International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.14 | Issue 4 | Oct - Dec -2025

www.ijpar.com

ISSN: 2320-2831

DOI : https://doi.org/10.61096/ijpar.v14.iss4.2025.1080 -1095

Research

# RP-HPLC METHOD FOR ESTIMATION OF ABACAVIR, LAMIVUDINE AS PER ICH GUIDELINES

**Gollapalli Vandana[1*], Dr.l. Harikiran[2], Dr. l. Harikiran[2]**

[1,2]*Department Of Pharmaceutical Analysis, Princeton College Of Pharmacy In Narapally, Ghatkesar, Telangana.*

*Author for Correspondence: Gollapalli Vandana
Email: princeton.pharmacy@gmail.com

Published on:

Published by:
Futuristic Publications



**Abstract**

A novel, precise, accurate, rapid and cost effective isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method was developed, optimized and validated for the estimation of Abacavir and Lamivudine in bulk and pharmaceutical dosage forms. The drugs were estimated using Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size column. A mobile phase composed of tri ethylamine buffer and methanol in proportion of 32:68 v/v, at a flow rate of 1.0 ml/min was used for the separation. Detection was carried out at 248 nm. The linearity range obtained was 30-70 µg/ml for Darunavir and 10-50 µg/ml for Cobicistat with retention times (Rt) of 3.297 min and 5.405 min for Abacavir and Lamivudine respectively. The correlation coefficient values were found to be 0.999 & 0.999. Precession studies showed % RSD values less than 2 % for both the drugs in all the selected concentrations. The percentage recoveries of Abacavir and Lamivudine were found to be 100.1873% for Darunavir and 100.748% for Cobicistat respectively. The assay results of Abacavir and Lamivudine were found to be 99.82%. The limit of detection (LOD) and limit of quantification (LOQ) were 2.6µg/ml and 7.8µg/ml for Darunavir and 3.4µg/ml 10.2µg/ml for Cobicistat respectively. The proposed method was validated as per the International Conference on Harmonization (ICH) guidelines. The proposed validated method was successfully used for the quantitative analysis of commercially available dosage form.

**Keywords:** Abacavir and Lamivudine, RP-HPLC, ICH Guidelines, Validation.

# 1. INTRODUCTION

## 1.1 Chromatography [2]

### 1.1.1 Introduction

The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma, and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

" *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns.

"Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)"

### 1.1.2. Types of Chromatography

The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, there are two other modes that use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also called Thin-Layer Chromatography, TLC), or (b) electro osmotic flow, as in the case of Capillary Electro Chromatography (CEC).

**1. Adsorption chromatography**

Chromatography in which separation is based mainly on difference between the adsorption affinities of the sample components for the surface of an active solid. The analyte interact with solid stationary surface and are displaced with eluent for active sites on surface.

**2. Partition chromatography**

This method results from a thermodynamic distribution of analytes between two liquid phases. On the basis of relative polarities of stationary and mobile phase, partition chromatography can be divided in to normal phase and reverse phase chromatography. In normal phase chromatography, the stationary phase bed is strongly polar in nature (e.g. Silica gel) and the mobile phase is non-polar (such as n-hexane or tetrahydrofuran). Polar sample are thus retained on polar surface of the column packing longer than polar material while in reverse phase chromatography, the stationary bed is non-polar (hydrophobic in nature, while the mobile phase is polar liquid, such as mixture of water and methanol or Acetonitrile. Here the more non polar the material is, the longer it will retain.

**3. Size-exclusion chromatography**

This involves a solid stationary phase with controlled pore size. Solids are separated according to molecular size, with the large molecule unable to enter the pores eluted first.

**4. Ion- exchange chromatography**

Involves a solid stationary phase with anionic or cationic groups on the surface to separation, HPLC and HPTLC methods have widely been exploited in pharmaceutical analysis because of its simplicity, precision, accuracy and reproducibility of result.

