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DEVELOPMENT AND VALIDATION OF A NEW RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE, DORAVIRINE AND TENOFOVIR DISOPROXIL FUMARATE IN COMBINED DOSAGE FORM

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ABSTRACT

A new, simple, accurate and specific RP-HPLC method was developed for the simultaneous estimation of the Lamivudine, Doravirine and Tenofovir disoproxil fumarate in combined solid dosage form. The chromatogram was run through Kromasil C18 250x4.6mm, 5□ column. Mobile phase containing a buffer and acetonitrile in the ratio of 60:40 v/v was pumped through the column at a flow rate of 1ml/min. Buffer used in this method was 0.1% OPA. Temperature of system was maintained at 30°C. The optimized wavelength for Lamivudine, Doravirine and Tenofovir disoproxil fumarate was 272.0 nm. Retention time of Lamivudine, Doravirine and Tenofovir disoproxil fumarate were found to be 2.314 min, 4.616 min and 3.196 min. % RSD of system precision for Lamivudine, Doravirine and Tenofovir disoproxil fumarate were found to be 1.0, 0.9 and 1.0 respectively. % RSD of method precision for Lamivudine, Doravirine, Tenofovir were found to be 0.5, 0.7 and 0.5 respectively. % Recovery was obtained as 99.57%, 99.89% and 99.74% for Lamivudine, Doravirine and Tenofovir disoproxil fumarate respectively. LOD values obtained from regression equations of Lamivudine, Doravirine and Tenofovir disoproxil fumarate were 0.27 ppm, 0.09 ppm and 0.18 ppm, and LOQ values obtained from regression equations of Lamivudine, Doravirine and Tenofovir disoproxil fumarate were 0.82ppm, 0.28 ppm and 0.54ppm respectively. Regression equation of Lamivudine was y = 14959x + 1184, Doravirine was y = 28129x + 2766 and of Tenofovir was y = 17361x + 6030.

Key Words: Lamivudine, Doravirine, Tenofovir disoproxil fumarate, RP-HPLC, method development, validation.

INTRODUCTION

Analytical chemistry deals with both theoretical and practical aspects of science and used by laboratories and pharmaceutical industries. It involves separation of components, identification and determination of composition of chemical compounds, drug substances, medicinal components, raw materials, finished drug products and other substances using modern analytical instruments by establishing authenticity through analytical instruments and methods. For this, methods were developed and validated. Analytical methods are used to characterize and study the composition of the drug substance during different phases of pharmaceutical drug production.

ANALYTICAL TECHNIQUES

Quantitative determination of concentration of a compound can be done by using different techniques like Titrations, Chromatography, Spectroscopy and Gravimetric analysis. They are used for the analysis of drugs in bulk, formulations and biological samples. The physical, chemical or electrical properties of a sample substance are analysed and measured that helps in the identification, separation and characterization of the compound of interest. Most of the analytical techniques use an advanced and practically efficient instrument for the intended purpose. Though the analytical techniques involve instrumental methods, electro chemical methods, chemical methods, radioactive methods, biological methods etc., in pharmaceutical industry, Spectroscopic and Chromatographic techniques are being used in larger scale. The introduction of highly advanced technology to the analytical techniques allowed the use of highly improvised and technically efficient modern analytical instruments of spectroscopy and chromatography like HPLC, HPTLC, and UPHPLC etc. Nowadays the coupling of spectroscopic methods with the chromatographic methods is done and the use of these techniques such as LC-MS, GC-MS etc. provided promising results.

SPECTROSCOPY

Spectroscopy is a technique of analysis that embraces the measurement of absorption of radiant energy by different chemical species at definite and narrow wavelength. It is the measuring and interpreting of electromagnetic radiation that is absorbed, scattered or emitted by the atoms, molecules or any other chemical species. Since each chemical species have a different and characteristic energy state, the absorption or emission of the radiation by interacting species can be studied and can be used as a identification tool.

ABSORPTION SPECTROPHOTOMETRY

At the point when a particle is presented to an electromagnetic radiation, certain measure of vitality related with the specific radiation is consumed by the atom. As an atom retains this vitality; an electron is advanced from the involved orbital to an abandoned orbital of more potential vitality and the most plausible change is from the most elevated involved sub-atomic orbital (HOMO) to the least abandoned sub-atomic orbital (LUMO). The vitality contrasts between electronic levels of many atoms differ from 125 to 650 KJ/mole.

ULTRA VIOLET – VISIBLE SPECTROSCOPY

The UV region of the electromagnetic spectrum ranges between 200-400 nm. Spectroscopy estimates the amount of electromagnetic radiation that is transmitted or consumed by the analyte molecules during the electronic transitions occurring due to the interaction with light. The visible region of the electromagnetic spectrum ranges between 400-780 nm. The degree of absorption of the UV-Visible light by the substance and the wavelength at which it is absorbed is recorded and then measured. The intensity of the absorption depends on the concentration and path length of the sample, hence explained using the Beer- Lambert's Law. The results of the Spectroscopic analysis are obtained in the form of absorption curves, which is a plot between energy absorbed by the molecule or absorbance on Y-axis and wavelength on X-axis. The absorption curve gives the wavelength of maximum absorption (λmax). The λmax of every substance is unique and specific that determines the character of a substance.

CHROMATOGRAPHY

Chromatography is an analytical technique that is used for the separation, purification and identification of the compounds of mixtures using two distinct phases called as stationary and mobile phase. The separation column allows a wide choice of materials for stationary and mobile phase and makes it suitable for the separation of hundreds of molecules with different physical and chemical properties. Chromatography was found useful in pharmaceutical field for separating and isolating active constituents, for removing impurities from drug substances etc. It is also used for the fractionation of complex mixture, separation of closely related compound such as isomers and in the isolation of unstable substances.

MODES OF CHROMATOGRAPHIC SEPARATION

There are mainly three modes of chromatographic operations are as follows:

• Elution techniques

1. Isocratic method

2. Gradient method

• Frontal techniques

• Displacement techniques

INTRODUCTION TO HPLC

High-pressure liquid chromatography / High-performance liquid chromatography is a method of choice for separations and analysis in many fields. Any substance that can be dissolved can be separated on a specific type of HPLC column. Most of the drugs in multi component dosage form can be analyzed by HPLC method. It is a form of column chromatography that is extensively used in the field of biochemistry and analytical chemistry.

There two elution principles for HPLC are as follows:

• Isocratic elution

• Gradient elution

Isocratic HPLC:

In Isocratic HPLC, the analyte or the sample is forced through the column of stationary phase by introducing it in a liquid at high pressure. The use of high pressure gives the components less time to diffuse within the column, which leads to improved resolution in the resulting chromatogram. The Solvents used in this include miscible combination of water or various organic liquids (most common are methanol and acetonitrile). The water may contain buffers or some salts to assist the separation of analyte components or compounds.

Gradient HPLC:

A further modification of HPLC by varying the mobile phase composition during the analysis is known as gradient elution. Here the adsorbent is kept constant (solid phase), and the eluting solvent (mobile phase) polarity is increased until complete elution is achieved. A normal gradient program for the reverse phase chromatography might start with 5% methanol and progress linearly to 50% methanol over a time of 25 minutes, depending on the hydrophobic nature of the analyte. The increasing order of polarity is as follows: Light petroleum solvents (hexane, heptanes, petroleum ether) < cyclohexane < toluene <dichloromethane < chloroform < ethyl ether < ethyl acetate < acetone < n-propanol < ethanol < water.

Advantages of HPLC method

- Efficient, extremely selective, widely applied.
- Non-destructive of sample.
- Readily adapted in quantitative analysis of compounds.
- Solely tiny sample needed.
- Higher resolving power.
- Larger sensitivity, speed and improved resolution.
- Reusable columns (expensive, but can be used for several analysis).
- Ideal for low volatile substances.
- Easier recovery of sample, simple handling and maintenance.
- Instrumentation is automatable (less time and labor cost).
- Precise and consistent, Calculations are done by integrator itself.
- Appropriate for the preparative liquid chromatography on large scale.

Modes of HPLC

1) Normal phase chromatography: Normal phase chromatography uses polar stationary phase and a non-polar mobile phase for the detachment of polar mixes. Usually the column is filled with silica particles. The elution order within the normal phase chromatography is that the non-polar components elute first and then the polar components elute. The polarity of packing is high and the polarity of solvent is low in Normal phase chromatography, when polarity of the solvent is increased; the retention time gets decreased.

2) Reversed phase chromatography: Reversed phase chromatography is a term given to the chromatographic condition in which a non-polar stationary phase is utilized along with a polar mobile phase. The column is filled with modified non-polar silica particles and this helps in faster elution of the polar molecules. Here the elution takes place in the order that polar components eluted first and then non-polar components. Here, the packing polarity is low and the solvent polarity is very high.

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

Reversed phase HPLC or RP-HPLC consists of a non-polar stationary phase and a polar mobile phase. One commonly used stationary phase is silica which is treated with RMe2SiCl, where R is a straight chain alkyl group such as C18H37, C8C17 etc. Therefore, the retention time is longer for molecules which are more non-polar in nature, which allows the elution of polar molecules more readily. The effect of increasing polarity of solvents can increase the retention time. The elution time is increased by the addition of less polar solvent. This method is mainly used for separation and analysis of large biomolecules like steroids, nucleic acids, amino acids, antibiotics etc.

COMPONENTS OF RP-HPLC INSTRUMENT

• Mobile phase

• Pumps

• Injection port

• Stationary phase

• Detector

MOBILE PHASE

The mobile phase in RP-HPLC has great influence on retention of solutes and separation of components from a mixture. The primary constituents of reverse phasemobile phase are water miscible solvents such as methanol, ethanol, acetonitrile, digoxin, and tetra hydrofuran which are added to adjust the polarity of mobile phase. The water used in the system should be of high quality, either distilled or demineralised. The most widely used organic modifiers for RP-HPLC are methanol, acetonitrile and tetrahydrofuran. Methanol and acetonitrile have comparable polarities but tetrahydrofuran is an aprotic solvent. When any inorganic salts or ionic surfactants are used, the mobile phase should be filtered before use since these additives frequently contain a significant amount of water insoluble contaminants that may damage the column. Mobile phases of the RP-HPLC system are generally non-inflammable due to the involvement of high-water content. Degassing of the mobile phase is an essential step that has to be followed in HPLC.

HPLC PUMPS:

Pump system in HPLC is constructed by materials that are inert to all mobile phases and solvent systems. Commonly used materials are glass, stainless steel, Sapphire and Teflon . Generally the pumps deliver a constant flow of mobile phase at a pressure from 1 to 550 bar and 14.6 to 800 psi. High capacity Pumps are also available that are capable of generating the pressure upto 8000 psi which provides a wide range of flow rate of mobile phases typically from 0.01 to 10 ml.The solvent flow from the pump to the system should be pulseless or it should be dampened to remove the pulses. For this purpose, certain pulse dampeners are used in the system.

INJECTION PORT FOR HPLC:

The samples that are to be analysed are injected into HPLC system via an injection port. The injection port of an HPLC system commonly consist of an injection valve and a sample loop. The sample is typically dissolved in a suitable mobile phase, drawn into a syringe and then it is injected into the sample loop via injection valve. One rotation of valve rotor closes the valve and opens the loop for injection of sample into the mobile phase stream. The loop volumes can range from 10 µl to over 500 µl. In modern HPLC systems, the sample injection process is automated.

STATIONARY PHASE (COLUMN):

The stationary phase in HPLC is a solid support that is contained within the column over which the mobile phase continuously flows. The chemical interactions of the stationary phase and the sample within the mobile phase, determines the degree of migration and separation of components that is contained in the sample. The column efficiency parameters are the number of the theoretical plates (N), HETP, velocity of the mobile phase and factors like Eddy's diffusion, longitudinal diffusion and resistance to mass transfer. The columns that are secondary to the stationary phase are guard, derivatizing, capillary, fast, and preparatory columns.

DETECTORS:

The detector emits a response due to the eluting sample component and subsequently signals a peak on the chromatogram. The bandwidth and height of the peaks may usually be adjusted using the coarse and fine tuning controls, and the detection and sensitivity parameters may also be controlled .Types of detectors that can be used with HPLC are Refractive Index (RI), Ultra-Violet (UV), Fluorescent, Radiochemical, Electrochemical, Near-Infrared (Near-IR), Mass Spectroscopy (MS), Nuclear Magnetic Resonance (NMR), Light Scattering (LS) detectors.

QUANTITATION METHODS OF HPLC

• Peak height or peak area measurements only provide a response in terms of detector signal. This response must be related to the concentration or mass of the compound of interest. To accomplish this, some type of calibration must be performed.

• The four primary techniques for quantitation are;

1. Normalized peak area method

2. External Standard method

3. Internal Standard method

4. Method of Standard addition

Normalized peak area method

The area percentage of any individual peak is referred to the normalized peak area. It is used to estimate the relative amount of small impurities or degradation compounds that are present in a purified material and in this method, the response factor for each component is identified.

External Standard method

This method includes the injection of both standard and unknown to the system and the unknown are determined graphically from the calibration plot or numerically by using some response factors. A response factor (Rf) can be determined for each standard as follows:

$$Rf = Standard\ Area\ (Peak\ height) / Standard\ Concentration$$

The external standard approach is preferred for most of the samples in HPLC that do not require an extensive sample preparation. For good quantitation using external standards, all the chromatographic conditions must remain constant during the separation of all the standards and samples. External standards are often used to ensure that the total chromatographic system is performing properly and that can provide reliable results.

Internal Standard method

In this approach, a selected, known compound of fixed concentration is added to the known amount of sample to obtain separate peaks in the chromatogram that compensate for the loss of compounds of interest during the sample pre-treatment steps. Any loss in the amount of the component of interest will be accompanied by the loss of an equivalent fraction of internal standard.

Primary requirements for an internal standard are:

• It must possess a completely resolved peak with no interferences.

• It must elute close to the elution of compound of interest.

• It must be stable and un-reactive with the sample components

• The internal standard should be added to the sample prior to the sample preparation procedure and then homogenized with it.

• It should be able to recalculate the concentration of a sample component in the original sample.

The response factor (RF) is the ratio of the peak areas of sample component (Ax) and the internal standard (AISTD) obtained by injecting both into the system in same quantity. It can be calculated by using the formula below:

$Rf = Ax / AISTD$

On the basis of the response factor and the strength of the internal standard (NISTD), the amount of the analyte in the original sample can be calculated using a formula. The calculations can be used after proving the linearity of the calibration curve for the internal standard and the analytical reference standard of the compound of interest.

Method of Standard addition

The method of standard addition can be used to provide a calibration plot for the quantitative analysis. It is most often used in the trace analysis. An important aspect of method of standard addition is that the response prior to the spiking of the additional analytes should be high enough to provide a reasonable S/N ratio (>10), otherwise the results produced will have a poor precision.

ANALYTICAL METHOD DEVELOPMENT

As the Separation, identification and estimation of each ingredient in a formulation is complicated due to the presence of excipients, additives and decomposition products therefore, analytical development is done for new drugs where no methods are available officially or alternate method development is done for an existing (non-pharmacopoeia) product to reduce the cost and time of analysis and for results of better precision and ruggedness. Best column, best mobile phase, best detection wavelength and true efforts in the separation can make a world of difference while developing HPLC method for routine analysis. Determining the ideal combination of these factors assures a validated method of separation.

a) The Mobile Phase : Mobile phase in these system is usually mixtures of two or more individual solvents with or without additives or organic solvent modifiers. The usual approach is to choose what appears to be the most suitable and appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system.

b) The Detector: UV-visible detectors are the most popular detectors that are used along with the system as they can detect a broad range of compounds and have a fair degree of selectivity for many analytes.

c) The Column Length: Many chromatographers make the mistake of simply using the column that is easily available. Since the length of the column is directly proportional with the resolving power, the greater the column length, greater will be the resolving power. When theoretically speaking, higher number of theoretical plates makes the column proportionally lengthy. Also the increased column length causes the increased broadening of the analysis peak. Here the selected column is a 250 cm × 4.6 mm length C18 column. These columns are highly able to resolve a wide variety of compounds and found to be stable with most of the compounds. More specifically many reverse phase

separations can be carried out on this column. The Method development can be streamlined by starting with shorter columns; 150, 100 or 50 cm long and then followed by the most appropriate selection.

d) The Stationary Phase: Selecting an appropriate stationary phase can also help to improve the efficiency of HPLC method development. For example, a C8 phase (reversed phase) can provide a further time saving analysis over a C18 phase, but it does not retain analytes as strongly as the C18 phase. Therefore, here we have selected the C18 stationary phase column. The particle size of the stationary phase is important as the decreased particle size provides more efficient separation. For normal phase applications, the cyano (nitrile) stationary phases are most versatile.

e) The Internal Diameter: By selecting a longer column with shorter internal diameter and filled with appropriate stationary phase, run times can be minimized so that an elution order and an optimum mobile phase can be quickly determined for the sample substance.

f) Gradient Programming: The fastest and easiest way to develop a faster HPLC method is to use a mobile phase gradient. Always start with a weak solvent strength and then move on to a higher solvent strength. In the reverse phase high performance chromatography, the gradient elution follows the increasing order of the polarity scale.

g) Retention: Analyte components may be too strongly retained sometimes (producing long run times). If this occurs, the solvent strength should be increased. In reverse phase analysis this means the application of a higher % of organic solvent in the mobile phase.

h) Separation: Analytes often co-elute with each other or sometimes with other impurities during the separation. To overcome this, the analysis should be run at both higher and lower solvent strengths, so the best separation conditions could be determined.

i) Peak Shape: Peak shape variation is a major problem, especially for basic compounds analyzed by reversed phase HPLC. To minimize any kind of potential problems, the use of high purity silica phases such as Wakosil II in the chromatographic system is advised.

j) Buffer selection: In reverse phase HPLC, the retention of analytes is related to their nature of hydrophobicity. The more hydrophobic the analyte, the longer it is retained. When an analyte is ionized, it becomes less hydrophobic and therefore, its retention decreases. Buffers play an additional role in the reproducibility of the separation. The buffer salts reduce peak tailing for basic compounds by effectively masking the silanols. They also reduce potential ion-exchange interactions with the un- protonated silanols.

k) Selection of pH: The pH that is frequently utilized for reversed phase HPLC is 1 - 8 and it can be separated into low pH (1 - 4) and middle pH (4 - 8). Each range has various points of interest. Low pH has the upside of making a domain in which top following is limited and the strategy toughness is amplified. Hence, working at low pH is prescribed. At a portable stage, pH may be more prominent than 7, which may cause the disintegration of silica and can seriously abbreviate the lifetime of segments that are stuffed with silica-based stationary stages. The pKa esteem (corrosive separation [ionization] consistent) for a compound is the pH at which it rises to the groupings of acidic and the fundamental type of atom that is available in the watery arrangements. Analytes at times may show up as wide or following pinnacles when the versatile stage pH is at or close to their pKa esteems. A more tough versatile stage pH will be not less than 1 pH unit that is not quite the same as the analyte pKa. The outcome is a steady chromatography.

l) Pressure range: The appropriate pressure is selected by considering the nature of the sample substance to be analyzed and the materials used for the stationary phase column. Usually high pressures are associated with the HPLC Pumps and the pressure of 550 bars or 800 psi may be applied in this reversed phase HPLC Technique.

STEPS TO BE FOLLOWED IN METHOD DEVELOPMENT

The Method development starts with documentation of the developed studies. All the available data related to the study must be established and they must be recorded in the laboratory notebook or in an electronic database.

Standard Analyte Characterization: - Collection of information about the analyte or drug should be started from the structure, physical, chemical properties, toxicity, purity, hygroscopic nature, solubility and stability. The Reference standard for the sample should be obtained. In case of multiple components in the sample are to be analyzed, the number of components should be noted, the data is assembled and the availability of reference standards for each component is determined. Proper storage conditions are set for the reference standards by the use of refrigerators, dessicators, freezers or any other techniques that are implemented for appropriate storage of materials. The most suitable method for the sample is considered.

❖ **Method Requirements:** - The aim and objectives or the primary requirement of the analytical method that need to be developed is studied and all the related factors are considered. Merits of the analytical method to be developed are well defined. The required detection limits, selectivity, linearity, range, accuracy, precision and other parameters involved are defined.

❖ **Literature Search and prior Methodology: -** The literature, articles, journals and all other types of information related to the analyte is surveyed. Literature is also done for the synthesis, physical and chemical properties, solubility and other information which are related to the specified analytical method. Information can be obtained also from the official standard books such as USP/NF, AOAC standard books, periodicals, chemical manufacturers along with the chemical abstract services and the computer data banks.

❖ **Choosing of Method: -** Any of the reported method from the literature that is adaptable to the current laboratory setting and future needs is determined. From the various sources available a suitable methodology is adopted. The methods are then modified. If there are no sufficient details reported for the drug or chemical, then the data of drugs are investigated thoroughly and are worked out.

VALIDATION

Validation can be defined as "Establishing of the documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes". It has been made mandatory by the regulatory bodies to prove the safety, efficacy, purity and effectiveness of the drug product, medical devices and biologics in the market and in the health care system.

IMPORTANCE OF VALIDATION

- Increased throughput and reduction in utility costs.
- More rapid and reliable automation and start-up of new equipment.
- Reduced testing in-process and finished goods of pharmaceutical industry.
- Fewer complaints about the process related failures.
- Avoidance of capital expenditure etc.

ANALYTICAL METHOD VALIDATION

Analytical method validation is a kind of process that is used for confirming that the analytical procedures that are employed for a specific test is suitable for the intended purpose. The results from the method validation can be used to judge the quality, reliability and consistency of the results that are produced in the analytical methods. It is an integral part of any analytical practices.

The main strategy and the steps for analytical method validation are as follows;

1. Develop a protocol, an operating procedure or a validation master plan for the

validation of the method.

2. Define the responsibilities for a specific validation project.

3. Develop a validation project plan.

4. Define the purpose, application and scope of the method chosen.

5. Define the performance parameters and the acceptance criteria.

6. Define the validation of experiments.

7. Verify the relevant performance characteristics of equipment used.

8. Qualify materials; e.g.: standards and reagents for purity, in accurate amount and of

sufficient stability.

9. Perform pre-validation of the experiments.

10. Adjust method parameters and acceptance criteria if necessary.

11. Perform full of the internal and external validation of the experiments.

12. Develop a standard operation procedure for executing the method in the routine

manner.

13. Define the criteria for revalidation.

14. Define type and frequency of system suitability, tests and analytical quality control

checks for the routine analysis.

15. Document the validation experiments and their results in the validation report.

For analytical method validation of the pharmaceuticals, guidelines from the International Conference on Harmonization (ICH), United States Food and Drug Administration (US FDA), American Association of Official Analytical Chemists (AOAC), United States Pharmacopoeia (USP) and International Union of Pure and Applied Chemists (IUPAC) are used. They provide a framework for performing such validations in an efficient and productive manner.

Performance characteristics examined while carrying out the method validation;

- Specificity
- Linearity
- Range
- Accuracy
- Precision (Repeatability and Ruggedness)
- Detection and Quantitation limit
- Robustness.
- System suitability

The different parameters of analytical method validation are discussed below as per

ICH guidelines**: -**

1) Specificity:

Specificity is the ability of an analytical procedure that can assess the analyte unequivocally in the presence of other components which may be expected to be present. Typically, these might include materials such as impurities, degradants, matrix, etc. An investigation of specificity of the procedure should be conducted during the validation of identification test or during the determination of impurities or the assay. Forced degradation studies are used for the specificity analysis. There are two types of degradation studies called as solid state forced degradation studies and solution state forced degradation studies.

2) Linearity:

The linearity of an analytical procedure is the ability of it within a given range to obtain the test results, that are directly proportional to the concentration of the analyte present in the sample. Linearity is evaluated graphically in addition to or as an alternative method to the mathematical evaluation. The evaluation is done by visually inspecting a plot of signal height or peak area with the analyte concentration.

3) Range:

The range of an analytical procedure can be explained as the interval between the upper and lower concentration of analyte that is present in the sample. This parameter including the concentrations of lower and higher for which it demonstrates that the analytical procedure has a suitable level of precision, accuracy and linearity. Normally the specified range is derived from the linearity studies done and it depends on the intended application of the procedure.

