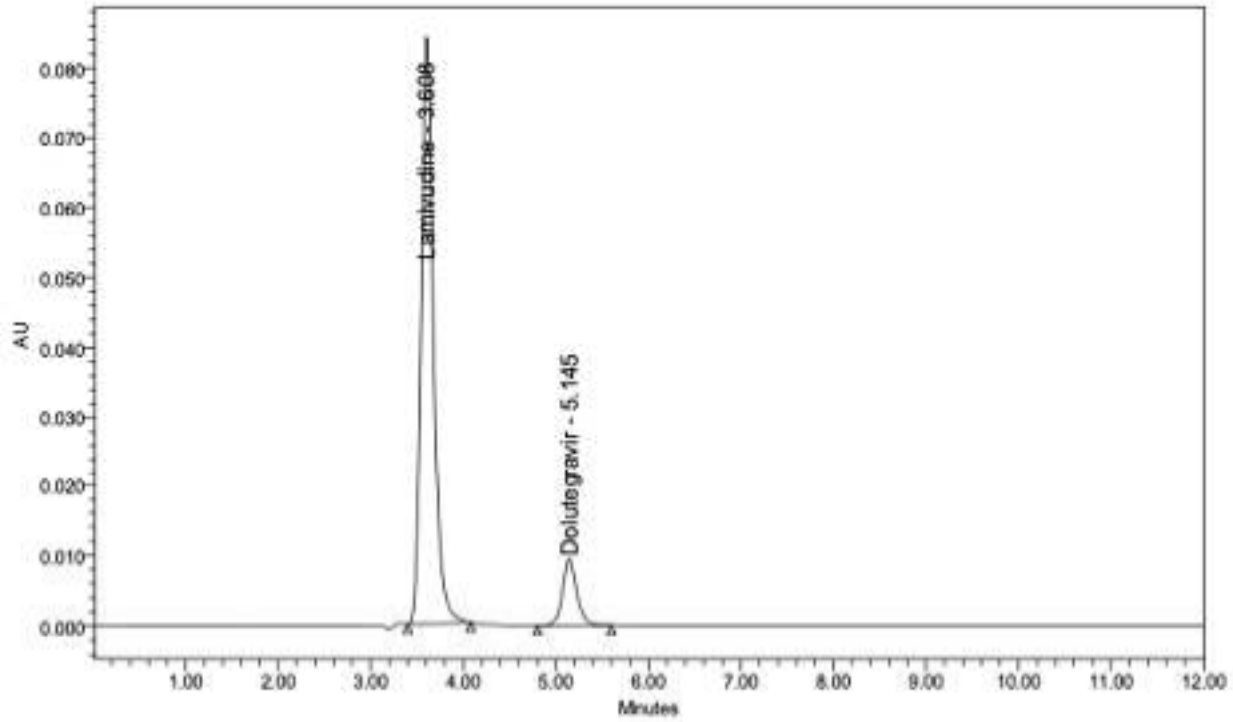

Lamivudine - 3.608
Dolutegravir - 5.148
AU
Minutes
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00


Lamivudine - 3.604
Dolutegravir - 5.141
AU
Minutes


Lamivudine - 3.602
Dolutegravir - 5.137
AU
Minutes


Lamivudina - 3.602
Dolutegravir - 5.138
AU
Minutes


Lamivudina - 3.605
Dolutegravir - 5.143
AU
Minutes

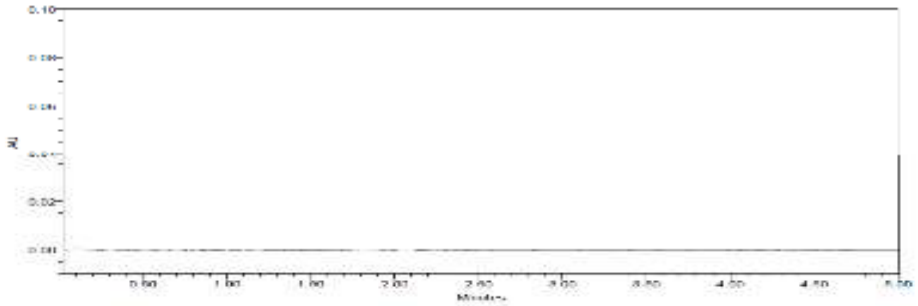


INTERMEDIATE PRECISIION OF SIX SAMPLES

Acceptance Criteria: The area of six standard injection results should have an RSD of no more than 2%.

SPECIFICITY:

Injections of Blank and Standard are made into the system for the Specificity research. The retion time of the analytical peaks are not interfered with by any peak in the blank.



ACCURACY:

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Transfer 50 mg of lamivudine and 6.25 mg of dolutegravir, both working standards, into a 10 ml clean, dry volumetric flask. Add roughly 7 mL of diluent, sonicate to completely dissolve it, and then add more of the same solvent to get the volume up to the target. (Stock answer)

Pipette 0.6 ml of the aforementioned stock solutions into a volumetric flask with a 10 ml capacity. Add diluent to the mark.

For preparation of 100% solution (With respect to target Assay concentration):

In a 10 ml clean, dry volumetric flask, accurately weigh and transfer 100 mg of lamivudine and 12.5 mg of dolutegravir as the working standard. Add around 7 mL of diluent, sonicate to completely dissolve it, and then add enough liquid to make the volume up to the target with the same solvent. (Stock answer)
Pipette 0.6 ml of the aforementioned stock solutions into a volumetric flask with a 10 ml capacity. Add diluent to the mark.

For preparation of 150% solution (With respect to target Assay concentration):

In a 10 ml clean, dry volumetric flask, accurately weigh and transfer 150 mg of lamivudine and 18.75 mg of dolutegravir as the working standard. Add around 7 mL of diluent, sonicate to completely dissolve it, and then add enough liquid to make the volume up to the target with the same solvent. (Stock answer)
Pipette 0.6 ml of the aforementioned stock solutions into a volumetric flask with a 10 ml capacity. Add diluent to the mark.

Procedure:

Inject the accuracy -50%, accuracy -100%, and accuracy -150% solutions along with the regular solution.

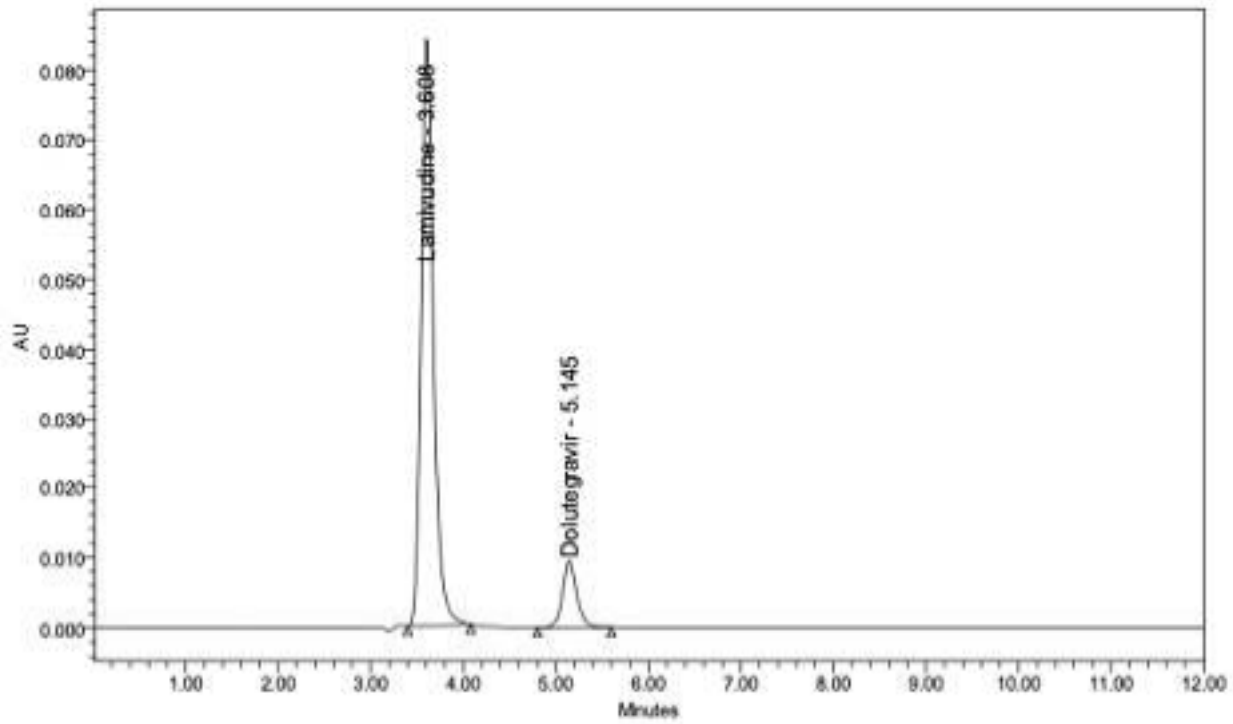
Calculate the individual recovery and mean recovery values as well as the Amount found and Amount added for Lamivudine and Dolutegravir.