**5. Solid-Phase Extraction [SPE]**

A sample preparation technique that uses LC principles to isolate, enriches, and/or purifies analytes from a complex matrix applied to a miniature chromatographic bed. *Offline* SPE is done with larger particles in individual plastic cartridges or in micro-elution plate wells, using low positive pressure or vacuum to assist flow. *Online* SPE is done with smaller particles in miniature HPLC columns using higher pressures and a valve to switch the SPE column online with the primary HPLC column, or offline to waste, as appropriate. SPE methods use step gradients to accomplish bed conditioning, sample loading, washing, and elution steps. The goal is to remove matrix interferences and to isolate the analyte in a solution, and at a concentration, suitable for subsequent analysis.

### 1.2.1. High Performance Liquid Chromatography (HPLC) [6]

The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called *high pressure liquid chromatography*, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as *parts per trillion* (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals.

**Polymer based columns**

Polymer based stationary phases show higher pH stability but lower mechanical stability, compared to silica based columns. Also, polymer based packing's are not compatible with all organic solvents. They swell or shrink in some organic solvents. Unfortunately, the pressure stability and solvent compatibility are different for the different nature of polymers and from manufacturer to manufacturer. Therefore, no general rules for the column care of polymer based materials can be given. Always read the instructions for the use of those columns. In case of doubt please contact the corresponding manufacturer.

## EXPERIMENTAL METHODS

### INSTRUMENTS USED

| Instruments and Glass wares | Model |
|---|---|
| HPLC | WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector. |
| pH meter | Lab India |
| Weighing machine | Sartorius |
| Volumetric flasks | Borosil |
| Pipettes and Burettes | Borosil |

### CHEMICALS USED:

| Chemical | Brand names |
|---|---|
| Abacavir (Pure) | Sura labs |
| Lamivudine (Pure) | Sura labs |
| Water and Methanol for HPLC | LICHROSOLV (MERCK) |
| Acetonitrile for HPLC | Merck |

## RESULTS AND DISCUSSION

**Trail 5 (Optimized Chromatogram):**

| | |
|---|---|
| Column | : Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size |
| Column temperature | : 38˚C |
| Wavelength | : 248nm |
| Mobile phase ratio | : Methanol: TEA buffer pH 4.8 (32:68v/v) |
| Flow rate | : 1ml/min |
| Injection volume | : 20µl |
| Run time | : 7minutes |

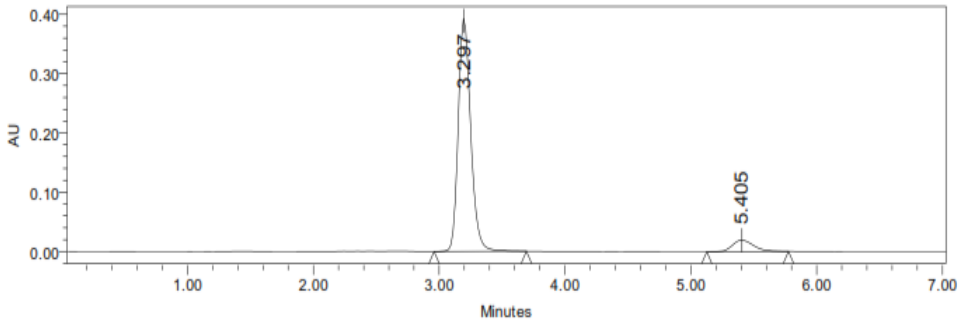


**Figure-: Optimized Chromatogram (Standard)**

**Table-: Optimized Chromatogram (Standard)**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Abacavir | 3.297 | 859856 | 42569 | 1.24 | 7896 | |
| 2 | Lamivudine | 5.405 | 5698 | 3652 | 1.36 | 6582 | 6.8 |

**Observation:** From the above chromatogram it was observed that the Abacavir and Lamivudine peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