4) Accuracy:

The Accuracy of an analytical procedure is the one that express the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value that is found out. This is also termed as trueness sometimes. The Accuracy should be established across the specified range of the analytical procedure.

5) Precision:

The precision of an analytical procedure is a measure of it that can express the closeness of agreement (degree of scatter) between the series of measurements which are obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The Presicion provides an indication of random errors that can occur. It can be broken down into two terms called repeatability and the intermediate presicion. This procedure should be performed only when the entire analytical method procedure is finalized.

6) Limit of Detection (LOD):

The detection limit of an analytical procedure is said to be the the lowest amount of analyte present in a sample, which can be detected ,but that is not quantitated necessarily under the stated experimental conditions.

7) Limit of Quantitation (LOQ):

The quantitation limit of an analytical procedure is defined as the lowest amount of analyte that is present in a sample, which can be determined quantitatively with suitable precision and accuracy. It can be also said as the minimum injected amount of analyte that produces a quantitative measurement in the target matrix with acceptable precision in chromatography, typically requiring peak heights that are 10-20 times higher than the baseline.

8) Robustness:

The robustness of an analytical procedure can be said as a measure of its capacity to remain unaffected by small, but deliberate variations in the method parameters and that can provide an indication of its reliability during the normal usage. These tests can examine the effect of operational parameters on the analysis results. For the determination of robustness of an analytical procedure, some method parameters are varied within a realistic range and then the quantitative influence of the parameter on the procedure is studied, if it is within a previously specified tolerance limit, the parameter is said to be within the robustness of the method range.

9) System Suitability Testing:

The working system has to be tested for its suitability for the intended purpose. System suitability testing is an integral part of many different analytical procedures. The tests for system suitability study is mainly based on the concept that the sections of electronics, equipment, analytical operations and the samples that are to be analyzed in a whole constitute an integral system that can be evaluated as such. Here, some of the parameters such as Theoretical plates count(N), Retention time (Rt), Tailing factor(T), Resolution(R), Capacity factor (K), Reproducibility (% RSD retention time and the area for six repetitions) etc are determined and then compared against the specifications which are set prior for the proposed method. The following table lists the terms that are generally used in method validation and their recommended limits which are obtained from the analysis of the system suitability sample. System suitability parameters and Characteristics that are to be validated in the HPLC method are given in Table 1.1 and 1.2, respectively.

Table no.1.1: System suitability parameters and their recommended limits

Parameter	Recommendation
Capacity Factor (K')	The peak should be well-resolved from other peaks and the void volume, generally K' > 2
Repeatability	RSD ≤ 1% N ≥ 5 is desirable
Relative Retention	Not essential as the resolution is stated
Resolution(Rs)	Rs of > 2 between the peak of interest and the closest eluting potential interferent
Tailing Factor(T)	T ≤ 2
Theoretical Plates(N)	In general should be > 2000

Table no.1.2: Characteristics to be validated in HPLC

Characteristics	Acceptance Criteria
Accuracy/trueness	Recovery 98-102% (individual)
Precision	RSD < 2%
Repeatability	RSD < 2%
Intermediate Precision	RSD < 2%
Specificity / Selectivity	No interference
Detection Limit	S/N > 2 or 3
Quantitation Limit	S/N > 10

Linearity	Correlation coefficient $R^2 > 0.999$
Range	80 –120 %

DRUG PROFILE

(a) LAMIVUDINE

Description:

Lamivudine is a nucleoside reverse transcriptase inhibitor. Structurally; a sulfur atom replaces the 3' carbon of the pentose ring. It is used to treat Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV).It is official in IP, BP and USP.

Structure:

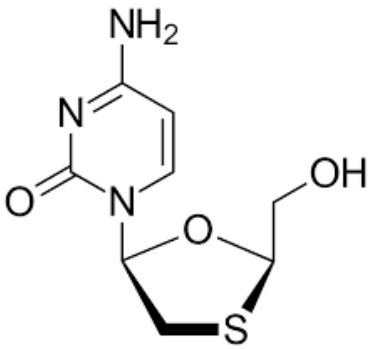

Fig no. 1.1: Structure of Lamivudine

Table no.1.3: Drug Profile of Lamivudine

CAS Number	134678-17-4
Molecular Weight	Average: 229.256
Monoisotopic	229.052111923
Molecular Formula	C8H11N3O3S
Appearance	Powder
Physical State	Solid
Solubility	2.76 mg/mL
Melting Point	160-162 °C
pK Values	pKa: 14.29 (Acidic), -0.16 (Basic).
IUPAC Name	4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1,3- oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one

Indication:

For the HIV infection treatment and chronic hepatitis B (HBV) treatment.

Pharmacodynamics:

Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV) to disrupt viral DNA synthesis. After it is phosphorylated, Lamivudine can form active metabolites that can compete for incorporation into viral DNA. Via DNA incorporation, Lamivudine metabolites competitively inhibit the activity of the HIV reverse transcriptase enzyme and act as a chain terminator of DNA synthesis. Due to the lack of a 3'-OH group, incorporated nucleoside analogues prevent the formation of a 5' to 3' phosphodiester linkage that is essential for DNA chain elongation.

Mechanism of action:

Lamivudine is chemically a synthetic nucleoside analogue and it is phosphorylated intracellularly to its active 5'-triphosphate metabolite, Lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into the viral DNA by HIV reverse transcriptase and HBV polymerase, that result in the viral DNA chain termination.

Absorption:

Lamivudine was rapidly absorbed after oral administration in HIV-infected patients. Absolute bioavailability in 12 adult patients was 86% ± 16% (mean ± SD) for the 150-mg tablet and 87% ± 13% for the oral solution. The peak serum Lamivudine concentration (Cmax) was 1.5 ± 0.5 mcg /mL when an oral dose of 2 mg/kg twice a day was given to HIV-1 patients. When given with food, absorption is slower, compared to the fasted state.

Metabolism:

Metabolism of Lamivudine is through a minor route of elimination. In human, the only known metabolite of Lamivudine is the trans-sulfoxide metabolite. The biotransformation of lamivudine is catalyzed by sulfotransferases.

Route of elimination:

The majority part of Lamivudine is eliminated as unchanged through urine by a process called active organic cationic secretion. 5.2% ± 1.4% (mean ± SD) of the administered dose was excreted as trans-sulfoxide metabolite in the urine. Lamivudine is also excreted in the human breast milk and it enters into the milk of lactating rats.

b) DORAVIRINE

Description:

In particular, Doravirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is intended to be administered in combination with other antiretroviral medicines. Doravirine is subsequently available as itself or as a more effective combination product of Doravirine (100 mg), Lamivudine (300 mg), and Tenofovir disoproxil fumarate (300 mg).

Structure:

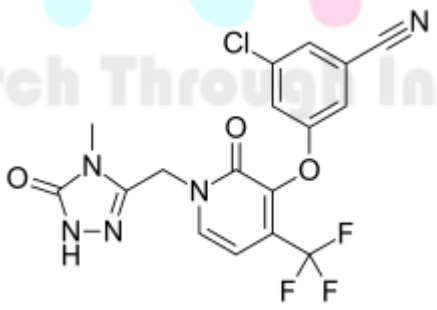

Fig no.1.2: Structure of Doravirine

Table no: 1.4: Drug profile of Doravirine

CAS Number	1338225-97-0
Molecular Weight	Average: 425.749
Monoisotopic:	425.050251565
Molecular Formula :	C17H11ClF3N5O3
Appearance	Powder
Physical State	Solid
Solubility	0.0115 mg/ml.
Storage	68°F to 77°F (20°C to 25°C)
Melting Point	283.1°C
pK Values	pKa : 7.34 (Acidic), 1.12 (Basic).
IUPAC Name	3-chloro-5-({1-[(5-hydroxy-4-methyl-4H-1,2,4- triazol-3-yl)methyl]-2-oxo-4-(trifluoromethyl)-1,2-dihydropyridin-3-yl}oxy)benzonitrile

Indication:

Doravirine is indicated for treating of HIV-1 infection in adult patients with no prior antiretroviral treatment history. It is usually used in combination with other antiretroviral agents.

Pharmacodynamics

In a clinical Phase 2 trial, when evaluating over a dose range of 0.25 to 2 times the recommended dose of Doravirine (in combination with Tenofovir) in HIV-1 infected subjects who has no antiretroviral treatment history, a kind of no exposure-response relationship for efficacy was identified for Doravirine.

Mechanism of action:

Doravirine is a pyridinone class of non-nucleoside reverse transcriptase inhibitor of HIV-1. As reverse transcriptase is the principal virally encoded enzyme with which retroviruses like HIV convert their RNA genomes into DNA for the purposes of proliferation within the host genome of infected cells, Doravirine subsequently functions by inhibiting HIV-1 replication by the non-competitive inhibition of HIV-1 reverse transcriptase (RT). However, the Doravirine does not inhibit the human cellular DNA polymerases α, ß, and the mitochondrial DNA polymerase.

Absorption:

The absolute bioavailability of the Doravirine is reported to be 64% with a Tmax of 2 hours. Following oral administration of Doravirine, all of the administered dose was recovered and it is considered to be well absorbed. The co-administration of Doravirine with food did not greatly change its pharmacokinetic profile during the clinical studies.

Metabolism:

Once administered and absorbed into the plasma circulation, the unchanged Doravirine is the major circulating component, followed by its M9 metabolite - a product of Cytochrome P450 3A 4/5 mediated oxidative metabolism, that resulting in the addition of oxygen to Doravirine's triazolone ring – which is the most abundant Doravirine metabolite present.

Route of elimination:

The primary route of elimination of Doravirine is via cytochrome P450 3A 4/5 metabolism. Only 6% of the total administered dose of Doravirine is found in the urine, unchanged. Minor levels of the unchanged drug are found in biliary or fecal elimination routes.

Dosage forms: Tablet

c) TENOFOVIR DISOPROXIL FUMARATE

Description:

Tenofovir disoproxil fumarate is in a class of medications that are called nucleoside reverse transcriptase inhibitors (NRTIs). It acts by decreasing the amount of HIV and HBV present in the blood. Tenofovir disoproxil fumarate may prevent the spread of hepatitis B to other people.

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Structure:

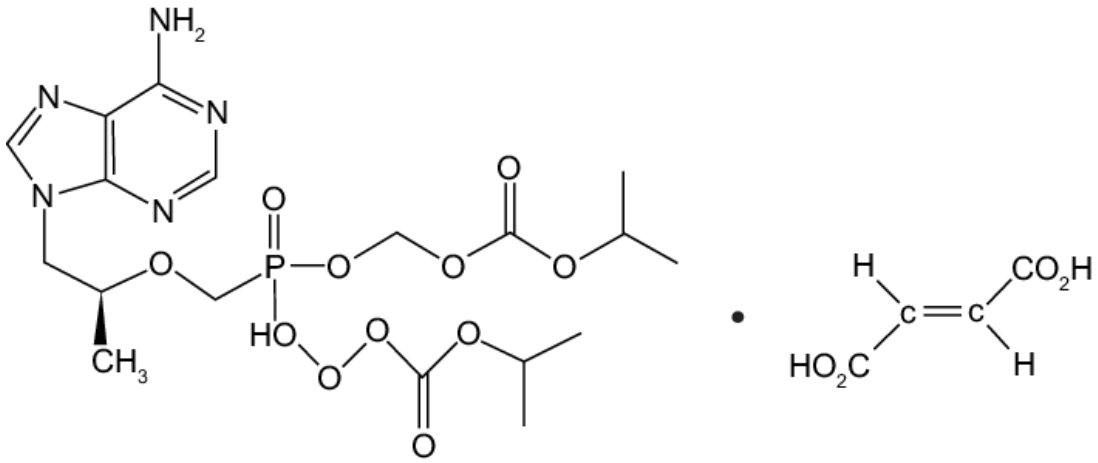


Fig no.1.3: Structure Tenofovir disoproxil fumarate

Table no.1.5: Drug profile of Tenofovir disoproxil fumarate

CAS Number	147127-20-6
Molecular Weight	Average: 287.2123
Monoisotopic	287.078340473
Molecular Formula	C9H14N5O4P
Appearance	Powder
Physical State	Solid
Solubility	1.87mg/mL
Storage	room temperature
Melting Point	279 0C
pK Values	pKa: 1.35 (Acid). 3.74(Base)
IUPAC Name	({[(2R)-1-(6-amino-9H-purin-9-yl) propan2-yl]oxy}methyl)phosphonic acid

Indication: Tenofovir disoproxil fumarate is used for HIV-1 infection and chronic hepatitis B treatment. For HIV-1 infection, Tenofovir is indicated in combination with other antiretroviral agents for people 2 years of age and older. For chronic hepatitis B patients, Tenofovir disoproxil fumarate is indicated for patients 12 years of age and older.

Pharmacodynamics; This drug prevents viral DNA chain elongation through inhibition of enzymes necessary for host cell infection viral replication in HIV-1 and Hepatitis B infections.

Mechanism of action: Tenofovir disoproxil fumarate is first hydrolysed to Tenofovir, which is then phosphorylated to its active form, Tenofovir diphosphate. The Tenofovir diphosphate inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate, deoxyadenosine 5'-triphosphate, and by DNA chain termination.

Absorption: After oral administration of the Tenofovir disoproxil fumarate to the patients with HIV infection, It is quickly absorbed and then metabolized to tenofovir. The administration of Tenofovir disoproxil 300 mg tablets after a high-fat meal increases the oral bioavailability of this drug and the administration of Tenofovir disoproxil with a light meal did not exert a relevant effect on the pharmacokinetics of Tenofovir when compared to administration under fasting conditions. The presence of ingested food slows the time to tenofovir Cmax by approximately 1 hour. Cmax and AUC of Tenofovir are 0.33 ± 0.12μg/mL and 3.32 ± 1.37μg/mL after several doses of Tenofovir disoproxil fumarate 300 mg once daily in the fed state when meal content is not controlled.

Metabolism:

Tenofovir disoproxil fumarate is the fumarate salt of prodrug called Tenofovir disoproxil. Tenofovir disoproxil is absorbed and then converted to its active form, Tenofovir, which is a nucleoside monophosphate (nucleotide) analog. The Tenofovir is converted to the active metabolite, that is Tenofovir diphosphate, a chain terminator, which uses constitutively expressed enzymes in the cell. Two phosphorylation steps are needed for convertion of Tenofovir disoproxil to its active drug form.

Route of elimination: Following the IV administration of Tenofovir disoproxil fumarate, approximately 70–80% of the dose is recovered in the urine as unchanged Tenofovir within 72 hours of the dosing. Tenofovir disoproxil fumarate is eliminated by combination processes of glomerular filtration and the active tubular secretion.

MATERIALS AND METHODS

The following materials and instruments used in the study were either AR/HPLC grade or the best possible Pharma grade available from well-known manufacturers or suppliers in the industrial and laboratory equipment market.

4.1: Drug Samples used:

The drug samples of Lamivudine, Doravirine and Tenofovir Disoproxil fumarate in combined (Delstrigo) and pure form were obtained from;

- Spectrum pharma research solutions pvt. Ltd, Hyderabad

Table no.4.1: List of Chemicals and Standards used in the study:

SL No:	Chemicals	Manufacturer	Grade
1.	Milli-Q water	Ranchem pvt Ltd	HPLC grade
2.	Methanol	Ranchem pvt Ltd	HPLC grade
3.	Acetonitrile	Ranchem pvt Ltd	HPLC grade
4.	Ortho phosphosphoric acid	Ranchem pvt Ltd	AR grade
5.	Triethylamine	Ranchem pvt Ltd	AR grade
6.	Potassium dihydrogen ortho phosphate	Ranchem pvt Ltd	AR grade
7.	0.45 µ PVDF syringe filter	MSP Lab Instruments	HPLC grade

Table no.4.2: List of Instruments used in the study:

SL No.	Instrument name	Model number	Software	Manufacturer
1.	HPLC System with quaternary pumps, Auto sampler and PDA detector	2695 Series	Empower Version 2	Waters
2.	UV double beam spectrophotometer	UV 3000 +	UV Win 5	Lab India
3.	Digital pH meter	Table top type with resolution 0.1		BVK Technology services, India
4.	Ultrasonic probe Sonicator	PKS 750 F		BVK Technology services, India
5.	Electronic Balance	PI series		Denver

4.4: Sample Processing

Diluents: Based up on the solubility of the drug, 2 diluent solutions were selected, Acetonitrile and Water (1:1) and Methanol and water (50:50)

Preparation of Standard stock solutions: Accurately weighed 37.5 mg of Lamivudine, 12.5 mg of Doravirine and 37.5 mg of Tenofovir disoproxil fumarate are transferred to three 50ml volumetric flasks separately. 10 ml of Diluent-1 (Acetonitrile and water) was added to each flask and then sonicated for 20 minutes. Flasks were made up to the mark with diluent-2 (Methanol and water) and filtered using 0.45 micron PVDF filter,then the flasks are labeled as Standard stock solution 1, 2 and 3. (Thereby the concentrations made up were 750 ppm of Lamivudine, 250 ppm of Doravirine & 750 ppm of Tenofovir).

Preparation of Standard working solutions (100% solution): For this 1 ml of the solution was pipette out from each stock solutions that were prepared prior. Then it is taken into the 10ml volumetric flasks and the volume is made up with the diluent-1. (Thereby the concentrations were made to 75 ppm of Lamivudine, 25 ppm of Doravirine & 75 ppm of Tenofovir)

Preparation of Sample stock solutions: 5 tablets of the combined dosage form (Lamivudine 300 mg + Doravirine 100 mg +Tenofovir disoproxil fumarate 300 mg) were weighed and the average weight of each tablet is calculated and then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, then 25mL of diluent-1 is added to the flask and the solution is sonicated for 50 minutes, further the volume is made up with the diluent-2 and filtered using 0.45 µ PVDF syringe filter . (3000 ppm of Lamivudine, 1000 ppm of Doravirine 3000 & ppm of Tenofovir)

Preparation of Sample working solutions (100% solution): From the filtered solutions prepared, 0.25 ml of ach solution was pipette out into 10 ml volumetric flasks and then volume is made up to 10 ml using the diluent-1. (75 ppm of Lamivudine, 25 ppm of Doravirine &75 ppm of Tenofovir)

Preparation of buffer:

0.1% OPA Buffer: 1ml of Ortho phosphoric acid was taken into a volumetric flask and then diluted to 1000 ml using the HPLC grade Milli-Q water.

0.01 N KH_2PO_4 Buffer: Accurately weighed 1.36 g of Potassium dihydrogen ortho phosphate is taken into a 1000 ml volumetric flask and about 900 ml of HPLC grade Milli-Q water is added. Then it is degassed and then sonicated for 20 minutes. Finally, the volume is made up with Milli-Q water, then its PH is adjusted to 3.5 with dilute 0.1 % Ortho phosphoric acid solution.

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Lamivudine, Doravirine and Tenofovir disoproxil fumarate. Then the solutions were injected six times to the system and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections in the results should not be more than 2%.

Specificity:

Checking of the interference in the optimized method. We should not find any interfering peaks in blank and placebo chromatograms at retention times of the selected drugs in this method. Then the method is said to be specific.

Precision:

Preparation of Standard stock solutions: Accurately weighed 37.5 mg of Lamivudine, 12.5 mg of Doravirine and 37.5 mg of Tenofovir disoproxil fumarate were transferred to three 50ml volumetric flasks separately. 10ml of Diluent-1 was added to flasks and sonicated for 20 minutes. Flasks were made up with diluents-2 (water and methanol (50:50) and labeled as Standard stock solution 1, 2 and 3. (750 ppm of Lamivudine, 250 ppm of Doravirine and 750 ppm of Tenofovir).

Preparation of Standard working solutions (100% solution): 1 ml from each stock solution was pipette out and taken into a 10ml volumetric flask and the volume is made up with diluent-1 (water and Acetonitrile). (75 ppm of Lamivudine, 25 ppm of Doravirine & 75 ppm of Tenofovir)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, then 25 ml of the diluents-1 is added and then sonicated for 50 minutes, further the volume is made up with the diluents-2 and then filtered using 0.45-micron PVDF filter. (3000 ppm of Lamivudine, 1000 ppm of Doravirine & 3000 ppm of Tenofovir disoproxil fumarate)

Preparation of Sample working solutions (100% solution): 0.25 ml of the filtered sample stock solution was transferred to 10 ml volumetric flask and volume is made up with diluent-1. (75 ppm of Lamivudine,25 ppm of Doravirine & 75 ppm of Tenofovir disoproxil fumarate).

Linearity:

Preparation of Standard stock solutions: Accurately weighed 37.5 mg of Lamivudine, 12.5 mg of Doravirine and 37.5 mg of Tenofovir disoproxil fumarate were transferred to three 50 ml volumetric flasks separately. 10 ml of Diluent-1 was added to the flasks and then sonicated for 20 minutes. Flasks were made up to the volume with diluent-2 (water and methanol (50:50) and labeled as Standard stock solution 1, 2 and 3.

25% Standard solution: 0.25 ml each from three standard stock solutions was pipette out to three volumetric flasks and then made up to 10ml using diluent-1. (18.75 ppm of Lamivudine, 6.25 ppm of Doravirine & 18.75 ppm of Tenofovir disoproxil fumarate).

50% Standard solution: 0.5 ml each from three standard stock solutions was pipette out to three volumetric flasks and then made up to 10 ml using diluent-1. (37.5 ppm of Lamivudine, 12.5 ppm of Doravirine & 37.5 ppm of Tenofovir disoproxil fumarate).

75% Standard solution: 0.75 ml each from three standard stock solutions was pipette out to volumetric flasks and made up to 10 ml with diluent-1. (56.25ppm of Lamivudine, 18.75ppm of Doravirine & 56.25ppm of Tenofovir disoproxil fumarate).

100% Standard solution: 1.0 ml each from three standard stock solutions was pipette out to volumetric flasks and made up to 10 ml with diluent-1. (75 ppm of Lamivudine, 25 ppm of Doravirine & 75 ppm of Tenofovir disoproxil fumarate).

125% Standard solution: 1.25 ml each from three standard stock solutions was pipette out to volumetric flasks and made up to 10 ml with diluent-1. (93.75 ppm of Lamivudine, 31.5 ppm of Doravirine & 93.75 ppm of Tenofovir disoproxil fumarate).

150% Standard solution: 1.5 ml each from three standard stock solutions was pipette out and volume is made up to 10 ml with diluent-1. (112.5 ppm of Lamivudine, 37.5 ppm of Doravirine & 112.5 ppm of Tenofovir disoproxil fumarate).

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 37.5 mg of Lamivudine, 12.5 mg of Doravirine and 37.5 mg of Tenofovir disoproxil fumarate are transferred to three 50 ml volumetric flasks separately. 10 ml of Diluent-1 was added to flasks and sonicated for 20 minutes. Flasks were made up to volume with diluent-2 (water and methanol (50:50) and labeled as Standard stock solution 1, 2 and 3.

Preparation of 50% Spiked Solution: 0.5 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipette out, and then made up to the mark with diluent-1.

Preparation of 100% Spiked Solution: 1.0 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipette out, and made up to the mark with diluent-1.

Preparation of 150% Spiked Solution: 1.5 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipette out, and made up to the mark with diluent-1.

Acceptance Criteria: The % Recovery for each level should be between 98.0 and 102.

Robustness:

Small deliberate changes were made in the method like Flow rate, mobile phase ratio, and temperature, but there were no recognizable change in the result and are within the range as per the ICH Guide lines.

Robustness conditions like Flow minus (0.9 ml/min), Flow plus (1.1 ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and then the samples were injected in duplicate manner. The System suitability parameters were not much affected and all the parameters were passed. % RSD of the system was within the limit.

LOD sample Preparation: 0.25 ml each from three standard stock solutions was pipette out and transferred to 3 separate 10 ml volumetric flask and made up with diluent-1. From the above solutions, 0.1 ml of Lamivudine, 0.1 ml of Doravirine and 0.1 ml of Tenofovir disoproxil fumarate solutions were transferred respectively to 10 ml volumetric flasks and then the volume is made up with the same diluent.