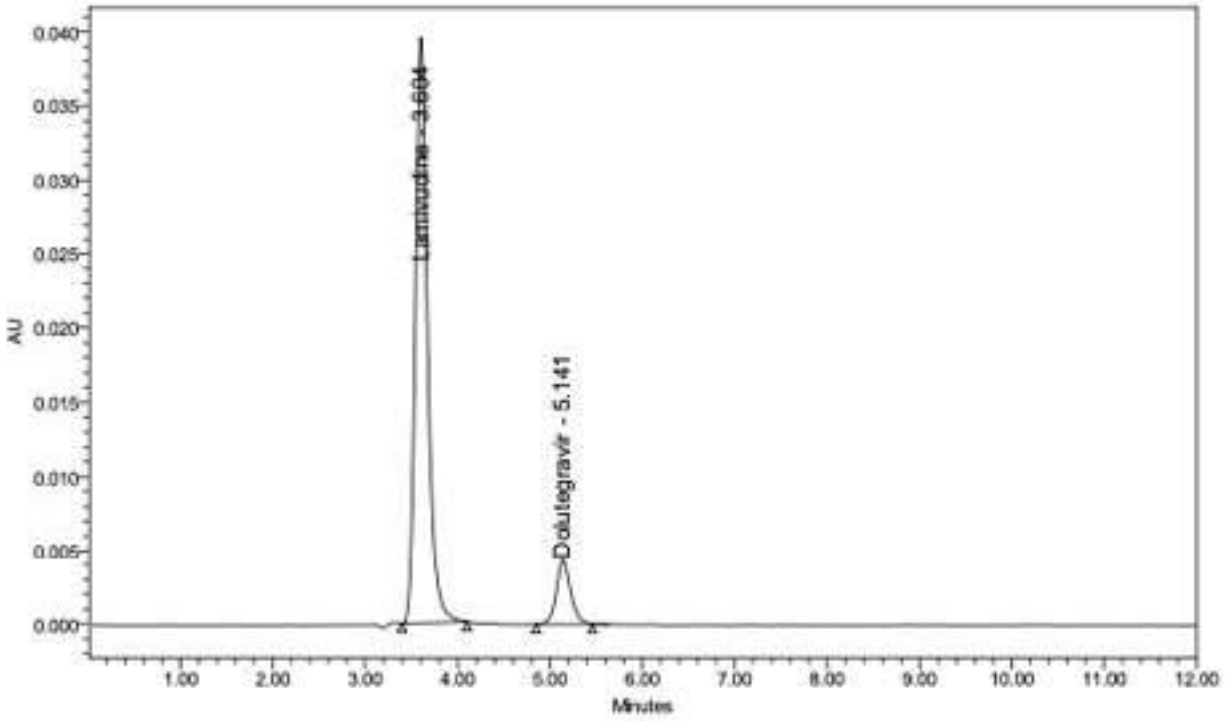
The accuracy results for Lamivudine

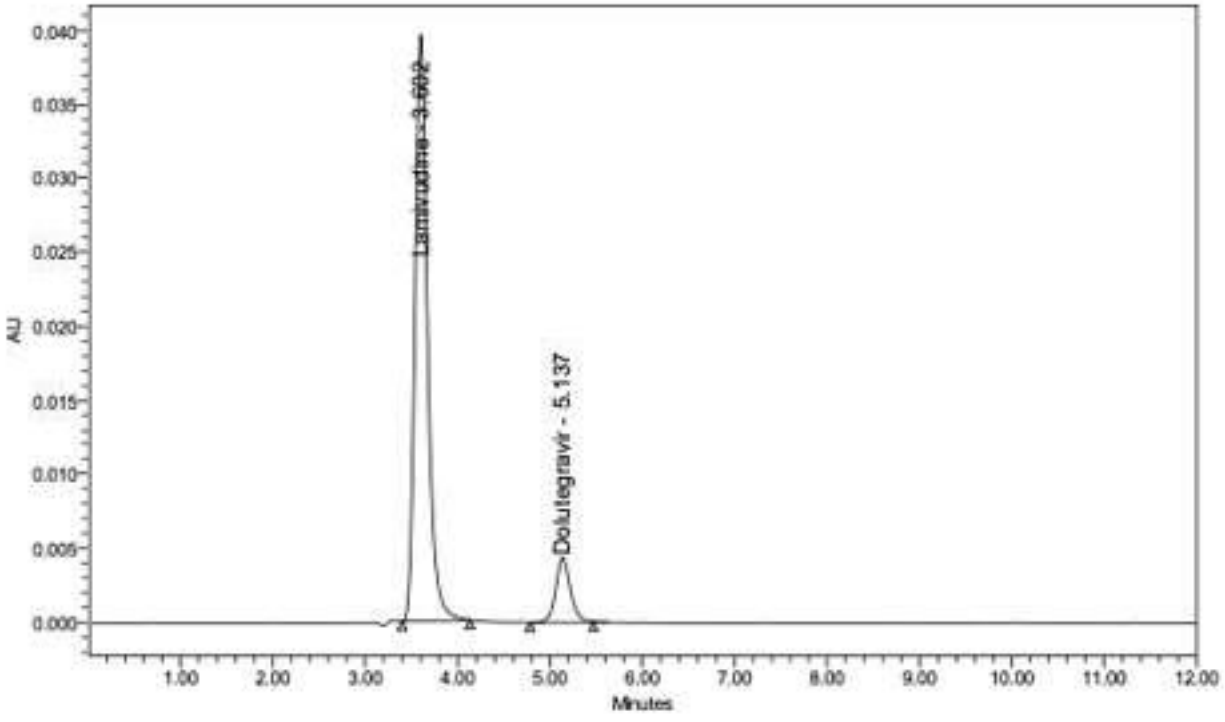
Concentration	**Area**	**Amount taken (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean Recovery**
50%	427928	50	49.98	99.96	99.86
100%	854989	100	99.86	99.86	
150%	1281399	150	149.66	99.77	

The accuracy results for Dolutegravir

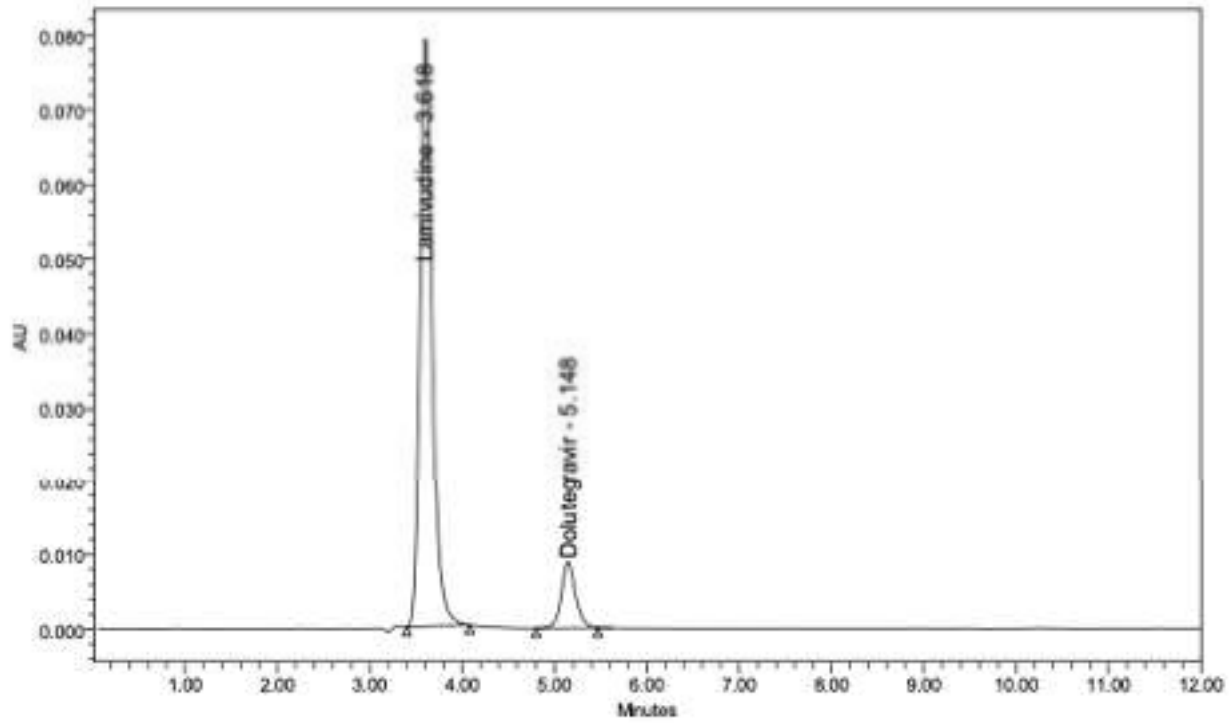
Concentration	**Area**	**Amount taken (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean Recovery**
50%	57620	6.25	6.27	100.26	99.96
100%	114986	12.5	12.51	100.04	
150%	171648	18.75	18.67	99.56	


