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Analytical Method Development and Validation of Abacavir & Lamivudine

Bimon Routray[1] , **Debya Sarita**[2],

1-Executive Accenture Tech ,Bangalore , 2-Executive Bioclinica, Bangalore

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Corresponding author address

Mr Bimon Routray,
Accenture Tech,Bangalore,
Karnataka,
India

ABSTRACT

Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry. In this present studyA sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of lamivudine and acavir tablet .In case of RP-HPLC various columns are available, but here THERMOSIL C18 150X4.6mm,.5µ or equivalent column was preferred because using this column peak shape, resolution and absorbance were good. A recovery of 101.1% for lamivudine and 99.9% abacavir was found in assay from tablet formulation

Keyword: RP-HPLC, Abacavir, Lamivudine

Introduction

Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry. Pharmaceutical analysis derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative analysis reveals the chemical identity of the sample. Quantitative analysis establishes the relative amount of one or more of these species or analytes in numerical terms. Qualitative analysis is required before a quantitative analysis can be undertaken. A separation step is usually a necessary part of both a qualitative and quantitative analysis. The results of typical quantitative analysis can computed from two measurements. One is the mass or volume of sample to be analyzed and second is the measurement of some quantity that is proportional to the amount of analyte in that.

Basic criteria for new method development of drug analysis:

- The drug or drug combination may not be official in any pharmacopoeias,
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations,
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,
- Analytical methods for the quantitation of the drug in biological fluids may not be available,
- Analytical methods for a drug in combination with other drugs may not be available,
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Importance Of Analytical Methods

Drug analysis reveals identification characterization & determination of the drugs in mixtures like dosage forms & biological fluids. The number of drugs introduced in to the market has been

increasing at very fast rate. These drugs may be either new entities in the market or partial structural modification of the existing drugs. Newer analytical methods are developed for these drugs or drug combination of the below reasons: -

- Official pharmacopoeia may not reveal an analytical procedure for the drugs or its combination.
- Analytical method may not be available for the drug combination due to interference caused by excipients.
- Analytical method for the quantification of the drug or drug combination from biological fluids may not be available.

The newly developed analytical methods having their importance in different fields: -

- Research & Development Centre
- Quality control Department
- Approved Testing Laboratories
- Chemical Analysis Laboratories sample and normally completes the analysis.

Materials And Method

UV analysis for detection of wavelength:

Preparation of 20ppm stock solution of abacavir & lamivdine:

Accurately weighed about 2 mg of abacavir & lamivudine and transferred to 100 ml volumetric flask. In this 5 ml methanol was added and sonicated todissolve the drug completely. In this mixture further added about 70 ml of mobilephase and Sonicated for 5-10 min then it was diluted to mark with mobile phase.

The sample was scanned in the wavelength range of 200-400. Abacavir & lamivudine shows the maximum absorption at the wavelength 232 nm.

Hence,

Max= 232nm was selected.

METHOD DEVELOPMENT & VALIDATION

Trial – 1

Preparation of Phosphate buffer:

Weighed 7.0grams of potassium di hydrogen ortho phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the Ph 4.5 with Orthophosphoric acid.

Preparation of mobile phase:

Mixed a mixture of above buffer 400 mL (40%) and 450 mL of Acetonitrile HPLC (15%) and 150mL of HPLC Water(45%) and degassed in ultrasonic water bath for 5 minutes. Filtered through 0.45 µ filter paper under vacuum filtration.

Diluent Preparation:

Mobile phase used as Diluent.

Standard Solution Preparation:

Weighed accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask add about 7mL of diluent and sonicated to dissolved it completely and make volume up to the mark with the same solvent.

Stock solution:

Further pipetted 1.0ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

20 µL of the standard , sample Injected into the chromatographic system and measured the areas for the Lamivudine and Abacavir peaks and observed the retention time, peak area, peak heights. The trial 1 was performed using THERMOSIL C18 150X4.6mm,.5µ column and mobile phase by using water :acetonitrile:phosphate buffer with PH4.5 in the ratio of 25 :35:40 with the flow rate of 1.0µl/min at wave length 232nm.

Trial – 2

Preparation of Phosphate buffer:

Weighed 7.0grams of potassium di hydrogen ortho phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH 4.5 with Orthophosphoric acid.

Preparation of mobile phase:

Mixed a mixture of above buffer 400 mL (40%) and 450 mL of Acetonitrile HPLC (15%) and 150mL of HPLC Water(45%) and degassed in ultrasonic water bath for 5 minutes. Filtered through 0.45 µ filter paper under vacuum filtration.