LOQ sample Preparation: 0.25 ml each from three standard stock solutions was pipette out and transferred to 3 separate 10 ml volumetric flasks and the volume is made up with diluent-1. From the above solutions, 0.3 ml of Lamivudine, 0.3 ml of Doravirine and 0.3 ml of Tenofovir disoproxil fumarate solutions were transferred respectively to three 10 ml volumetric flasks and then volume is made up with the same diluent.

Degradation studies:

Oxidation:

To 1 ml of stock solutions of Lamivudine, Doravirine and Tenofovir disoproxil fumarate, 1 ml of 20% hydrogen peroxide (H_2O_2) solution was added separately. These solutions were kept at 60^0C for 30 minutes. For the HPLC study, the resultant solutions were diluted to obtain concentrations of 75µg/ml, 25µg/ml and 75µg/ml of all components and 10 µl of the solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stocks solutions of Lamivudine, Doravirine and Tenofovir disoproxil fumarate, 1ml of 2N Hydrochloric acid was added and refluxed for 30 minutes at 60^0C. The resultant solutions were diluted to obtain concentrations of 75µg/ml, 25µg/ml and 75µg/ml of all components and 10µl of the solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Alkaly Degradation Studies:

To 1 ml of stock solution Lamivudine, Doravirine and Tenofovir disoproxil fumarate, 1 ml of 2N sodium hydroxide was added and refluxed for 30 minutes at 60^0C. The resultant solutions were diluted to obtain concentrations of 75µg/ml, 25µg/ml and 75µg/ml of all components and 10µl of the solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105^0c for 1 hour to study dry heat degradation. For HPLC study, the resultant solutions were diluted to obtain concentrations of 75µg/ml, 25µg/ml and 75µg/ml of all components and 10µl of the solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the concentrations of 750µg/ml, 250µg/ml and 750µg/ml solutions of Lamivudine, Doravirine and Tenofovir disoproxil fumarate to UV Light by keeping the solution contained beaker in the UV Chamber for 1 day or 200 Watt hours/m^2 in the photo stability chamber. For HPLC study, the resultant solutions were diluted to obtain concentrations of 75µg/ml, 25µg/ml and 75µg/ml of all components and 10µl of the solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing was done under neutral conditions and was studied by refluxing the drug in the water for 6 hours at a temperature of 60ºC. For HPLC study, the resultant solutions were diluted to obtain concentrations of 75µg/ml, 25µg/ml and 75µg/ml of all components and 10µl of the solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

5.1: SOLUBILITY STUDIES

The solubility study was carried out at 25 degree Celsius.

Table no.5.1: Solubility studies

Solvent name	Lamivudine	Doravirine	Tenofovir disoproxil fumarate
Water	soluble	insoluble	Slightly soluble
Methanol	Sparingly soluble	Slightly soluble	soluble
Acetonitrile	soluble	soluble	soluble

5.2: SELECTION OF WORKING WAVELENGTH (LAMDA MAX)

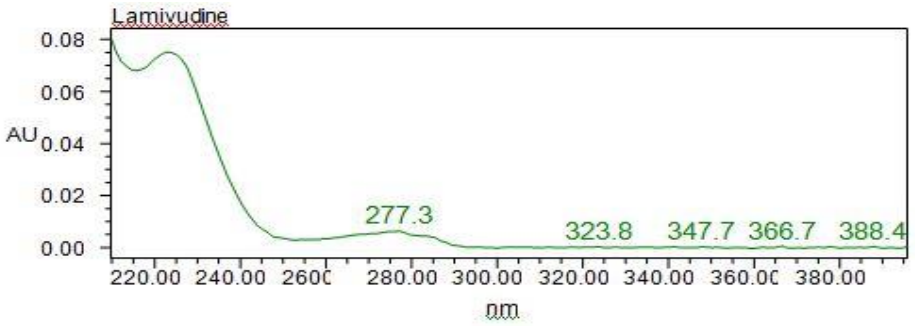


Fig no. 5.1: UV Spectrum of Lamivudine

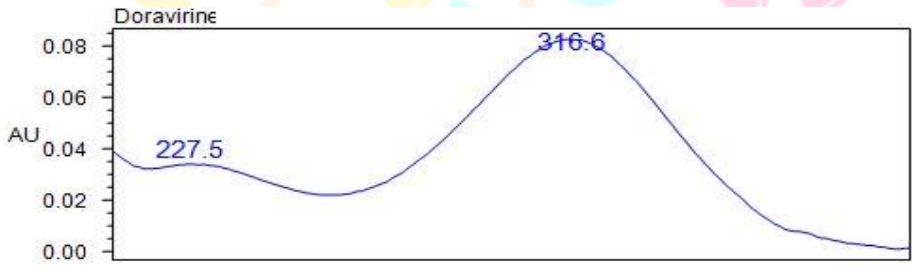


Fig no. 5.2: UV Spectrum of Doravirine

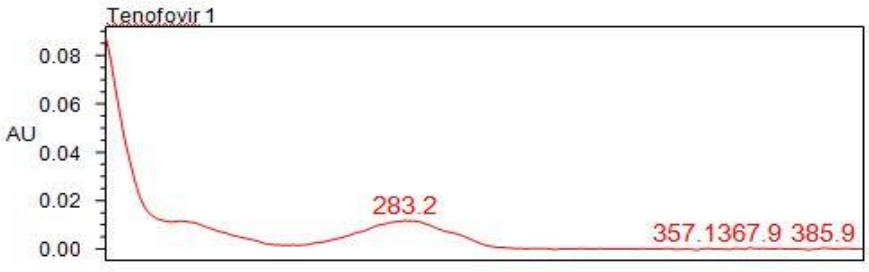


Fig no.5.3: UV Spectrum of Tenofovir disoproxil fumarate

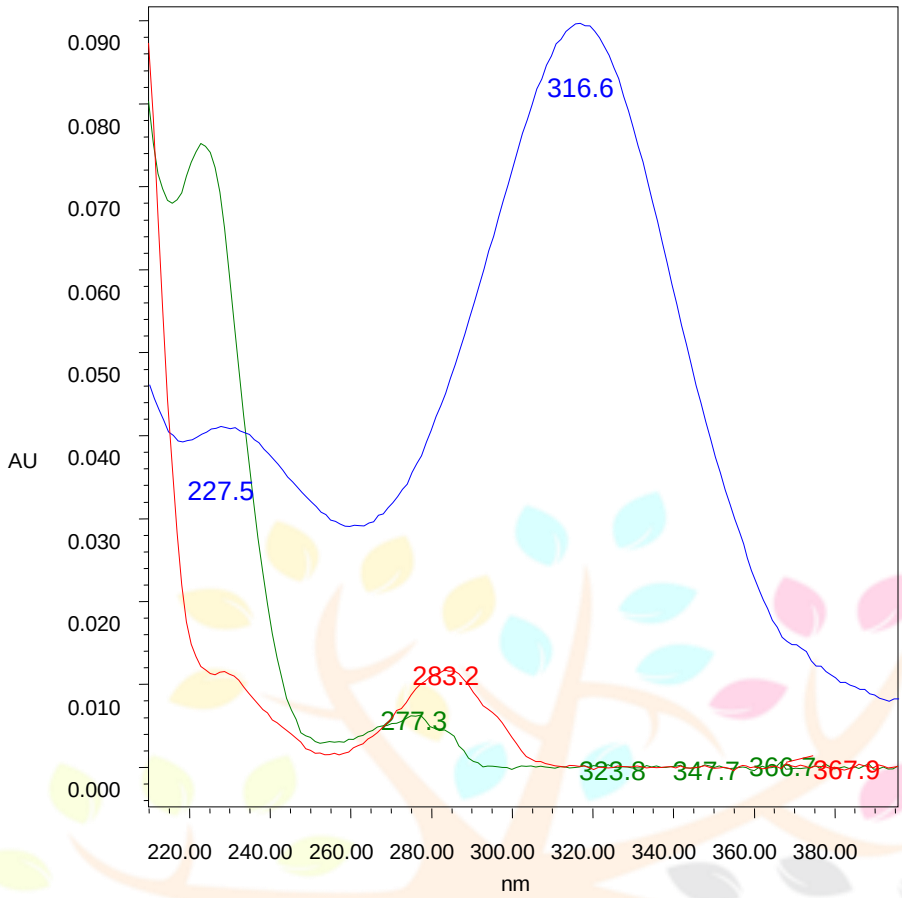


Fig no.5.4: Overlay UV spectrum of Lamivudine, Doravirine and Tenofovir.

Result: The wavelength of 272 nm has been selected.

5.3: RP-HPLC METHOD DEVELOPMENT

Method development: Method development for RP-HPLC was done by changing various columns, mobile phase ratios, buffers and its pH etc.

Trial 1:

Chromatographic conditions:

Mobile phase : 0.01N KH_2PO_4 : Acetonitrile (50:50 V/v)

Flow rate : 1 ml/min

Column : Discovery C18 150 x 4.6 mm, 5µ.

Detector wave length : 272.0 nm

Column temperature : 30°C

Injection volume : 10µL

Run time : 7 min

Diluent : 0.01 N KH_2PO_4 : Acetonitrile (50:50 v/v).

Results : In This trail only two peaks was eluted and the Doravirine peak was not eluted and also all system suitability parameters was not within the limit. So further trail was carried out

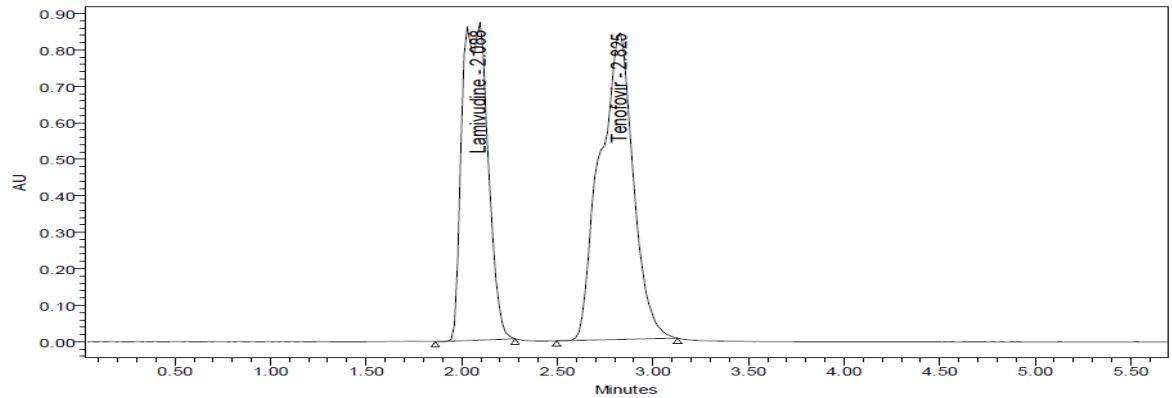


Fig no.5.5: Trial chromatogram 1

Trial 2:

Chromatographic conditions:

Mobile phase : Acetonitrile: 0.01N KH_2PO_4 (50:50 v/v)

Flow rate : 1 ml/min

Column : BDS C18 150 x 4.6 mm, 5µ.

Detector wave length : 272.0 nm

Column temperature : 30°C

Injection volume : 10.0µL

Run time : 8 min

Diluent : 0.01 N KH_2PO_4: Acetonitrile (50:50\v/v).

Results : In this trail all peaks were eluted but all peak shape was not good and system suitability parameters were not good and Lamivudine and Tenofovir was eluted with void volume so further trail was carried out.

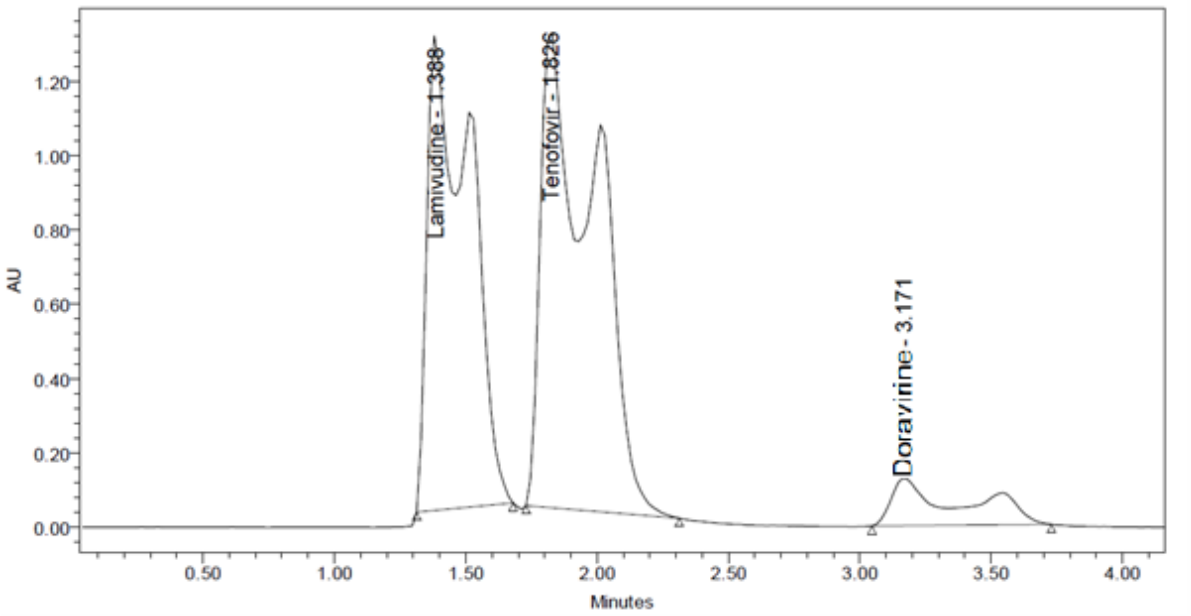


Fig no. 5.6: Trial chromatogram 2

Trial 3:

Chromatographic conditions:

Mobile phase : 0.01N KH_2PO_4 and Acetonitrile (50:50 v/v)

Flow rate : 1 ml/min

Column : Symmetry C18 150 x 4.6 mm, 5µ.

Detector wave length : 272.0 nm

Column temperature : 30°C

Injection volume : 10.0µL

Run time : 10 min

Diluent : 0.01 N KH_2PO_4: Acetonitrile (50:50 v/v).

Results : In this Trail only two peaks were released and the Doravirine peak was not eluted, so further trial is carried out.

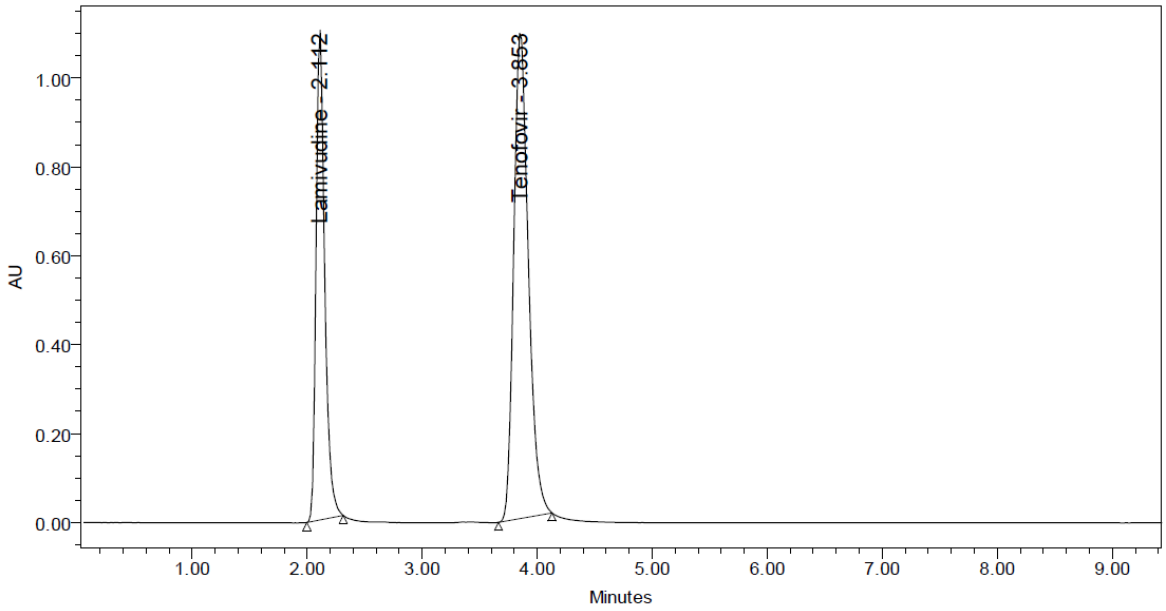


Fig no.5.7: Trial chromatogram 3

Trial: 4

Mobile phase : 0.01N KH_2PO_4: ACN (50:50 V/v)

Flow rate : 1ml/min

Column Used : Altima C18 150 x 4.6 mm, 5µ.

Wavelength : 272.0 nm

Temperature : 30°C

Injection Volume : 10.0µl

Buffer : 0.01N KH_2PO_4

Run tim : 8.0 min

Diluent : 0.01 N KH_2PO_4 : Acetonitrile (50:50 V/v).

Observation : In this trail also only two peaks were eluted Doravirine

Peak was not eluted so, further trail is carried out.

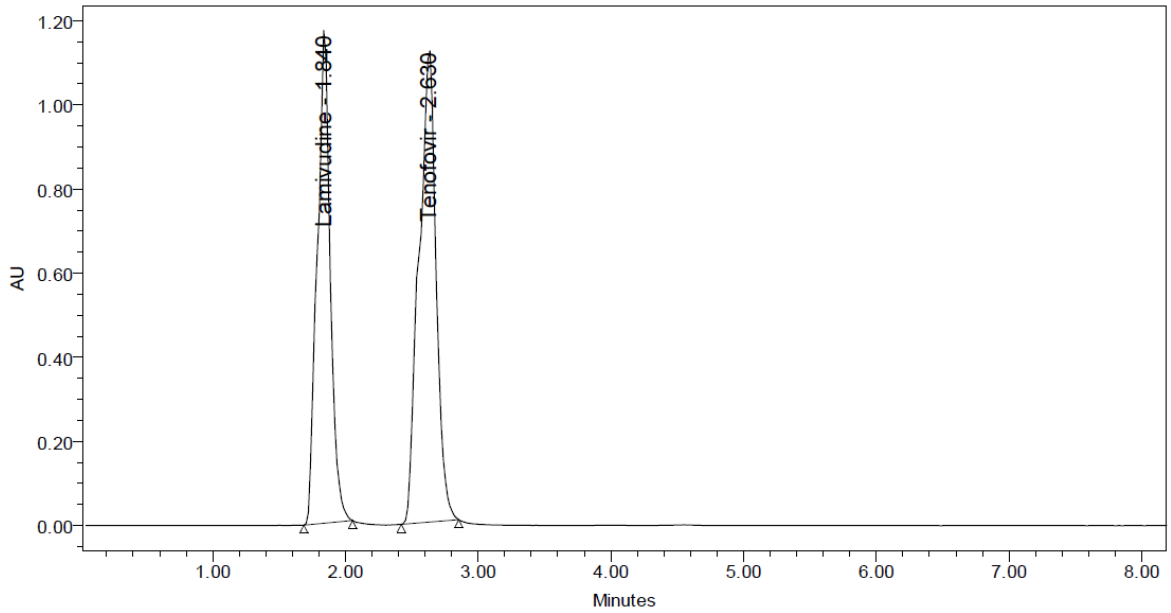


Fig no.5.8: Trial chromatogram 4

Trial: 5

Mobile phase : 0.01N KH2PO4: ACN (50:50 v/v)

Flow rate : 1ml/min

Column Used : Kromosil C18 150 x 4.6 mm, 5µ.

Wavelength : 272.0 nm

Temperature : 30°C

Injection Volume : 10.0µl

Buffer : 0.01N KH_2PO_4

Run time : 7.0 min

Diluent : 0.01 N KH_2PO_4: Acetonitrile (50:50 v/v).

Observation : In this trail all peaks were eluted with good peak shape but

More retention time so, further trial was carried out.

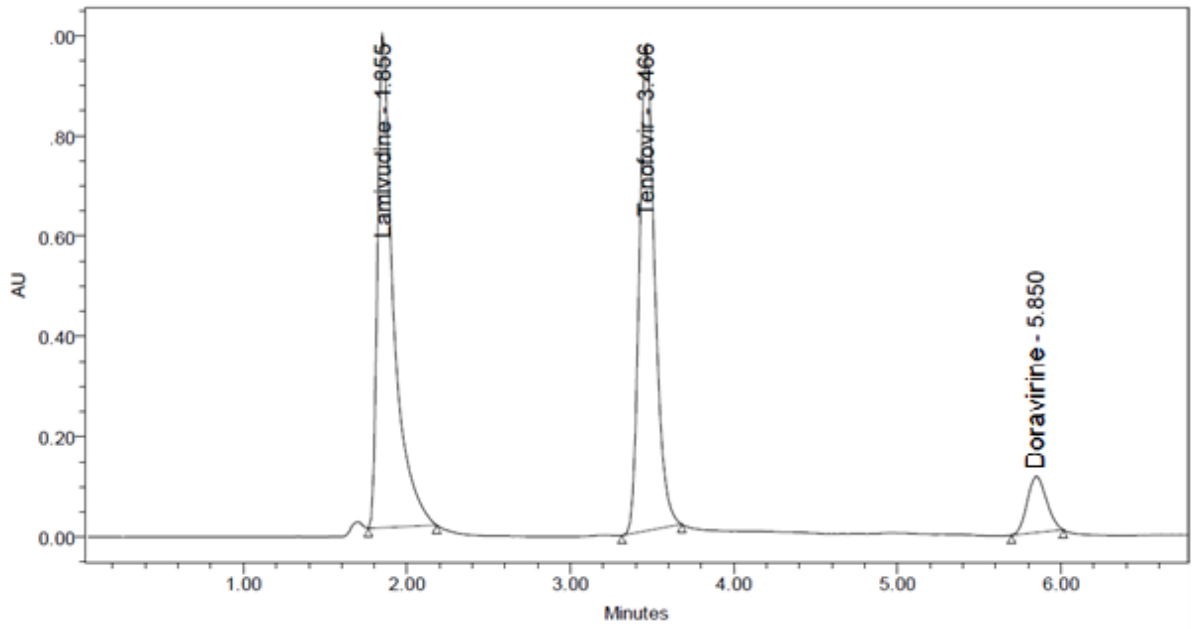


Fig no.5.9: Trial chromatogram 5

Optimized Method

Column Used : Kromasil C18 250 x 4.6 mm, 5µ.

Buffer : 0.1% OPA

Mobile phase : Buffer : Acetonitrile (60:40 v/v)

Flow rate : 1.0 ml/min

Diluent : 0.1% OPA : Acetonitrile (50:50 v/v).

Wavelength : 272.0 nm

Temperature : 30°C

Injection Volume : 10µl

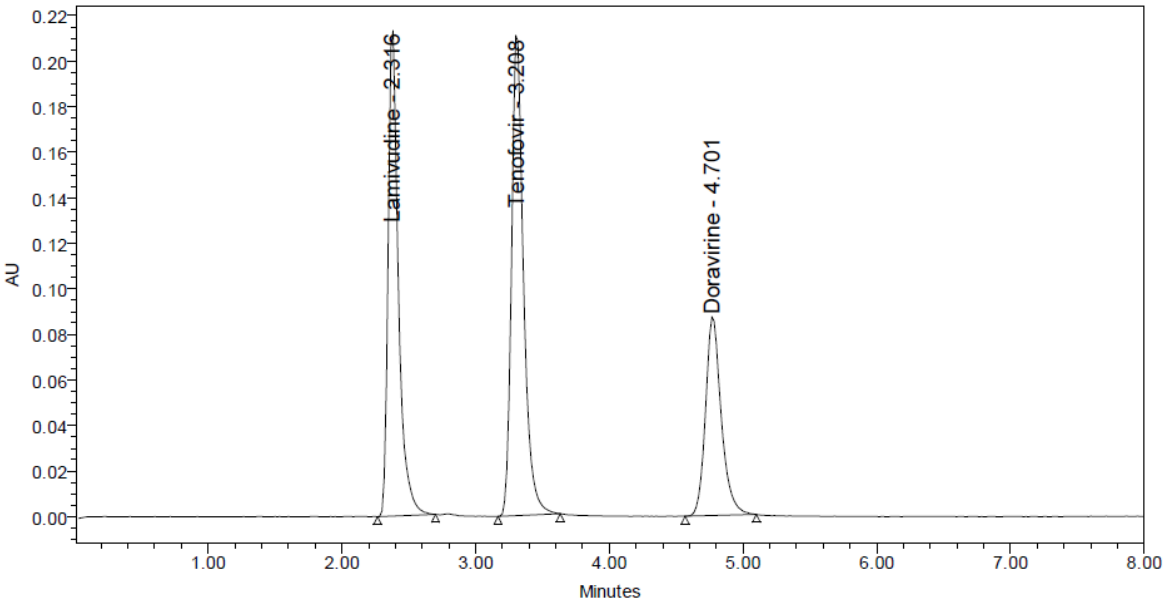


Fig no.5.10: Optimized chromatogram

Observation: Lamivudine, Doravirine and Tenofovir disoproxil fumarate were eluted at 2.316 min, 4.701 min and 3.208 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Optimized Method: Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

5.4: METHOD VALIDATION PARAMETERS

5.4.1: System suitability:

Table no.5.2: System suitability parameters for Lamivudine,Doravirine and Tenofovir.