Lamivudine - 3.608
Dolutegravir - 5.145
AU
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
Minutes





ACCURACY 50% CHROMATOGRAM


Lamivudine - 3.618
Dolutegravir - 5.148
AU
Minutes
0.080
0.070
0.060
0.050
0.040
0.030
0.010
0.000
1.00
2.00
3.00
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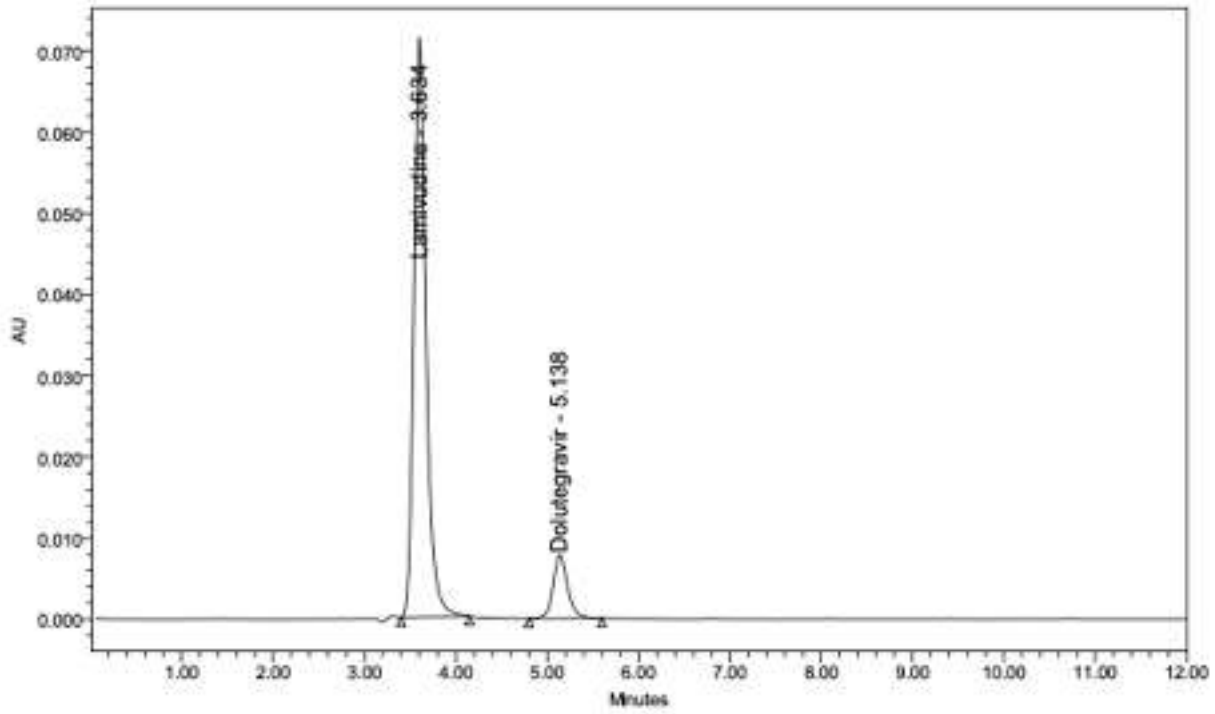
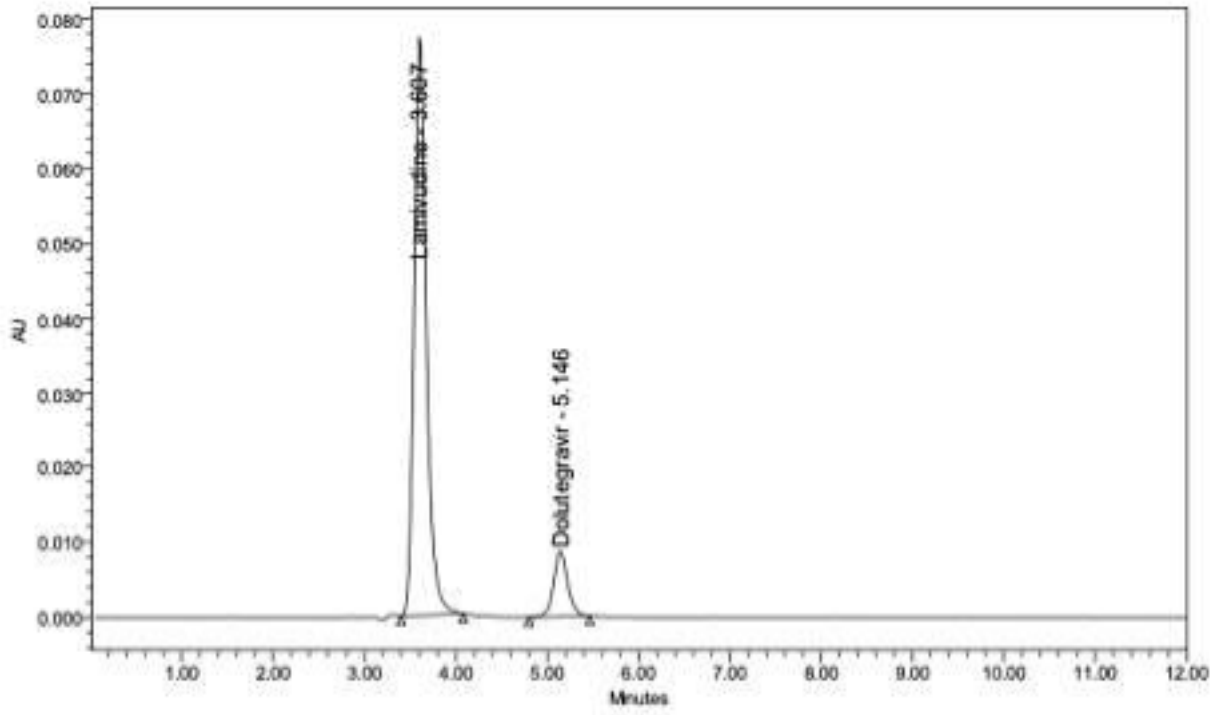
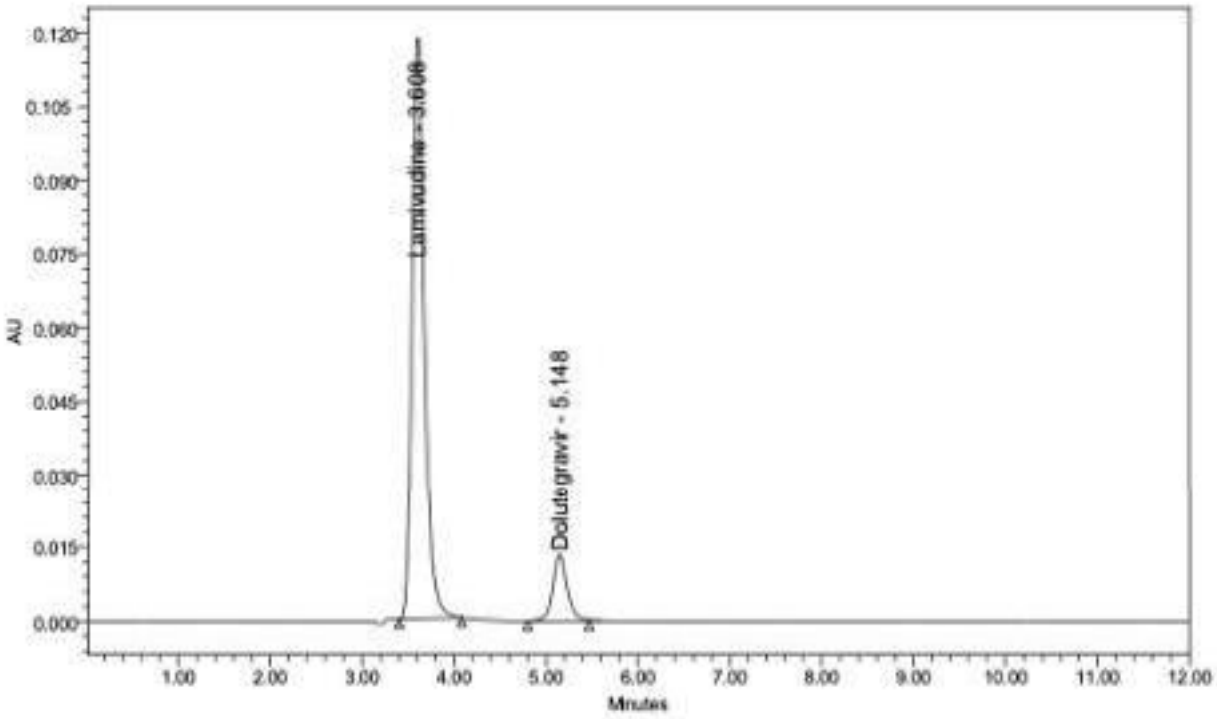
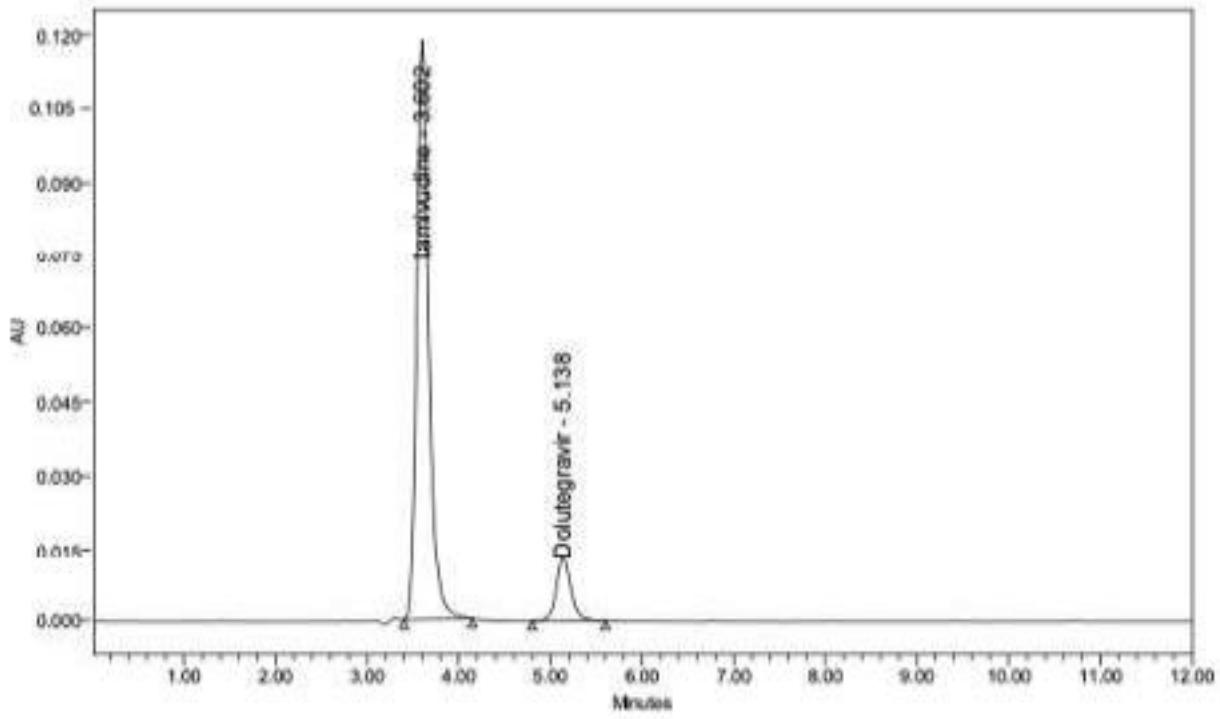
6.00
7.00
8.00
9.00
10.00
11.00
12.00

