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DEVELOPMENT AND VALIDATION FOR THE METHOD SIMULTANEOUS DETERMINATION OF DOLUTIGRAVIR AND LAMIVUDINE IN API FORM AND MARKETED PHARMACEUTICAL TABLET DOSAGE FORM BY USING RP-HPLC

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Abstract:

Analytical Method Development and Validation for Dolutegravir and Lamivudine in bulk and Combine Dosage Form by RP-HPLC, New method was established for simultaneous estimation of Dolutegravir and Lamivudine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Dolutegravir and Lamivudine by using Symmetry C18 5µm (4.6 x 150mm), flow rate was 1.0 ml/min, mobile phase ratio was Phosphate buffer (0.02M) pH-3.8: Methanol: Acetonitrile (60:20:20%v/v), detection wavelength was 260nm. The retention times of Dolutegravir and Lamivudine were found to be 2.324mins and 4.314mins respectively. The % purity of Dolutegravir and Lamivudine was found to be 99.865% and 99.658% respectively. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Dolutegravir and Lamivudine was found in concentration range of 0µg-36µg and 0µg-39µg and correlation coefficient (r2) was found to be 0.9995 and 0.9998, % recovery was found to be 100.280, %RSD for repeatability was 0.174 and 0.709, % RSD for intermediate precision was 0.093 and 0.937 respectively. The precision study was precise, robust, and repeatable. LOD value was 1.377 and 1.079, and LOQ value was 4.174 and 3.272 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Dolutegravir and Lamivudine in API and Pharmaceutical dosage form.

Keywords: *Dolutegravir and Lamivudine, Method Development, Validation.*

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INTRODUCTION:

Analytical chemistry is used to determining the qualitative and quantitative composition of material under study. Both these aspects are necessary to understand the sample material. Analytical chemistry is divided into two branches quantitative and qualitative. A qualitative analysis gives us the information about the nature of sample by knowing about the presence or absence of certain components[1]. A quantitative analysis provides numerical information as to the relative amount of one or more of this component. For analyzing the drug samples in bulk, pharmaceutical formulations and biological fluids, different analytical methods are routinely being used. In non-instrumental, the conventional and physicochemical property are used to analyze the sample. The instrumental methods of analysis are based upon the measurements of some physical property of substance using instrument to determine its chemical composition. The instrumental methods are simple, precise and reproducible as compared to classical methods. Therefore, analytical methods developed using sophisticated instruments such as spectrophotometer, HPLC, GC and HPTLC have wide applications in assuring the quality and quantity of raw materials and finished products.[2]

Chromatography: Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.[1]Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive[3]

Introduction to chromatography, its types and classification Chromatography: Chromatography is a set of techniques in which separation of chemical substances takes place quantitatively as well as qualitatively.

Terminology used in Chromatography Mobile Phase: In chromatography the substance which is introduced with or along with the sample and causes elution of the contents of the sample. It may be liquid or gas.

Stationary phase: Stationary phase of the chromatographic system refers to that part which is present before the introduction of sample or solute in the column (as in column chromatography) or on a solid support (as in paper or similar chromatography). It may be liquid or solid.

Eluent: The substance which separates the components of the mixture in chromatographic technique. Eluent is that part which brings separation when the solution is passed either from the column or from the solid support.

Eluate: The substance which is separated as an individual component of the mixture is called eluate.

Important types of Chromatographic Techniques: Following are some important types of Chromatographic separation techniques. They are defined thoroughly by explaining their general principle, application, and a brief outline of their instrumentation for a complete understanding. Following are some commonly utilized types of techniques:[4]

i. Gas Chromatography

ii. High Pressure Liquid Chromatography

iii. Supercritical fluid Chromatography

iv. Gel Exclusion Chromatography.

High Pressure Liquid Chromatography (HPLC): High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). The sample is carried by a moving carrier gas stream of helium or nitrogen. HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics, and chemicals.[5]

Sample retention time will vary depending on the interaction between the stationary phase, the molecules being analyzed, and the solvent, or

solvents used. As the sample passes through the column it interacts between the two phases at different rate, primarily due to different polarities in the analytes. Analytes that have the least amount of interaction with the stationary phase or the most amount of interaction with the mobile phase will exit the column faster.

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

HPLC has been used for manufacturing (e.g., during the production process of pharmaceutical and biological products), legal (e.g., detecting performance enhancement drugs in urine), research (e.g., separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (e.g., detecting vitamin D levels in blood serum) purposes.[1]

High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

Types of HPLC: There are following variants of HPLC, depending upon the phase system (stationary) in the process:

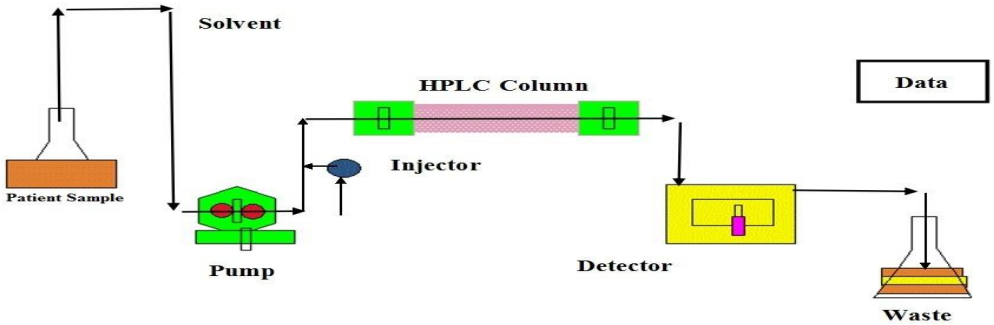
1. Normal Phase HPLC: This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these. [6] Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

2. Reverse Phase HPLC: The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.

3. Size-exclusion HPLC: The column is filled with material having precisely controlled pore sizes, and the particles are separated according to its their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

4. Ion-Exchange HPLC: The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples.The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

Instrumentation of HPLC [8]



Schematic diagram of HPLC instrumentation

As shown in the schematic diagram in Figure above, HPLC instrumentation includes a pump, injector, column, detector and integrator or acquisition and display system. The heart of the system is the column where separation occurs.

1. Solvent Reservoir: Mobile phase contents are contained in a glass reservoir. The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.

2. Pump: A pump aspirates the mobile phase from the solvent reservoir and forces it through the system's column and detector. Depending on a number of factors including column dimensions, particle size of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated.

3. Sample Injector: The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).

4. Columns: Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 3–10 µm.Columns with internal diameters of less than 2 mm are often referred to as micro bore columns. Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.

5. Detector: The HPLC detector, located at the end of the column detects the analytes as they elute from the chromatographic column. Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.

6. Data Collection Devices: Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

Applications of HPLC: The information that can be obtained by HPLC includes resolution, identification and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include:

Pharmaceutical Applications

1. To control drug stability.
2. Tablet dissolution study of pharmaceutical dosages form.
3. Pharmaceutical quality control.

Environmental Applications

1. Detection of phenolic compounds in drinking water.
2. Bio-monitoring of pollutants.

Applications in Forensics

1. Quantification of drugs in biological samples.
2. Identification of steroids in blood, urine etc.
3. Forensic analysis of textile dyes.
4. Determination of cocaine and other drugs of abuse in blood, urine etc.

Food and Flavour

1. Measurement of Quality of soft drinks and water.
2. Sugar analysis in fruit juices.
3. Analysis of polycyclic compounds in vegetables.
4. Preservative analysis.

Applications in Clinical Tests

1. Urine analysis, antibiotics analysis in blood.
2. Analysis of bilirubin, biliverdin in hepatic disorders.
3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.[9]

Method Development

Steps for HPLC Method Development: Analytical method development is considered as a critical process in pharmaceuticals. Availability of the different types of columns, operating parameters and mobile phase composition, diluent and pH values make it critical to develop an analytical method. A good analytical method should be simple, used column, mobile phase and buffer should be common. It can be done easily step by step.

Following are the common HPLC method development steps.

1. Selection of HPLC Analytical Method
2. Selection of Chromatographic Conditions
3. Parameter Optimization

1. Selection of HPLC Analytical Method: First of all consult the literature that is available on the product. It will help you to understand the nature of the product that will help to select the different parameters.

A. Sample Preparation: Select method to prepare the sample according to its solubility, filtration requirements, extraction requirements or other special requirements to make a clear solution of HPLC analysis.

B. Chromatography: Reverse phase chromatography is used for most of the samples but when acidic or basic molecules are present in the sample then reverse phase ion suppression (for weak acid or base) or reverse phase ion pairing (for strong acid or base) should be used. The stationary phase should be C18 bonded. Normal phase is used for low or medium polarity analyte especially when it is required to separate the product isomers. Choose

cyano bonded phase for normal phase separations. Ion exchange chromatography is best to use for inorganic anion or Cation analysis. If analyte has higher molecular weight than size exclusion chromatography is the best to use.[10]

C. Gradient/Isotonic HPLC: Gradient HPLC is helpful in the analysis of complex samples having a number of components. It will help to get higher resolution than isotonic HPLC having constant peak width while in isotonic HPLC peak width increases with the retention time. Gradient HPLC has great sensitivity, especially for the products having longer retention time.

D. Column Size: 100-150 mm columns are used for most of the samples. It reduces the method development and analysis time for the sample. Bigger columns are used for complex samples those take more time in separation. Initially, a flow rate should be kept between 1 and 1.5 ml/min and column particle size should be between 3 and 5 µm.

E. HPLC Detectors: If the analyte has chromophores that enable the compound to be detected by UV than it is better to use UV detector. It is always better to use a UV detector than others. Fluorescence and electrochemical detectors should be used for trace analysis. Samples having high concentration should be analyzed using refractive index detectors.

F. Wavelength: λmax of the sample has the greatest sensitivity to the UV light. It detects the sample components that have chromophores. A wavelength above 200 nm gives greater sensitivity than the lower wavelengths. Wavelengths lower than 200 nm gives more noise, therefore, it should be avoided.

2. Selection of Chromatographic Conditions: After selection of analytical method, different chromatographic conditions are selected. The flow of the analytes through the column depends upon the concentration of the solvent in the mobile phase. The concentration of solvent is generally used to control the retention time. Mobile phase pH and ion pairing reagents also affect the retention time of the sample. Samples having a large number of components are analyzed using the gradient to avoid the large retention time while the samples containing one or two components are analyzed on an isotonic system.

3. Parameter Optimization: After taking the same sample runs some parameters including column dimensions, particle size, run time and flow rate are optimized. It is done to get the best resolution and minimum run time. After proper optimization of the analysis method, it is validated to ensure the consistency of the analytical method. Analytical method validation is now done mandatory by all regulatory authorities.[11]

Parameters for HPLC:

Performance Calculations: Calculating the following values (which can be included in a custom report) used to access overall system performance.

1. Relative retention
2. Theoretical plates
3. Capacity factor
4. Resolution
5. Peak asymmetry
6. Plates per meter

The following information furnishes the parameters used to calculate these system performance values for the separation of two chromatographic components. (Note: where the terms w and t both appear in the same equation they must be expressed the same units).

System suitability parameters: The theory of chromatography has been used as the basis for system-suitability tests, which are set of quantitative criteria that test the suitability of the chromatographic system to identify and quantify drug related samples by HPLC at any step of the pharmaceutical analysis.

1. Relative retention: The time elapsed between the injection of the sample components in to the column and their detection is known as the retention time (Rt).

$$\alpha = (t_2-t_a) / (t_1-t_a)$$

Where,

α =Relative retention.

t_1= Retention time of the peak measured from point of injection.

t_2 = Retention time of the second measured from point of injection.

t_a = Retention time of an inert peak not retained by the column, measured from point of injection.

2. Theoretical plates:

$$n = 16 (t_R / w)^2$$

Where,

n =Theoretical plates.

t_R = Retention time of the component.

W = Width of the base of the component peak using tangent method.

3. Capacity factor: The capacity factor describes the thermodynamic basis of the separation and its definition is the ratio of the amounts of the solute at the stationary and mobile phases within the analyte band inside the chromatographic column.

$$K^1 = (t_2/t_a)-1$$

Where,

K^1 = Capacity factor.

t_a = Retention time of an inert peak not retained by the column, measured from point of injection.

4. Resolution: the gap between two peaks $R = 2 (t_2-t_1) / (w_2-w_1)$

Where,

R =Resolution between a peak of interest (peak 2) and the peak preceding it (peak 1).