SL No	Lamivudine.				Doravirine				Tenofovir			
Ini	RT (min)0	TP	Tailing	Reso	RT (mi	TP	Tailing	Resolution	RT (min)	TP	Tailing	Resoluti
1	2.304	5130	1.39	4.1	4.537	7967	1.29	7.8	3.185	6756	1.31	6.1
2	2.314	5172	1.35	4	4.614	7881	1.24	7.7	3.196	6994	1.3	6
3	2.335	5152	1.41	4.2	4.616	7953	1.24	7.7	3.233	6748	1.33	6.2
4	2.353	5132	1.38	4	4.686	8077	1.24	7.5	3.247	6739	1.27	6
5	2.362	5255	1.35	4.3	4.688	8096	1.23	7.6	3.253	6957	1.31	5.9
6	2.364	5101	1.38	4	4.701	7917	1.25	7.8	3.267	6924	1.33	6.2

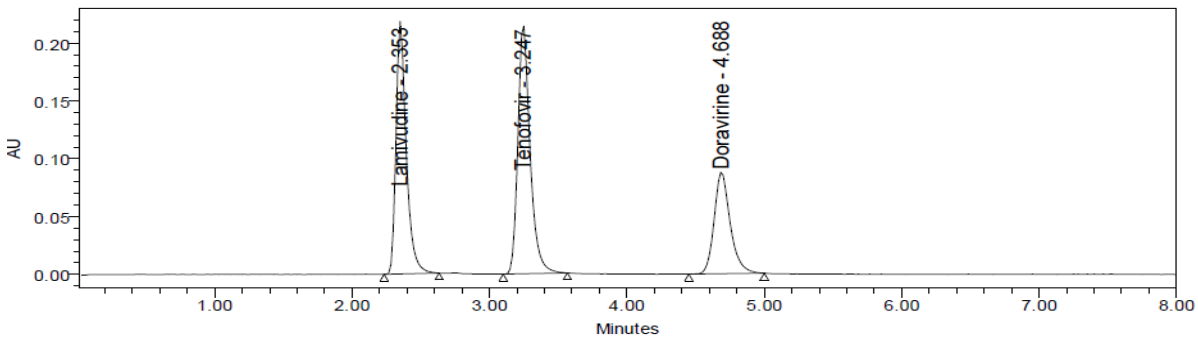


Fig no.5.11: System suitability chromatogram

Discussion: plate count of the Lamivudine was 5240±54, Doravirine was 7982±87 and of Tenofovir disoproxil fumarate was 6853±118, and tailing factor of Lamivudine was 1.4±0.023, Doravirine was 1.2±0.021and of Tenofovir disoproxil fumarate was 1.3±0.022, resolution between Lamivudine and Tenofovir disoproxil fumarate was 6.1±0.121 and resolution between Tenofovir disoproxil fumarate and Doravirine was 7.7±0.117.According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitability parameters were passed and were within the limits.

5.4.2: Specificity:

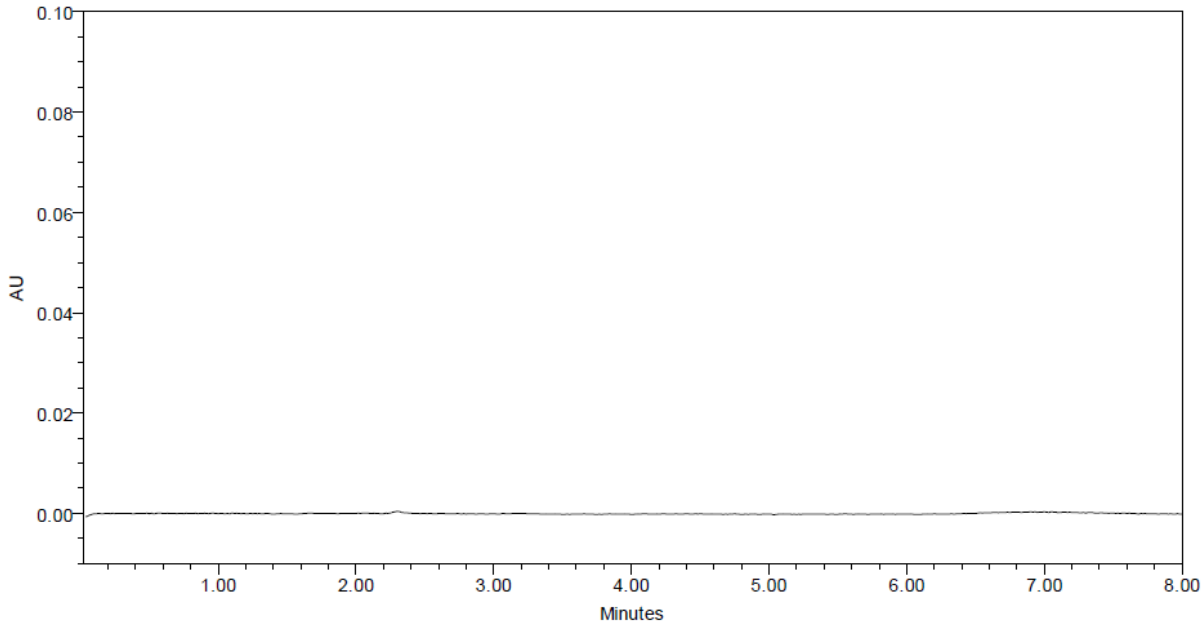


Fig no.5.12: Blank chromatogram.

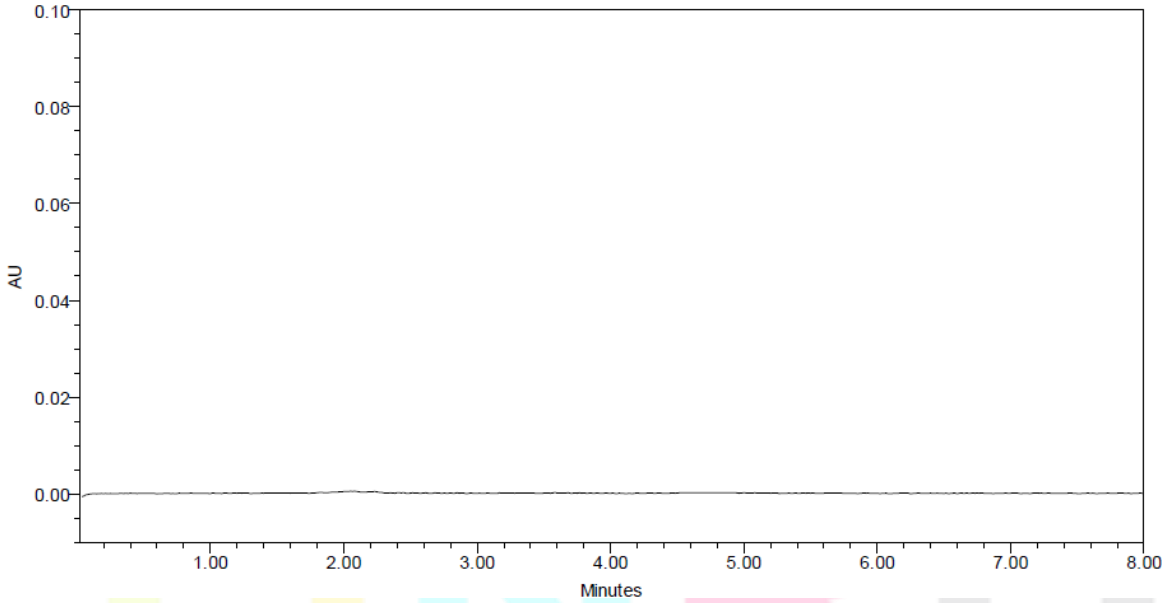


Fig no.5.13 : Placebo chromatogram

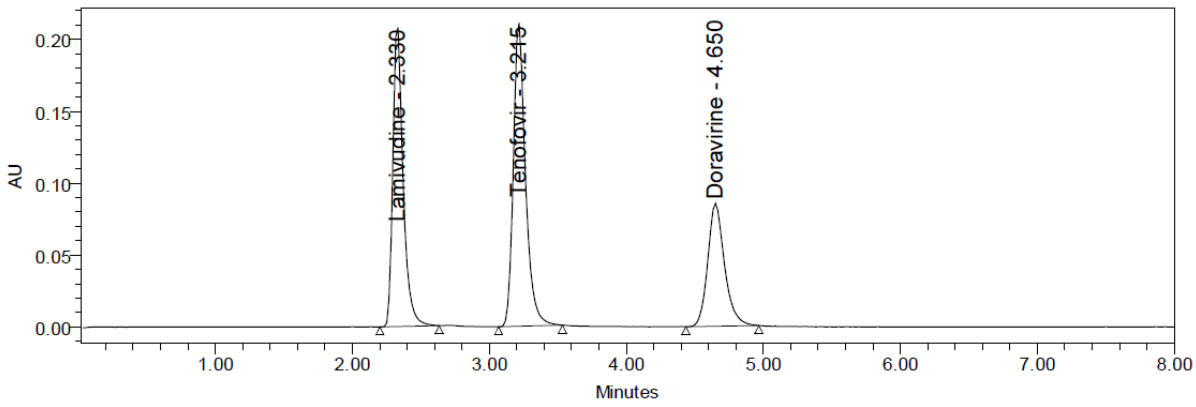


Fig no.5.14 : Optimized chromatogram

Discussion: Retention times of Lamivudine, Tenofovir disoproxil fumarate and Doravirine were 2.330 min, 3.215 min and 4.650min respectively. We did not found any interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

5.4.3: Linearity:

Table no.5.3: Linearity table for Lamivudine, Tenofovir and Doravirine.

Lamivudine.		Tenofovir		Doravirine	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
18.75	283811	18.75	324530	6.25	176255
37.5	558736	37.5	658776	12.5	355168
56.25	854208	56.25	996858	18.75	536825
75	1105420	75	1326351	25	706756
93.75	1413962	93.75	1618531	31.25	885352
112.5	1682085	112.5	1952918	37.5	1050923

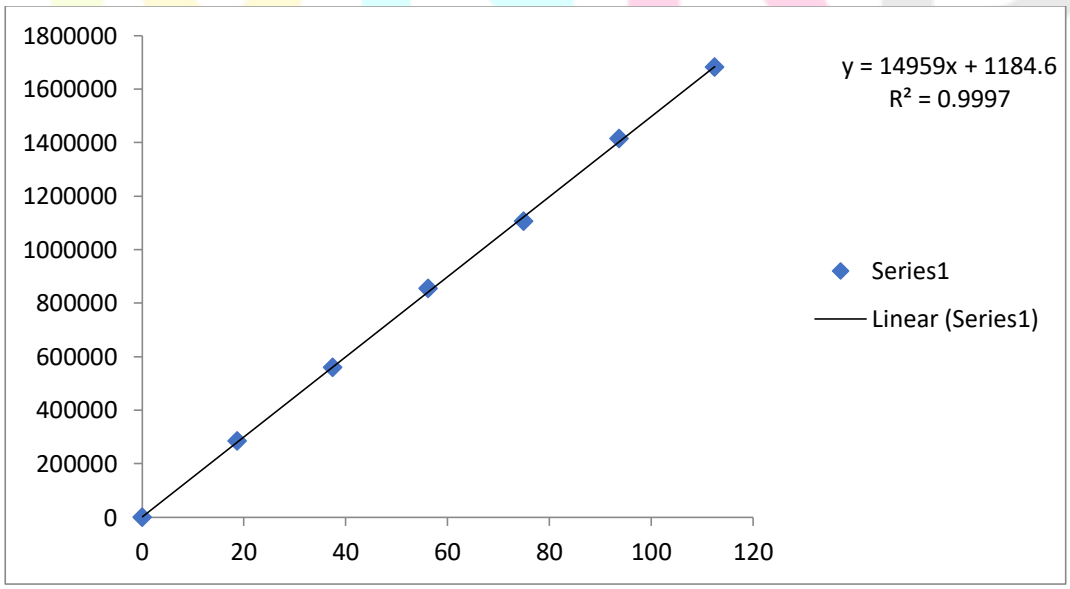


Fig no.5.15: Calibration curve of Lamivudine.

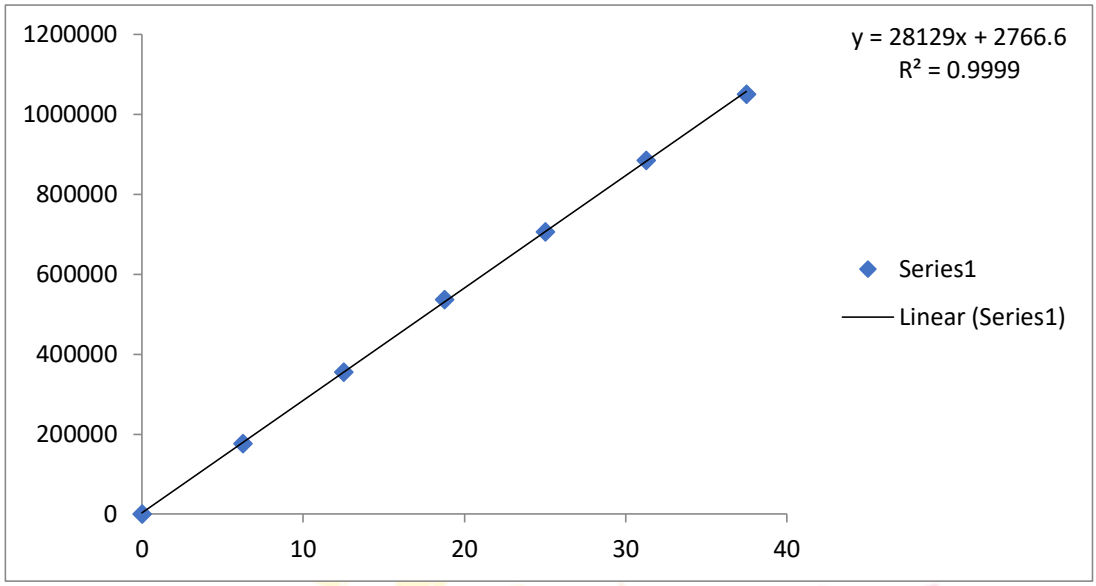


Fig no.5.16: Calibration curve of Doravirine

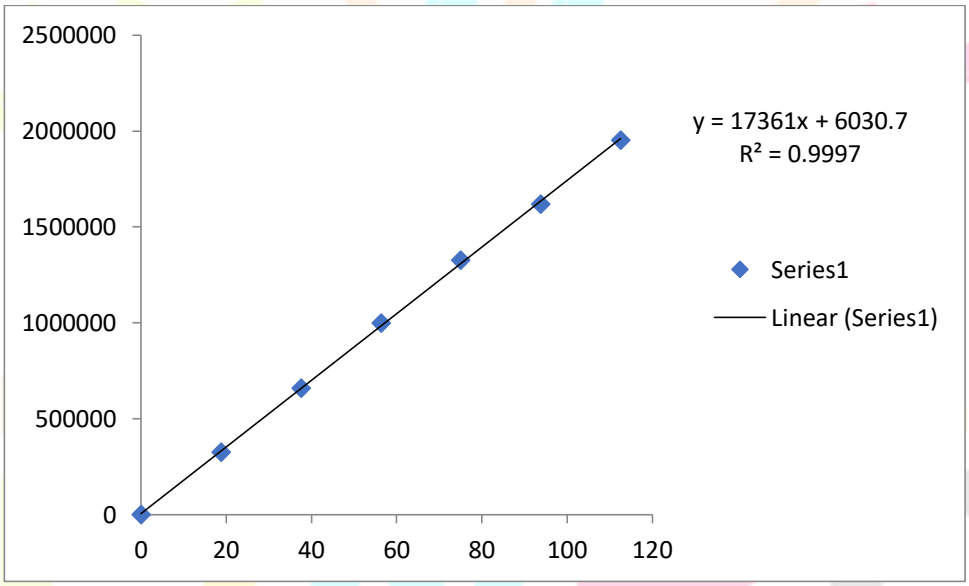


Fig no. 5.17: Calibration curve of Tenofovir disoproxil fumarate

Discussion:

Six linear concentrations of Lamivudine (18.75-112.5µg/ml), Tenofovir disoproxil fumarate (18.75-112.5µg/ml) and Doravirine (6.25-37.5µg/ml) were injected in a triplicate manner. Average areas were mentioned above and linearity equations obtained for Lamivudine was y = 14959x + 1184., Tenofovir disoproxil fumarate was y = 17361x + 6030. and of Doravirine was y = 28129x + 2766. Correlation coefficient obtained was 0.999 for all the three drugs.

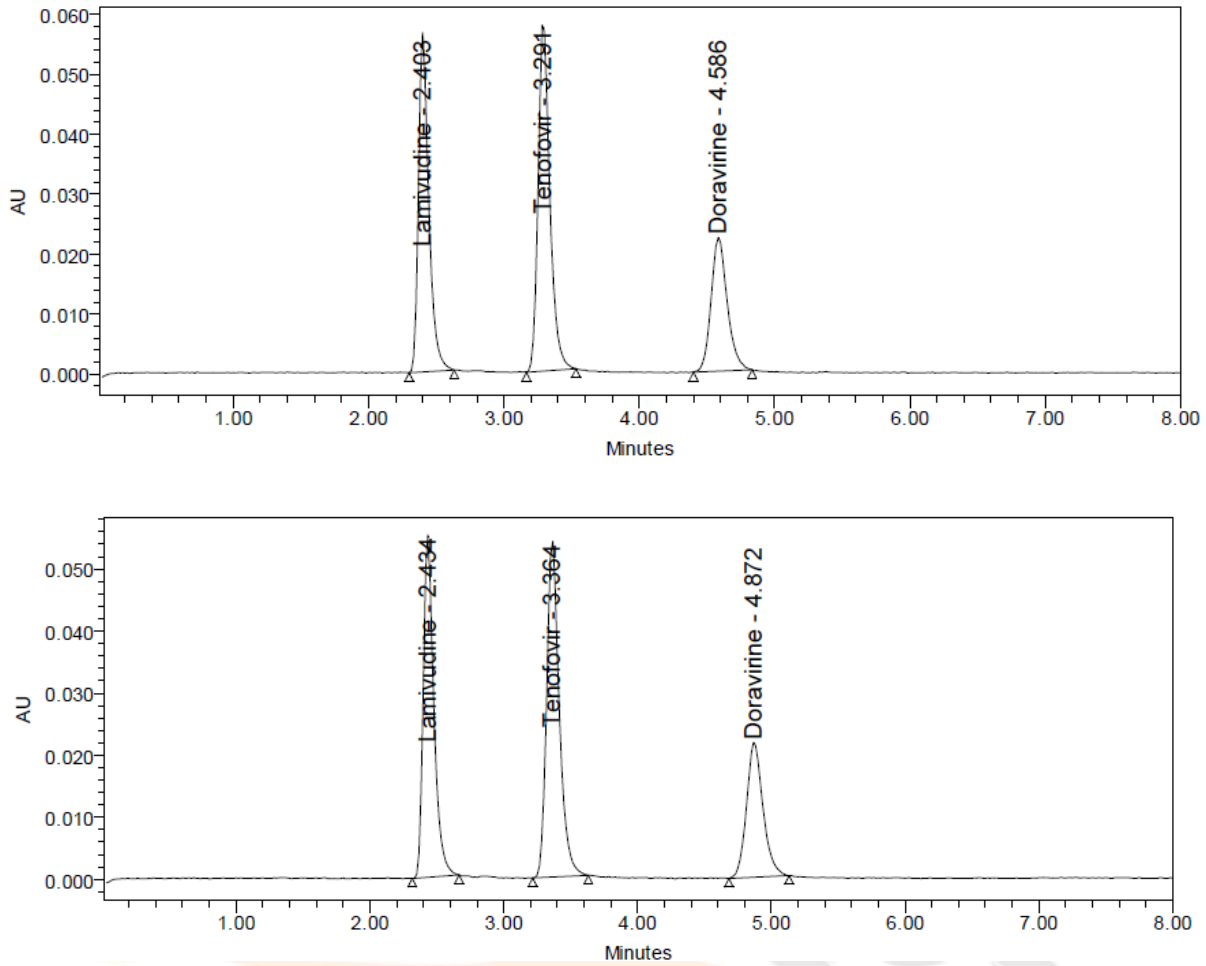
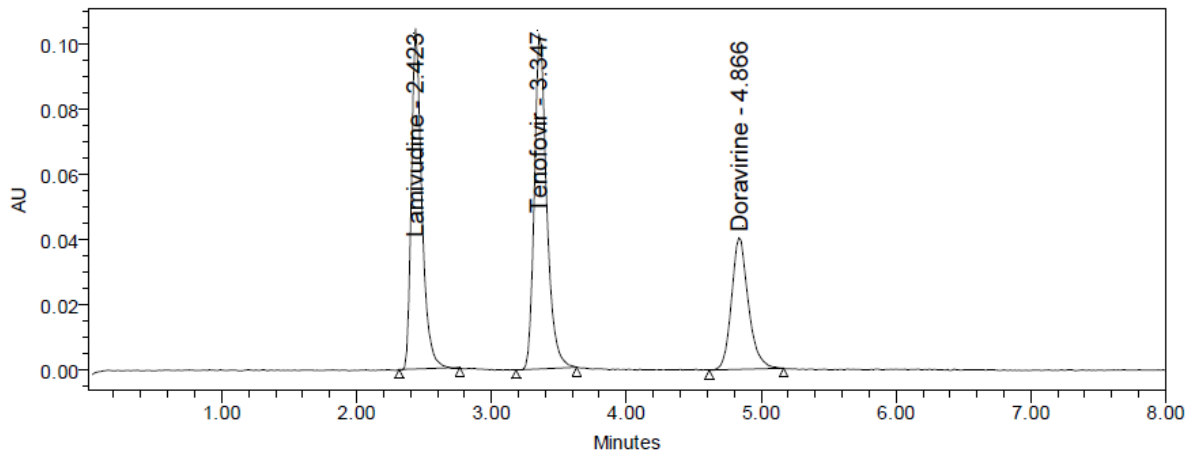