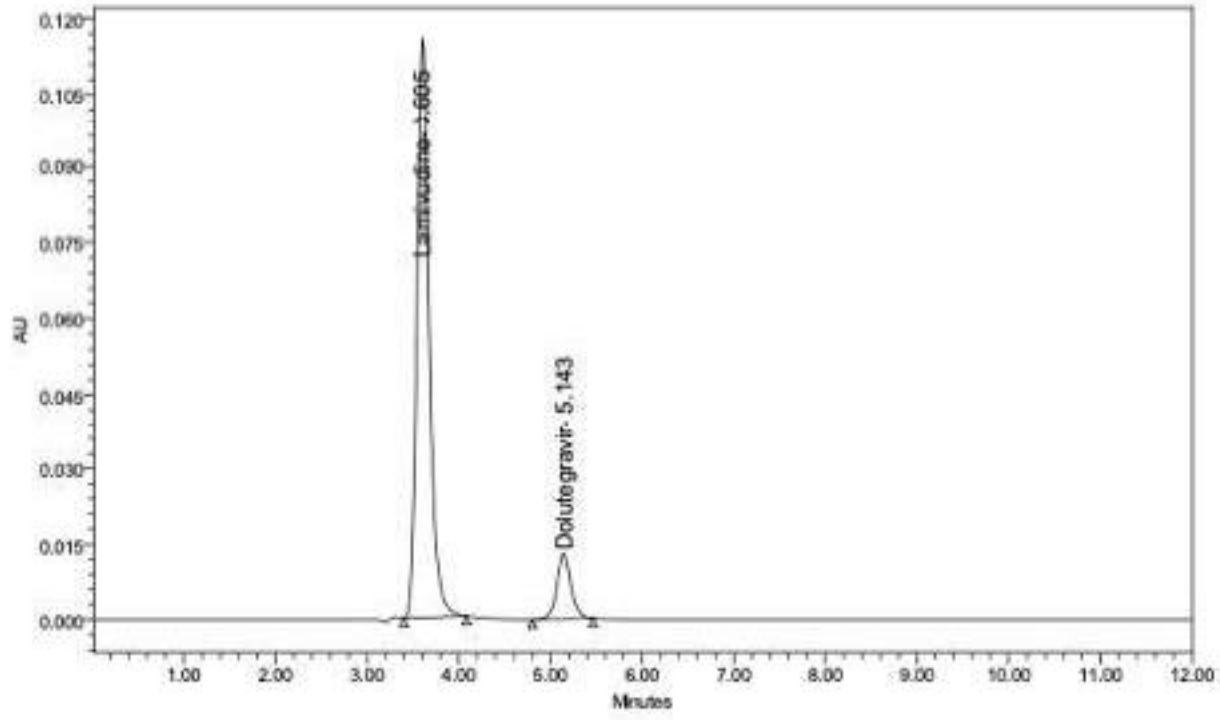




ACCURACY 100 % CHROMATOGRAM


0.120
0.105
0.090
0.075
0.060
0.045
0.030
0.015
0.000
AU
Lamivudine - 3.606
Dolutegravir - 5.148
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
Minutes





ACCURACY 150% CHROMATOGRAMS

Acceptance Criteria:

Each level's percentage recovery should range from 98.0 to 102.0%.

LINEARITY:

Preparation of stock solution:

In a 10 ml clean, dry volumetric flask, accurately weigh and transfer 100 mg of lamivudine and 12.5 mg of dolutegravir as the working standard. Add around 7 mL of diluent, sonicate to completely dissolve it, and then add enough liquid to make the volume up to the mark using the same solvent. (Stock answer)

Preparation of Level – I:

10 ml of volumetric flask were filled with 0.2 ml of the stock solution mentioned above.

Preparation of Level – II:

10 ml of volumetric flask were filled with 0.4 ml of the stock solution mentioned above.

Preparation of Level – III:

10 ml of volumetric flask were filled with 0.6 ml of the stock solution mentioned above.

Preparation of Level – IV:

10 ml of volumetric flask were filled with 0.8 ml of the stock solution mentioned above.

Preparation of Level – V:

10 ml of volumetric flask were filled with 1.0 ml of the stock solution mentioned above.