Diluent Preparation:

Mobile phase used as Diluent.

Standard Solution Preparation: Weighed accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask add about 7mL of diluent and sonicated to dissolved it completely and make volume up to the mark with the same solvent.

Stock solution:

Further pipetted 1.0ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

20 µL of the standard , sample Injected into the chromatographic system and measured the areas for the Lamivudine and Abacavir peaks and observed the retention time, peak area, peak heights. The trial 2 was performed using THERMOSIL C18 150X4.6mm,.5µ column and mobile phase by using water :acetonitrile:phosphate buffer with ph4.5 in the ratio of 35 :35:30 with the flow rate of 1.0µl/min at wave length 232nm.

Trial –3

Preparation of Phosphate buffer:

Weighed 7.0grams of potassium di hydrogen ortho phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH 3.5 with Orthophosphoric acid.

Preparation of mobile phase:

Mixed a mixture of above buffer 400 mL (40%) and 450 mL of Acetonitrile HPLC (15%) and 150mL of HPLC Water(45%) and degassed in ultrasonic water bath for 5 minutes. Filtered through 0.45 µ filter paper under vacuum filtration.

Diluent Preparation:

Mobile phase used as Diluent.

Standard Solution Preparation:

Weighed accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask add about 7mL of diluent and sonicated to dissolved it completely and make volume up to the mark with the same solvent.

Stock solution:

Further pipetted 1.0ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

20 µL of the standard , sample Injected into the chromatographic system and measured the areas for the Lamivudine and Abacavir peaks and observed the retention time, peak area, peak heights.

The trial 3 was performed using THERMOSIL C18 150X4.6mm,.5 µ column and mobile phase by using water :acetonitrile:phosphate buffer with PH3.5 in the ratio of 15:30:55 with the flow rate of 1.0µl/min at wave length 232nm.

Trial – 4

Preparation of Phosphate buffer:

Weighed 7.0grams of potassium di hydrogen ortho phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase:

Mixed a mixture of above buffer 400 mL (40%) and 450 mL of Acetonitrile HPLC (15%) and 150mL of HPLC Water(45%) and degassed in ultrasonic water bath for 5 minutes. Filtered through 0.45 µ filter paper under vacuum filtration.

Diluent Preparation:

Mobile phase used as Diluent.

Standard Solution Preparation:

Weighed accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask add about 7mL of diluent and sonicated to dissolved it completely and make volume up to the mark with the same solvent.

Stock solution:

Further pipetted 1.0ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

20 µL of the standard , sample Injected into the chromatographic system and measured the areas for the Lamivudine and Abacavir peaks and observed the retention time, peak area, peak heights.

The trial 4 was performed using THERMOSIL C18 150X4.6mm,.5 µ column and mobile phase by using water :acetonitrile:phosphate buffer with PH 3.0 in the ratio of 25:35:40 with the flow rate of 1.0µl/min at wave length 232nm.

Trial – 5

Preparation of Phosphate buffer:

Weighed 7.0grams of potassium di hydrogen ortho phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH 2.5 with Orthophosphoric acid.

Preparation of mobile phase:

Mixed a mixture of above buffer 400 mL (40%) and 450 mL of Acetonitrile HPLC (15%) and 150mL of HPLC Water(45%) and degassed in ultrasonic water bath for 5 minutes. Filtered through 0.45 µ filter paper under vacuum filtration.

Diluent Preparation:

Mobile phase used as Diluent.

Standard Solution Preparation:

Weighed accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask add about 7mL of diluent and sonicated to dissolved it completely and make volume up to the mark with the same solvent.

Stock solution:

Further pipetted 1.0ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

20 µL of the standard , sample Injected into the chromatographic system and measured the areas for the Lamivudine and Abacavir peaks and observed the retention time, peak area, peak heights.

The trial 5 was performed using THERMOSIL C18 150X4.6mm,.5 µ column and mobile phase by using water :acetonitrile:phosphate buffer with PH 2.5 in the ratio of 35:20:45 with the flow rate of 1.0µl/min at wave length 232nm.

Trial –6

Preparation of Phosphate buffer:

Weighed 7.0grams of potassium di hydrogen ortho phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the PH 2.5 with Orthophosphoric acid.

Preparation of mobile phase:

Mixed a mixture of above buffer 400 mL (40%) and 450 mL of Acetonitrile HPLC (15%) and 150mL of HPLC Water(45%) and degassed in ultrasonic water bath for 5 minutes. Filtered through 0.45 µ filter paper under vacuum filtration.