**Optimized Chromatogram (Sample)**

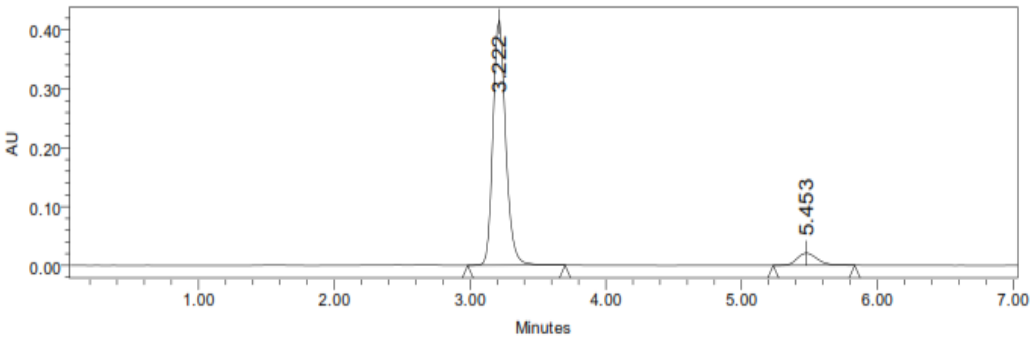


**Figure-: Optimized Chromatogram (Sample)**

**Table-: Optimized Chromatogram (Sample)**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Abacavir | 3.222 | 865898 | 43659 | 1.26 | 7985 | |
| 2 | Lamivudine | 5.453 | 5789 | 3785 | 1.38 | 6659 | 7.0 |

**Acceptance Criteria:**

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

## METHOD VALIDATION

**Blank:**

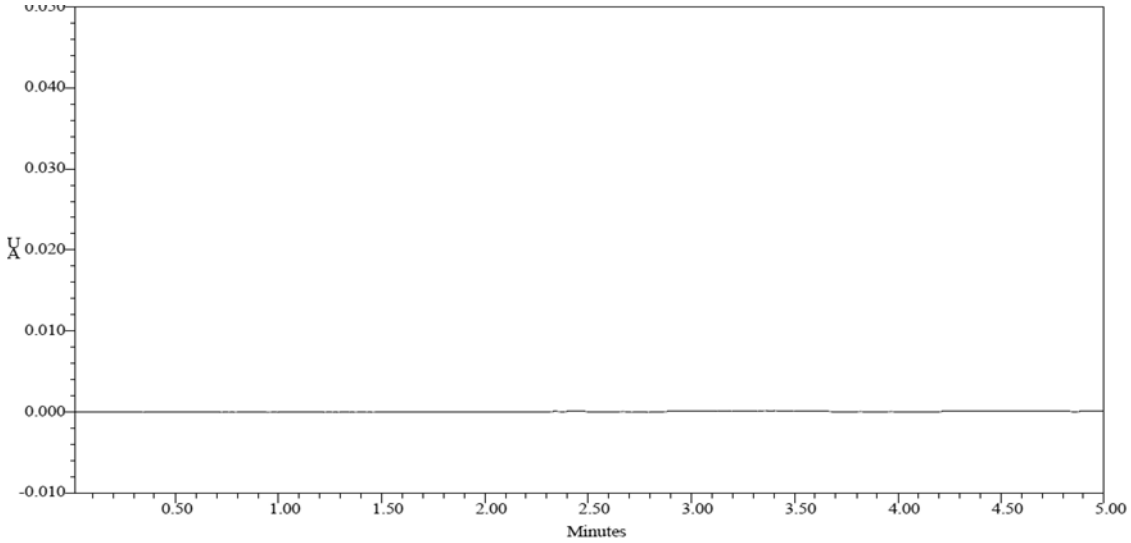


**Fig: Chromatogram showing blank (mobile phase preparation)**

**System Suitability:**

**Table-: Results of system Suitability for Abacavir**

| S.No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Abacavir | 3.200 | 859865 | 42568 | 7895 | 1.24 |
| 2 | Abacavir | 3.248 | 859788 | 42587 | 7859 | 1.24 |
| 3 | Abacavir | 3.299 | 857984 | 42659 | 7869 | 1.24 |
| 4 | Abacavir | 3.297 | 854879 | 42875 | 7849 | 1.24 |
| 5 | Abacavir | 3.297 | 857896 | 42487 | 7859 | 1.23 |
| **Mean** | | | 858082.4 | | | |
| **Std. Dev.** | | | 2024.409 | | | |
| **% RSD** | | | 0.235922 | | | |