W_2 = Width of the base of component peak 2.
W_1 = Width of the base of component peak 1.

5. Peak Asymmetry

$$T = W_{0.05} / 2f$$

Where,
T = Peak asymmetry, or tailing factor.
$W_{0.05}$ = Distance from the leading edge to the tailing edge of the peak, measured at a point 5 % of the peak height from the baseline.
f= Distance from the peak maximum to the leading edge of the peak.

6. Plate per Meter:

$$N = n/L$$

Where,
N = plates per meter.
L = column length in meters.

Advantages:

- HPLC separations can be accomplished in a minutes, in some cases even in seconds.
- High resolution of complex sample mixture into individual components.
- Rapid growth of HPLC is also because of its ability to analyze substances that are unsuitable for gas liquid chromatographic (GLC) analysis due to non-volatility or thermal-instability.
- Quantitative analysis is easily and accurately performed and errors of less than 1 % are common to most HPLC methods.
- Depending on sample type and detector used, it is frequently possible to measure 10^{-9} g or 1 ng of sample. With special detectors, analysis down to 10-12 pg has been reported.
- As HPLC is versatile, it can be applied to wide variety of samples like organic, inorganic, high molecular weight liquids, solids, and ionic-nonionic compounds.

Disadvantages:

- HPLC instrumentation is expensive and represents a major investment for many laboratories.
- HPLC cannot handle gas samples.
- HPLC is poor identifier. It provides superior resolution but it does not provide the information that identifies each peak.
- Only one sample can be analyzed at a time.
- Finally, at present there is no universal and sensitive detector.

Method Validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Analytical methods need to be validated or revalidated.

- Before their introduction into routine use;
- Whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and
- Whenever the method is changed and the change is outside the original scope of the method.[12]

1. Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s). This definition has the following implications: Identification: to ensure the identity of an analyte. Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc. Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

2. Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

3. Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

3.1. Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

3.2. Intermediate precision: Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

3.3. Reproducibility: Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

4. Detection Limit: The detection limit of an individual analytical procedure is the lowest amount

of analyte in a sample which can be detected but not necessarily quantitated as an exact value.[13]

5. Quantitation Limit: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

6. Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

7. Range: The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

8. Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Aim and Objectives

Review of literature for Dolutegravir and Lamivudine gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other drugs.

Literature survey reveals that certain spectrophotometric methods were reported for estimation of Dolutegravir and Lamivudine and there is a method is available for such estimation by RP-HPLC.

In view of the need for a suitable RP-HPLC method for routine analysis of Dolutegravir and Lamivudine in formulations, attempts were made to develop simple, precise and accurate analytical method for estimation of Dolutegravir and Lamivudine and extend it for their determination in formulation.

Validation is a necessary and important step in both framing and documenting the capabilities of the developed method.[14]

The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient.

The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness, and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.

The primary objective of proposed work is:

To develop new simple, sensitive, accurate and economical analytical method for the estimation of Dolutegravir and Lamivudine in bulk form and marketed pharmaceutical dosage form.

To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Dolutegravir and Lamivudine in bulk and its dosage form.

Plan of Work

1. Collection of literature for the selected drug.
2. Extensive literature survey for selection of appropriate solvents to dissolve respective selected drug and preparation of stock solution.
3. Study of drug profile
4. Procurement of samples, standards and other chemicals.
5. Selection of chromatographic conditions
6. Selection of mobile phase
7. Method trials on HPLC by using different solvents and columns.
8. Development of RP-HPLC method which is different from the finished articles.
9. Optimization of the developed method by varying mobile phase conditions, temperature.
10. Validation of the developed method for the following parameters:

Accuracy, Precision, Specificity, Limit of detection, limit of quantitation, Linearity Robustness, System Suitability.[15]

MATERIALS AND METHODS:

List of Equipment's and List of Chemicals

S.No.	Instrument	Model No.	Software	Manufacturer's Name
1	HPLC Alliance	Waters2695	Empower	Waters
2	UV Double Beam Spectrophotometer	UV3000	UVWin5	Lab India
3	Digital Weighing Balance	BSA224SCW	-	Sartorius
4	Ph meter	AD102U	-	Lab India
5	Ultra Sonicator	SE60US	-	-
6	Suction Pump	VE115N	-	-

S.No.	Chemical	Manufacturer	Grade
1	Water	Merck	HPLCGrade
2	Methanol	Merck	HPLCGrade
3	Acetonitrile	Merck	HPLCGrade
4	Potassium dihydrogen ortho phosphate	Merck	A.R

Wave length Detection (Or) Selection of Wave length: The detection wavelength was selected by dissolving the drug in mobile phase togeta concentrationof10µg/mlforindividualandmixedstanda rds.Theresulting solution wasscanned in U.V range from200-400nm. The overlay spectrum of Dolutegravir and Lamivudine was obtained and the isobestic point of Dolutegravir and Lamivudineshowedabsorbance'smaximaat260 nm.[16]

Results and Discussions

Method Development

Preparations and Procedures:

Preparation of Phosphate buffer: (pH:3.8): Weighed 0.136086grams of KH_2PO_4 was taken into a1000ml beaker, dissolved and dilutedto1000mlwithHPLCwater,adjustedthepHto3.8 withorthophosphoric acid.[17]

Preparation of Mobile Phase: AmixtureofpH3.8Phosphatebuffer600mL(60%),200m LofMEOH(20%) and 200mLofAcetonitrileare taken and degassed in ultrasonic water bath for 15minutes. Then thissolutionisfilteredthrough0.45µfilterundervacuumf iltration.[19]

Diluent Preparation: Mobile phase issued as Diluent.

PreparationoftheindividualDolutegravirstandar dpreparation:10mgofDolutegravirw o r k i n g standardwasaccuratelyweighedandtransferredintoa10 mlcleandryvolumetricflaskandabout2mlofDiluentisad ded.Thenitissonicatedtodissolveitcompletelyandmade volumeup to the mark with the diluent.(Stock solution). Further 10.0 ml from the above stock solutionispipetteintoa100ml volumetricflaskandwasdiluteduptothemarkwithdiluen t.[20]

Preparation of the individual Lamivudine standard preparation: 10mg of Lamivudine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of Diluent is added.Thenitissonicated to dissolve it completely and made volume up to the mark with the diluent.(Stock solution). Further 10.0mlfromtheabovestock solutionispipetteintoa100ml volumetricflaskandwasdiluteduptothemarkwithdiluen t.[21]

Preparation of Sample Solution: (Tablet) Accurately10tabletsare
Weighed and crushed in mortar and pestle andweight equivalentto10mgofDolutegravir and Lamivudine (marketed formulation)sample intoa10mLcleandryvolumetric flask and about7mLofDiluents is added and sonicated to dissolve it complete lyandmade volumeup to the mark with the same solvent. (Stocksolution)Further 3mlofabovestocksolutionwaspipetted intoa10mlvolumetricflaskanddiluteduptothemarkwith diluent.[22]

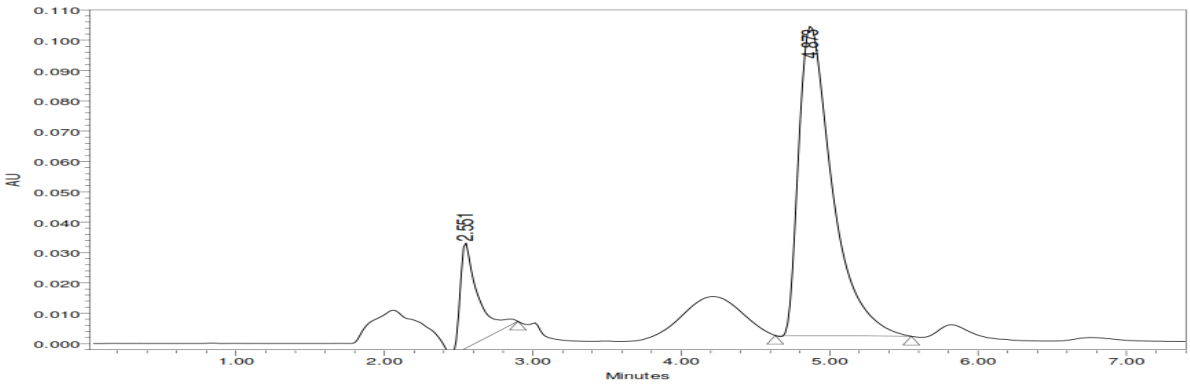
Procedure:20µLofthestandard,sampleareinjectedinto thechromatographic systemandthe areas for Dolutegravir and Lamivudine peaks are measured and the %Assayis calculated by using the formulae.

TRIALS FOR THE METHOD DEVELOPMENT

Trial-1

Chromatographic conditions

Column : AgilentC18(4.6*150mm)5µm
Mobilephaseratio : Water:Methanol(40:60%v/v) Detection wavelength : 260nm
Flowrate : 1ml/min
Injectionvolume : 10µl
Columntemperature : Ambient
Autosamplertemperature : Ambient



Chromatogram of Trail-1

Table-: Details of Trial-1

S.No.	PeakName	Rt	Area	Height	USPPlate count	USP Tailing	USP Resolution
1	Dolutegravir	2.551	8671924	460798	745	2.19	
2	Lamivudine	4.879	2283694	179357	1911	2.79	1.45

Observation: Dolutegravir and Lamivudine were separated and two individual peaks are displayed. Buttheyarenotclear.

Trial-2[25]

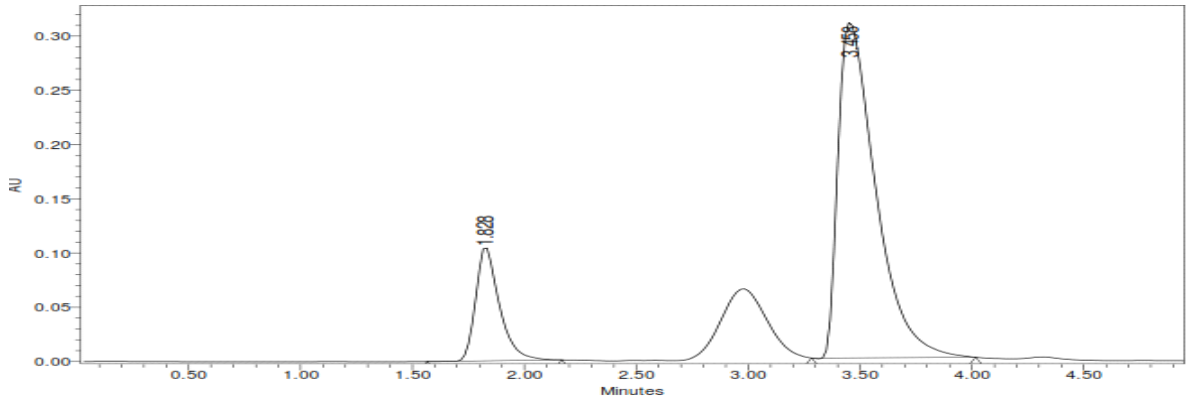
Chromatographicconditions:

Column :ThermosilC18(4.6*150mm)5µm

Mobile phase ratio : Water:Methanol(40:60%v/v) Detection wavelength : 260nm

Flowrate : 1ml/min Injectionvolume : 10µl

Autosampler temperature : Ambient

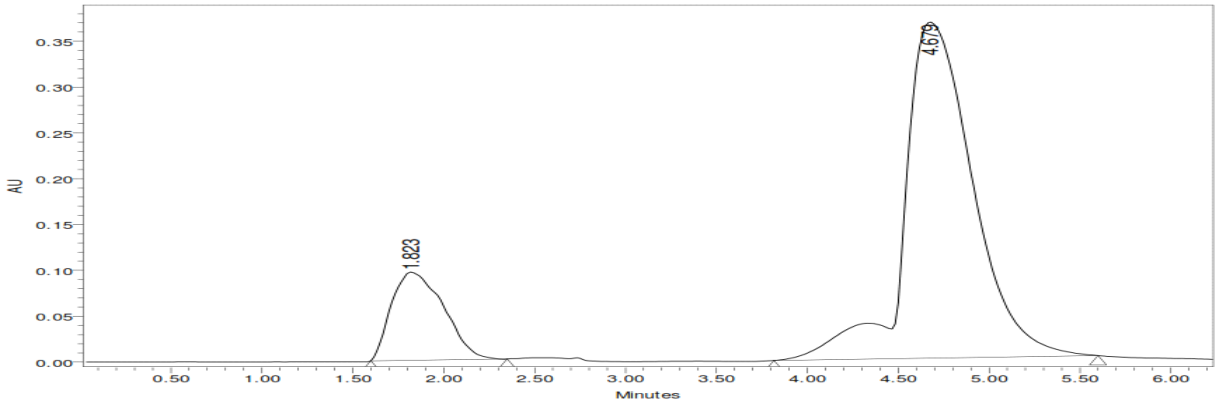


ChromatogramofTrial-2

DetailsofTrial-2

S.No.	PeakName	R_t	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Dolutegravir	1.828	7913799	394185	722	2.21	
2	Lamivudine	3.458	1853381	162758	2614	2.85	1.52