Diluent Preparation:

Mobile phase used as Diluent.

Standard Solution Preparation:

Weighed accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask add about 7mL of diluent and sonicated to dissolved it completely and make volume up to the mark with the same solvent.

Stock solution:

Further pipetted 1.0ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

20 µL of the standard , sample Injected into the chromatographic system and measured the areas for the Lamivudine and Abacavir peaks and observed the retention time, peak area, peak heights.

The trial 6 was performed using THERMOSIL C18 150X4.6mm,.5µ column and mobile phase by using water :acetonitrile:phosphate buffer with PH 2.5 in the ratio of 40:20:40 with the flow rate of 1.0µl/min at wave length 232nm.

Trial –7

Preparation of Phosphate buffer:

Weighed 7.0grams of potassium di hydrogen ortho phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH as 2.5 with Orthophosphoric acid.

Preparation of mobile phase:

Mixed a mixture of above buffer 400 mL (40%) and 450 mL of Acetonitrile HPLC (15%) and 150mL of HPLC Water(45%) and degassed in ultrasonic water bath for 5 minutes. Filtered through 0.45 µ filter paper under vacuum filtration.

Diluent Preparation:

Mobile phase used as Diluent.

Standard Solution Preparation:

Weighed accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask add about 7mL of diluent and sonicated to dissolved it completely and make volume up to the mark with the same solvent.

Stock solution:

Further pipetted 1.0ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

20 µL of the standard , sample Injected into the chromatographic system and measured the areas for the Lamivudine and Abacavir peaks and observed the retention time, peak area, peak heights.

The trial 7 was performed using THERMOSIL C18 150X4.6mm,.5µ column and mobile phase by using water :acetonitrile:phosphate buffer with PH 2.5 in the ratio of 45:15:40 with the flow rate of 1.0µl/min at wave length 232nm.

VALIDATION OF DEVELOPED RP-HPLC METHOD

Preparation of Phosphate buffer:

Weighed 7.0grams of potassium di hydrogen ortho phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 2.5 with Orthophosporic acid

Preparation of mobile phase

Mixed a mixture of above buffer 400 mL (40%) and 450 mL of Acetonitrile HPLC (15%) and 150 mL of HPLC Water(45%) and degas in ultrasonic water bath for 5 minutes. Filtered through 0.45µ filter under vacuum filtration.

Diluent Preparation:

Mobile phase used as Diluent.

Preparation of the Lamivudine & Abacavir Standard & Sample Solution:

Standard Solution Preparation:

weighed accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask & about 7mL of Diluent was added and sonicated to dissolved it completely and make volume upto the mark with the same solvent.

(Stock solution)

Further pipetted 0.5ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Sample Solution Preparation:

weighed accurately and transfered 100 mg of Lamivudine and Abacavir tablet powder into a 10mL clean dry volumetric flask & about 7mL of Diluent was added and sonicated to dissolved it completely and filtered with 0.45µ filter paper and make volume upto the mark with the same solvent.

(Stock solution)

Further pipetted 0.5ml&1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Lamivudine and Abacavir peaks and calculate the % Assay by using the formulae.

System Suitability:

Tailing factor for the peaks due to Lamivudine & Abacavir in Standard solution Should not be more than 1.5

Theoretical plates for the Lamivudine & Abacavir peaks in Standard solution Should not be less than 2000

Calculation: (For Lamivudine)

Assay % =

$$\frac{AT}{} \times \frac{WS}{} \times \frac{DT}{} \times \frac{P}{} \times \frac{Avg.\ Wt}{} \times 100$$

AS DS WT 100 Label Claim

Where:

AT = average area counts of sample preparation.

AS= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

WT= Weight of sample taken in mg.

DS= Dilution factor of standard solution.

DT= Dilution factor of sample solution.

P = Percentage purity of working standard.

LC = LABEL CLAIM OF Lamivudine mg/ml.

Calculation: (For Abacavir)

Assay % =

AT WS DT P Avg. Wt

---------- x ----------x --------- x ----------x---------- X 100

AS DS WT 100 Label Claim

Where:

AT = average area counts of sample preparation.

AS= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

WT= Weight of sample taken in mg.

DS= Dilution factor of standard solution.

DT= Dilution factor of sample solution.

P = Percentage purity of working standard.