Procedure:

Measure the peak area after injecting each level into the chromatographic apparatus.

Plot a graph showing peak area vs concentration (peak area on the Y axis and concentration on the X axis).

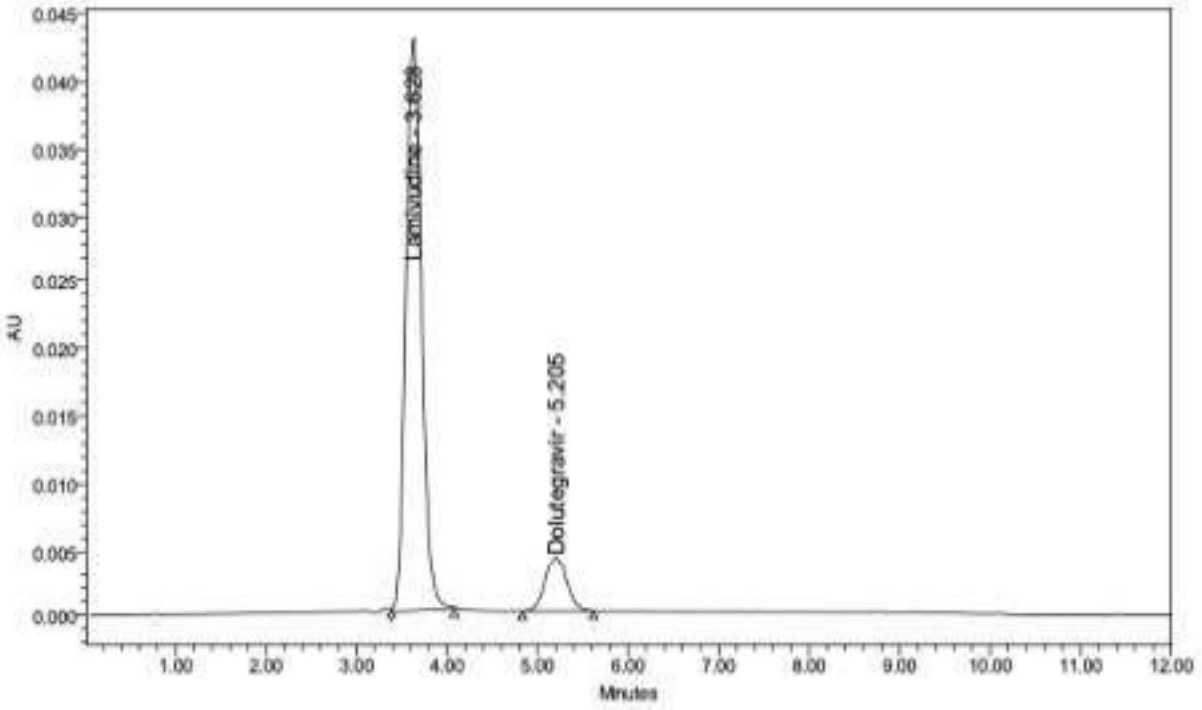
and figure out the correlation factor.

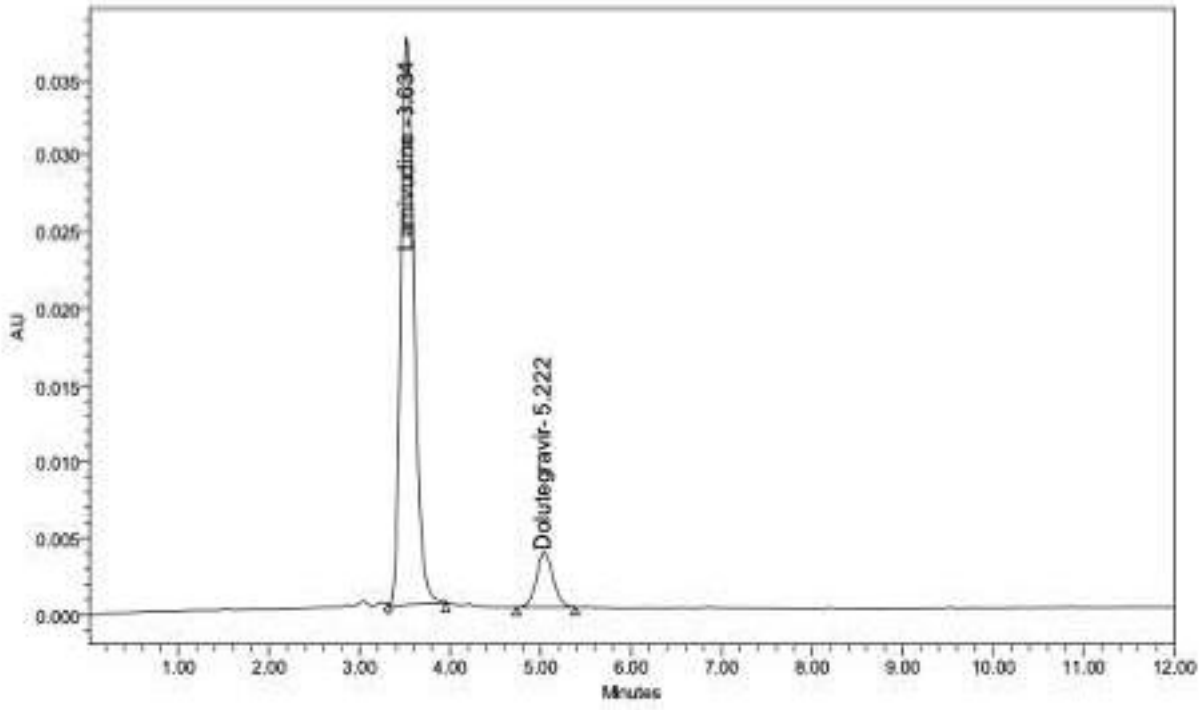
Linearity Results: (for Lamivudine)

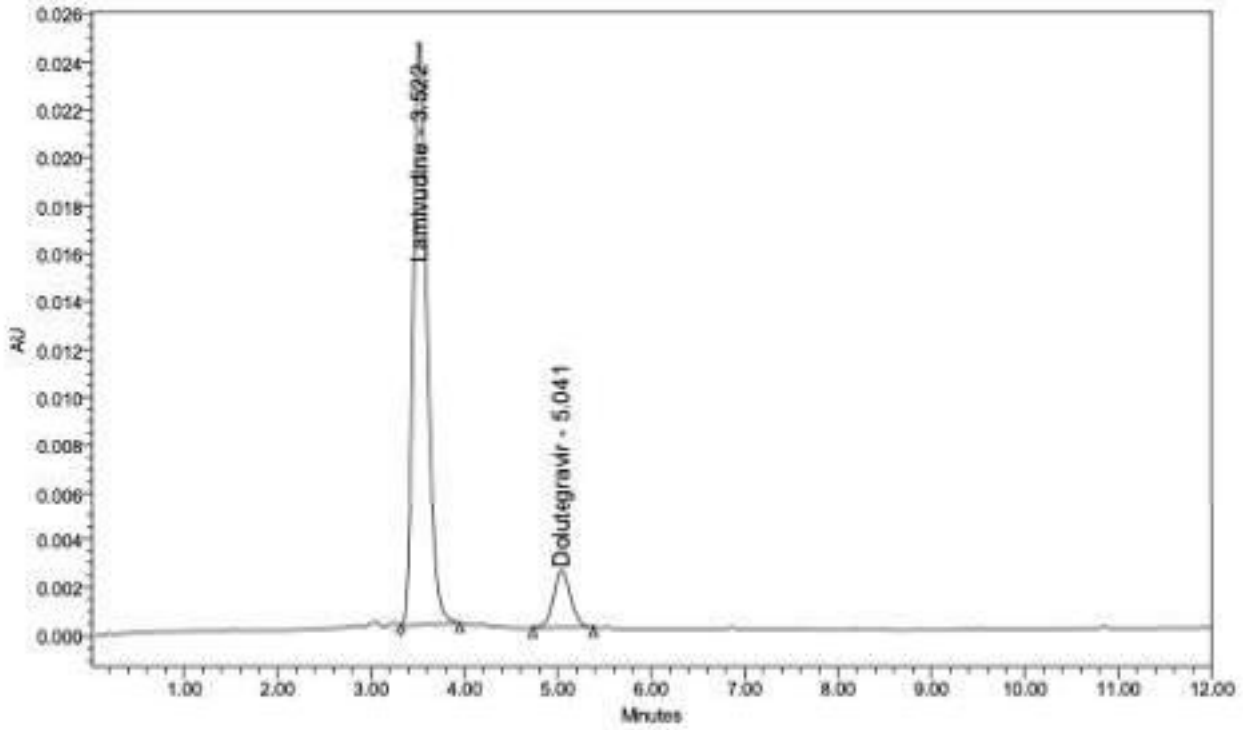
S. No	Linearity Level	Conc	Area
1	I	200	244841
2	II	400	525756
3	III	600	856654
4	IV	800	1150925
5	V	1000	1435608
Correlation/regression coefficient			0.999