- **Observation:** Peaks symmetry is beingi improved when compared to the previous trial. Further trials areconducted for better re solution.
- **Trail-3**
- **Chromatographic conditions:**
- Column : AgilentC185µm(4.6*250mm)
- Mobile phase ratio : Phosphate buffer(0.05m)pH5.0:Methanol (50:50%v/v)
- Detection wavelength : 260nm
- Flowrate : 1ml/min
- Injection volume : 10µl



ChromatogramofTrial-3

DetailsofTrail-3

S.No.	PeakName	R_t	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Dolutegravir	1.823	9849287	482363	198	1.97	
2	Lamivudine	4.679	3272312	356630	5036	1.15	4.23

- **Observation:** Thereisnoticeableimprovementinresolution.Butpeaksymmetryisnotachieved.[28]
-

- **Trial-4**
- **Chromatographic conditions:**
- Column : Inertsil ODSC185µm(4.6*250mm)
- Mobilephaseratio : Phosphate buffer(0.05M)pH4.6:MeOH: ACN (50: 30: 20)
- Detectionwavelength : 260nm
- Flowrate : 1ml/min Injectionvolume : 20µl
- Autosamplertemperature : Ambient

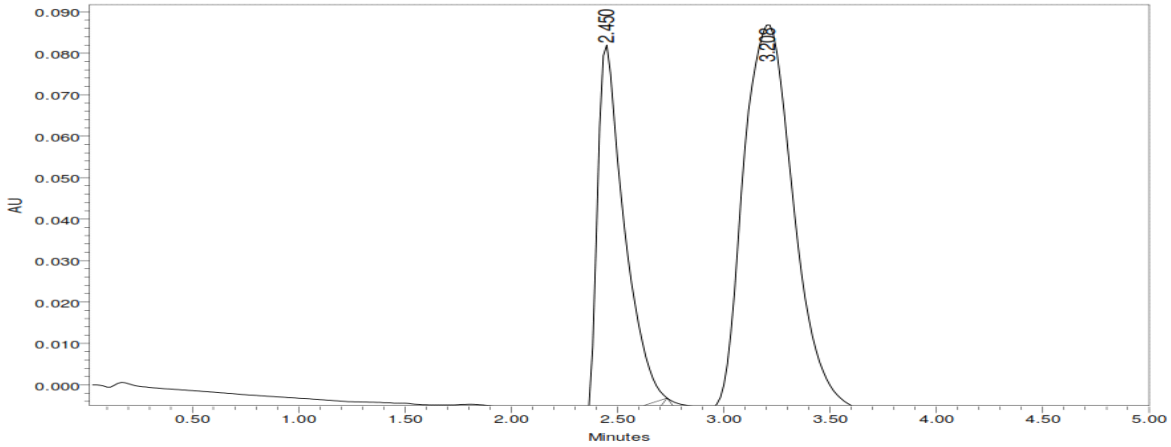


Fig-4.4 :Chromatogram of Trial-4[28]

Table-: DetailsofTrial-4

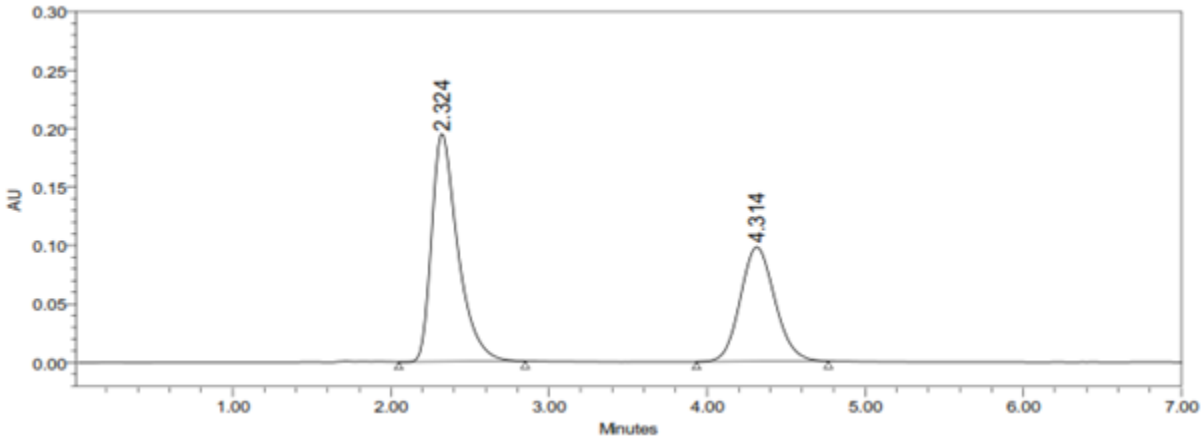
S.No.	Peakname	R_t	Area	Height	Platecount	Tailing	Resolution
1	Dolutegravir	3.191	11286305	813690	1587	1.46	
2	Lamivudine	3.945	3443649	160557	616	1.80	1.46

Observation: The tailing factor is within the limit. But theo therparameters are not within the limit

Optimized Chromatographic Method:

Chromatographic conditions:

- Column : SymmetryC18 5µm(4.6 x 150mm)
- Mobile phase ratio: Phosphate buffer(0.02M)pH-3.8:Methanol: Acetonitrile (60:20:20%v/v)
- Detection wavelength : 260nm
- Flowrate : 1ml/min
- Injection volume : 20µl
- Column temperature : Ambient
- Autosampler temperature: Ambient

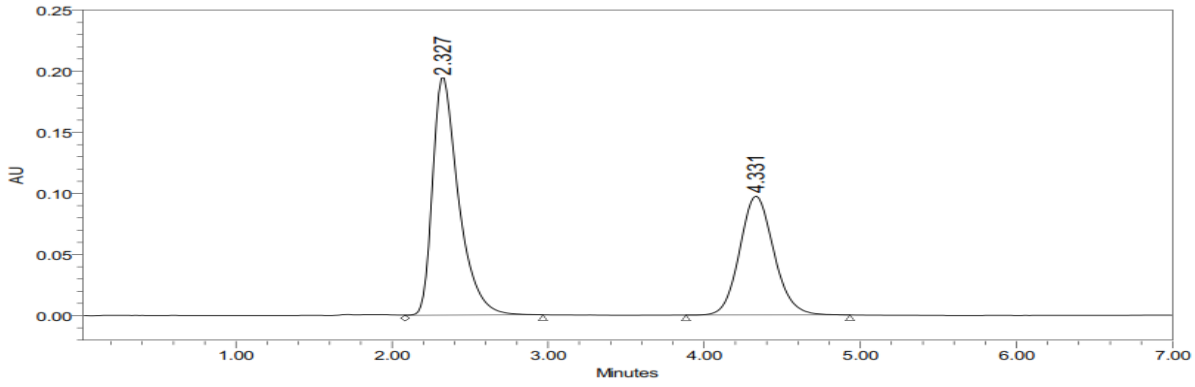


Chromatogram of Optimized Method

Details of Optimized Method

S.No	Peakname	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Dolutegravir	2.324	946124	155429	5105	1.3	8.1
2	Lamivudine	4.314	111541	13239	3788	1.4	

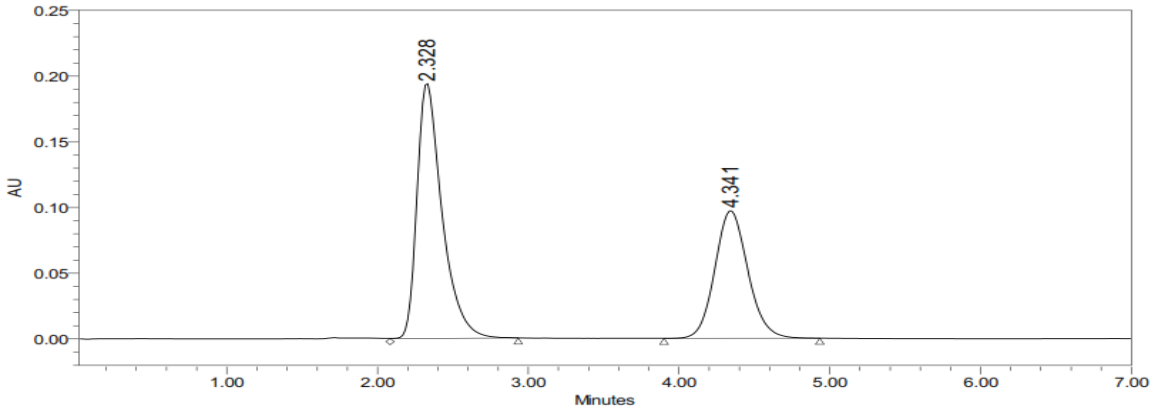
System Suitability: System Suitability was the checking of a system to ensure system performance before or during the analysis of unknowns. Parameters such as tailing factor, resolution, plate count and reproducibility are determined and compared against the specification suitable for the method.[29]



Chromatogram for System Suitability Injection-1

Results for System Suitability Injection-1

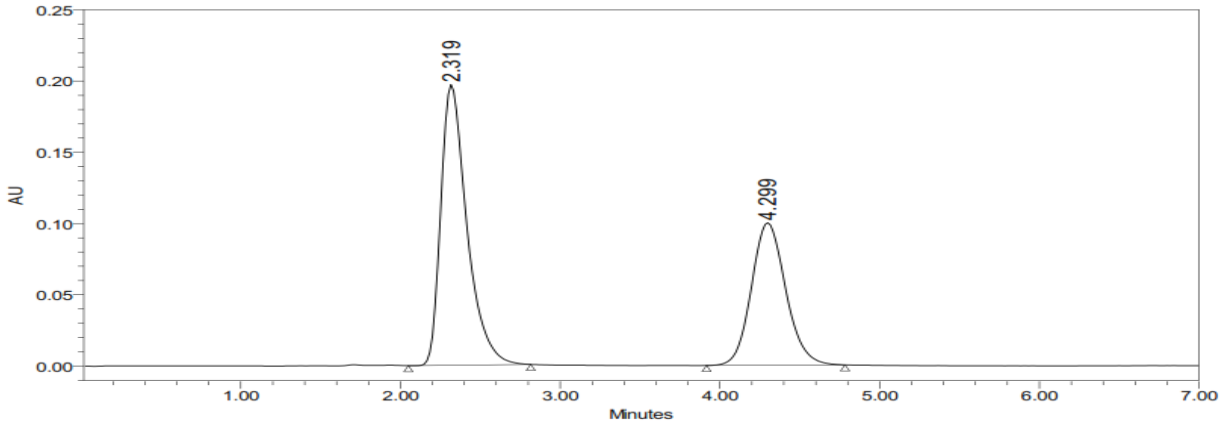
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.327	946257	1.3	5245	8.6
2	Lamivudine	4.331	112543	1.4	3854	



Chromatogram for System Suitability Injection-2

Results for System Suitability Injection-2

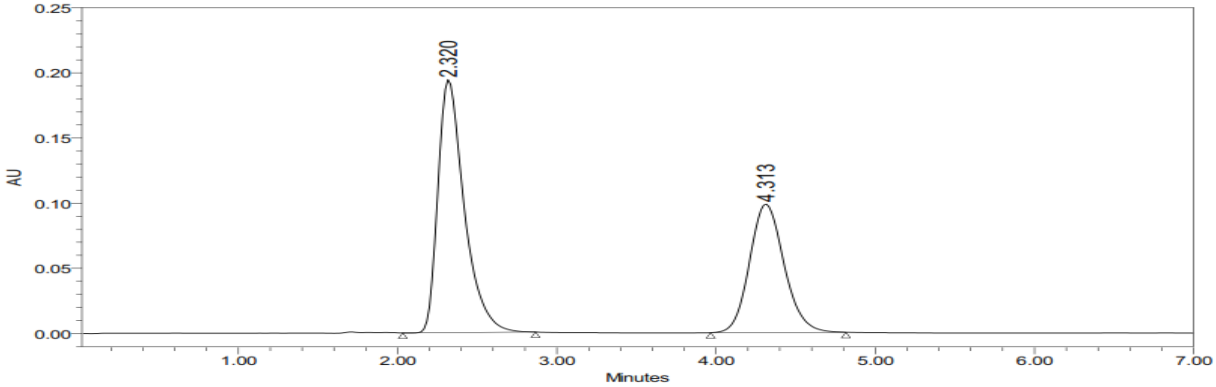
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.328	946325	1.2	5326	8.7
2	Lamivudine	4.341	111652	1.5	3965	