LC = LABEL CLAIM OF Abacavir **mg/ml.**

METHOD VALIDATION SUMMARY:

Precision:

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multipleSamplings of a homogenous sample. Precision of an analytical method isusually expressed as standard deviation or relative standard deviation.

Preparation of stock solution:

weighted accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolved it completely and make volume up to the mark with the same solvent.

Accurately weighted and transfer 100mg of lamivudine and abacavir working standard into a 10ml volumetric flask

(Stock solution)

Further pipette 0.5ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be with in the specified limits.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Preparation of stock solution:

Weighed accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask, about 7mL of Diluent was added and sonicate to dissolved it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 0.5ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Accuracy:

The accuracy of an analytical method is the closeness of test resultsobtained by that method to the true value. Accuracy may often the expressed aspercent recovery by the assay of known added amounts of analyte.

Preparation of Standard stock solution:

Weighed accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask & about 7mL of Diluent was added and sonicate to dissolved it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 0.5ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation Sample solutions:

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

Weighed accurately and transfered 50.0mg of Lamivudineand 50.0mg of Abacavir working standard into a 10mL clean dry volumetric flask & about 7mL of Diluent was added and sonicate to dissolved it completely and make volume up to the mark with the same solvent.

(Stock Solution).

Further pipette 0.5ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted upto the mark with diluent.

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

Weighed accurately and transfered 102.0mg of Lamivudine and 100.0mg of Abacavir working standards into a 10mL clean dry volumetric flask & about 7mL of Diluent was added and sonicate to dissolved it completely and make volume up to the mark with the same solvent. (Stock solution):

Further pipette 0.5ml & 1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

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

Weighed accurately and transferred .0mg of Lamivudine and 150.0mg of Abacavir working standards into a 10mL clean dry volumetric flask & about 7mL of Diluent was added and sonicate to dissolved it completely and make volume up to the mark with the same solvent.

(Stock solution).

Further pipetted 0.5ml&1.0ml of Lamivudine & Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Lamivudine & Abacavir and calculate the individual recovery and mean recovery values.

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0%

LINEARITY:

Preparation of stock solution:

Weighed accurately and transfered 100 mg of Lamivudine and Abacavir working standard into a 10mL clean dry volumetric flask & about 7mL of Diluent was added and sonicate to dissolved it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (300ppmof Lamivudine&600ppmof Abacavir):

0.3ml &0.6ml of stock solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – II (400ppmof Lamivudine&800ppmof Abacavir):

0.4ml &0.8ml of stock solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – III (500ppmof Lamivudine&1000ppmof Abacavir):

0.5ml &1.0ml of stock solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – IV (600ppmof Lamivudine&1200ppmof Abacavir):

0.6ml &1.2ml of stock solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – V (700ppmof Lamivudine&1400ppmof Abacavir)

0.7ml &1.4ml of stock solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Procedure:

Injected each level into the chromatographic system and measured the peak area.Ploted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculated the correlation coefficient.

Acceptance Criteria:

Correlation coefficient should be not less than 0.999.

LIMIT OF DETECTION: (for Lamivudine)

Preparation of 300µg/ml solution:

Weighed accurately and transfered 100mg of Lamivudineworking standard into a 10mL clean dry volumetric flask & about 7mL of Diluent was added & sonicated to dissolved it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipetted 0.3mlof Lamivudinethe above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 1.12% solution At Specification level (0.06µg/ml solution):

Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted upto the mark with diluent.

Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted upto the mark with diluent.

Further pipetted 1.0ml of the above stock solution into a 10ml volumetric flask and diluted upto the mark with diluent.

Pipette 1.12mL of solution into a 10 ml of volumetric flask and diluted upto the mark with diluent.

Acceptance Criteria:

- S/N Ratio value shall be 3 for LOD solution.

LIMIT OF DETECTION: (for Abacavir)

Preparation of 1000µg/ml solution:

Weighed accurately and transfered 100mg of Abacavir working standard into a 10mL clean dry volumetric flask & about 7mL of Diluent was added and sonicated to dissolved it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipetted 1ml of Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 0.02% solution At Specification level (0.02µg/ml solution):

Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted upto the mark with diluent

Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted upto the mark with diluent

Pipetted 0.02ml of solution into a 10 ml of volumetric flask and diluted upto the mark with diluent

Acceptance Criteria:

- S/N Ratio value shall be 3 for LOD solution.