Linearity Results: (for Dolutegravir)

Sl. No	Linearity level	Conc	Area
1	I	25%	29672
2	II	50%	68336
3	III	75%	113345
4	IV	100%	159680
5	V	125%	204473
Correlation/regression coefficient			0.999


Lamivudine - 3.628
Dolutegravir - 5.205
AU
Minutes


Lamivudine - 3.634
Dolutegravir- 5.222
AU
Minutes


Lamivudine - 3.522
Dolutegravir - 5.041
AU
Minutes
0.026
0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
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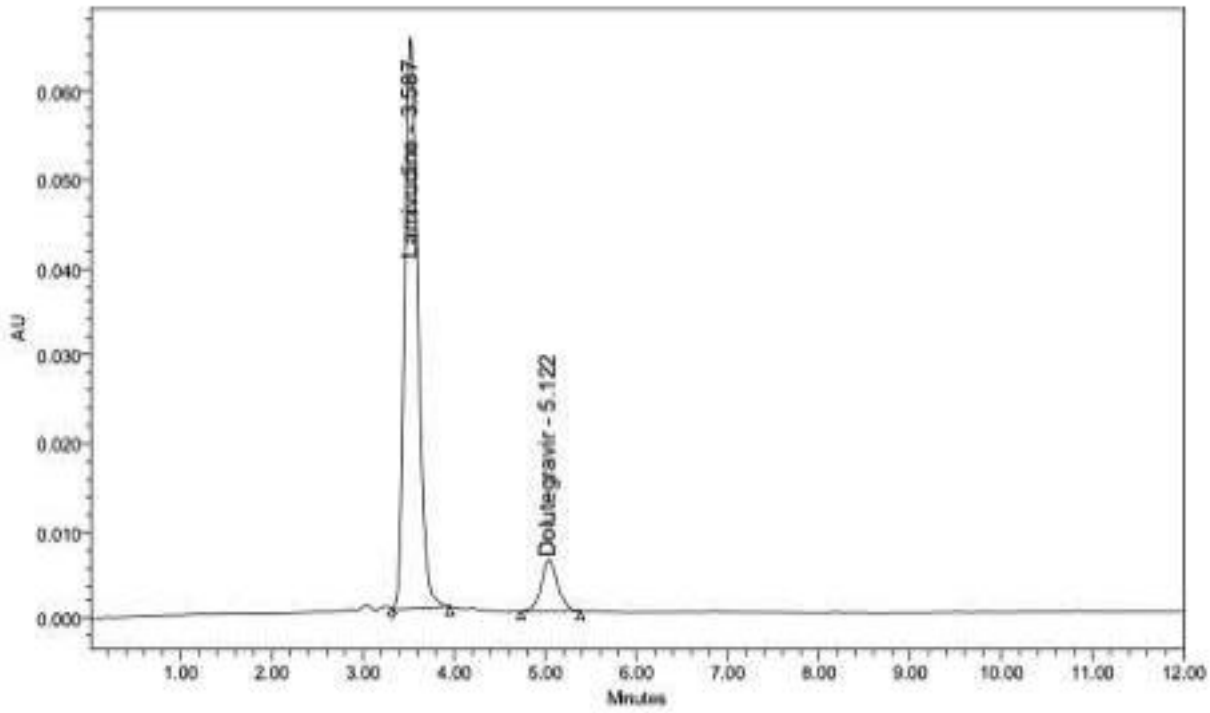
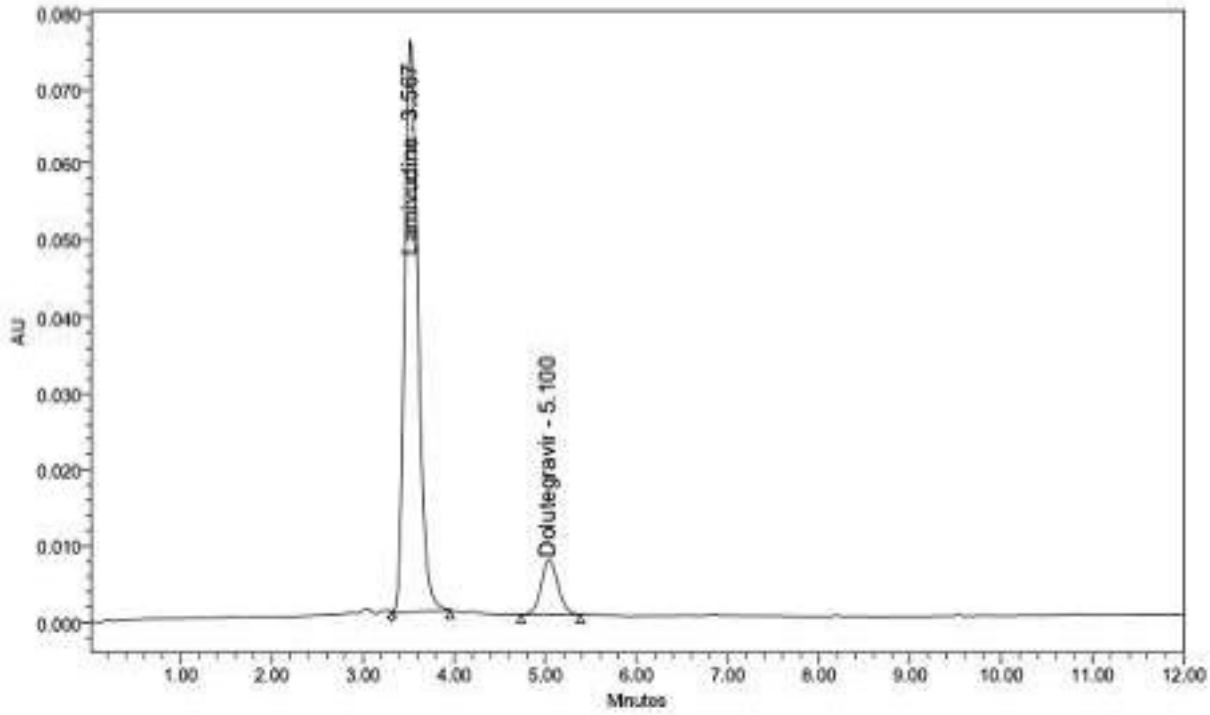

Lamivudine - 3.587
Dolutegravir - 5.122
AU
Minutes


Lamivudine - 3.567
Dolutegravir - 5.100
AU
Minutes

LINEARITY CHROMATOGRAMS

Acceptance Criteria: Correlation coefficient should be not less than 0.99.

DETECTION LIMIT

LIMIT OF DETECTION: (for Lamivudine) Preparation of 600µg/ml solution:
Transfer 100 mg of the Lamivudine working standard, accurately weighed, into a 10 ml clean, dry volumetric flask. Add around 7 mL of the diluent, sonicate to completely dissolve it, and then add enough of the same solvent to bring the volume up to the target level. (Stock answer)
Pipette 0.6 ml of the aforementioned stock solutions into a volumetric flask with a 10 ml capacity. Add diluent to the mark.

Preparation of 1.68µg/ml solution:

Pipette 1 ml of the aforementioned stock solution into a volumetric flask with a 10 ml capacity, then add diluent to the mark.
Pipette 0.28 ml of the aforementioned stock solution into a 10 ml volumetric flask and add diluent to get the desired concentration.

Calculation of S/N Ratio:

Avg Baseline Noise obtained from the Blank : 66 µV Signal Obtained from the LOD solution : 198 µV
S/N = 198/66 = 3 . 0 0

Acceptance Criteria:

S/N Ratio value should be 3 for LOD solution.

LIMIT OF QUANTIFICATION:

Preparation of 600 µg/ml of the solution:
Precisely weigh and transfer 12.5 mg of the Dolutegravir working standard into a 10 ml clean, dry volumetric flask. Add approximately 7 mL of the diluent, sonicate to completely dissolve it, and then add enough of the same solvent to bring the volume up to the target. (Stock answer)
Pipette 0.6 ml of the aforementioned stock solutions into a volumetric flask with a 10 ml capacity. Add diluent to the mark.