Chromatogram for System Suitability Injection-3

Results for System Suitability Injection-3

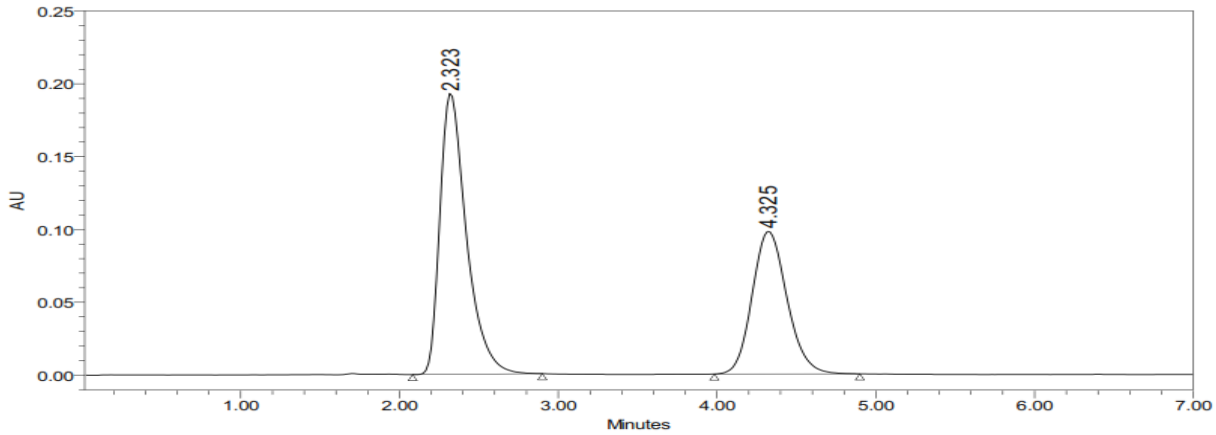
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.319	946859	1.3	5124	8.9
2	Lamivudine	4.299	112854	1.3	3874	



Chromatogram for System Suitability Injection-4

Results for System Suitability Injection-4

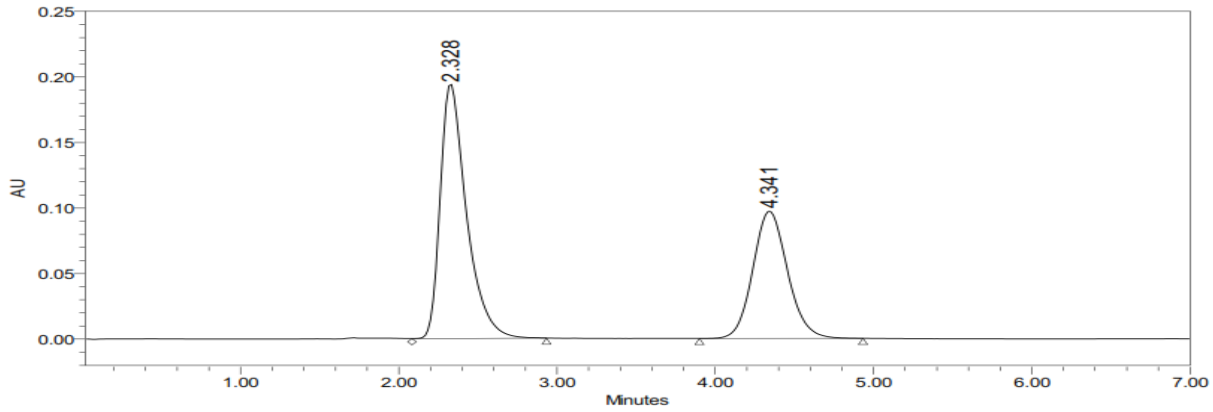
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.320	945875	1.3	5296	8.6
2	Lamivudine	4.313	111485	1.5	3698	



Chromatogram for System Suitability Injection-5

Results for System Suitability Injection-5

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.323	946396	1.2	5248	8.9
2	Lamivudine	4.325	113526	1.4	3785	



Chromatogram for System Suitability Injection-6

Results for System Suitability Injection-6

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.328	946548	1.3	5295	8.7
2	Lamivudine	4.341	112985	1.6	3965	

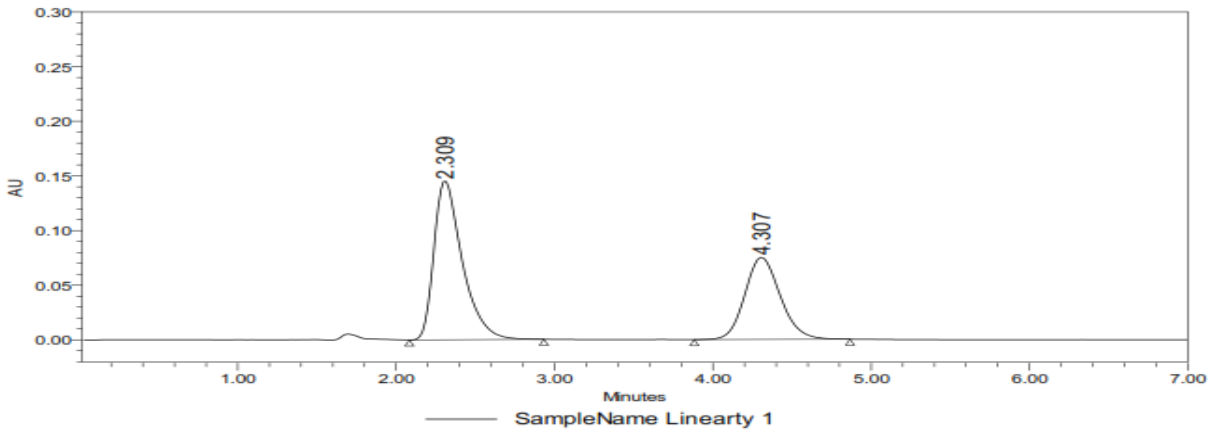
Data of System Suitability Test for Dolutegravir

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing	Resolution
1	Injection 1	2.327	946257	5245	1.3	8.6
2	Injection 2	2.328	946325	5326	1.2	8.7
3	Injection 3	2.319	946859	5124	1.3	8.9
4	Injection 4	2.320	945875	5296	1.3	8.6
5	Injection 5	2.323	946396	5248	1.2	8.9
6	Injection 6	2.328	946548	5295	1.3	8.7
Mean			**946376.7**			
S.D			**325.8936**			
%RSD			**0.034436**			

Data of System Suitability Test for Lamivudine

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing
1	Injection 1	4.331	112543	3854	1.4
2	Injection 2	4.341	111652	3965	1.5
3	Injection 3	4.299	112854	3874	1.3
4	Injection 4	4.313	111485	3698	1.5
5	Injection 5	4.325	113526	3785	1.4
6	Injection 6	4.341	112985	3965	1.6
Mean			**112507.5**		
S.D			**795.4945**		
%RSD			**0.707059**		

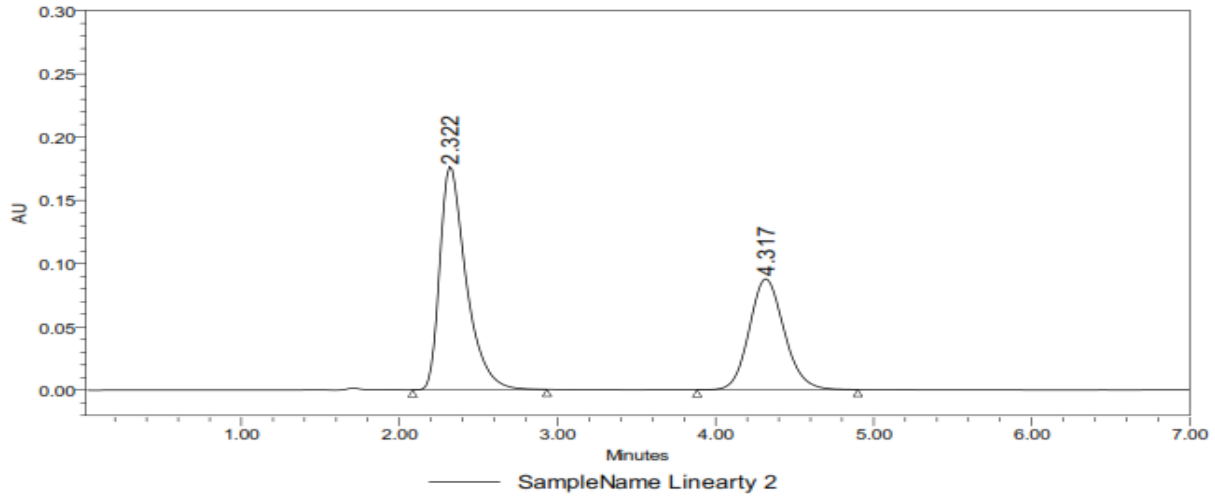
Linearity: Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line.[30]



Chromatogram for Injection Level-I

Results of Linearity Injection Level-I

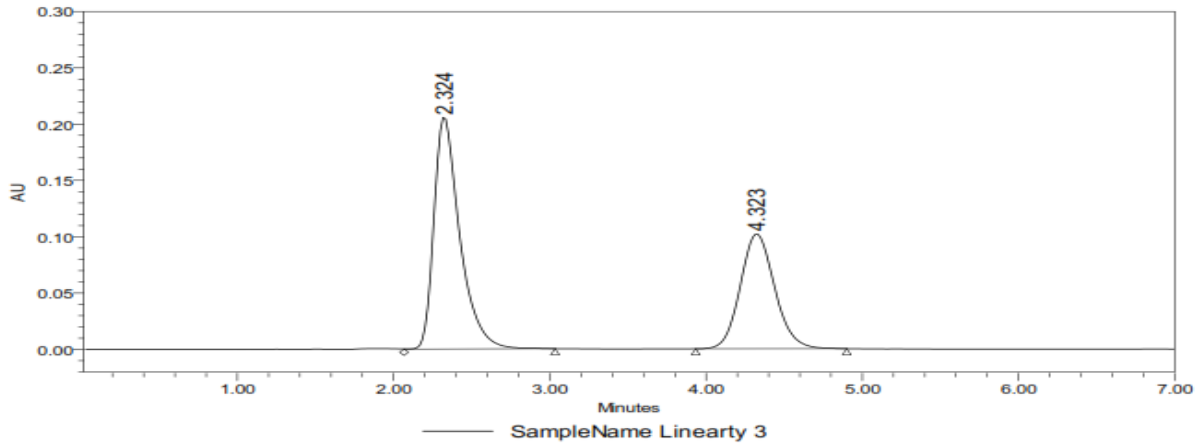
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.309	523864	1.36	5246	8.75
2	Lamivudine	4.307	65698	1.42	3896	