**Acceptance Criteria:**

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

**Table-: Results of System Suitability for Lamivudine**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Lamivudine | 5.413 | 5689 | 3659 | 6583 | 1.36 |
| 2 | Lamivudine | 5.484 | 5687 | 3648 | 6592 | 1.37 |
| 3 | Lamivudine | 5.405 | 5682 | 3698 | 6549 | 1.37 |
| 4 | Lamivudine | 5.405 | 5649 | 3675 | 6571 | 1.36 |
| 5 | Lamivudine | 5.409 | 5674 | 3649 | 6529 | 1.36 |
| **Mean** | | | 5676.2 | | | |
| **Std. Dev.** | | | 16.2696 | | | |
| **% RSD** | | | 0.286628 | | | |

**Acceptance Criteria:**

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

## SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Abacavir and Lamivudine in drug product.

**Assay (Standard):**

**Table-: Peak Results for Assay Standard**

**Abacavir**

| S.No. | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Abacavir | 3.211 | 859785 | 42598 | 1.25 | 7856 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2 | Abacavir | 3.222 | 859865 | 42895 | 1.24 | 7859 |
| 3 | Abacavir | 3.254 | 857849 | 42578 | 1.25 | 7869 |

**Lamivudine**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Lamivudine | 5.414 | 5699 | 3685 | 1.36 | 6598 | 6.9 |
| 2 | Lamivudine | 5.453 | 5687 | 3659 | 1.37 | 6537 | 6.9 |
| 3 | Lamivudine | 5.424 | 5689 | 3649 | 1.36 | 6582 | 7.0 |

**Assay (Sample):**

**Table-: Peak Results for Assay sample**

**Abacavir**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Abacavir | 3.297 | 865985 | 43659 | 1.26 | 7985 |
| 2 | Abacavir | 3.294 | 865798 | 43875 | 1.26 | 7925 |
| 3 | Abacavir | 3.295 | 865456 | 43659 | 1.27 | 7946 |

**Lamivudine**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Lamivudine | 5.435 | 5789 | 3659 | 1.37 | 6659 | 6.9 |
| 2 | Lamivudine | 5.417 | 5798 | 3684 | 1.38 | 6689 | 7.0 |
| 3 | Lamivudine | 5.434 | 5749 | 3695 | 1.38 | 6648 | 6.9 |

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Abacavir and Lamivudine in pharmaceutical dosage form was found to be 99.82%.

## LINEARITY

**CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:**

**Abacavir**

| Concentration µg/ml | Average Peak Area |
|---|---|
| 20 | 164436 |
| 30 | 255571 |
| 40 | 348687 |
| 50 | 439024 |
| 60 | 534830 |

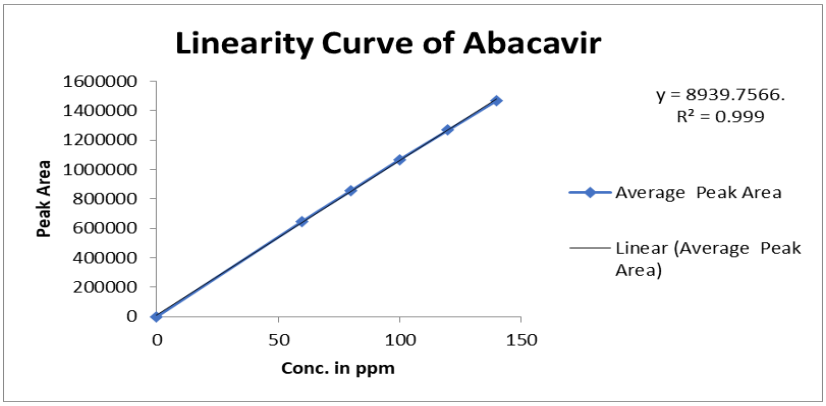


**Fig-: Calibration Curve of Abacavir**

**LINEARITY PLOT:**

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

Y = mx + c

Slope (m) = 8939

Intercept (c) = 9566

Correlation Coefficient (r) = 0.999

**VALIDATION CRITERIA:** The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

**CONCLUSION:** Correlation Coefficient (r) is 0.99, and the intercept is 7566. These values meet the validation criteria.