Preparation of 5.58 µg/ml solution:

Pipette 1 ml of the aforementioned stock solution into a 10 ml volumetric flask, and then add diluent to the mark.
Pipette 0.93 ml of the aforementioned stock solution into a 10 ml volumetric flask and add diluent to reach the desired concentration.

Calculation of S/N Ratio:

Avg Baseline Noise obtained from Blank : 66 µV Signal Obtained from the LOQ solution : 659µV

S/N = 659/66 = 9.98

Acceptance Criteria:

S/N Ratio value should be 10 for LOQ solution.

LIMIT OF DETECTION: (Dolutegravir) Preparation of 75 µg/ml solution:

Precisely weigh and transfer 12.5 mg of the Dolutegravir working standard into a 10 ml clean, dry volumetric flask. Add approximately 7 mL of the diluent, sonicate to completely dissolve it, and then add enough of the same solvent to bring the volume up to the target. (Stock answer)

Pipette 0.6 ml of the aforementioned stock solutions into a volumetric flask with a 10 ml capacity. Add diluent to the mark.

Preparation of 2.03 µg/ml solution:

Further Pipette 1 ml of the aforementioned stock solution into a volumetric flask with a 10 ml capacity. Add diluent to the mark.

Added pipette Pour 2.7ml of the aforementioned stock solution into a 10ml volumetric flask, then diluent to the desired concentration.

Calculation of S/N Ratio:

Avg Baseline Noise obtained from Blank : 66 µV Signal Obtained from the LOD solution : 199 µV S/N = 199/66 = 3 . 0 2

Acceptance Criteria:

S/N Ratio value must be 3 for LOD of tbe solution.

LIMIT OF QUANTIFICATION: Preparation of 75 µg/ml solution:

Transfer 12.5 mg of the Dolutegravir working standard, accurately weighed, into a 10 ml clean, dry volumetric flask. Add about 7 mL of the diluent, sonicate to completely dissolve it, and then add enough of the same solvent to bring the volume up to the target. (Stock answer)

Pipette 0.6 ml of the aforementioned stock solutions into a volumetric flask with a 10 ml capacity. Add diluent to the mark.

Preparation of 6.57µg/ml solution:

Pipette 2 ml of the aforementioned stock solution into a 10 ml volumetric flask, and then add diluent to the mark.

Pipette 4.38 ml of the aforementioned stock solution into a 10 ml volumetric flask and add diluent to reach the desired concentration.

Calculation of S/N Ratio:

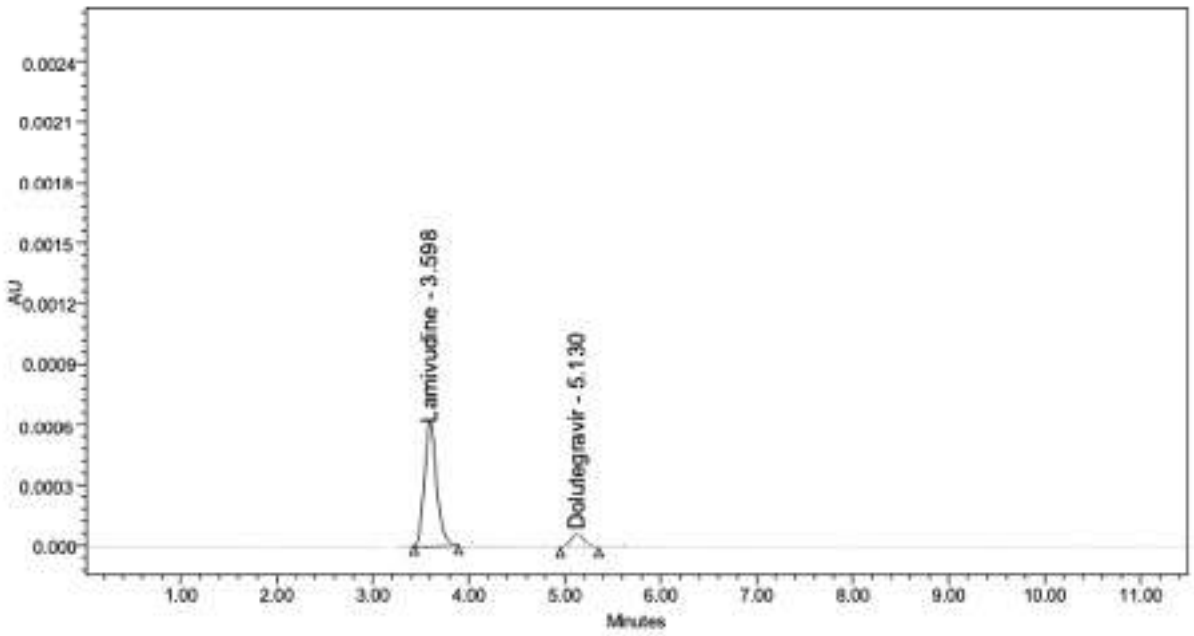
Avg Baseline Noise obtained from the Blank : 66 µV Signal Obtained from the LOQ solution : 660µV S/N = 660/66 = 10.00

Acceptance Criteria:

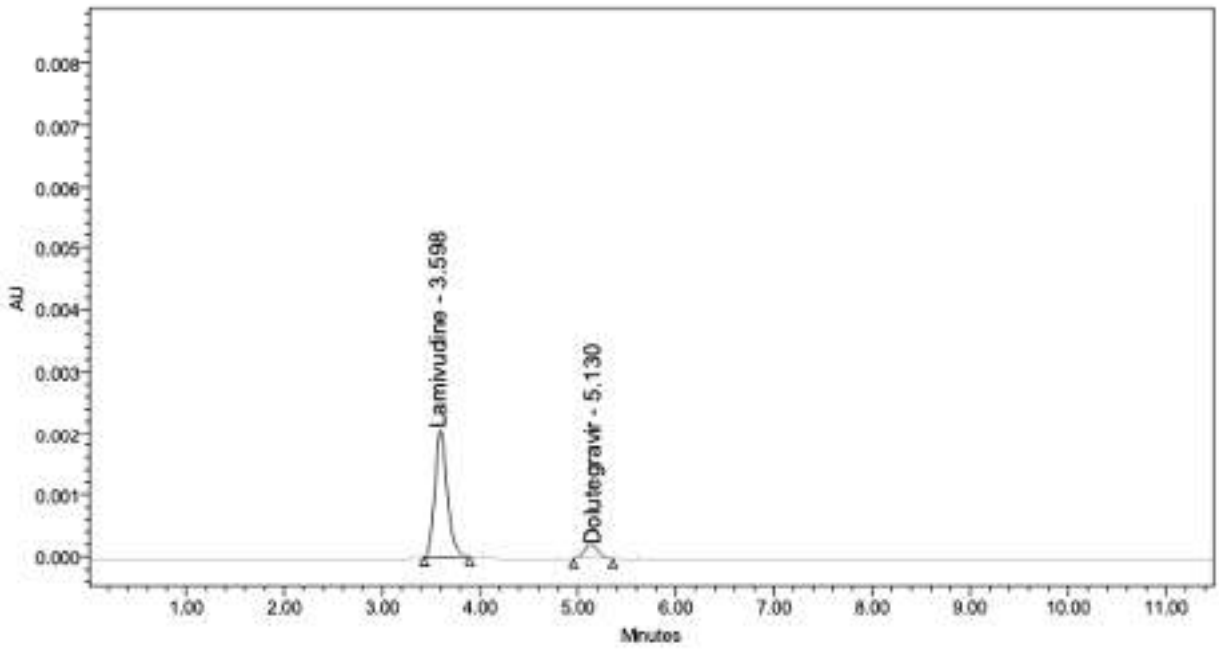
S/N Ratio value must be 10 for LOQ of the solution.

Procedure for LOD and LOQ:

The LOD and LOQ solutions that would be obtained were prepared for three injections, and the area of each injection was measured in the HPLC. The region of six duplicate injections' % RSD was confirmed to be within the prescribed bounds.