LIMIT OF QUANTIFICATION(lamivudine):

Preparation of 300µg/ml solution:

Weighed accurately and transfered 10mg of Lamivudine working standard into a 10mL clean dry volumetric flask & about 7mL of Diluent was added and sonicated to dissolved it completely and make volume up to the mark with the same solvent. (Stocksolution).

Further pipetted 0.3mlof Lamivudine the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 0.4% solution At Specification level (0.2µg/ml solution):

Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted upto the mark with diluent.Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted upto the mark with diluent. Pipetted 0.4mL of solution into a 10 ml of volumetric flask and diluted upto the mark with diluent.

Acceptance Criteria:

- S/N Ratio value shall be 10 for LOQ solution.

LIMIT OF QUANTIFICATION(abacavir):

Preparation of 1000µg/ml solution:

Weighed accurately and transfered 100mg of Abacavir working standard into a 10mL clean dry volumetric flask & about 7mL of Diluent was added and sonicated to dissolved it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipetted 1.0ml of Lamivudinethe above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 0.08% solution At Specification level (0.08µg/ml solution): Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted upto the mark with diluent. Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted upto the mark with diluents.Pipetted 0.08mL of solution into a 10 ml of volumetric flask and diluted upto the mark with diluent

ROBUSTNESS:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a). The flow rate was varied at 0.9 ml/min to 1.1ml/min.

Standard solution 500ppm of Lamivudine & 1000ppm Abacavir was prepared and analysed using the varied flow rates along with method flow rate.

b). The Organic composition in the Mobile phase was varied from 55% to 65%.

Standard solution 500 µg/ml of Lamivudine&1000 µg/ml Abacavir was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

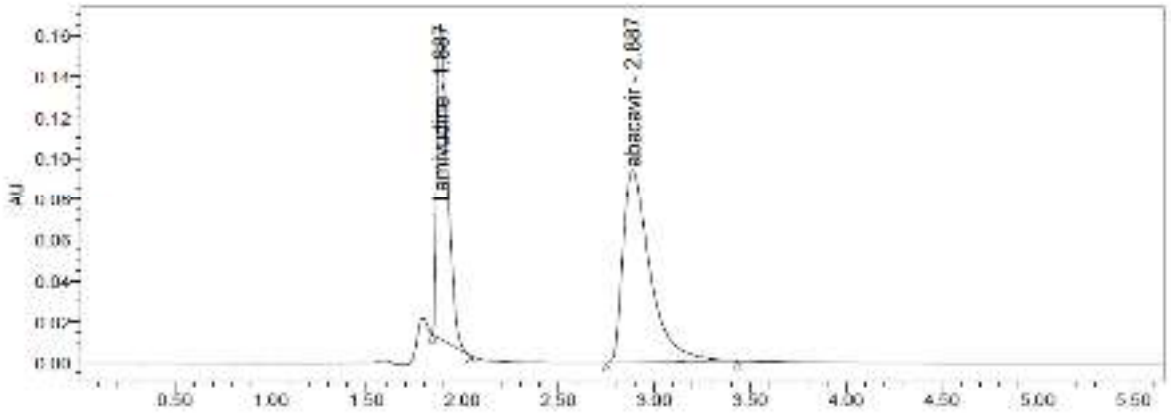
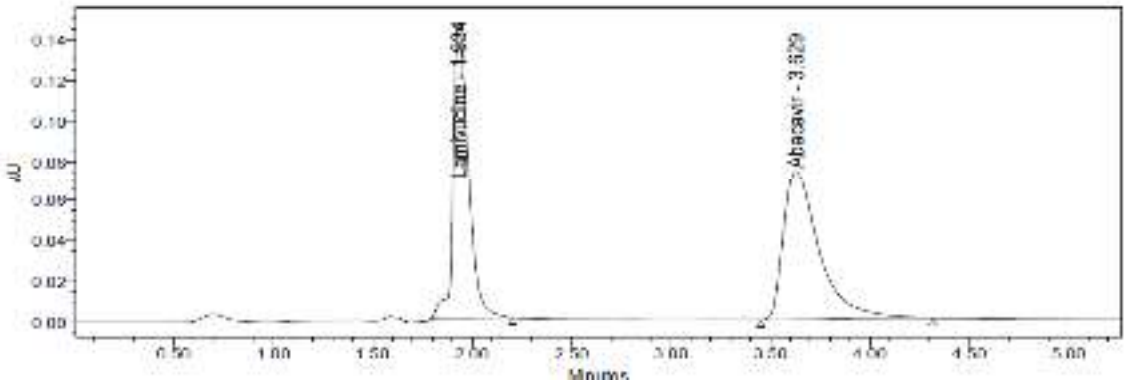


Fig. -6.1 : the chromatogram of first trial.