Fig no.5.18: Linearity 25% Chromatograms



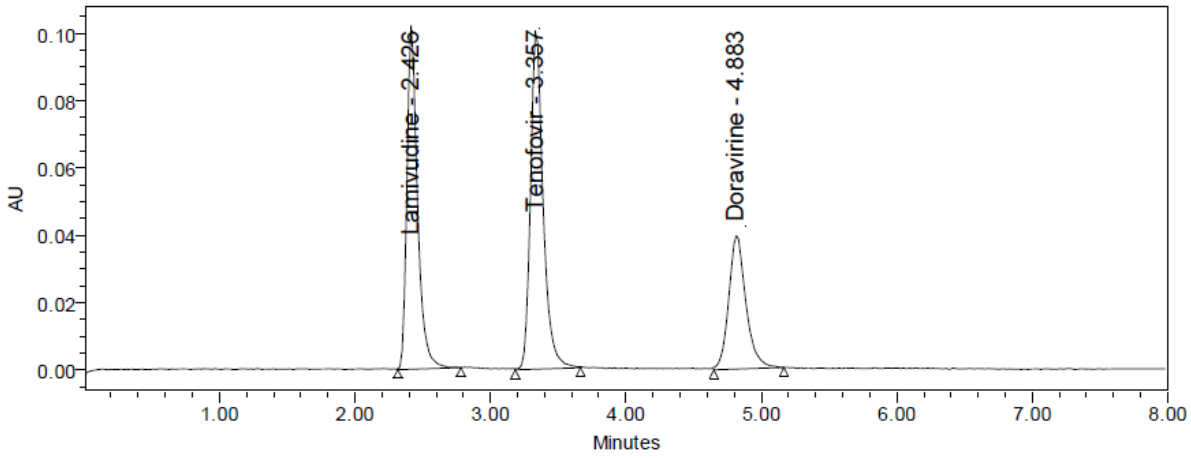


Fig no.5.19: Linearity 50% Chromatograms

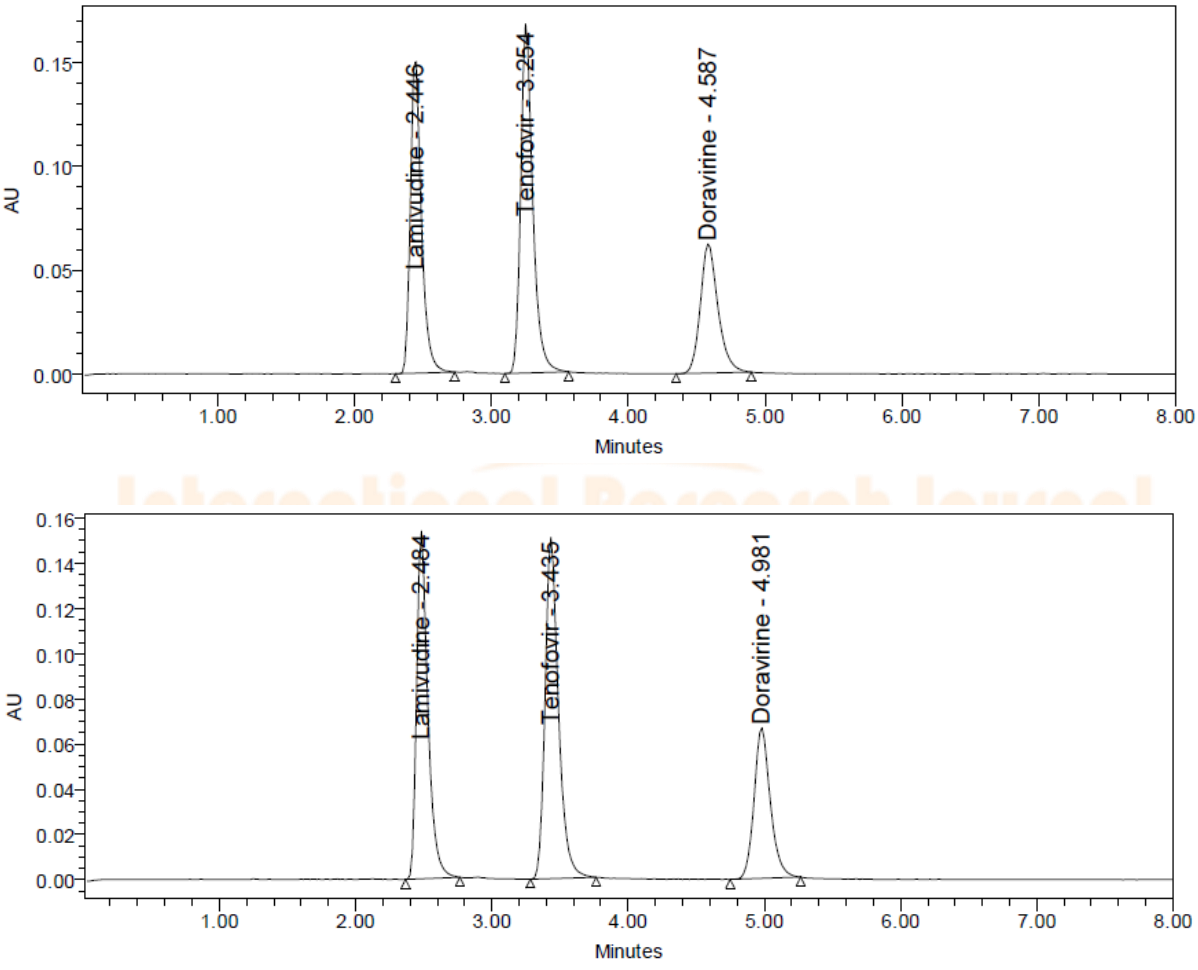


Fig no.5.20: Linearity 75% Chromatogram

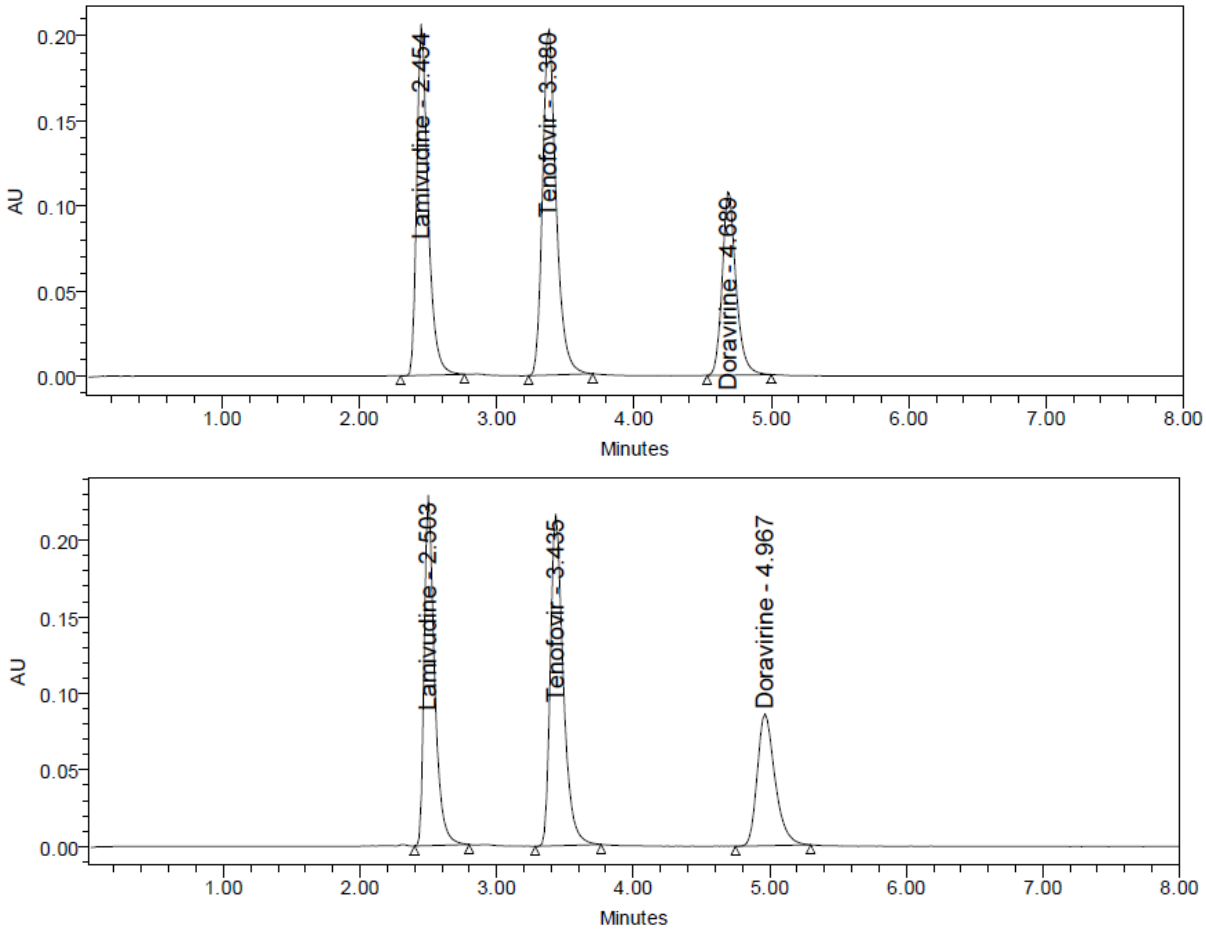


Fig no.5.21: Linearity 100% Chromatograms

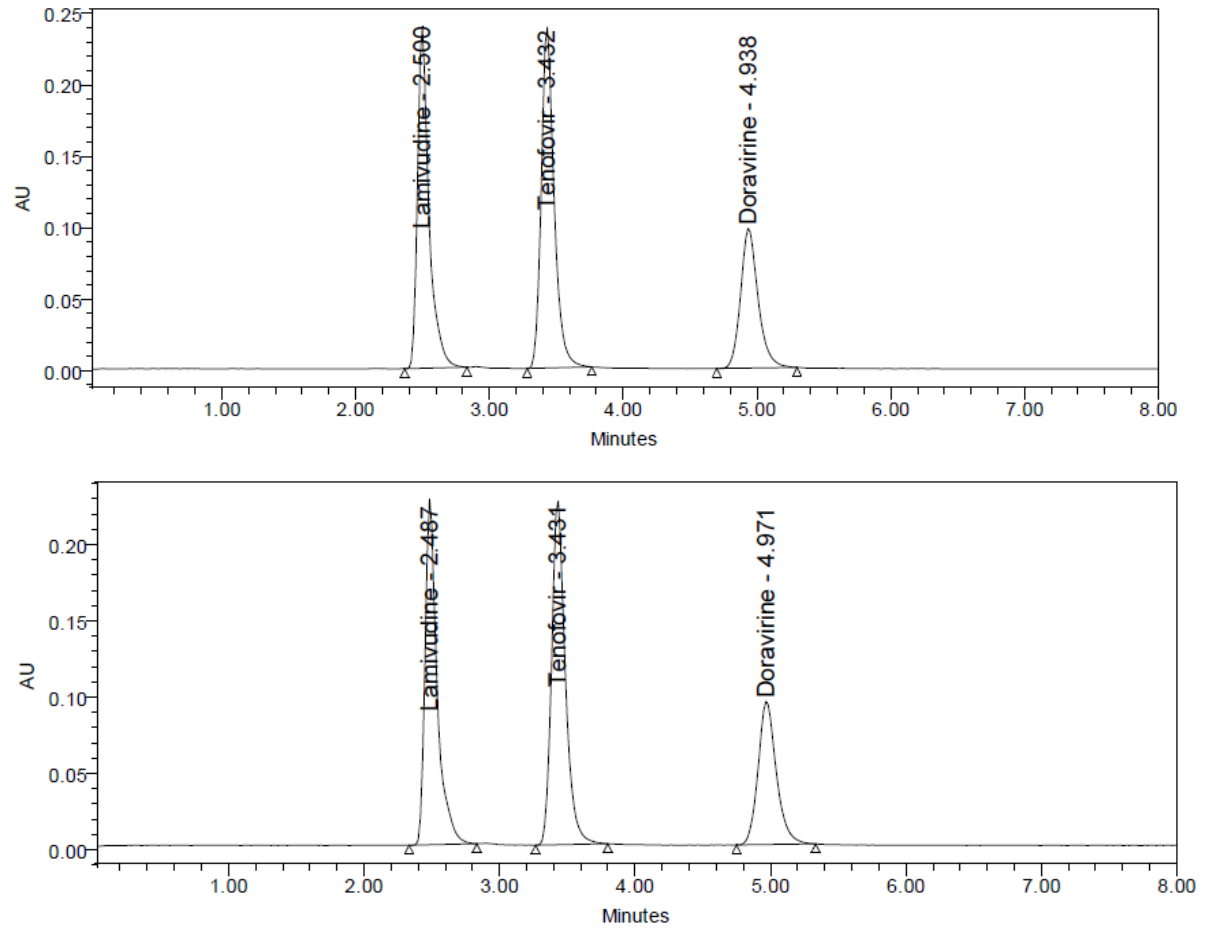


Fig no.5.22: Linearity 125% Chromatograms

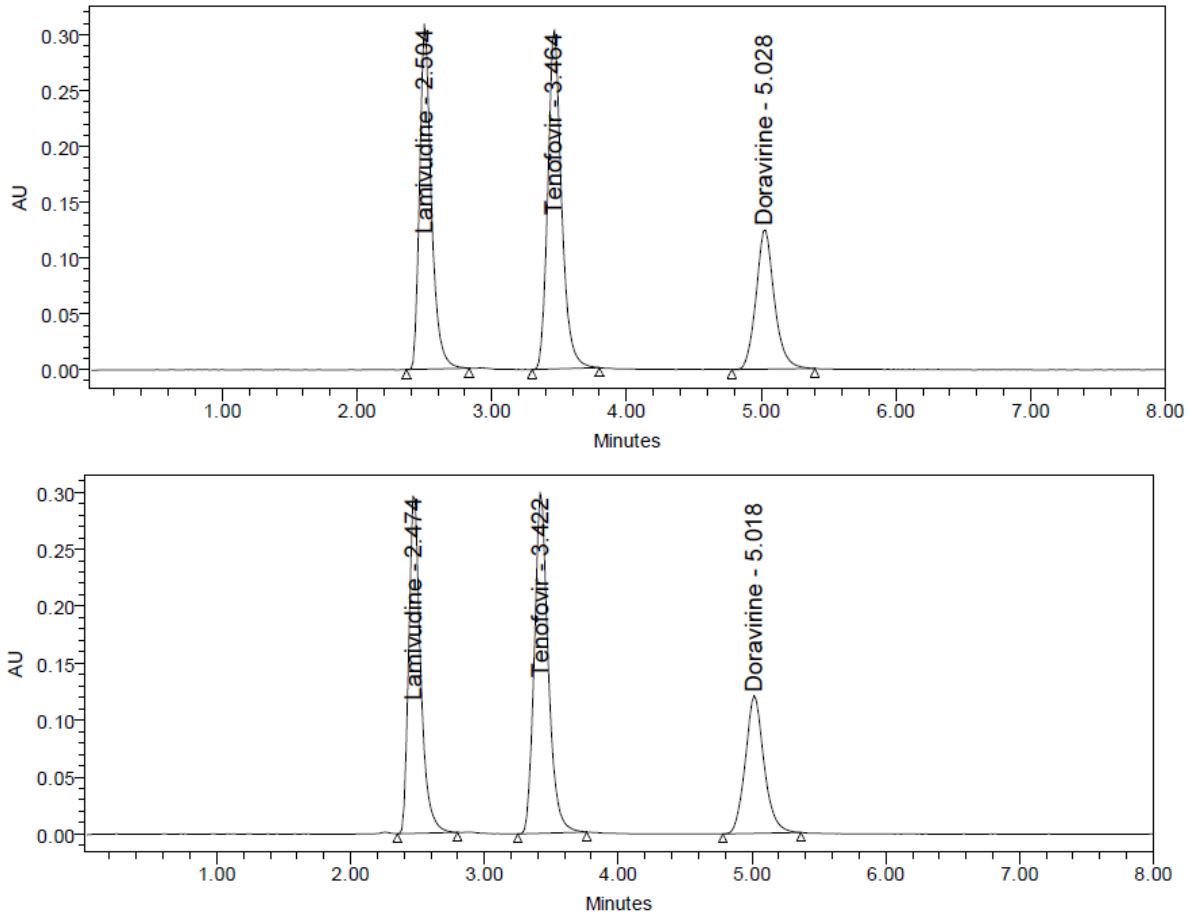


Fig no.5.23: Linearity 150% Chromatograms

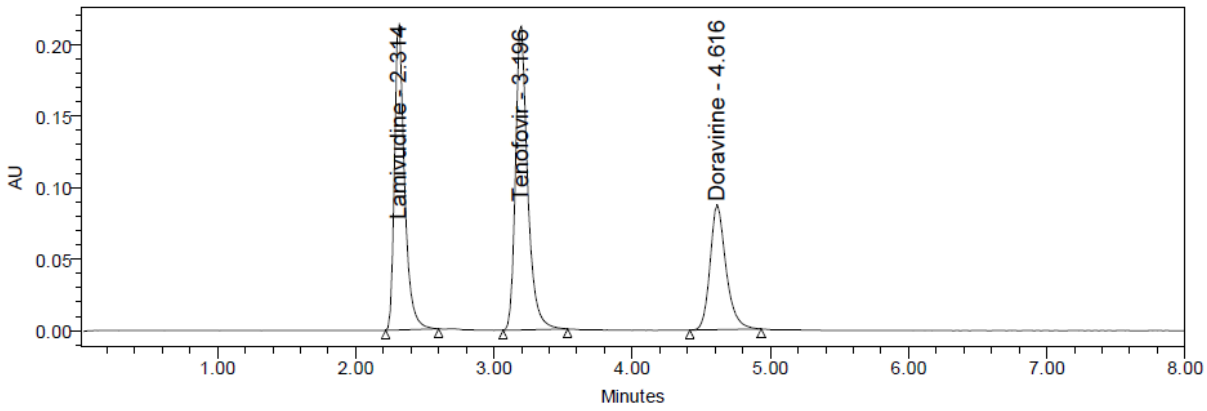
Precision:

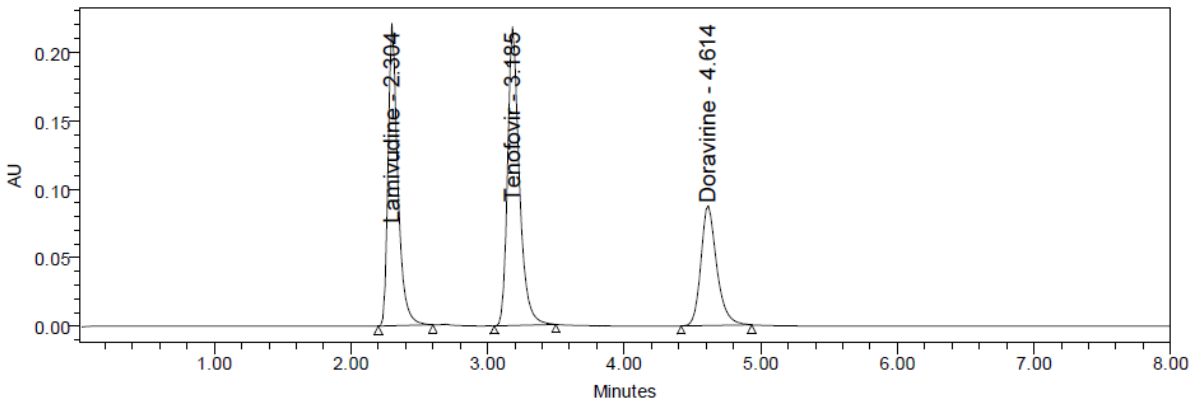
System Precision:

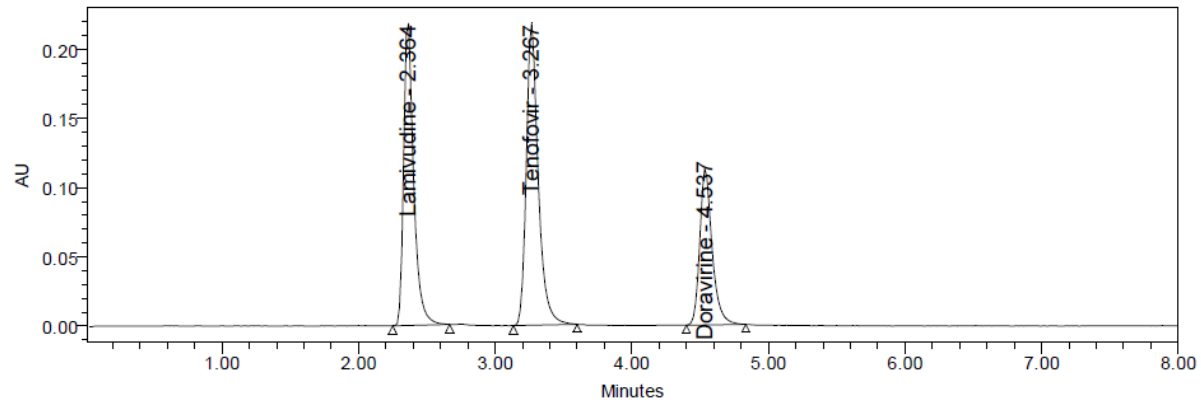
Table no.5.4 : System precision table of Lamivudine, Tenofovir disoproxil fumarate and Doravirine .

S. No	Area of Lamivudine.	Area of Tenofovir	Area of Doravirine
1.	1085597	1287919	708646
2.	1099294	1286219	703014
3.	1109959	1312269	700476
4.	1116113	1317900	699353
5.	1096317	1295165	713021

6.	1103838	1312755	715080
Mean	1105104	1302038	706598
S.D	10718.7	13913.7	6636.4
%RSD	1.0	1.1	0.9







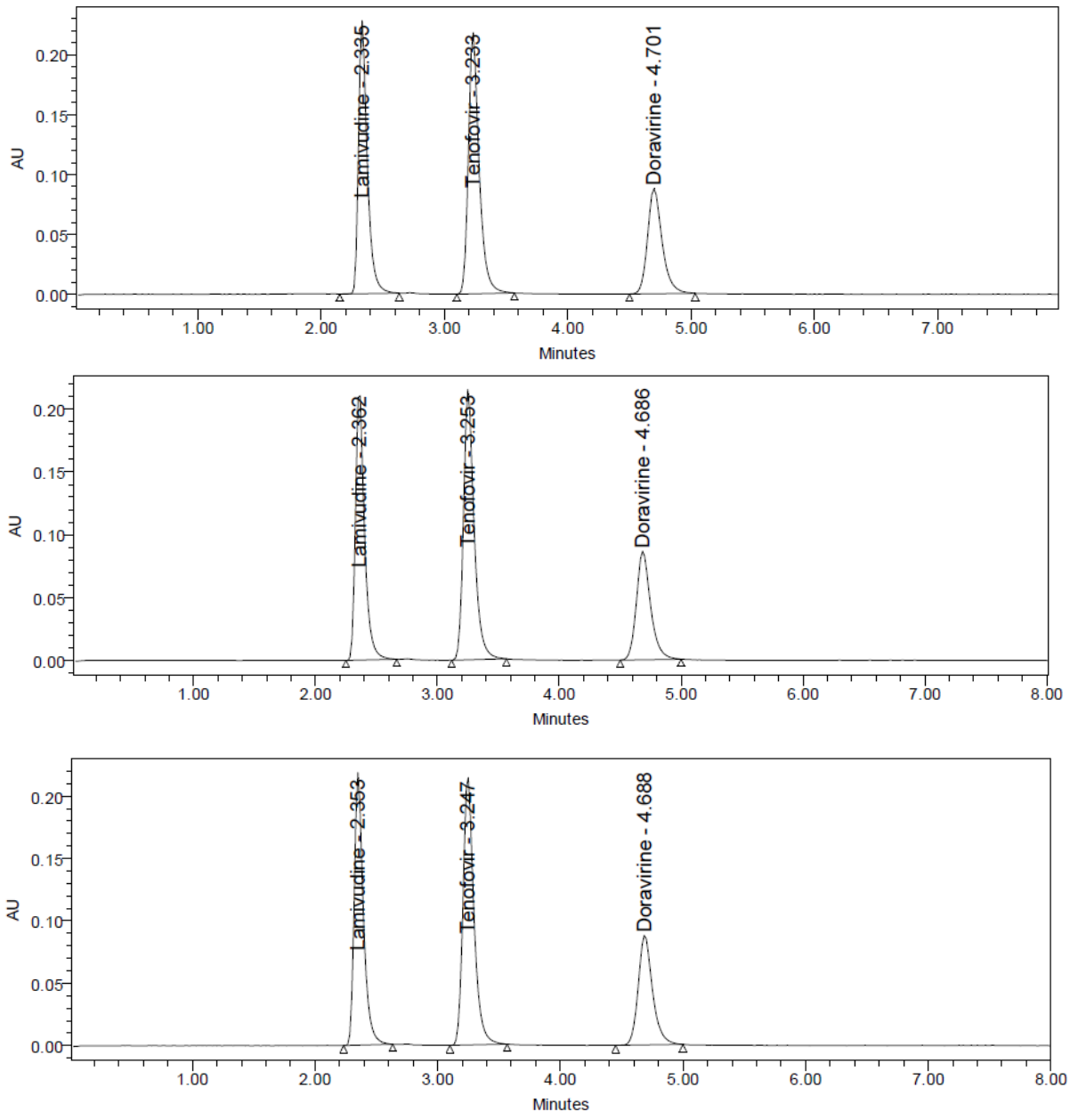


Fig no.5.24: System precision chromatograms

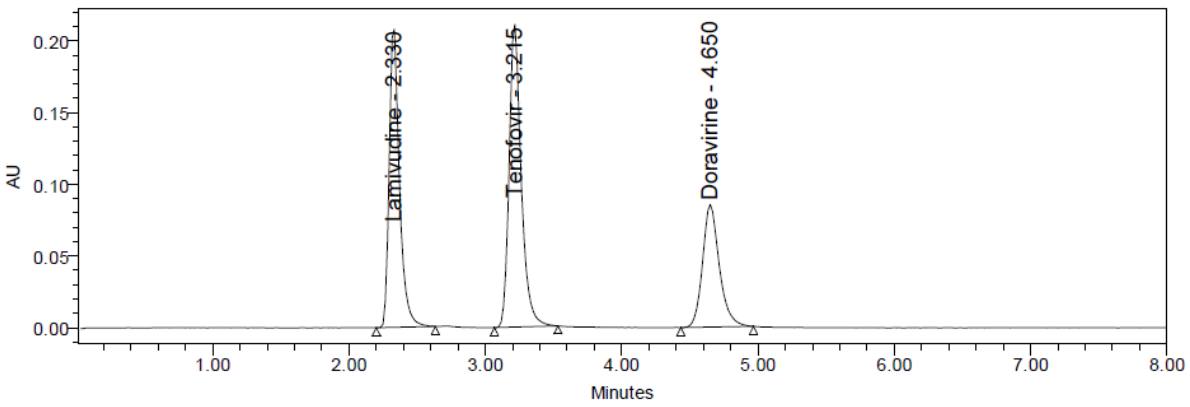
Discussion: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for three drugs and obtained as 1.0%, 1.1% and 0.9% respectively for Lamivudine, Tenofovir disoproxil fumarate and Doravirine. As the limit of Precision was less than "2" the system precision was passed in this method.