LOD FOR LAMIVUDINE AND DOLUTEGRAVIR



LOQ FOR LAMIVUDINE AND DOLUTEGRAVIR

ROBUSTNESS:

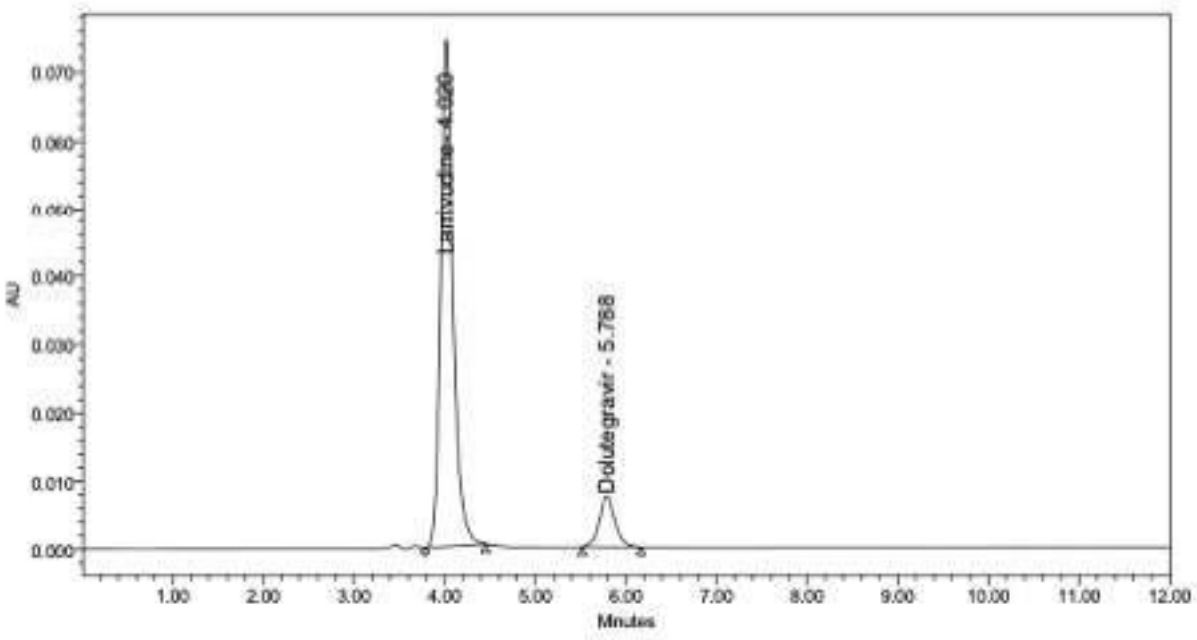
A deliberate modification in the Flow rate, Mobile Phase composition, and Temperature Variation was done as part of the Robustness to assess the influence on the approach.

A. The flow rate was ranged for 0.9 ml/min to 1.1 ml per minute.

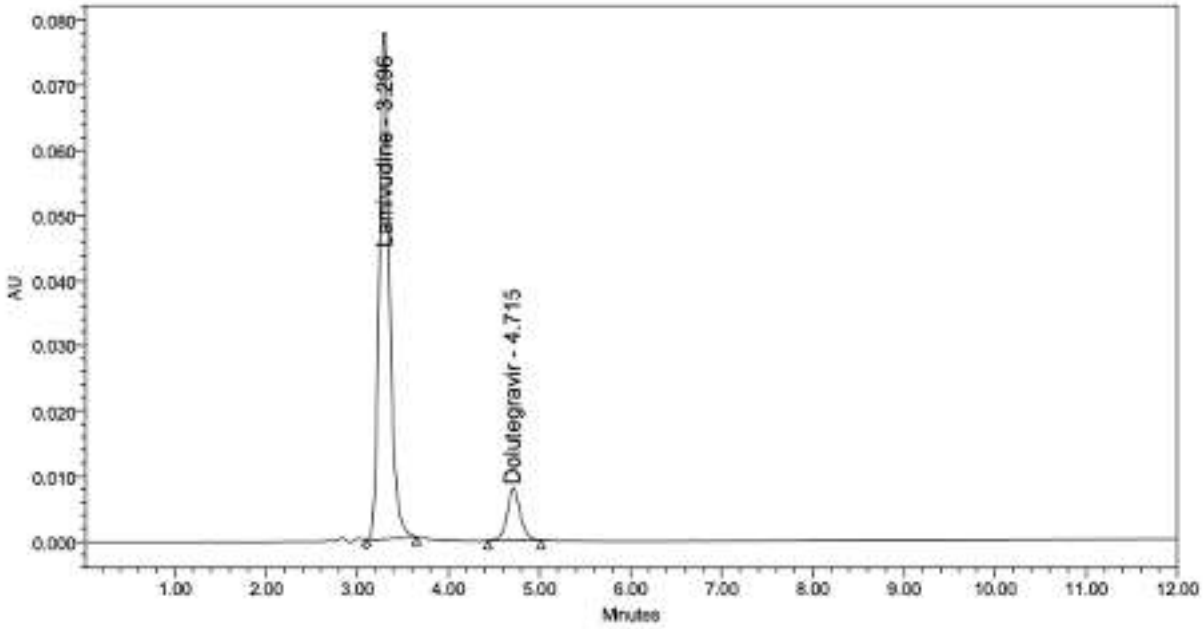
The standard solution with 600 ppm concentration of of Lamivudine & 75 ppm of Dolutegravir was made and analyzed using the varied flow rates.
Based on an analysis of the aforementioned findings, it can be said that the flow rate variation considerably impacted the approach. As a result, it shows that the procedure is reliable even when the flow rate changes by 10%.

B. The mobile phase composition was varied from ±10%.

Prepared standard solution of 600 ppm concentration of Lamivudine & 75 ppm of Dolutegravir, with the real mobile phase composition, and was analyzed using several mobile phase compositions.
Based on an analysis of the aforementioned findings, a 10% variation can be said to exist. The technique was greatly impacted by the organic composition of the mobile phase. The approach is therefore robust even when the mobile phase changes, according to this.



FLOW MINUS CHROMATOGRAM



FLOW PLUS CHROMATOGRAM

DEGRADATION STUDIES: Stress testing must be done in order to clarify the inherent stability properties of the active chemical, according to the International Conference on Harmonisation (ICH) regulation entitled stability testing of novel medicinal substances and products. The purpose of this research was to apply the suggested methodology to the stress degradation tests of lamivudine and dolutegravir.

Preparation of stock:

A 10 mL clean, dry volumetric flask should contain the equivalent of 100 mg of lamivudine and 12.5 mg of dolutegravir after precisely weighing and crushing 10 tablets in a mortar and pestle. To completely dissolve the 7 mL of diluent and make the volume up to the required level with the same solvent, sonicate the mixture for up to 5 minutes. The 0.44 micron Injection filter is then used to filter it. (Stock answer)

Hydrolytic degradation under acidic condition

Pipette 3 ml of 0.1N HCl and 3 ml of the aforementioned solution into a 10 ml volumetric flask. The volumetric flask was then maintained at 60 °C for 24 hours, neutralized with 0.1 N NaOH, and diluted to 10 ml. Place the filtered solution in vials using 0.44-micron syringe filters.

Hydrolytic degradation under alkaline condition

Pipette 3 ml of 0.1N NaOH and 3 ml of the aforementioned solution into a 10 ml volumetric flask. The volumetric flask was then maintained at 60 °C for 24 hours, neutralized with 0.1 N HCl, and diluted to 10 ml. Place the filtered solution in vials using 0.44-micron syringe filters.

Thermal induced degradation

Lamivudine and Dolutegravir sample were taken in petridish and preserved in Hot air oven at 110^0 C for 3 hrs. Then the sample was engaged and diluted with diluents and injected into HPLC and analyzed.

Sample Name	Lamivudine	
	Area	**% Degraded**
Standard solution	114736	
Acid	106691	7.0
Base	109733	4.4
Peroxide	109509	4.6
Thermal	109294	4.7
Photo	107294	6.5

Sample Name	Dolutegravir	
	Area	**% Degraded**
Standard	854796	
Acid	797354	6.7
Base	814877	4.7
Peroxide	805816	5.7
Thermal	814022	4.8

Oxidative degradation

Pipette 0.6 ml of the stock solution was added to a 10 ml volumetric flask, along with 1 ml of 12.5% w/v hydrogen peroxide, and the volume was diluted to the required amount. After that, for 15 minutes, the volumetric flask was left at room temperature. Fill vials with the filtered solution after filtering using 0.45-micron syringe filters.

Photo degradation:

Pipette out, 0.6 ml the stock solution into a 10ml volumetric flask and rendering to sunlight for one day and and then add diluent to the until it reaches the desired volume level. Using syringe filters with a 0.45-micron pore size, put the filtered solution in vials.

Results:

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