Table No.6.1 : observation table of the chromatogram of first trial.

Peak Name	RT	Area	% Area	Height
Lamivudine	1.887	593861	39.00	156895
abacavir	2.887	928701	61.00	94376

The retention time of lamivudine was found to be 1.887 min and abacavir was found to be 2.887 but the peak obtained are not intence and shows tailing effect. Thus the method was rejected.



Trial-2**Fig. –:** the chromatogram of second trial.

Table No.6.2: observation table of the chromatogram of second trial.

Peak Name	RT	Area	% Area	Height
Lamivudine	1.934	774276	45.73	146740
Abacavir	3.629	918691	54.27	74047

The retention time of lamivudine was found to be 1.934 min and abacavir was found to be 3.629 but the peak obtained was very low resolution. Thus the method was rejected.

Trial-3

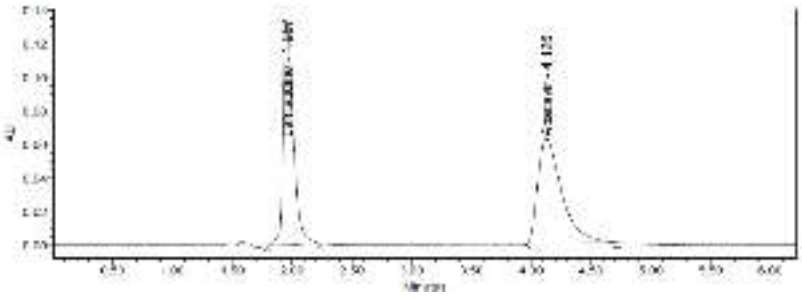


Fig. –: the chromatogram of third trial.

Table No: observation table of the chromatogram of third trial.

Peak Name	RT	Area	% Area	Height
Lamivudine	1.957	782736	47.35	134434
Abacavir	4.126	870450	52.65	62606

The retention time of lamivudine was found to be 1.957 min and abacavir was found to be 4.126 but the peak obtained was not intense. Thus the method was rejected.

Trial-4

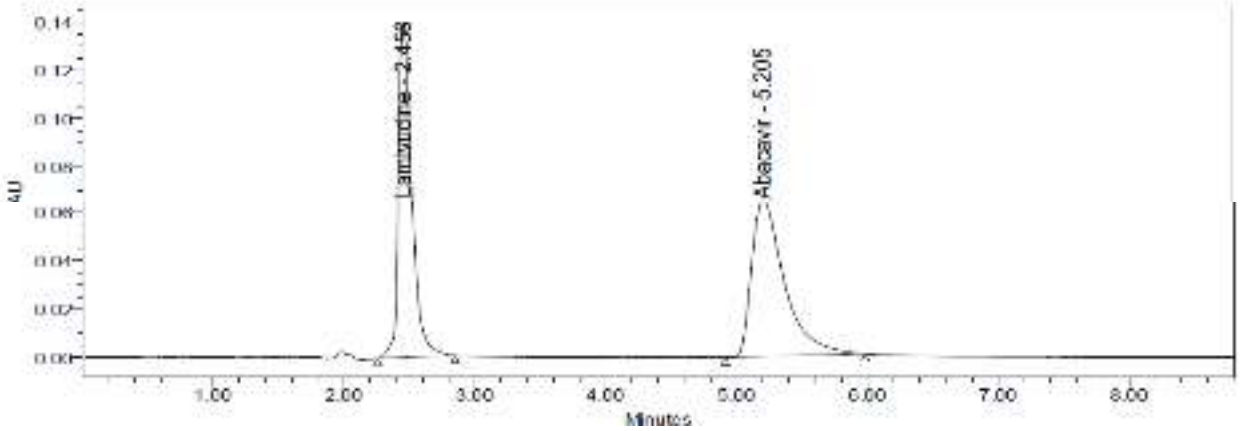


Fig. – 6.4 : the chromatogram of fourth trial .

Table No.6.4 : observation table of the chromatogram of fourth trial .

Peak Name	RT	Area	% Area	Height
Lamivudine	2.458	990403	46.95	139769
Abacavir	5.205	1118959	53.05	64833

The retention time of lamivudine was found to be 2.458 min and abacavir was found to be 5.205 but the peak obtained shows tailing and peak was not intense. Thus the method was rejected.