**Lamivudine**

| Concentration µg/ml | Average Peak Area |
|---|---|
| 25 | 1782454 |
| 37.5 | 2728974 |
| 50 | 3688678 |
| 62.5 | 4658022 |

| | |
|---|---|
| 75 | 5592695 |

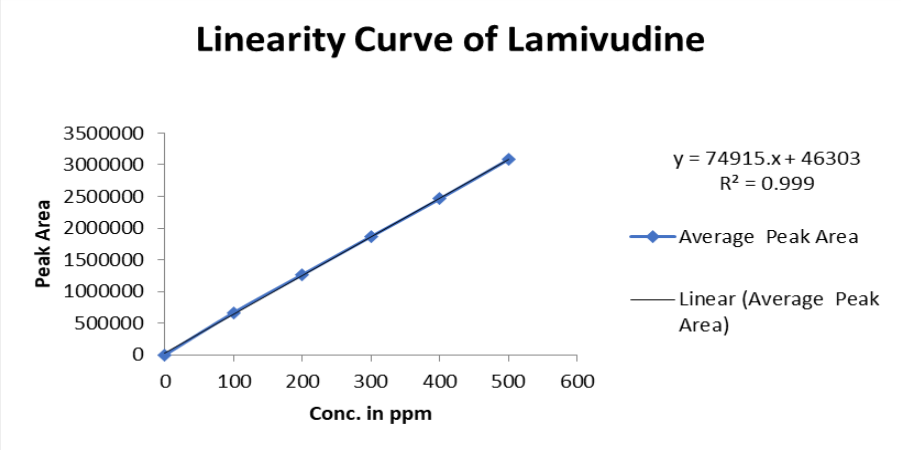


**Fig-: Calibration Curve of Lamivudine**

**LINEARITY PLOT:**

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

Y = mx + c

Slope (m) = 74915

Intercept (c) = 46303

Correlation Coefficient (r) = 0.999

**VALIDATION CRITERIA:** The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

**CONCLUSION:** Correlation Coefficient (r) is 0.99, and the intercept is 46303. These values meet the validation criteria.

**PRECISION:**

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

**REPEATABILITY**

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

**Table-: Results of Repeatability for Abacavir:**

| S. No. | Peak name | Retention time | Area(µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Abacavir | 3.213 | 859856 | 42659 | 7859 | 1.24 |
| 2 | Abacavir | 3.253 | 857985 | 42598 | 7869 | 1.24 |
| 3 | Abacavir | 3.297 | 856984 | 42587 | 7846 | 1.25 |
| 4 | Abacavir | 3.215 | 856987 | 42569 | 7819 | 1.25 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5 | Abacavir | 3.254 | 859878 | 42894 | 7856 | 1.24 |
| **Mean** | | | 858338 | | | |
| **Std.dev** | | | 1454.222 | | | |
| **%RSD** | | | 0.169423 | | | |

**Acceptance criteria:**

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

**Table-: Results of repeatability for Lamivudine:**

| S. No. | Peak Name | Retention time | Area(µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Lamivudine | 5.441 | 5697 | 3659 | 6592 | 1.36 |
| 2 | Lamivudine | 5.442 | 5689 | 3648 | 6539 | 1.36 |
| 3 | Lamivudine | 5.409 | 5698 | 3692 | 6584 | 1.37 |
| 4 | Lamivudine | 5.520 | 5639 | 3648 | 6579 | 1.36 |
| 5 | Lamivudine | 5.424 | 5688 | 3689 | 6549 | 1.36 |
| **Mean** | | | 5682.2 | | | |
| **Std.dev** | | | 24.57031 | | | |
| **%RSD** | | | 0.432408 | | | |

**Intermediate precision:**

**Day 1:**

**Table-: Results of Intermediate precision for Abacavir**

| S.No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Abacavir | 3.211 | 868956 | 43659 | 7985 | 1.26 |
| 2 | Abacavir | 3.211 | 869857 | 43985 | 7954 | 1.27 |
| 3 | Abacavir | 3.210 | 865983 | 43879 | 7946 | 1.26 |
| 4 | Abacavir | 3.212 | 866587 | 43865 | 7963 | 1.27 |
| 5 | Abacavir | 3.211 | 864256 | 43875 | 7964 | 1.26 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6 | Abacavir | 3.297 | 868974 | 43562 | 7942 | 1.26 |
| **Mean** | | | 867435.5 | | | |
| **Std. Dev.** | | | 2167.095 | | | |
| **% RSD** | | | 0.249828 | | | |