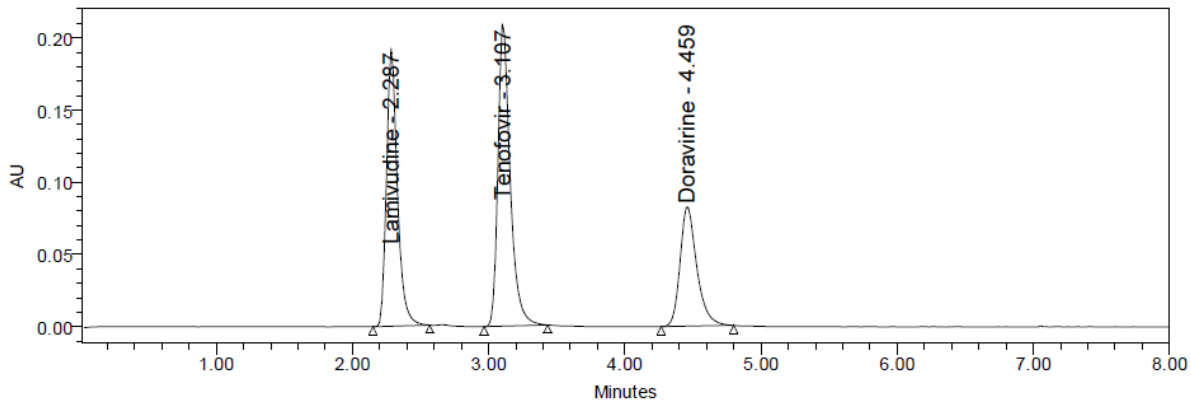
Repeatability:

Table no.5.5 : Repeatability table of Lamivudine, Tenofovir disoproxil fumarate and Doravirine .

S. No	Area of Lamivudine.	Area of Tenofovir	Area of Doravirine

1.	1089546	1299037	699114
2.	1105404	1284671	703230
3.	1099697	1297801	708036
4.	1094896	1301120	708933
5.	1101686	1289286	707985
6.	1096832	1295271	712907
Mean	1114685	1294531	706701
S.D	5545.3	6319.6	4827.8
%RSD	0.5	0.5	0.7





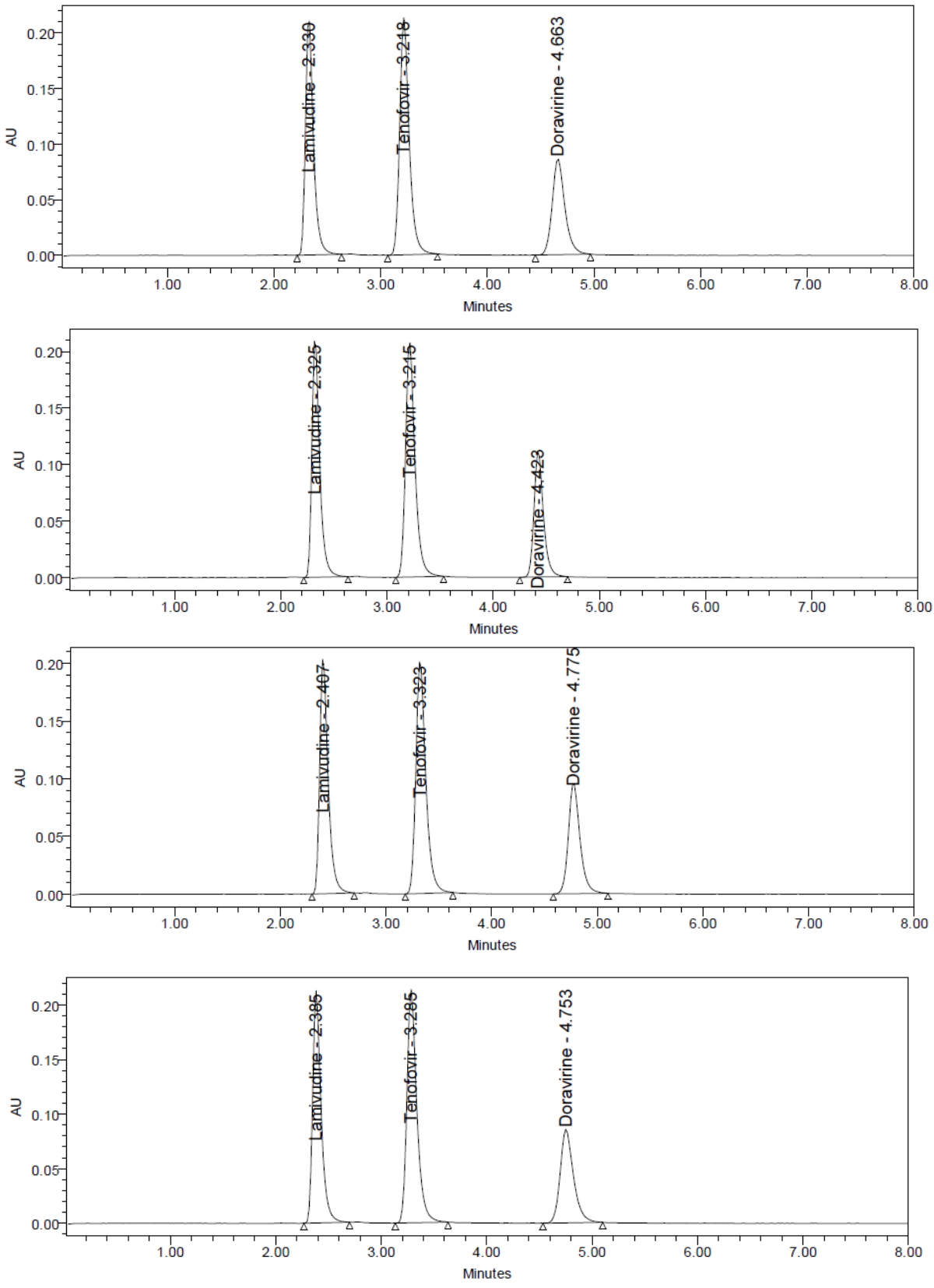


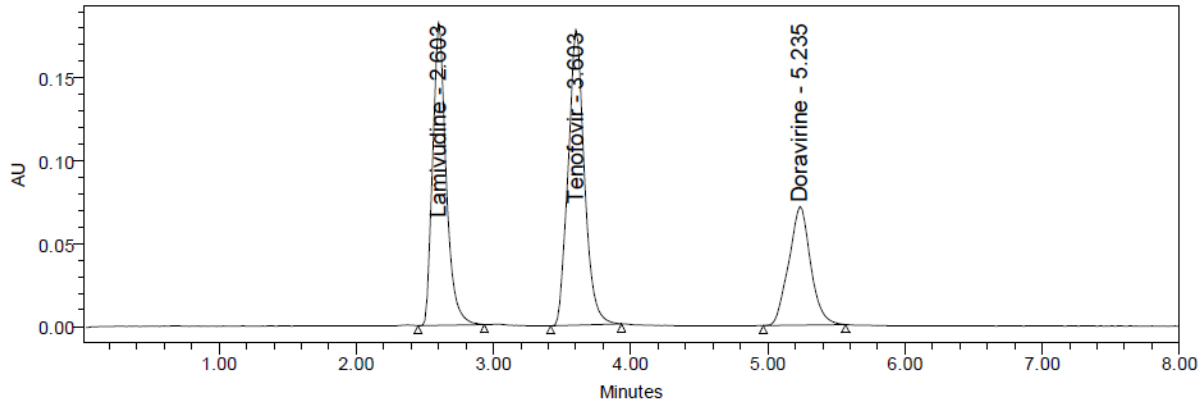
Fig no.5.25: Repeatability chromatograms

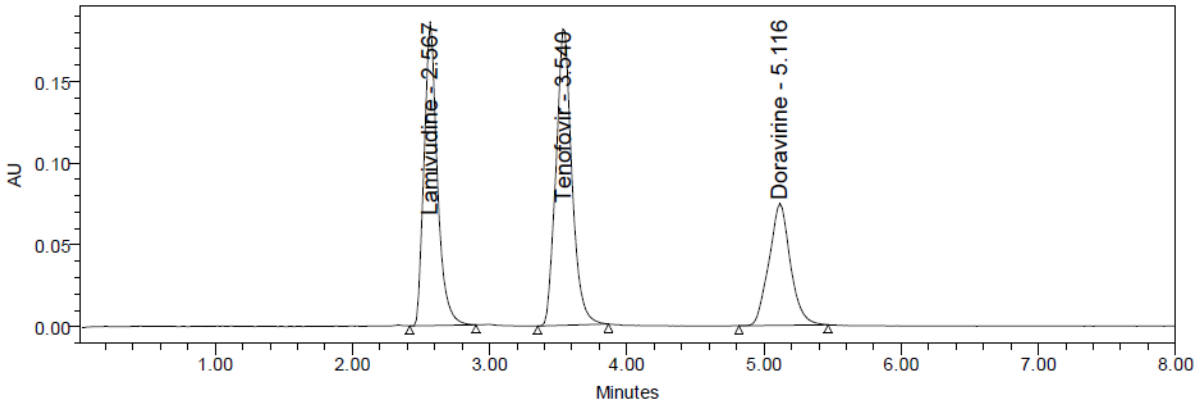
Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for three drugs and obtained as 0.5%, 0.5% and 0.7% respectively for Lamivudine, Tenofovir disoproxil fumarate and Doravirine .As the limit of Precision was less than "2" the system precision was passed in this method.

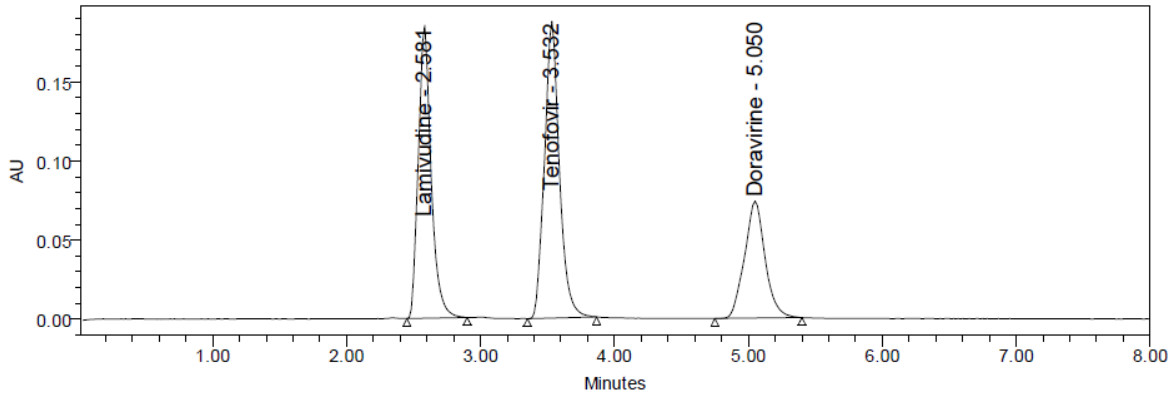
Intermediate precision (Day to Day Precision):

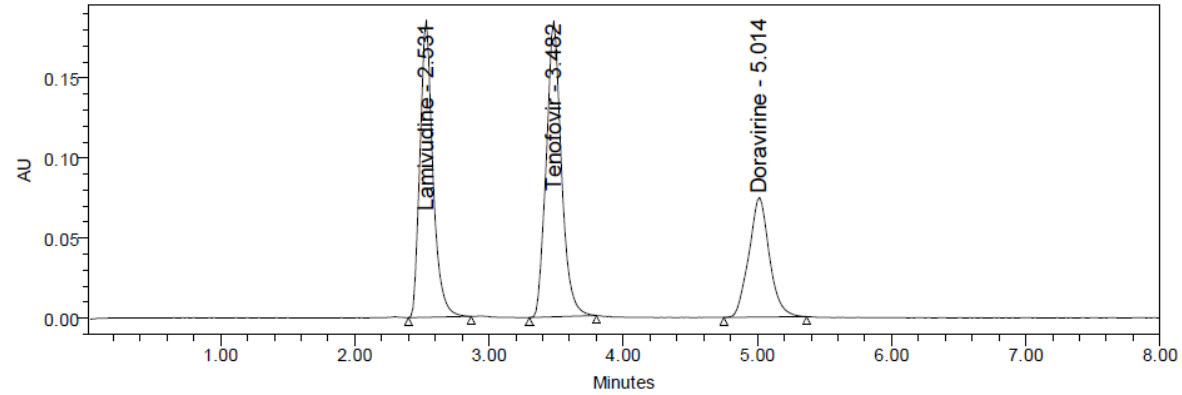
Table no. 5.6: Intermediate precision table of Lamivudine, Tenofovir disoproxil fumarate and Doravirine .

S. No	Area of Lamivudine.	Area of Tenofovir	Area of Doravirine
1.	1037303	1249501	678115
2.	1038063	1274900	689195
3.	1042509	1246346	687909
4.	1042509	1258108	705389
5.	1028570	1258108	695389
6.	1045016	1259893	688684
Mean	1038995	1257809	690780
S.D	5888.3	9970.4	9057.8
%RSD	0.6	0.8	1.3


Lamivudine - 2.603
Tenofovir - 3.603
Doravirine - 5.235
AU
Minutes


Lamivudine - 2.567
Tenofovir - 3.540
Doravirine - 5.116
AU
Minutes


Lamivudine - 2.584
Tenofovir - 3.532
Doravirine - 5.050
AU
Minutes


Lamivudine - 2.531
Tenofovir - 3.482
Doravirine - 5.014
AU
Minutes

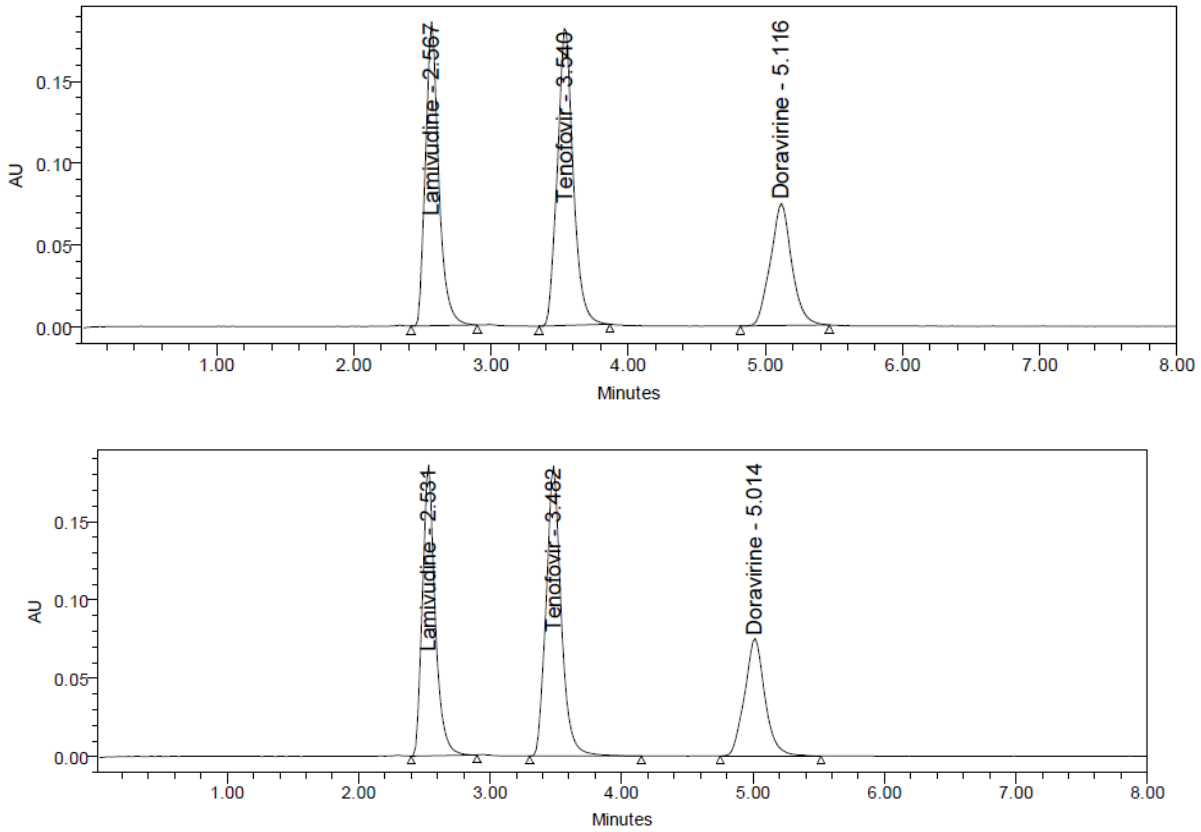


Fig no.5.26: Intermediate precision chromatograms

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for three drugs and obtained as 0.6%, 0.8% and 1.3% respectively for Lamivudine, Tenofovir disoproxil fumarate and Doravirine . As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy:

Table no.5.7: Accuracy table of Lamivudine.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	37.5	37.24	99.30	99.57%

	37.5	37.26	99.35	
	37.5	37.40	99.74	
100%	75	73.58	98.11	
	75	75.98	101.31	
	75	75.13	100.17	
150%	112.5	111.83	99.41	
	112.5	111.35	98.97	
	112.5	112.29	99.81	

Table no.5.8: Accuracy table of Doravirine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	12.5	12.55	100.40	99.89%
	12.5	12.58	100.62	

	12.5	12.54	100.35
100%	25	24.83	99.32
	25	24.86	99.45
	25	24.90	99.58
150%	37.5	37.52	100.06
	37.5	37.46	99.90
	37.5	37.24	99.31

Table no.5.9: Accuracy table of Tenofovir disoproxil fumarate

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	37.5	37.56	100.15	99.74%
	37.5	37.34	99.58	
	37.5	38.16	101.76	
100%	75	74.34	99.12	
	75	75.11	100.14	
	75	74.36	99.14	

150%	112.5	111.23	98.87	
	112.5	112.25	99.77	
	112.5	111.52	99.13	

Discussion: Three levels of Accuracy sample were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.57%, 99.74% and 99.89% for Lamivudine, Tenofovir disoproxil fumarate and Doravirine respectively.