Chromatogram for InjectionLevel-II

Results of Linearity Injection Level-II

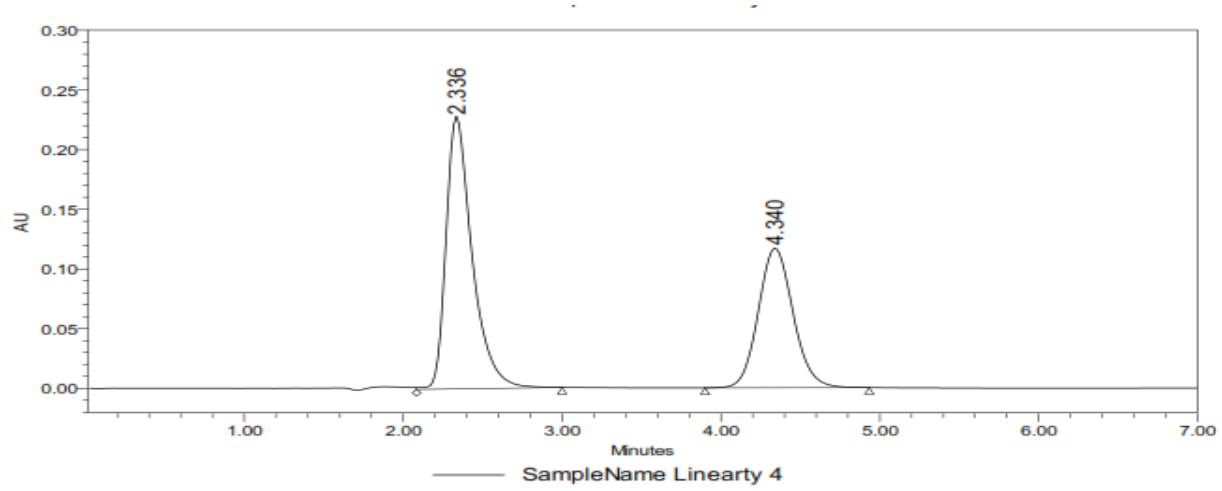
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.322	764875	1.38	5487	8.76
2	Lamivudine	4.317	98254	1.46	3964	



Chromatogram for InjectionLevel-III

Results of Linearity Injection Level-III

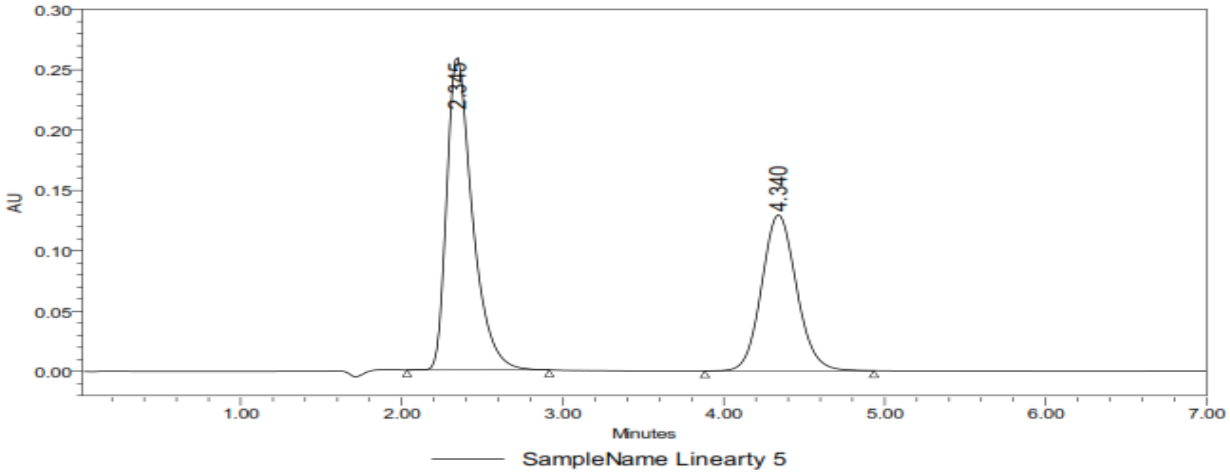
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.324	999874	1.32	5682	8.91
2	Lamivudine	4.323	128587	1.45	3981	



Chromatogram for Injection Level-IV

Results of Linearity Injection Level-IV

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.336	1235658	1.36	5658	8.63
2	Lamivudine	4.340	160648	1.43	3854	



Chromatogram for Injection Level-V

Results of Linearity Injection Level-V

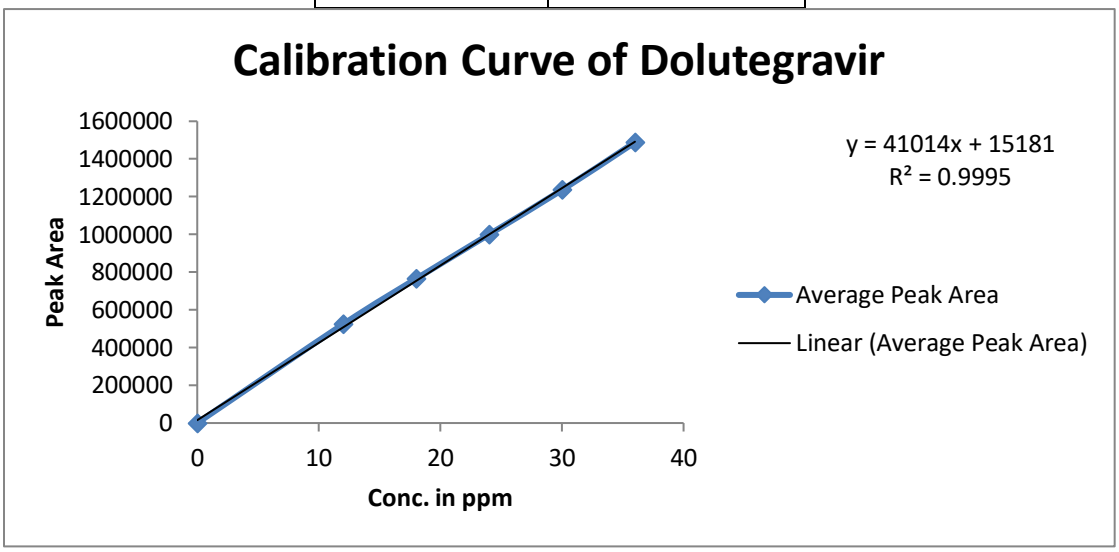
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.345	1488542	1.35	5748	8.62
2	Lamivudine	4.340	191874	1.45	3965	

chromatographic data for linearity study of dolutegravir:

Chromatographic Data for Linearity Study of Dolutegravir

Concentration µg/ml	Average Peak Area
0	0
12	523864

18	764875
24	999874
30	1235658
36	1488542



Calibration Curve of Dolutegravir

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Dolutegravir is a straight line.[31]

Y = mx + c

Slope (m) = 41014

Intercept (c) = 15181

Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 15181. These values meet the validation criteria.

Chromatographic Data for Linearity Study of Lamivudine:

Chromatographic Data for Linearity Study of Lamivudine

Concentration µg/ml	**Average Peak Area**
0	0
13	65698
19.5	98254

26	128587
32.5	160648
39	191874

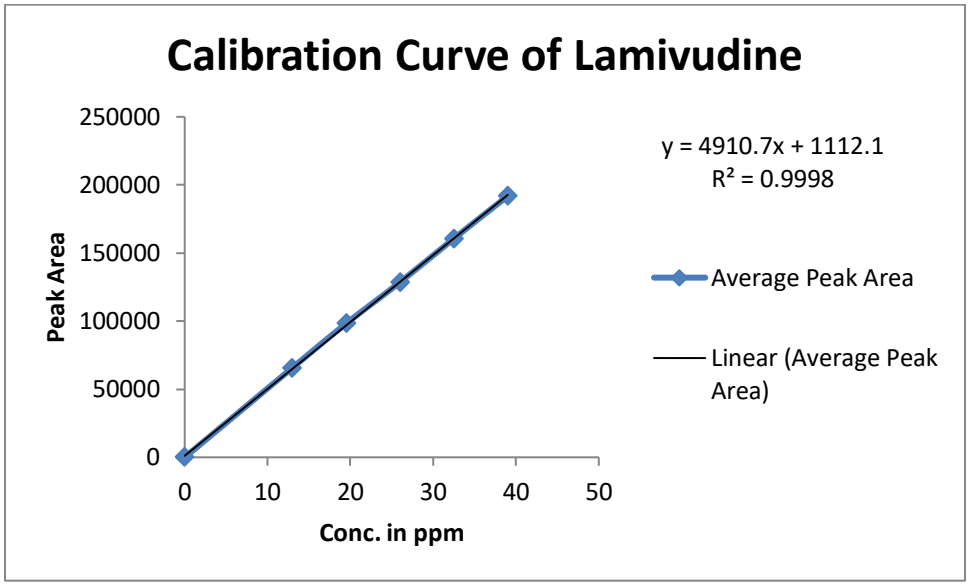


Fig-34: Calibration Curve of Lamivudine

Linearity Plot:

The plot of Concentration (x) versus the Average Peak Area (y) data of Lamivudine is a straight line.

Y = mx + c

Slope (m) = 4910.7

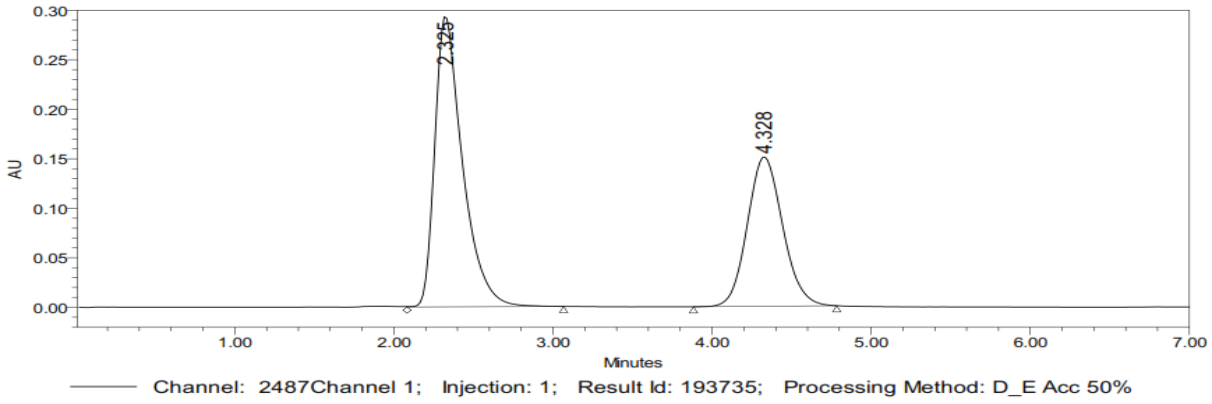
Intercept (c) = 1112.1

Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater. [31]

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 1112.1. These values meet the validation criteria.

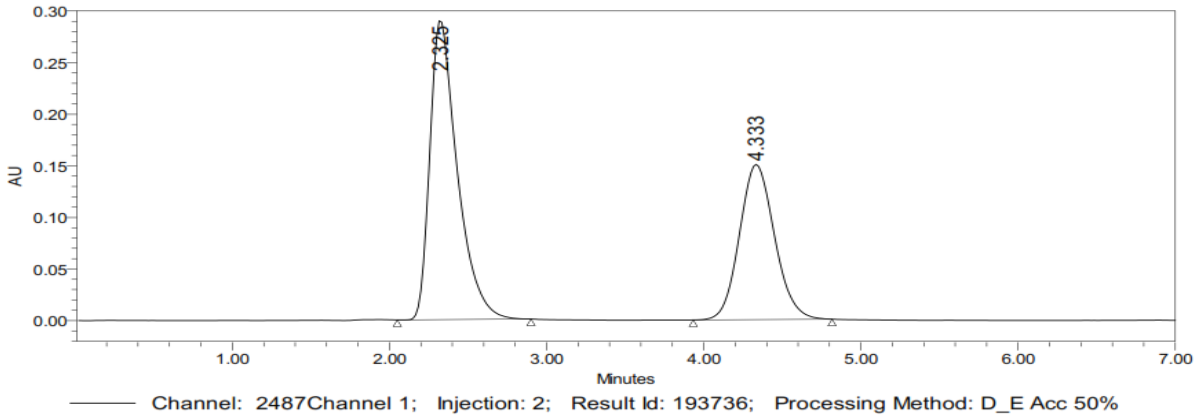
Accuracy:
Accuracy 50%



Chromatogramshowingaccuracy50%injection-1

Results of Accuracy 50% Injection-1

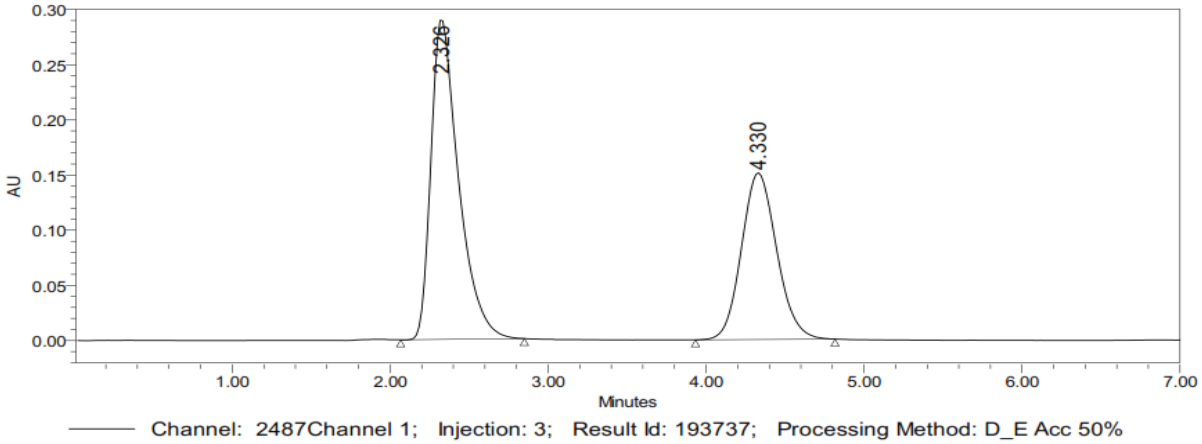
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.325	508568	1.34	5246	8.64
2	Lamivudine	4.328	64796	1.45	3895	