**Acceptance criteria:**

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

**Table-: Results of Intermediate precision for Lamivudine**

| S.No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Lamivudine | 5.411 | 5785 | 3789 | 6659 | 1.37 |
| 2 | Lamivudine | 5.410 | 5798 | 3758 | 6625 | 1.38 |
| 3 | Lamivudine | 5.420 | 5766 | 3746 | 6649 | 1.38 |
| 4 | Lamivudine | 5.423 | 5746 | 3795 | 6675 | 1.37 |
| 5 | Lamivudine | 5.419 | 5782 | 3761 | 6653 | 1.38 |
| 6 | Lamivudine | 5.409 | 5786 | 3752 | 6627 | 1.37 |
| **Mean** | | | 5777.167 | | | |
| **Std. Dev.** | | | 18.40018 | | | |
| **% RSD** | | | 0.318498 | | | |

**Acceptance Criteria:**

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

**Day 2:**

**Table-: Results of Intermediate precision Day 2 for Abacavir**

| S.No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Abacavir | 3.211 | 845985 | 44585 | 8025 | 1.27 |
| 2 | Abacavir | 3.233 | 847895 | 44895 | 8069 | 1.28 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | Abacavir | 3.244 | 848985 | 44758 | 8046 | 1.27 |
| 4 | Abacavir | 3.297 | 847859 | 44548 | 8094 | 1.28 |
| 5 | Abacavir | 3.297 | 845984 | 44865 | 8042 | 1.28 |
| 6 | Abacavir | 3.202 | 847898 | 44254 | 8076 | 1.27 |
| **Mean** | | | 847434.3 | | | |
| **Std. Dev.** | | | 1201.345 | | | |
| **% RSD** | | | 0.141763 | | | |

**Acceptance Criteria:**

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

**Table-: Results of Intermediate precision Day 2 for Lamivudine**

| S.No. | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Lamivudine | 5.411 | 5898 | 3986 | 6852 | 1.39 |
| 2 | Lamivudine | 5.410 | 5884 | 3955 | 6864 | 1.39 |
| 3 | Lamivudine | 5.420 | 5863 | 3956 | 6829 | 1.40 |
| 4 | Lamivudine | 5.405 | 5845 | 3945 | 6874 | 1.39 |
| 5 | Lamivudine | 5.409 | 5896 | 3925 | 6829 | 1.39 |
| 6 | Lamivudine | 5.463 | 5874 | 3962 | 6825 | 1.40 |
| **Mean** | | | 5876.667 | | | |
| **Std. Dev.** | | | 20.39281 | | | |
| **% RSD** | | | 0.347013 | | | |

**Acceptance Criteria:**

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

## ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

**Table-: The accuracy results for Abacavir**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 451144.3 | 25 | 24.998 | 99.992% | 100.1873% |
| 100% | 897248.3 | 50 | 50.104 | 100.208% | |
| 150% | 1344562 | 75 | 75.278 | 100.362% | |

**Acceptance Criteria:**

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

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

**Table-: The accuracy Results for Lamivudine**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 2895 | 15 | 15.084 | 100.560% | 100.748% |
| 100% | 5685.333 | 30 | 30.282 | 100.940% | |
| 150% | 8449 | 45 | 45.335 | 100.744% | |

**Acceptance Criteria:**

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

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

## LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

**Abacavir:**

**Result:**

=2.6µg/ml

**Lamivudine:**

**Result:**

=3.4µg/ml

**Quantitation limit**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

**LOQ=10×σ/S**

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

**Abacavir:**

**Result:**

=7.8µg/ml

**Lamivudine:**

**Result:**

=10.2µg/ml

**ROBUSTNESS**

**Table-: Results for Robustness**

**Abacavir**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0mL/min | 859856 | 3.297 | 7896 | 1.24 |
| Less Flow rate of 0.9mL/min | 915847 | 3.639 | 7251 | 1.20 |
| More Flow rate of 1.1mL/min | 842564 | 2.859 | 7415 | 1.21 |
| Less organic phase (about 5 % decrease in organic phase) | 825498 | 3.460 | 7365 | 1.23 |
| More organic phase (about 5 % Increase in organic phase) | 814578 | 3.022 | 7258 | 1.22 |