Trial-5

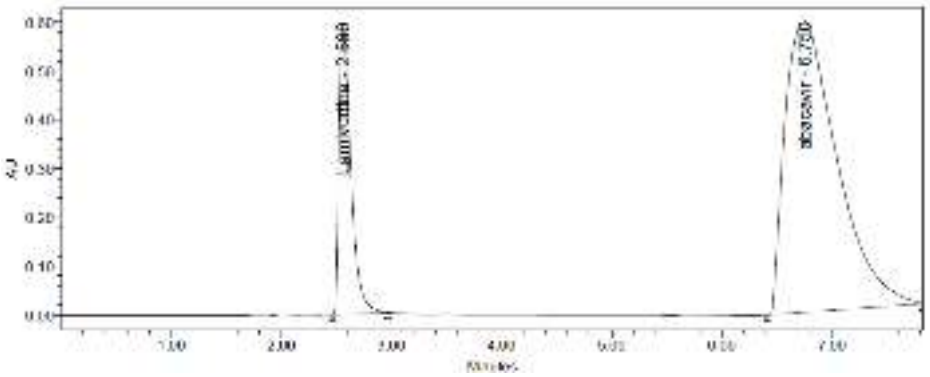


Fig. –: the chromatogram of fifth trial.

Table : observation table of the chromatogram of fifth trial.

Peak Name	RT	Area	% Area	Height
Lamivudine	2.560	4433784	18.52	597196
abacavir	6.750	19503609	81.48	594331

The retention time of lamivudine was found to be 2.560 min and abacavir was found to be 6.750 but the peak obtained shows broadening of abacavir. Thus this method was rejected.

Trial-6

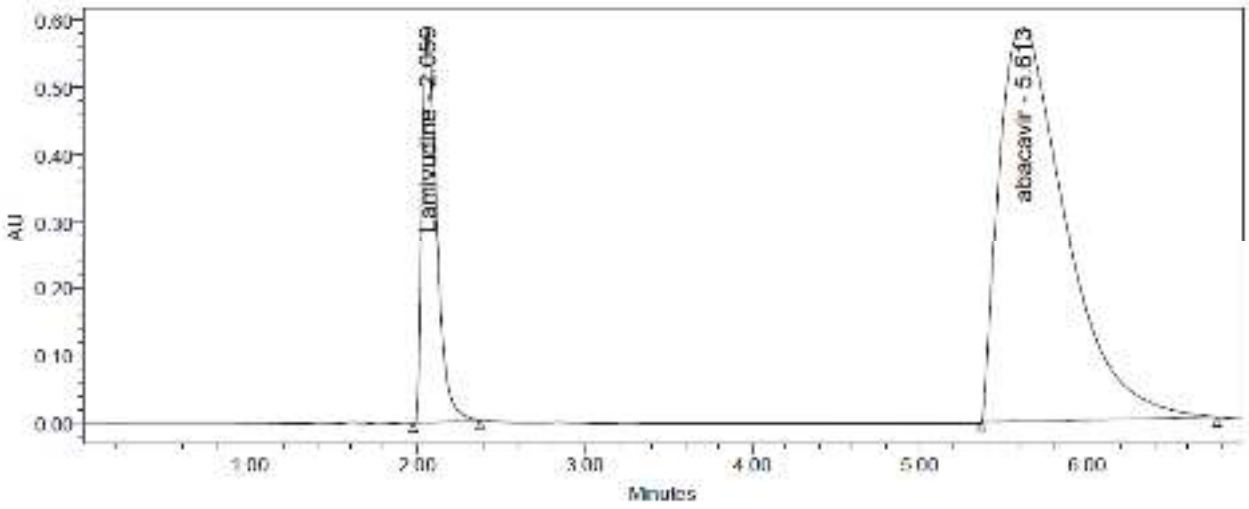


Fig. – : the chromatogram of sixth trial.

Table : observation table of the chromatogram of sixth trial.

Peak Name	RT	Area	% Area	Height
Lamivudine	2.059	3537693	17.97	587847
abacavir	5.613	16152391	82.03	585327

The retention time of lamivudine was found to be 2.059 min and abacavir was found to be 5.613 but the peak obtained shows broadening of abacavir. Thus this method was rejected.