Chromatogramshowingaccuracy50%injection-2

Results of Accuracy 50% Injection-2

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.325	507946	1.38	5474	8.75
2	Lamivudine	4.333	65547	1.46	3985	



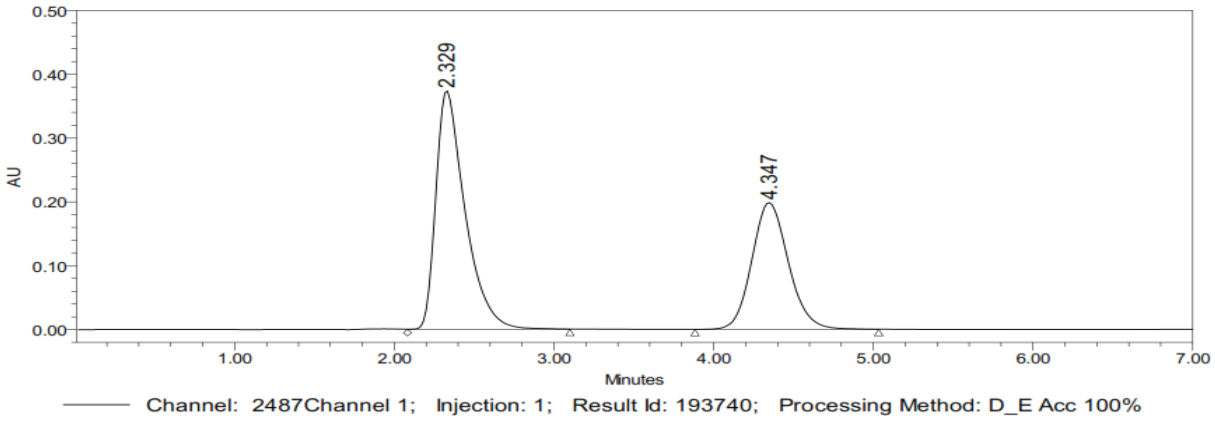
Chromatogramshowingaccuracy50%injection-3

Results of Accuracy 50% Injection-3

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.326	508587	1.37	5369	8.96
2	Lamivudine	4.330	64938	1.49	3854	

Results of Accuracy for concentration-50% Accuracy 100%

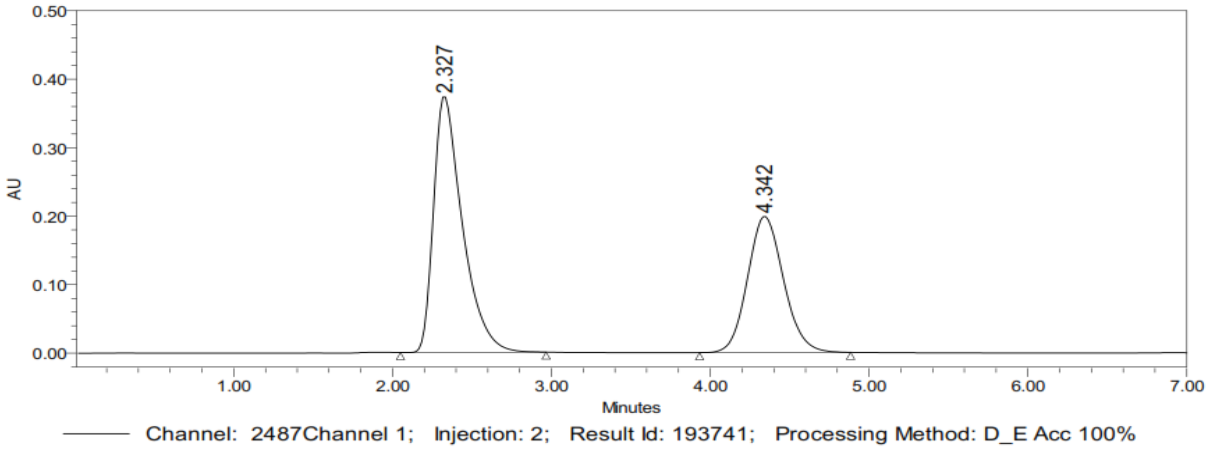
S.No.	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Dolutegravir	2.325	508568	136554	1.34	5246	1
2	Lamivudine	4.328	64796	11254	1.45	3895	1
3	Dolutegravir	2.325	507946	137484	1.38	5474	2
4	Lamivudine	4.333	65547	11247	1.46	3985	2
5	Dolutegravir	2.326	508587	136985	1.37	5369	3
6	Lamivudine	4.330	64938	11321	1.49	3854	3



Chromatogramshowingaccuracy100%injection-1

Results of Accuracy 100% Injection-1

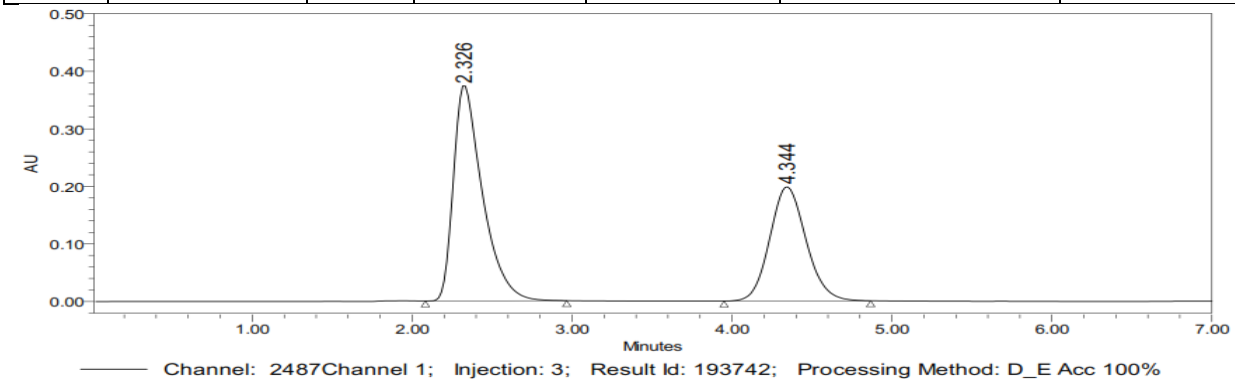
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.329	997954	1.48	6154	8.54
2	Lamivudine	4.347	128564	1.54	4568	



Chromatogramshowingaccuracy100%injection-2

Results of Accuracy 100% Injection-2

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.327	999452	1.46	6285	8.72
2	Lamivudine	4.342	129868	1.59	4695	



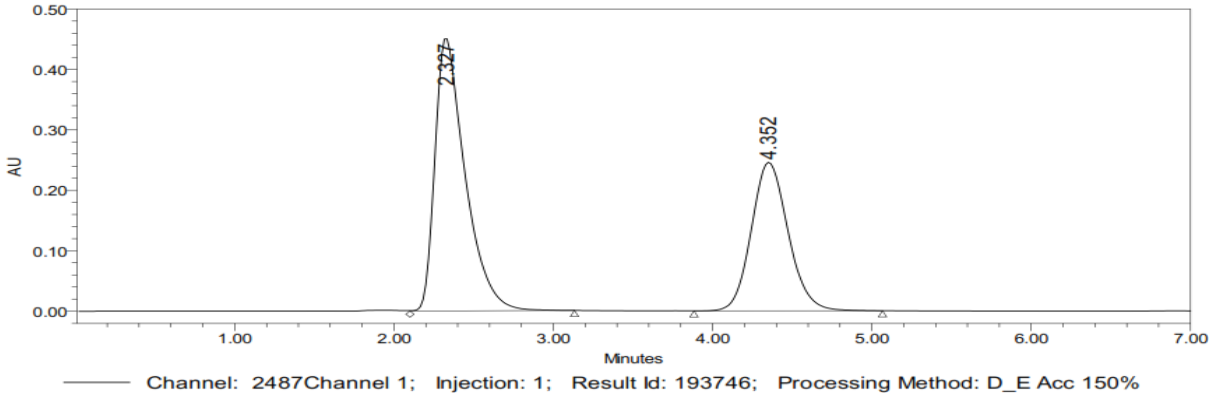
Chromatogramshowingaccuracy100%injection-3

Results of Accuracy 100% Injection-3

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.326	999895	1.49	6198	8.65
2	Lamivudine	4.344	129586	1.57	4875	

Results of Accuracy for concentration-100% Accuracy 150%

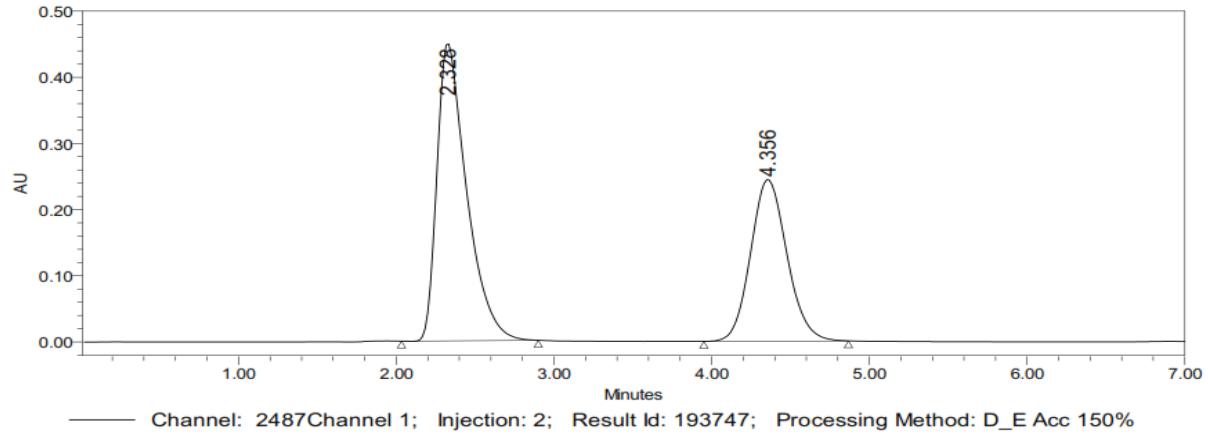
S.No.	Name	RT	Area	Height	USPTailing	USPPlateCou	Injection
1	Dolutegravir	2.329	997954	156452	1.48	6154	1
2	Lamivudine	4.347	128564	13254	1.54	4568	1
3	Dolutegravir	2.327	999452	157484	1.46	6285	2
4	Lamivudine	4.342	129868	13125	1.59	4695	2
5	Dolutegravir	2.326	999895	154896	1.49	6198	3
6	Lamivudine	4.344	129586	13265	1.57	4875	3