**Acceptance Criteria:**

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

**Table-: Results for Robustness**

**Lamivudine**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.1mL/min | 5698 | 5.405 | 6582 | 1.36 |
| Less Flow rate of 0.9mL/min | 6452 | 6.250 | 6785 | 1.32 |
| More Flow rate of 0.8mL/min | 5254 | 4.863 | 6365 | 1.34 |
| Less organic phase (about 5 % decrease in organic phase) | 5487 | 6.196 | 6254 | 1.38 |
| More organic phase (about 5 % Increase in organic phase) | 5369 | 5.010 | 6298 | 1.33 |

**Acceptance Criteria:**

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

## SUMMARY AND CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Abacavir and Lamivudine was done by RP-HPLC.

The TEA buffer was $p^H$ 4.8 and the mobile phase was optimized with consists of Methanol: TEA buffer mixed in the ratio of 32:68 % v/ v.

A Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size or equivalent chemically bonded to porous silica particles was used as stationary phase.

The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Abacavir and Lamivudine were found to be from 30-70µg/ml, 10-50µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Abacavir and Lamivudine. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear.

The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

## ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Princeton College of Pharmacy, Narapally, Ghatkesar, Telangana, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Pharma Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

## REFERENCES


1. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
2. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
3. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf
4. Detectors used in HPLC (online )URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
5. Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
6. Dr.Kealey and P.J.Haines, Analytical Chemistry, 1stedition, Bios Publisher,(2002),PP:1-7.
7. A.BraithWait and F.J.Smith, Chromatographic Methods, 5thedition, Kluwer Academic Publisher, (1996), PP 1-2.
8. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
9. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1stedition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
10. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
11. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, (1995), PP 1126.
12. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1- 8.
13. Introduction to analytical method validation (online), available from: URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
14. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
15. Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, (1997), PP 180-182.
16. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. (1994), PP 1-5.
17. Sharma B K, Instrumental method of chemical analysis Meerut. (1999), PP 175-203.
18. Willard, H. y. Merritt L.L, Dean J.A and Settle F.A "Instrumental methods of analysis" 7th edition CBS publisher and distributors, New Delhi, (1991), PP 436-439.
19. ICH Q2A, "validation of analytical methods, definitions and terminology", ICH Harmonized tripartite guideline, (1999).
20. https://go.drugbank.com/drugs/DB01048
21. https://en.wikipedia.org/wiki/Abacavir
22. https://en.wikipedia.org/wiki/Lamivudine
23. https://www.drugbank.ca/drugs/DB00709
24. Ahmed Serag , Mohamed A. Hasan , Enas H. Tolba , Ahmed M. Abdelzaher , Ayman Abo Elmaaty, Analysis of the ternary antiretroviral therapy dolutegravir, lamivudine and abacavir using UV spectrophotometry and chemometric tools, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy Volume 264, 5 January 2022, 120334
25. Srinivas Lella, P. Venkateswararao And N. Srinivasrao, A Stability Indicating Rp-Hplc Metods For Simultaneous Estimation Of The Abacavir, Lamivudine And Dolutegravir In Bulk And Pharmaceutical Dosage Form, INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY, 2019, 9(3), 146-153
26. Khaleel Noorbasha & Sharmila Nurbhasha, A new validated stability-indicating RP-HPLC method for simultaneous quantification of dolutegravir and lamivudine in bulk and pharmaceutical dosage form, Future Journal of Pharmaceutical Sciences volume 6, Article number: 39 (2020)
27. Sufiyan Ahmad Raees Ahmad,1 Lalit Patil,1 Mohammed Rageeb Mohammed Usman,2 Mohammad Imran,3 and Rashid Akhtar, Analytical Method Development and Validation for the Simultaneous Estimation of Abacavir and Lamivudine by Reversed-phase High-performance Liquid Chromatography in Bulk and Tablet Dosage Forms, Pharmacognosy Res. 2018 Jan-Mar; 10(1): 92–97.
28. Rajkumar Prava, Ganapathy Seru, Vamsi Krishna Pujala, RP-HPLC method development and validation for the simultaneous determination of lamivudine, abacavir and dolutegravir in pharmaceutical dosage forms, World Journal of Pharmaceutical Sciences, 26-04-2017.