Trial-7

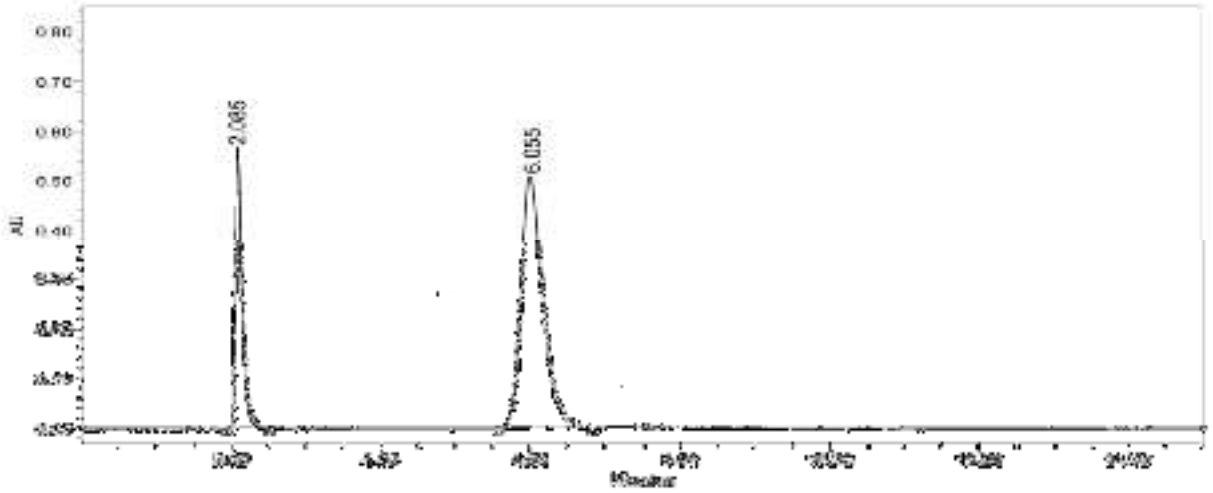


Fig. – : The chromatogram of seventh trial, this is the optimum one.

Table : observation table of the chromatogram of seventh trial, this is the optimum one.

The retention time of lamivudine was found to be 2.085 min and abacavir was found to be 6.055 but the peaks were found to be good.

Out of the 7 trials made in the lab, the 7th trial was accepted because when compare to the other trials 7th trial was found to be less in retention time & having good resolution due to the exact ratio of mobile phase, its pH and the flow rate adjustment. Thus the method was accepted.

Conclusion

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of lamivudine and acavir tablet.

- In case of RP-HPLC various columns are available, but here THERMOSIL C18 150X4.6mm,.5µ or equivalent column was preferred because using this column peak shape, resolution and absorbance were good.
- Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (acetonitrile, chloroform, methanol, water,etc).
- The drug was found to be highly soluble in acetonitrile & water. Thus Using of acetonitrile, water, phosphate buffer PH 2.5 solvents with appropriate composition newer methods can be developed and validated.
- The chromatograms for lamivudine and abacavir were found to be satisfactory on THERMOSIL C18 150X4.6mm,.5µ or equivalent column using Water:acetonitrile:buffer2.5 (45:15:40) as mobile phase with flow rate 1.0ml/minute.

- Wavelength detection was selected after performing UV-spectroscopy of the standard solution of drug at 232nm.
- The calibration curve for Lamivudine & Abacavir was obtained by plotting peak area versus concentration over the range of 300-700 ppm & 600-1400ppm respectively.
- The regression equation so obtained for calibration curve was y= 6156.x + 44283, R^2 = 0.998 & y=30832 x-8E + 06, R= 0.999 respectively for Lamivudine and Abacavir.
- Further the proposed RP-HPLC method has excellent results for all the validation parameters which are studied such as Precision, Accuracy, Linearity, Ruggedness, and Specificity shown in Table below.
- The result shows that the developed method is yet another suitable method for assay and validation parameter studies which can help in the analysis of lamivudine and abacavir in different formulations.
- A recovery of 101.1% for lamivudine and 99.9% abacavir was found in assay from tablet formulation. It can be concluded that the proposed rp-hplc method is sensitive and reproducible for the analysis of Lamivudine and Abacavir in pharmaceutical dosage forms within a short analysis of time.

Table No. : Summary of Results of lamivudine and abacavir

Parameter	Acceptance criteria	Result obtained from lamivudine	Result obtained from abacavir
Precision	% RSD should not be more than 2	0.380	1.290
Accuracy	% RSD should not be more than 2	101.7%	99.9%
Linearity	Correlation coefficient should not be less than 0.99	0.999	0.999

Ruggedness	% RSD should not be more than 2	0.585	1.76
Specificity	Should not interfere with placebo	pass	Pass

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