Chromatogramshowingaccuracy150%injection-1

Results of Accuracy 150% Injection-1

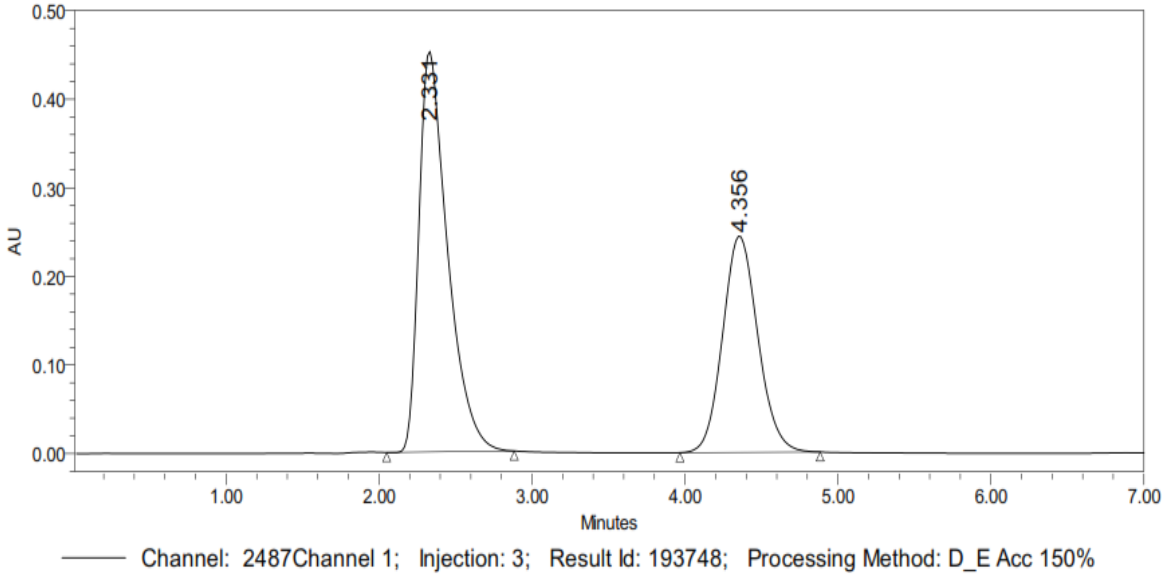
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.327	1492458	1.56	7584	8.74
2	Lamivudine	4.352	178954	1.63	5685	



Chromatogramshowingaccuracy150%injection-2

Results of Accuracy 150% Injection-2

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.328	1498657	1.57	7694	8.24
2	Lamivudine	4.356	15698	1.68	5578	



Chromatogramshowingaccuracy150%injection-3

Results of Accuracy 150% Injection-1

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.331	1497486	1.59	7598	8.35
2	Lamivudine	4.356	177929	1.69	5628	

Results of Accuracy for concentration-150%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Dolutegravir	2.327	1492458	165842	1.56	7584	1
2	Lamivudine	4.352	178954	15475	1.63	5685	1
3	Dolutegravir	2.328	1498657	168594	1.57	7694	2
4	Lamivudine	4.356	177845	15698	1.68	5578	2
5	Dolutegravir	2.331	1497486	165876	1.59	7598	3
6	Lamivudine	4.356	177929	15874	1.69	5628	3

Accuracy results of Dolutegravir

%Concentration (at specificationLevel)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	508367	12	12.024	100.200%	100.150%
100%	999100.3	24	23.989	99.954%	
150%	1496200.3	36	36.110	100.305%	

Accuracy results of Lamivudine

%Concentration (at specification Level)	Area	Amount Added(mg)	Amount Found(mg)	%Recovery	Mean Recovery
50%	65093.67	13	13.029	100.223%	100.280%
100%	129339.3	26	26.111	100.426%	
150%	178242.7	39	36.070	100.194%	

Acceptance Criteria:[32]

- The percentage recovery was found to be within the limit (98-102%).

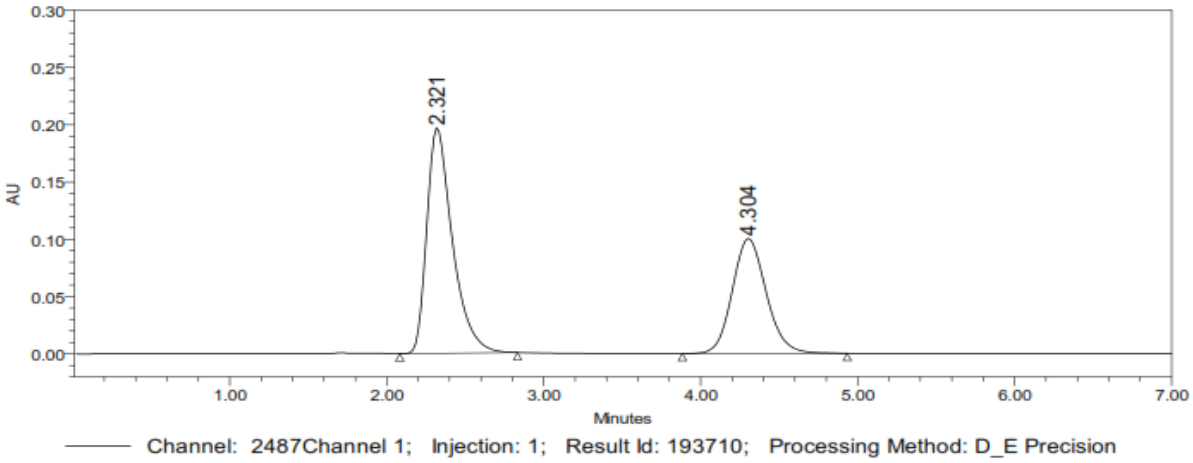
Observation: The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability[33]

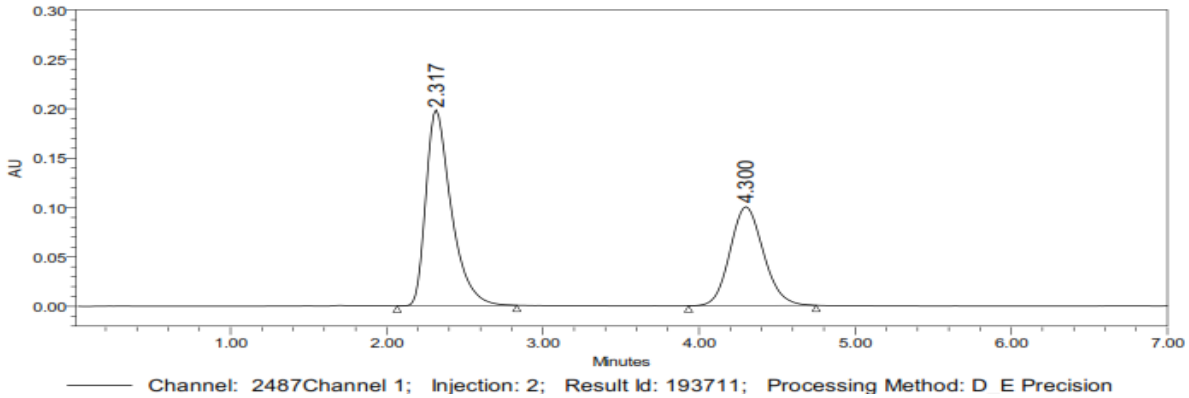
Obtained five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.



Chromatogram showing precision injection -1

Results showing precision injection -1

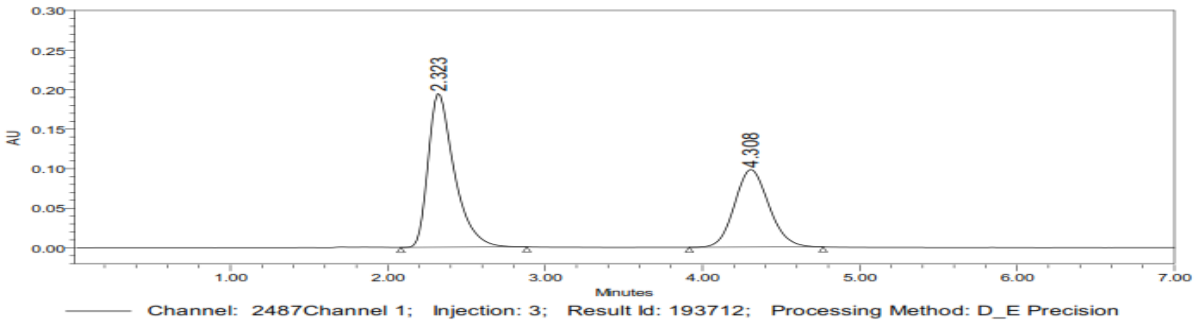
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.321	946253	1.36	5326	8.25
2	Lamivudine	4.304	111563	1.42	3869	



Chromatogram showing precision injection -2

Results showing precision injection -2

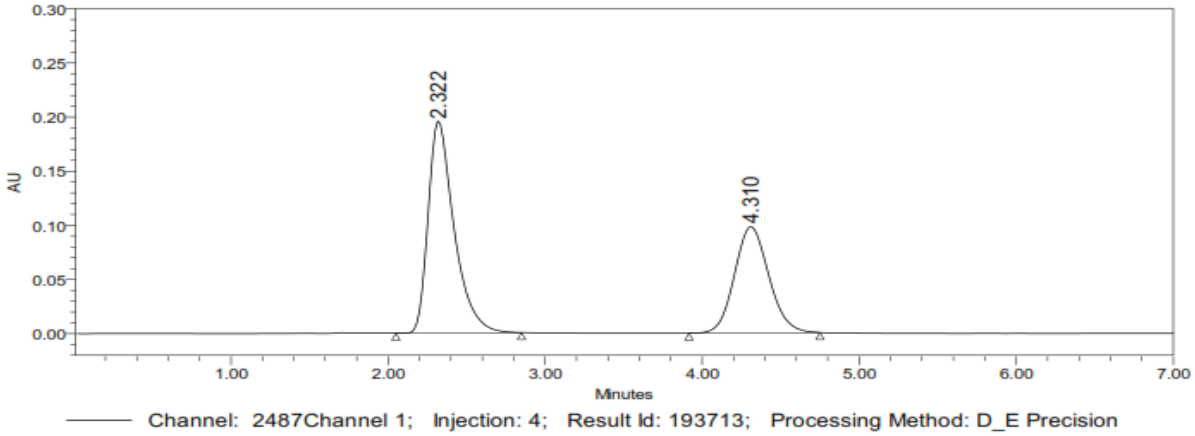
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.317	947845	1.37	5246	8.26
2	Lamivudine	4.300	111254	1.43	3852	



Chromatogram showing precision injection -3

Results showing precision injection -3

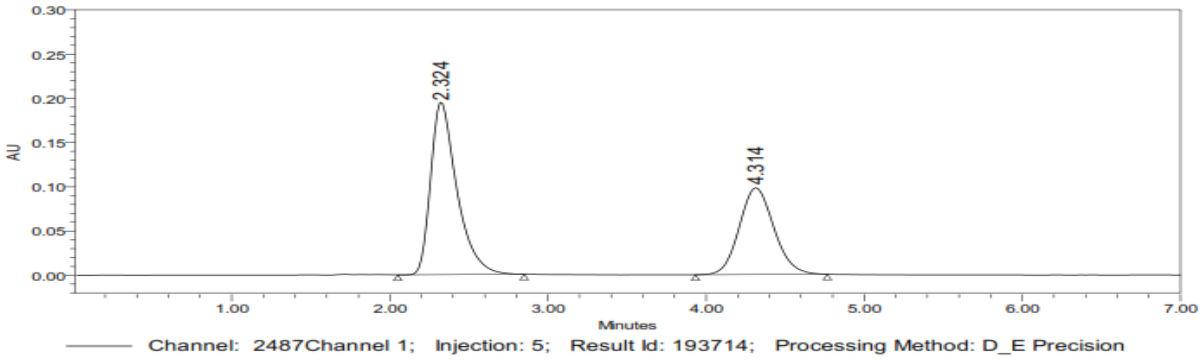
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.323	945867	1.35	5478	8.34
2	Lamivudine	4.308	111672	1.45	3896	



Chromatogram showing precision injection -4

Results showing precision injection -4

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.322	948572	1.38	5425	8.37
2	Lamivudine	4.310	112654	1.42	3962	



Chromatogram showing precision injection -5

Results showing precision injection -5

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.324	949857	1.36	5326	8.39
2	Lamivudine	4.314	113123	1.48	3874	

Results of repeatability for Dolutegravir:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Dolutegravir	2.321	946253	155465	5326	1.36	8.25
2	Dolutegravir	2.317	947845	154578	5246	1.37	8.26
3	Dolutegravir	2.323	945867	155845	5478	1.35	8.34
4	Dolutegravir	2.322	948572	155698	5425	1.38	8.37
5	Dolutegravir	2.324	949857	154857	5326	1.36	8.39
Mean			**947678.8**				
Std. Dev			**1649.66**				
%RSD			**0.174074**				