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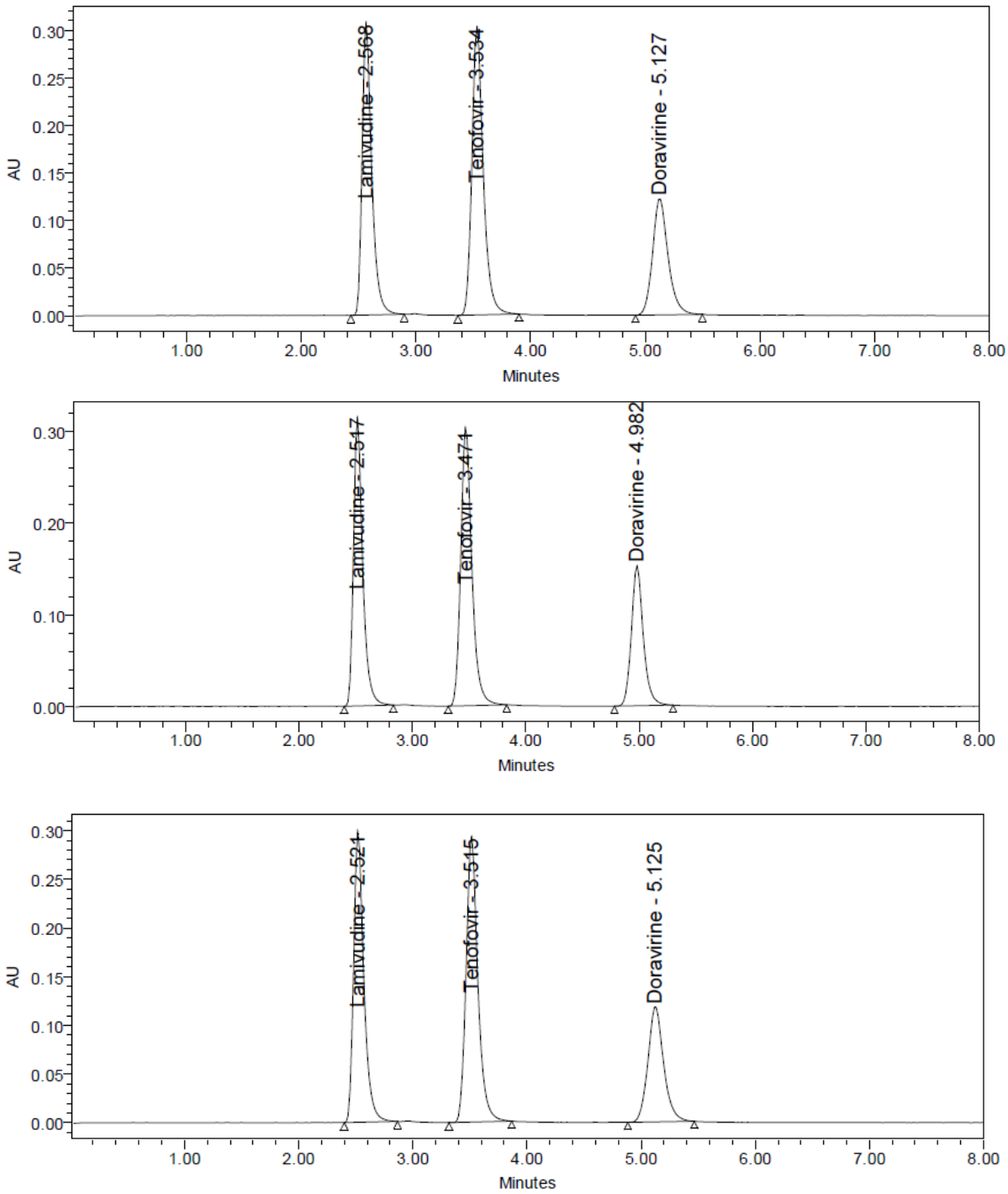

Fig no.5.27: Accuracy 50% chromatograms

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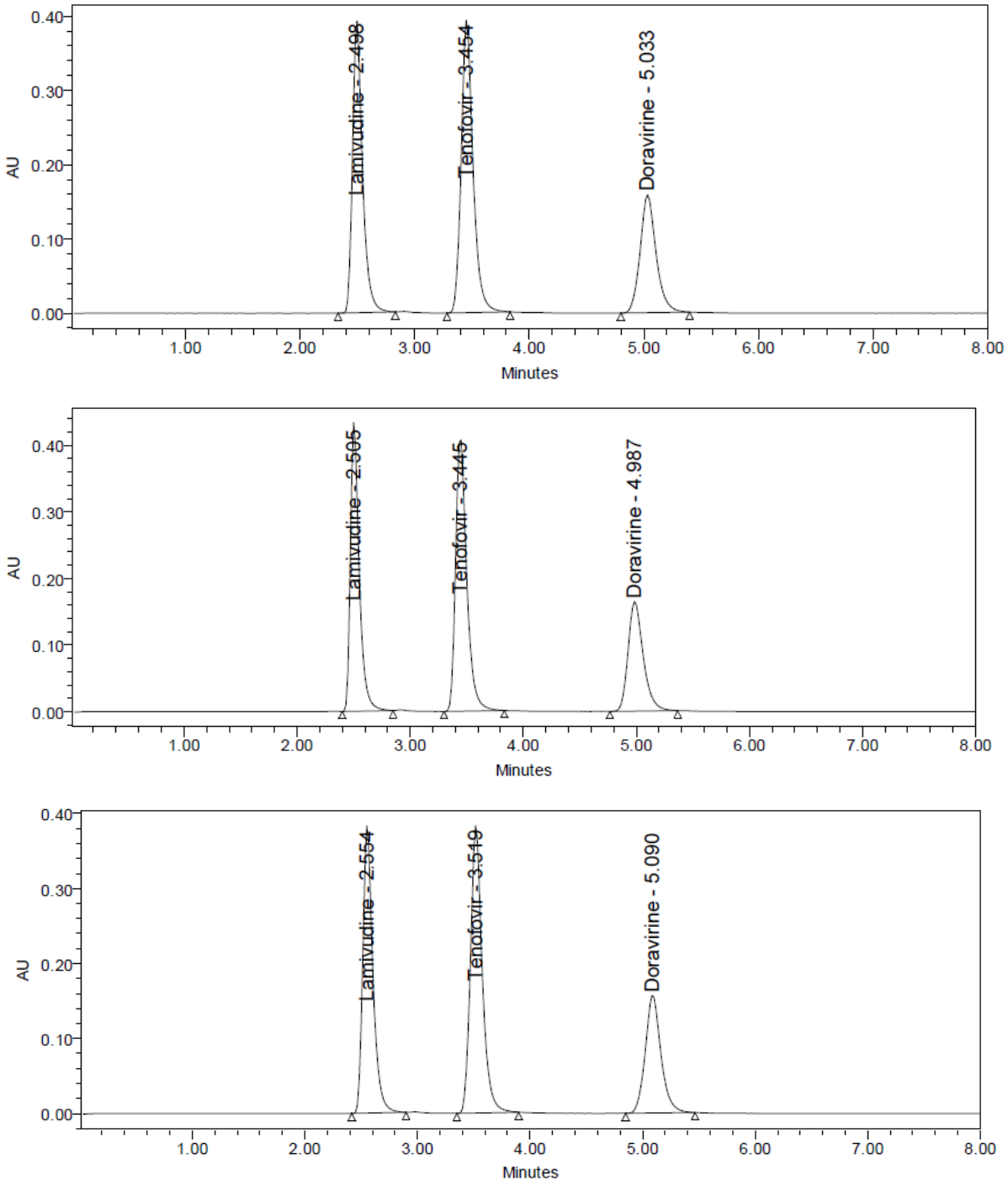

Fig no.5.28: Accuracy 100% chromatograms

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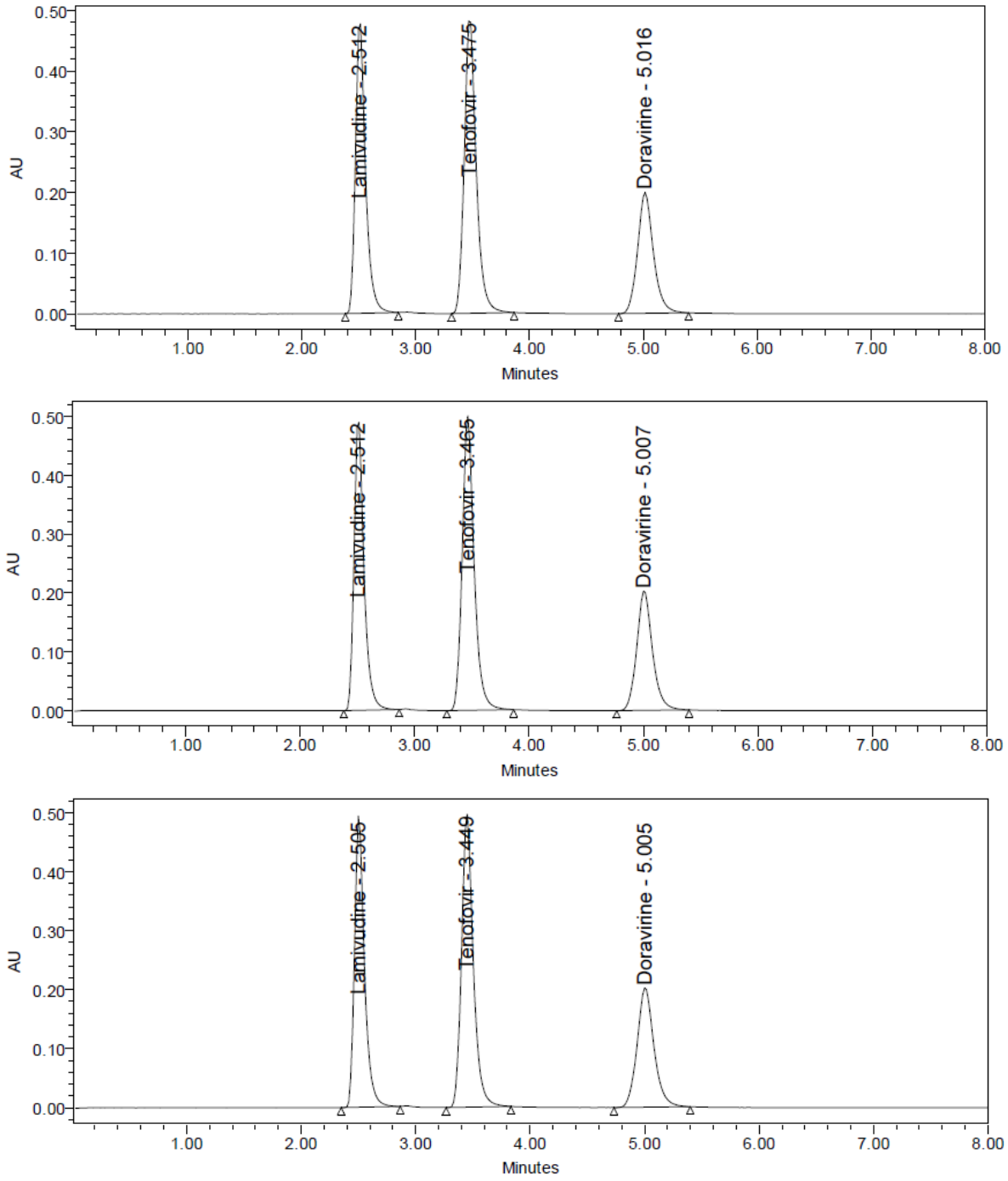

Fig no.5.29: Accuracy 150% chromatograms

Sensitivity:

Table no.5.10: Sensitivity table of Lamivudine, Tenofovir and Doravirine.

Molecule	LOD(µg/ml)	LOQ(µg/ml)
Lamivudine.	0.27 µg/ml	0.82 µg/ml

Tenofovir	0.18 µg/ml	0.54µg/ml
Doravirine	0.09 µg/ml	0.28 µg/ml

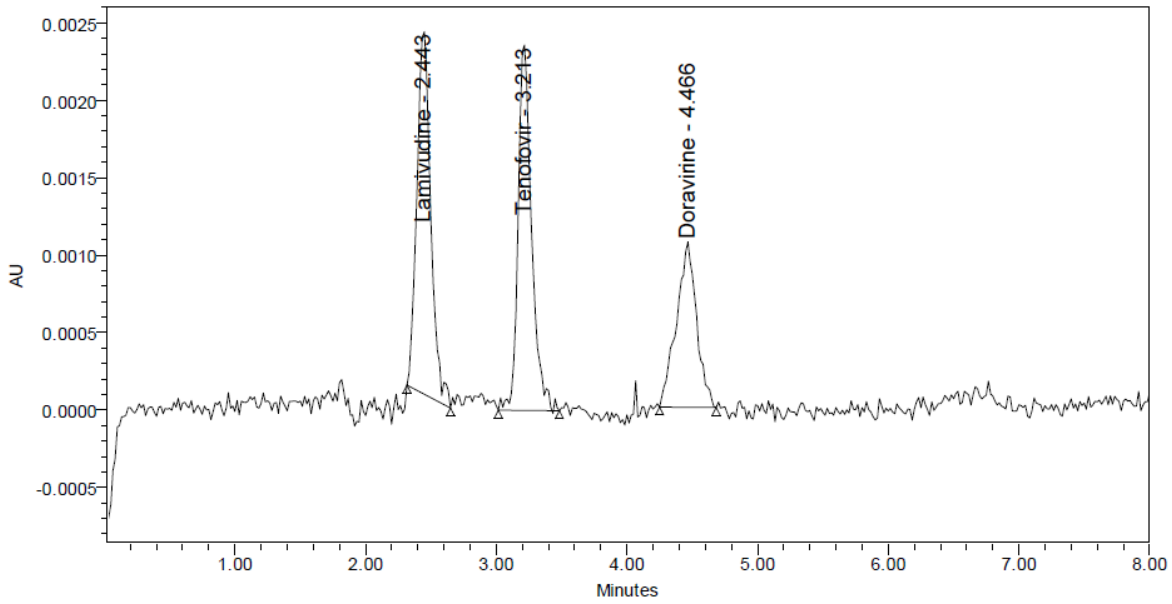

Fig no.5.30: LOD chromatogram of standard

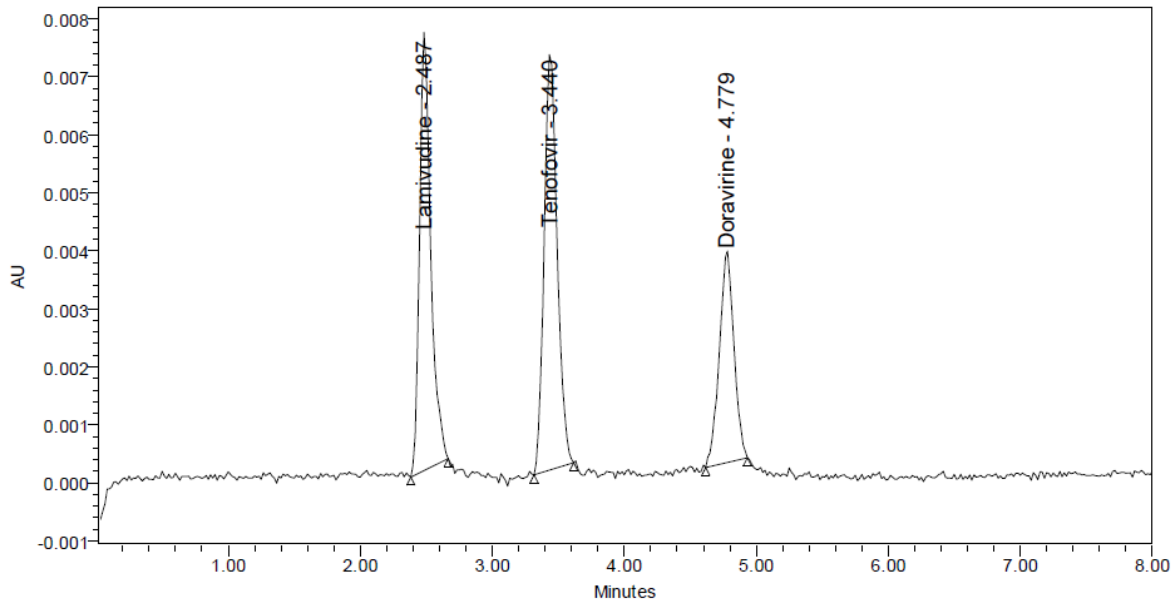

Fig no.5.31: LOQ chromatogram of standard

Robustness:

Table no.5.11: Robustness data for Lamivudine, Tenofovir disoproxil fumarate and Doravirine

S.no	Condition	%RSD of Lamivudine.	%RSD of Tenofovir	%RSD of Doravirine
1	Flow rate (-) 0.9ml/min	0.5	0.4	0.8
2	Flow rate (+) 1.1ml/min	1.3	0.7	0.5
3	Mobile phase (-) 55B:45A	0.1	1.2	0.1
4	Mobile phase (+) 65B:35A	0.1	1.2	1.2
5	Temperature (-) 25°C	1.1	1.2	1.5
6	Temperature (+) 35°C	0.2	0.1	0.5

Discussion: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (65B:35A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

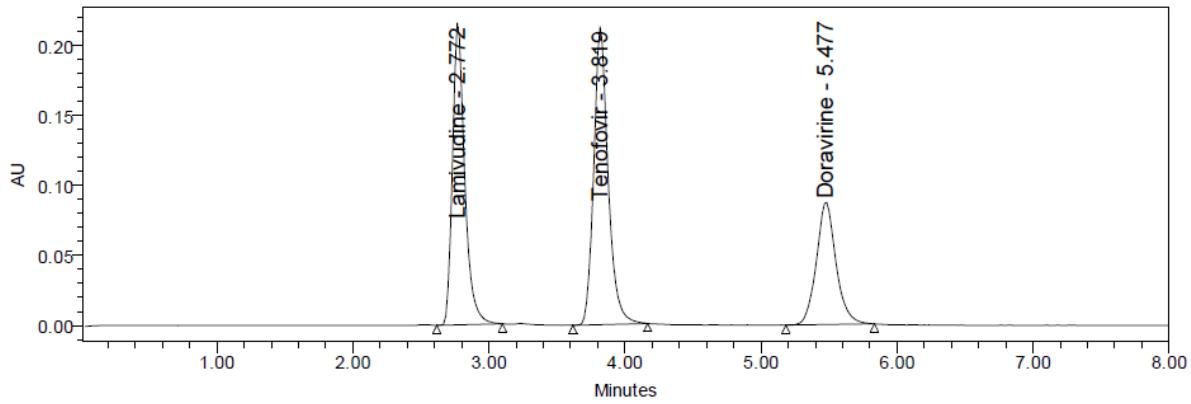

Fig no.5.32: Flow minus injection chromatogram

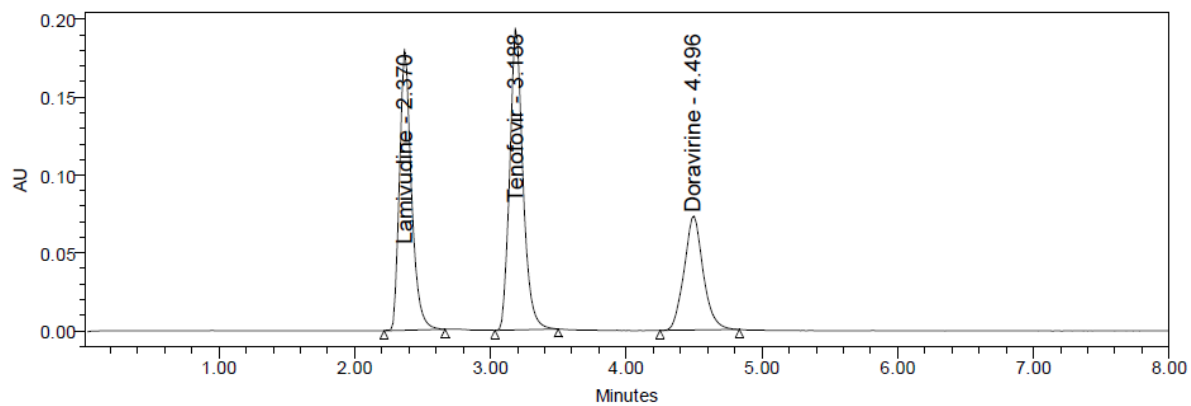

Fig no.5.33: Flow plus injection chromatogram

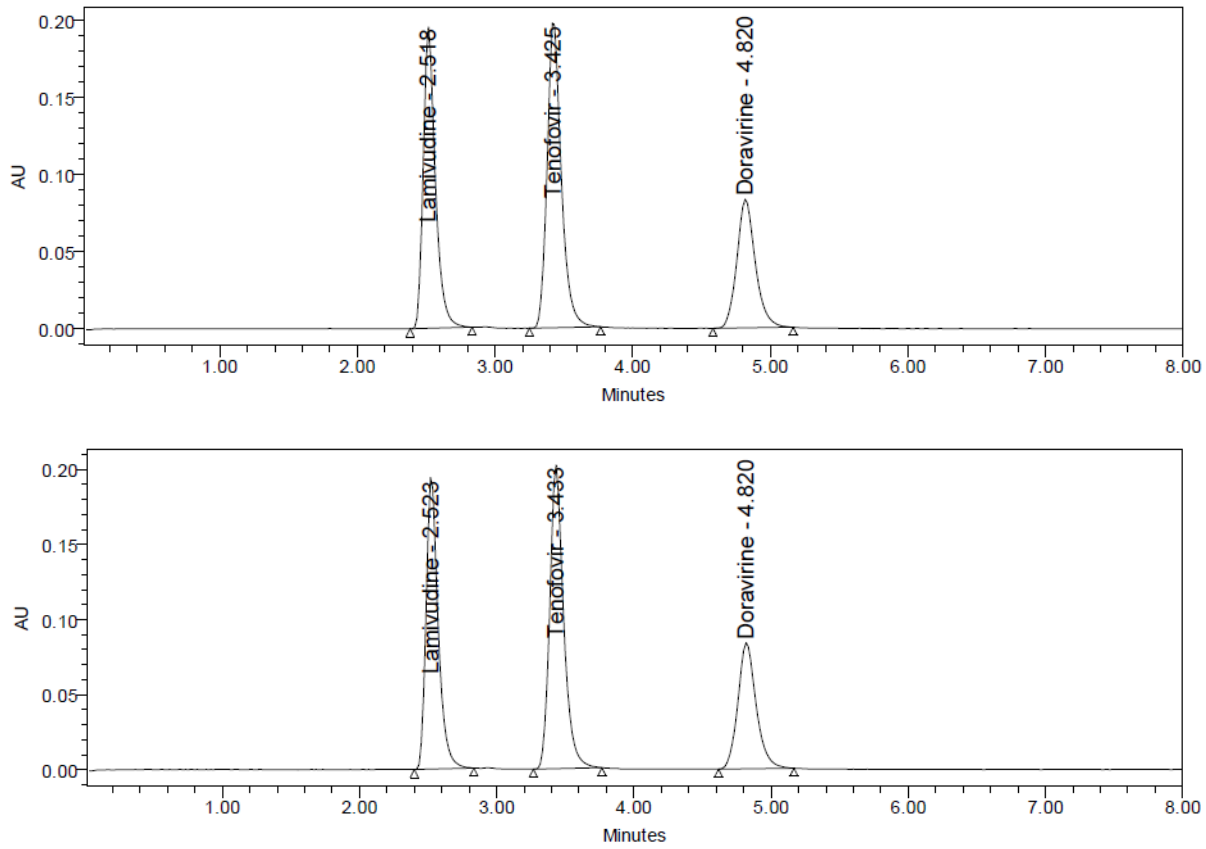

Fig no.5.34: Mobile phase minus injection chromatogram

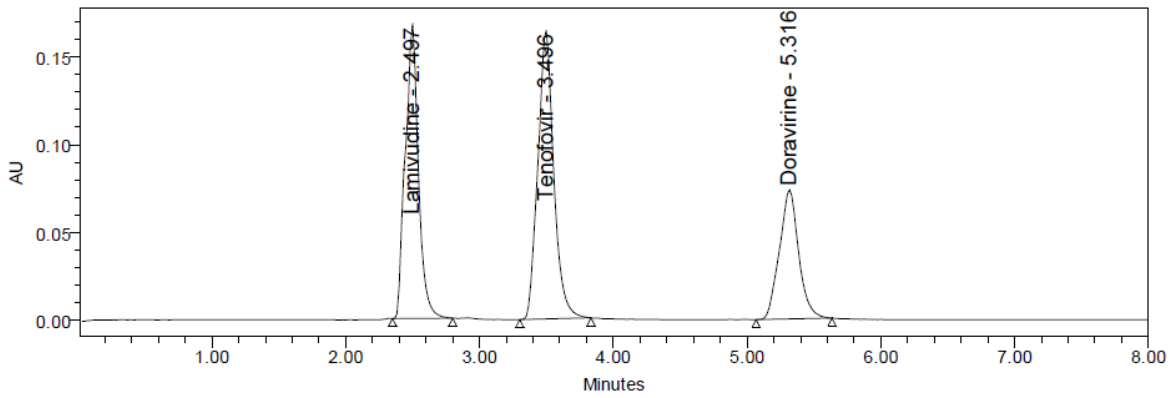

Fig no.5.35: Mobile phase plus injection chromatogram

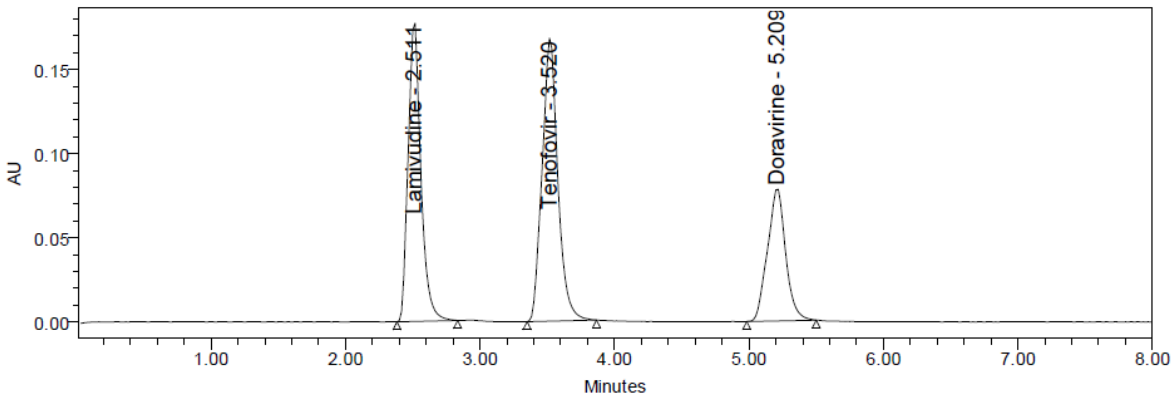

Fig no.5.36: Temperature minus injection chromatogram

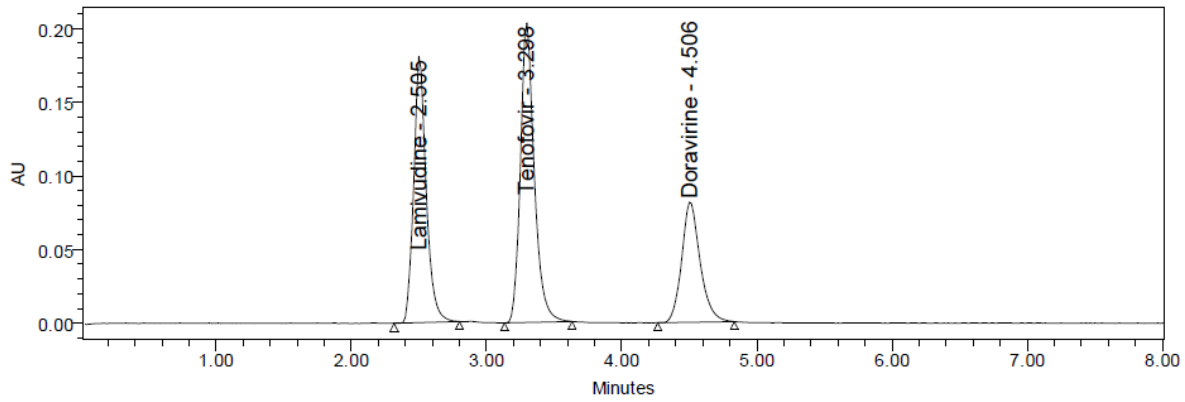

Fig no.5.37: Temperature plus injection chromatogram.