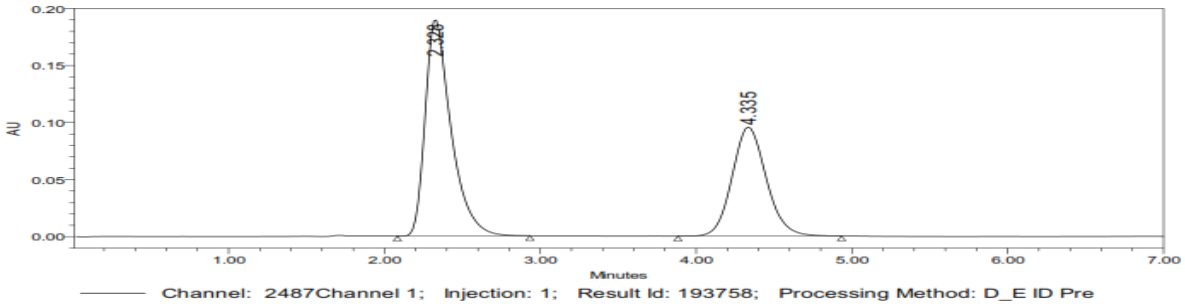
Results of Repeatability for Lamivudine:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Lamivudine	4.304	111563	13254	3869	1.42
2	Lamivudine	4.300	111254	13425	3852	1.43
3	Lamivudine	4.308	111672	13254	3896	1.45
4	Lamivudine	4.310	112654	13265	3962	1.42
5	Lamivudine	4.314	113123	13154	3874	1.48
Mean			**112053.2**			
Std. Dev			**795.2614**			
%RSD			**0.709718**			

Acceptance Criteria:[34]

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

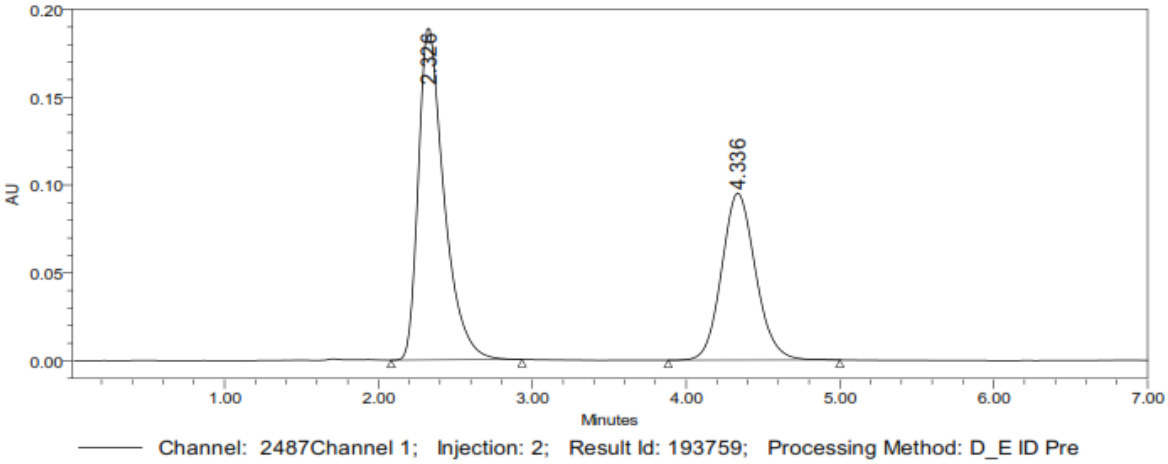
Intermediate Precision/Ruggedness:



ChromatogramofStandardInjection-1 (IDPrecision)

Results of StandardInjection-1 (IDPrecision)

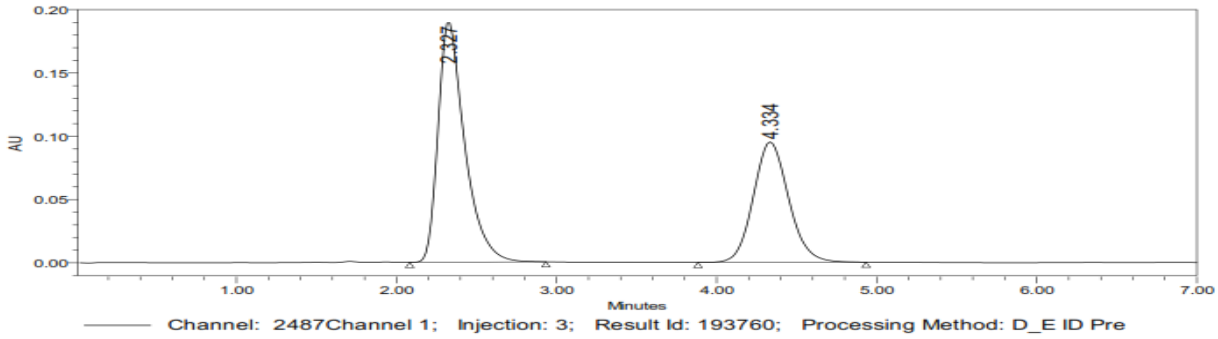
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.328	956325	1.35	5246	8.24
2	Lamivudine	4.335	121231	1.52	3896	



ChromatogramofStandardInjection-2 (IDPrecision)

Results of StandardInjection-2 (IDPrecision)

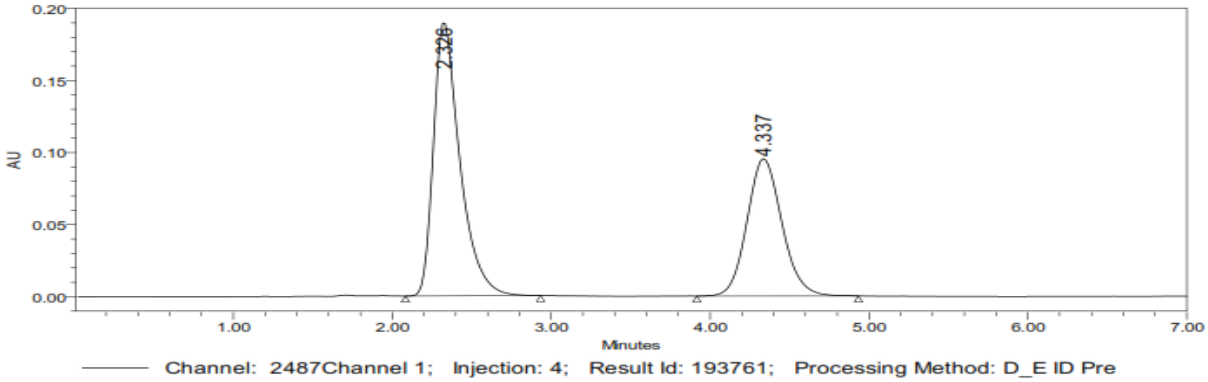
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.326	958741	1.38	5367	8.26
2	Lamivudine	4.336	121457	1.54	3785	



ChromatogramofStandardInjection-3 (ID Precision)

Results of StandardInjection-3 (ID Precision)

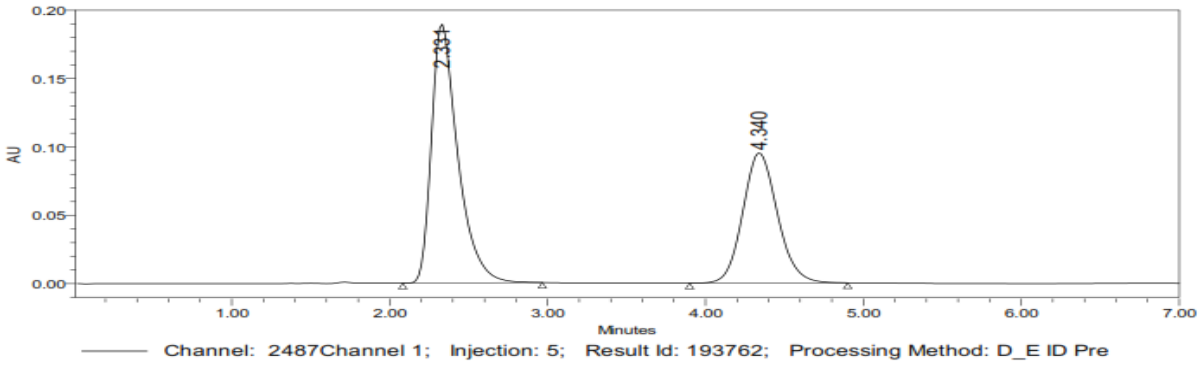
S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.327	957542	1.34	5265	8.47
2	Lamivudine	4.334	123142	1.58	3969	



ChromatogramofStandardInjection-4 (ID Precision)

Results of StandardInjection-4 (ID Precision)

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.326	956895	1.39	5384	8.29
2	Lamivudine	4.337	121325	1.57	3859	



ChromatogramofStandardInjection-5 (ID Precision)

Results of StandardInjection-5 (ID Precision)

S.No.	Peak Name	Rt	Peak Area	USP Tailing	USP Plate Count	Resolution
1	Dolutegravir	2.331	957486	1.35	5297	8.34
2	Lamivudine	4.340	123654	1.59	3789	

Results of Intermediate precision for Dolutegravir

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Dolutegravir	2.328	956325	156325	5246	1.35	8.24
2	Dolutegravir	2.326	958741	157854	5367	1.38	8.26
3	Dolutegravir	2.327	957542	156986	5265	1.34	8.47
4	Dolutegravir	2.326	956895	158547	5384	1.39	8.29
5	Dolutegravir	2.331	957486	156985	5297	1.35	8.34
Mean			**957397.8**				
Std.Dev.			**899.5091**				
%RSD			**0.093954**				

Results of Intermediate precision for Lamivudine

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Lamivudine	4.335	121231	13458	3896	1.52
2	Lamivudine	4.336	121457	13674	3785	1.54

Acceptance Criteria:[35]

- %RSD of five different sample solutions should not more than 2.

Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±5nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-4, % RSD < 2%) the developed RP-HPLC method for the analysis of Dolutegravir (API).

Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.96
Flow (0.9 ml/min)	0.84
Temperature (27^{0}C)	0.81
Temperature (23^{0}C)	0.94
Wavelength of Detection (265 nm)	0.56
Wavelength of detection (255 nm)	0.17

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±5nm) & acetonitrile content in mobile phase (±2%) studied to determine the

robustness of the method are also in favour of (Table-4, % RSD < 2%) the developed RP-HPLC method for the analysis of Lamivudine (API).

Result of method robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.58
Flow (0.9 ml/min)	0.64
Temperature (27^0C)	0.72
Temperature (23^0C)	0.91
Wavelength of Detection (265 nm)	0.86
Wavelength of detection (255 nm)	0.78

Limit of detection (LOD) & Limit of quantification (LOQ): [37]

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3 (SD/S).

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Result & Discussion: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 1.377µg/ml& 4.174µg/ml respectively for Dolutegravir.

The LOD was found to be 1.079µg/ml and LOQ was found to be 3.272µg/ml for Lamivudine which represents that sensitivity of the method is high.

Estimation of Dolutegravir&Lamivudine in TABLET Dosage Form

Dolutegravir&Lamivudine

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Finally the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra sonicator, Then the final volume was make up to the mark with the mobile phase. The final solution was filtered through a selected membrane filter (0.45 µm) and in order to sonicated to degas the mobile phase (Solvent system). From this above stock solution

(1 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system (Mobile phase).[38]

The prepared solutions were injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection (Blank Solution) of the standard solution also injected into the HPLC system and the chromatograms and peak areas were recorded and calculated. The obtained data are shown in Table 40.

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

Where:

AT = Test Preparation Peak Area

AS = Standard preparation Peak Area

WS = Working standard weight taken in mg

WT = Sample weight taken in mg

DS = Standard solution dilution

DT = Sample solution dilution

P = Working standard percentage purity

(1 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system (Mobile phase).

The prepared solutions were injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection (Blank Solution) of the standard solution also injected into the HPLC system and the chromatograms and peak areas were recorded and calculated. [38]

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

Where:

AT = Test Preparation Peak Area

AS = Standard preparation Peak Area

WS = Working standard weight taken in mg

WT = Sample weight taken in mg

DS = Standard solution dilution

DT = Sample solution dilution

P = Working standard percentage purity

CONCLUSION:

A sensitive & selective RP-HPLC method has been developed & validated for the simultaneous analysis of Dolutegravir and Lamivudine API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity & stability which can help in the simultaneous analysis of Dolutegravir and Lamivudine in different formulations.

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