Assay:

Delstrigo (300+300+100) the label claim Tenofovir disoproxil fumarate 300mg, Lamivudine 300mg and Doravirine 100mg per unit formulation Assay was performed with the above formulation. Average % Assay for Lamivudine, Tenofovir disoproxil fumarate and Doravirine.Obtained assay results were 99.78%, 99.81% and 100.07% respectively.

Table no.5.12: Assay Data of Lamivudine.

S.no	Standard Area	Sample area	% Assay
1	1085597	1089546	98.30
2	1099294	1105404	99.73
3	1109959	1099697	99.21
4	1116113	1094896	98.78
5	1096317	1101686	99.39
6	1103838	1096832	98.95
Avg	1105104	1114685	99.06

Stdev	10718.7	5545.3	0.500
%RSD	1.0	0.5	0.5

Table no. 5.13: Assay Data of Doravirine

S.no	Standard Area	Sample area	% Assay
1	708646	699114	99.42
2	703014	703230	100.10
3	700476	708036	100.23
4	699353	708933	100.10
5	713021	707985	100.79
6	715080	712907	100.13
Avg	706598	706701	0.487
Stdev	6636.4	4827.8	0.5
%RSD	0.9	0.7	99.42

Table no. 5.14: Assay Data of Tenofovir disoproxil fumarate

S.no	Standard Area	Sample area	% Assay
1	1287919	1299037	99.47

2	1286219	1284671	98.37
3	1312269	1297801	99.38
4	1317900	1301120	99.63
5	1295165	1289286	98.72
6	1312755	1295271	99.18
Avg	1302038	1294531	99.13
Stdev	13913.7	6319.6	0.48
%RSD	1.1	0.5	0.5

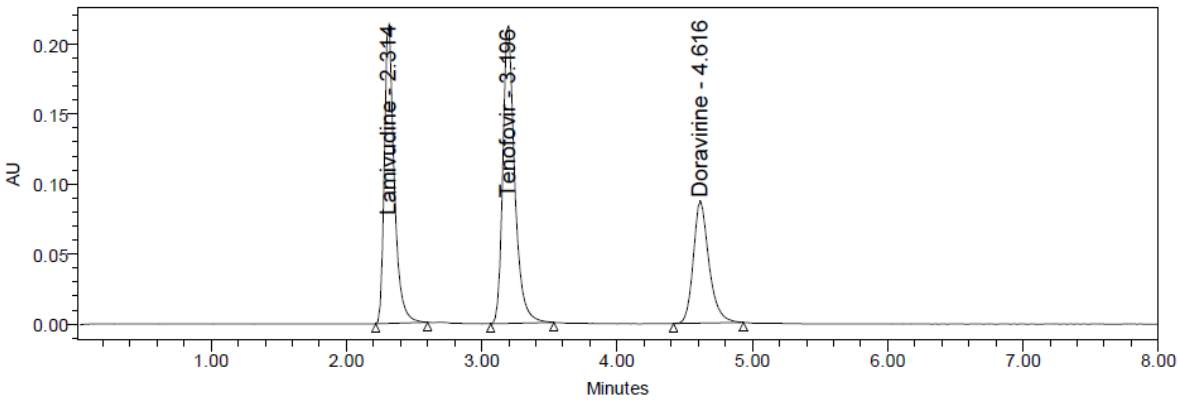

Fig no.5.38: Chromatogram of working standard solution

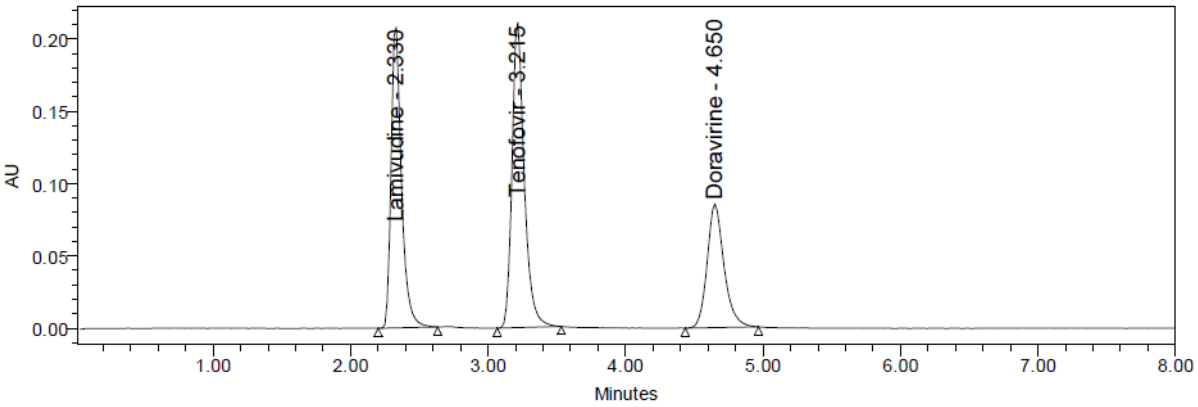

Fig no.5.39: Chromatogram of working sample solution

DEGRADATION

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table no. 5.15: Degradation Data of Lamivudine.

SL.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	3.93	0.243	0.27
2	Alkali	3.27	0.231	0.275
3	Oxidation	3.18	0.231	0.275
4	Thermal	2.94	0.256	0.264
5	UV	2.19	0.264	0.271
6	Water	0.43	0.171	0.264

Table no.5.16: Degradation Data of Doravirine

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.18	0.093	0.265
2	Alkali	4.05	0.123	0.271
3	Oxidation	3.62	0.107	0.264
4	Thermal	3.21	0.075	0.263
5	UV	2.24	0.071	0.268
6	Water	0.65	0.072	0.265

Table no. 5.17: Degradation Data of Tenofovir disoproxil fumarate

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	3.72	0.107	0.279
2	Alkali	4.99	0.225	0.31
3	Oxidation	5.66	0.218	0.299
4	Thermal	2.31	0.11	0.274
5	UV	1.34	0.101	0.279
6	Water	0.72	0.108	0.277

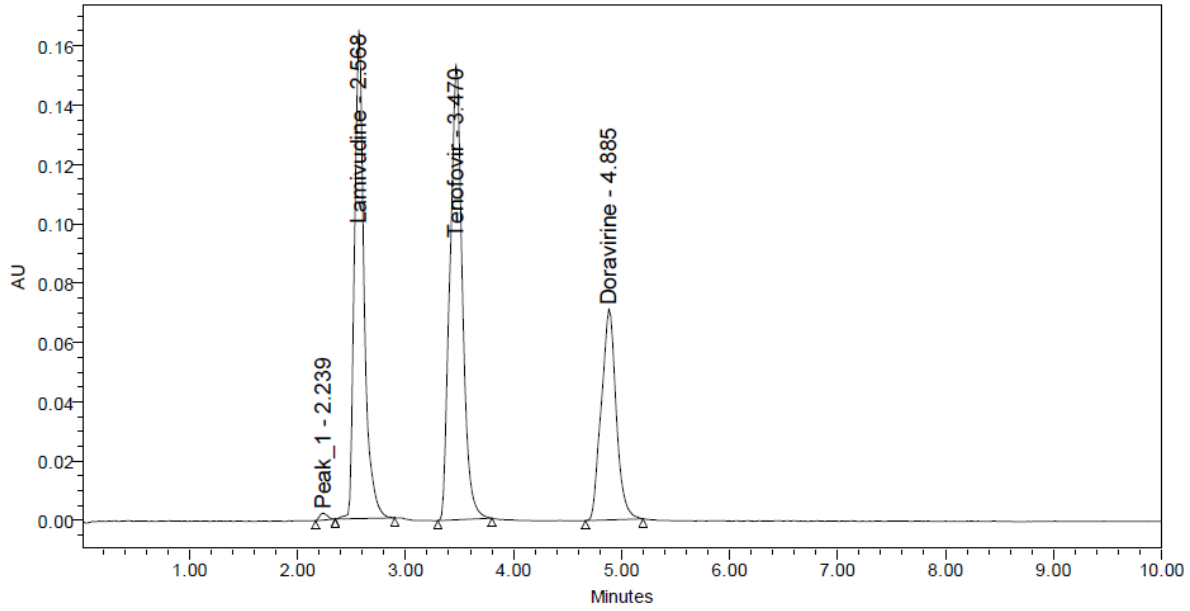

Fig no. 5.40: Acid Degradation chromatogram of Lamivudine, Tenofovir disoproxil fumarate & Doravirine

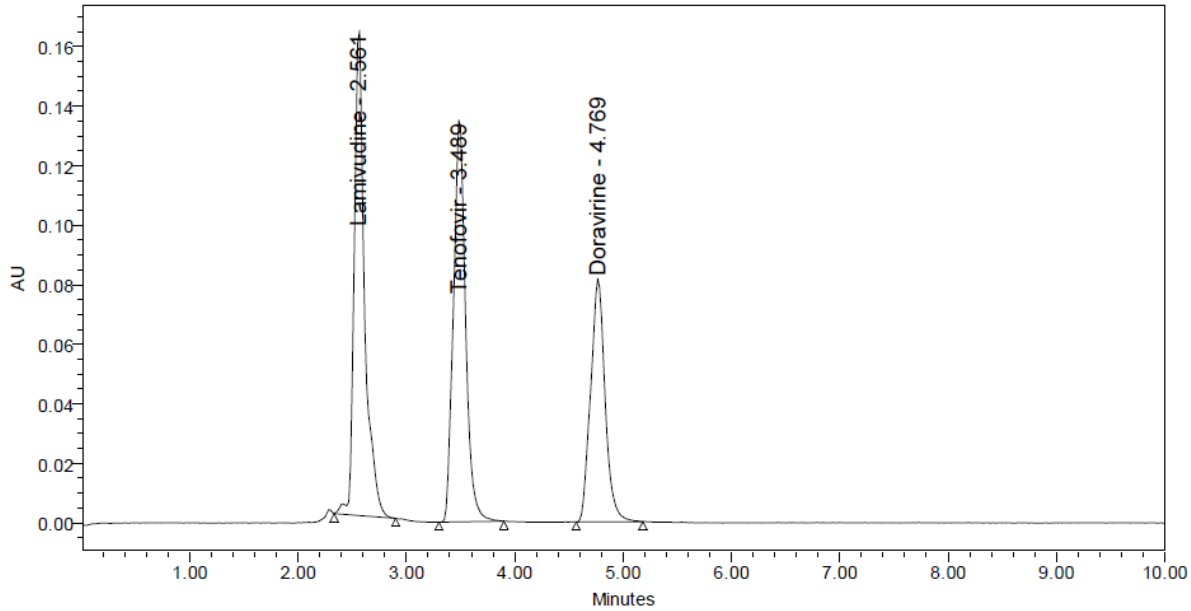

Fig no.5.41: Base Degradation chromatogram of Lamivudine, Tenofovir disoproxil fumarate & Doravirine

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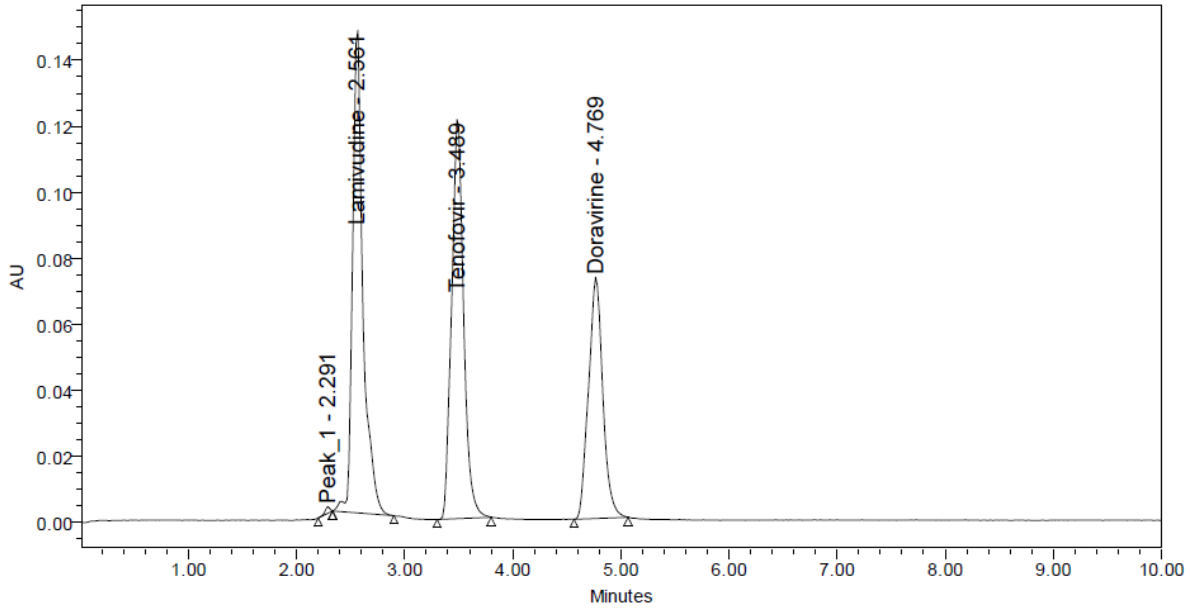

Fig no. 5.42: Peroxide Degradation chromatogram of Lamivudine, Tenofovir disoproxil fumarate & Doravirine.

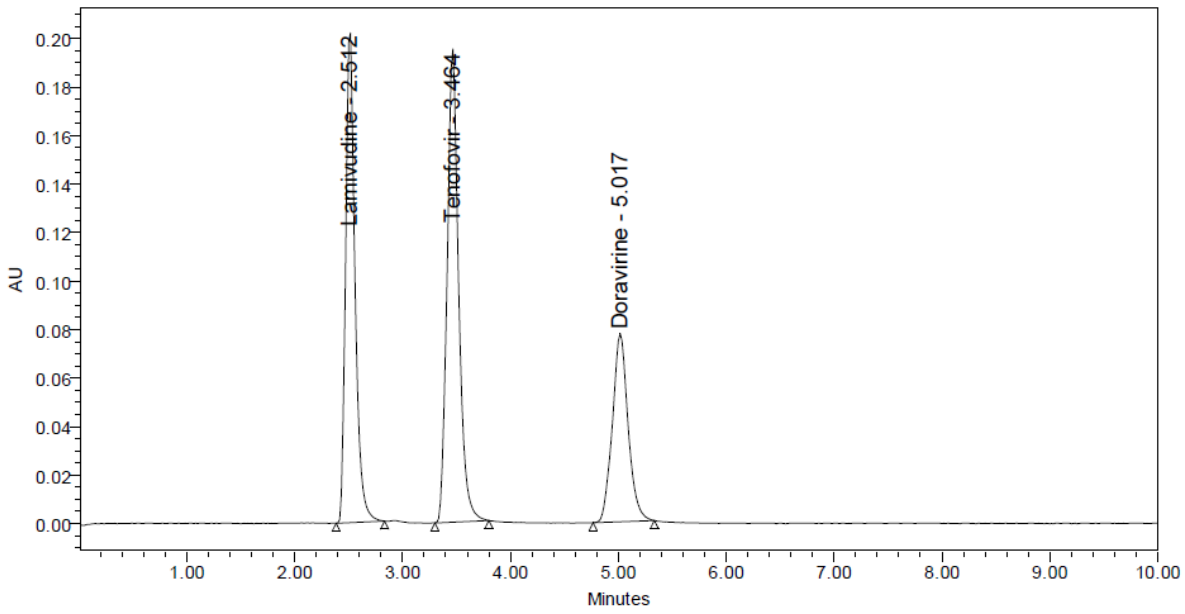

Fig no. 5.43: Thermal Degradation chromatogram of Lamivudine, Tenofovir & Doravirine

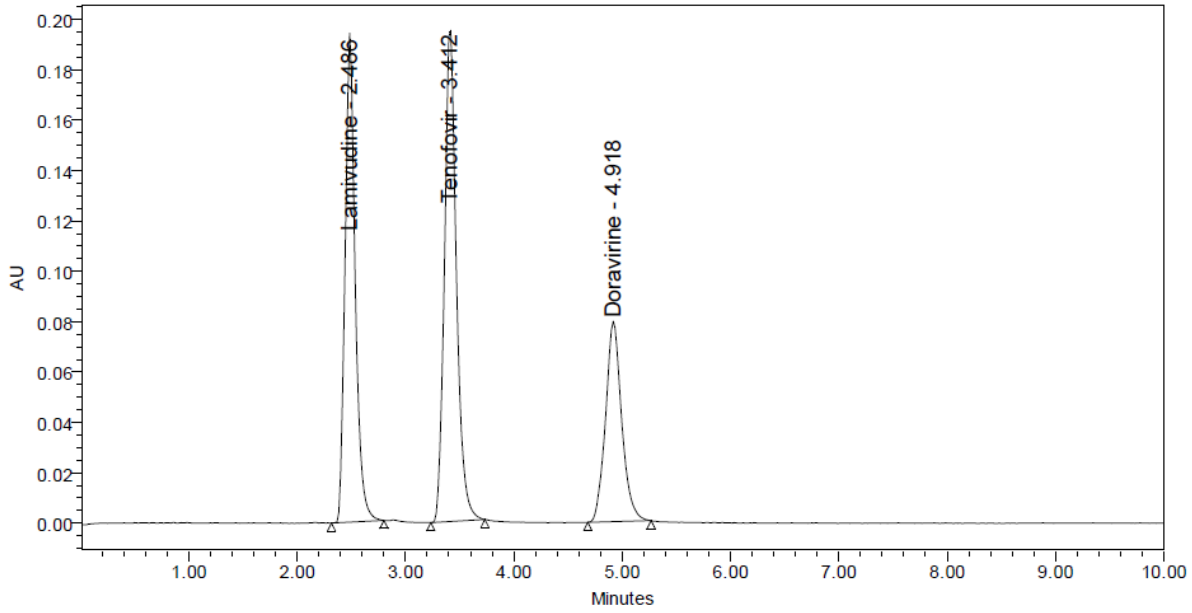

Fig no. 5.44: UV degradation chromatogram of Lamivudine,Tenofovir & Doravirine

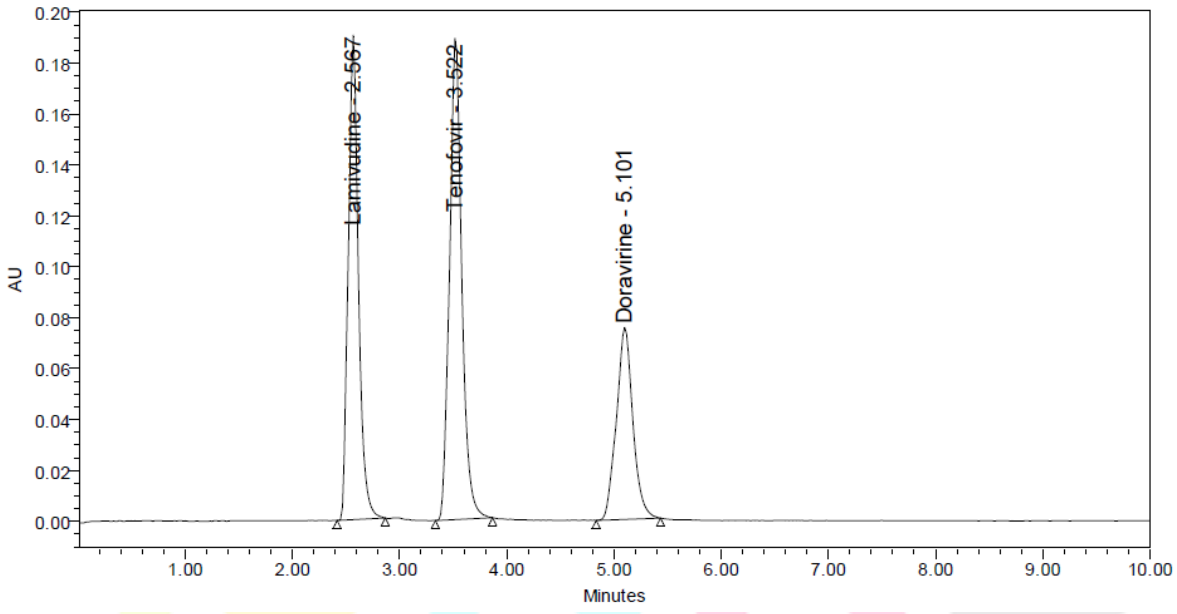

Fig no. 5.45: Water Degradation chromatogram of Lamivudine, Tenofovir disoproxil fumarate & Doravirine

SUMMARY OF THE STUDY

Table no.5.18: Summary of the study

Parameters	Lamivudine.	Tenofovir	Doravirine	LIMIT

Linearity		18.75-112.5µg/ml	18.75-112.5µg/ml	6.25-37.5µg/ml	R< 1
Regression coefficient		0.999	0.999	0.999	
Slope(m)		14959	17361	28129	
Intercept(c)		1184	6030	2766	
Regression equation		y = 14959x + 1184.	y = 17361x + 6030	y = 28129x + 2766.	
Assay (% mean assay)		99.06%	99.13%	100.13%	90-110%
Specificity		Specific	Specific	Specific	No interference of any peak
System precision %RSD		1.0	1.1	0.8	NMT 2.0%
Method precision %RSD		0.5	0.5	0.7	NMT 2.0%
Accuracy % recovery		99.57%	99.74%	99.89%	98-102%
LOD		0.27 µg/ml	0.18 µg/ml	0.09 µg/ml	NMT 3 µg/ml
LOQ		0.82 µg/ml	0.54 µg/ml	0.28 µg/ml	NMT 10µg/ml
Robustness	FM	0.5	0.4	0.8	%RSD NMT 2.0
	FP	1.3	0.7	0.5	
	MM	0.1	1.2	0.1	
	MP	0.1	1.2	1.2	
	TM	1.1	1.2	1.5	

	TP	0.2	0.1	0.5	

CONCLUSION

A simple, accurate, precise, selective and sensitive RP-HPLC method was developed for the simultaneous estimation of the Lamivudine, Doravirine and Tenofovir disoproxil fumarate in Tablet dosage form. Retention time of Lamivudine,Doravirine and Tenofovir disoproxil fumarate were found to be 2.314 min, 4.616 min and 3.196 min. % RSD of system precision for Lamivudine, Doravirine and Tenofovir disoproxil fumarate were and found to be 1.0,0.9 and 1.0 respectively. % RSD of method precision for Lamivudine, Doravirine and Tenofovir disoproxil fumarate were and found to be 0.5,0.7 and 0.5 respectively. % recovery was obtained as 99.57% 99.89% and 99.74% for Lamivudine,Doravirine and Tenofovir disoproxil fumarate respectively. LOD values obtained from regression equations of Lamivudine, Doravirine and Tenofovir disoproxil fumarate were 0.27ppm, 0.09ppm and 0.18ppm and LOQ values obtained from regression equations of Lamivudine, Doravirine and Tenofovir disoproxil fumarate were 0.82ppm, 0.28 ppm and 0.54ppm respectively. Regression equation of Lamivudine was y = 14959x + 1184, Doravirine was y = 28129x + 2766 and of Tenofovir was y = 17361x + 6030.Retention times are decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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