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# New analytical method development and validation of abacavir by RP-HPLC

**B Sravanthi***

Omega college of pharmacy, Moradabad, Uttar Pradesh, India

**Corresponding author:** B Sravanthi, ✉

Omega college of pharmacy, Moradabad, Uttar Pradesh, India



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## ABSTRACT

A simple, precise, accurate, specific and RP-HPLC method was developed for determination of Abacavir in pharmaceutical formulation. The presented method is simple, since diluted samples are directly used without any preliminary chemical dramatization or purification steps. The proposed method was validated for various ICH parameters like linearity, limit of detection, accuracy, precision, ruggedness, robustness, and system suitability. A RP-HPLC assay utilized Symmetry C18 (4.6 x 150mm, 5 m, Make: XTerra) or equivalent with mobile phase composition of ph 7 buffer: acetonitrile was used, and flow rate was 0.8 ml $min^{-1}$ with UV detection at 285 nm. The retention time Abacavir of was 2.573 min respectively. The total RP-HPLC run time was 5 min. Linearity was observed over concentration range of 20-60 µg/ml for Abacavir. Commercial tablet formulations and laboratory prepared dilutions were successfully analyzed using the developed methods.

**Keywords:** Abacavir, RP-HPLC, ph 7 buffer: Acetonitrile.

## INTRODUCTION TO ANALYSIS

Analysis is important in any product or service, but in drug it is very important as it involves life. In comparison to general consumer products, in drugs there is and there can be only quality/standard product and no other product this comes from series of tests from quality control, starting from raw materials in process during manufacture, finished product is the moral obligation to the patients, hence the manufacture and quality of drugs should be taken care off. These tests may vary from single entity or combination of several potent drugs in formulation these tests of quality control may belong to the following types:

**Chemical methods**
**Physicochemical methods**
**Microbiological methods**
**Biological methods**

## INTRODUCTION TO CHROMATOGRAPHY

**Chromatography**

A method of separating and identifying the components of a complex mixture by differential movement through a two-phase system, in which the movement is affected by a flow of a liquid or a gas (mobile phase) which percolates through an adsorbent (stationary phase) or a second liquid phase [1].

**Types of chromatography**

Paper Chromatography
Column Chromatography
Thin Layer Chromatography
Gas Chromatography
Ion Exchange Chromatography
Affinity Chromatography
Two-Dimensional Chromatography
High Performance (Pressure) Liquid Chromatography
High Pressure Thin Layer Chromatography
HPLC (High Pressure / Performance Liquid Chromatography)

**Principle**

The mainprinciple involved in HPLC is adsorption. When a mixture of components is introduced into a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary

phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated.

The technique of high-performance liquid chromatography is so called because of its improved performance when compared to classical column chromatography. It is also called as high pressure liquid chromatography since high pressure is used when compared to classical column chromatography [2].

**Types of HPLC techniques**

**Based on modes of chromatography**

Normal-phase chromatography

Reversed-phase chromatography (RPC)

BASED ON PRINCIPLE OF SEPARATION:

Adsorption chromatography

Chiral Phase chromatography

Size-exclusion (or) Gel Permeation chromatography

Ion-exchange chromatography

Ion – Pair chromatography

Affinity chromatography

**Elution technique**

Isocratic Separation

Gradient Separation

**Based on scale of operation**

Analytical HPLC

Preparative HPLC

Based on type of analysis

Qualitative Analysis

Quantitative Analysis

**Instrumental requirements**

Pumps- solvent delivery system

Mixing unit, gradient controller and solvent degassing

Injector- manual or auto injectors

Guard column

Analytical columns

Detectors

Recorders and Integrators

## The schematic diagram of HPLC

**Figure 1:** Instrumentation of HPLC

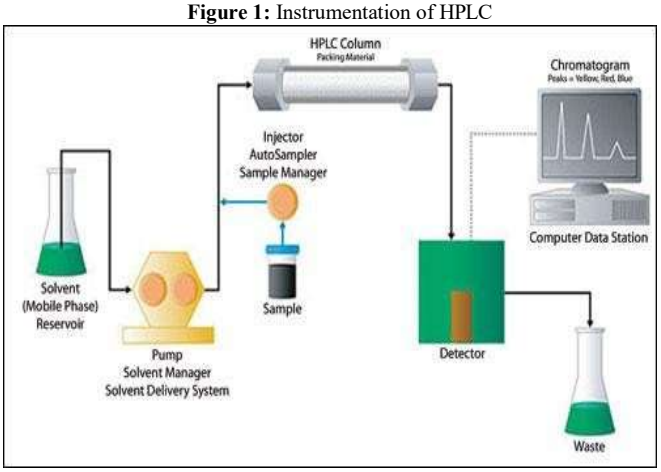


**Figure 2:** HPLC equipment

**Parameters used in HPLC**

Retention time

Retention volume

Separation factor

Resolution

Theoretical plate

HETP- Height Equivalent to a Theoritical Plate

Efficiency (no of theoretical plates)

Assymetry factor- Fronting Tailing

RP-HPLC (Reverse Phase High Pressure Liquid Chromatography) [3].

## INTRODUCTION

Reversed-phase chromatography (RP-HPLC) separates molecules on the basis of differences in their hydrophobicity. The components of the analyst mixture pass over stationary-phase particles bearing pores large enough for them to enter, where interactions with the hydrophobic[6] surface removes them from the flowing mobile-phase stream. The strength and nature of the interaction between the sample particles and the stationary phase depends on both hydrophobic interactions and polar interactions. As the concentration of organic solvent in the eluant increases, it reaches a critical value for each analyzed which desorbs it from the hydrophobic stationary-phase surface and allows it to elute from the column in the flowing mobile phase.

Since this elution depends on the precise distribution of hydrophobic residues in each species, each analyte elutes from the column at a characteristic time, and the resulting peak can be used to confirm its identity and quantify it.

Mobile phase solvents:

Main solvent: MeOH-H2O, CH3CN - H2O

Sub solvent: EtOH, IPA, THF, DMF

Additive: Acid, Salt, Ion-pairing agent

## MATERIALS AND METHODLOGY

DRUG: Abacavir

MOBILE PHASE**:** Acetonitrile + Buffer (60:40) [4].

## METHODOLOGY

### RP-HPLC method

**Determination of abacavir by reverse phase high performance liquid chromatography (RP-HPLC)**

A simple, precise, accurate, specific and RP- HPLC method was developed for determination of Abacavir in pharmaceutical formulation. The presented method is simple, since diluted samples are directly used without any preliminary chemical dramatization or purification steps. A RP-HPLC assay utilized Symmetry C18 (4.6 x 150mm, 5 □m, make: XTerra) or equivalent with mobile phase composition of phosphate buffer: acetonitrile [40:60] with ph 7 was used, and flow rate was 0.8 ml $min^{-1}$ with UV detection at 285 nm. The retention time of Abacavir was 2.573 min respectively. The total RP-HPLC run time was 5 min. Linearity was observed over concentration range of 20-60 µg/ml for Abacavir. The proposed method was validated for various ICH parameters like linearity, limit of detection, accuracy, precision, ruggedness, robustness, and system suitability. Commercial tablet formulations and laboratory prepared dilutions were successfully analyzed using the developed methods.

### Method validation

### Precision

**Preparation of stock solution**

Accurately weigh and transfer 10 mg of Abacavir Working standard into a 10 ML volumetric flask add about 7 ML of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

**Preparation of 40 µg/ML solution**

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. Mix well and filter through 0.45µm filter.

**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 within the specified limits [4, 5].

## RESULTS AND DISCUSSION

**Table 1:** Linearity studies of Abacavir in RPHPLC

| Linearity | Concentration | Area |
|---|---|---|
| I | 20µg/ml | 1446976 |
| II | 30µg/ml | 2032387 |
| III | 40µg/ml | 2668344 |
| IV | 50µg/ml | 3324757 |
| V | 60µg/ml | 3869812 |
| **Correlation coefficient** | | **0.999** |

**Table 2:** Precision studies of Abacavir in RPHPLC

| INJECTION | AREA |
|---|---|
| Injection-1 | 3065639 |
| Injection-2 | 2991185 |
| Injection-3 | 2992147 |
| Injection-4 | 3047484 |
| Injection-5 | 3015295 |
| **AVERAGE** | **3022350** |
| **STANDARDDEVIATION** | **33312.8** |
| **%RSD** | **1.10** |

**Table 3:** Intermediate Precision studies of Abacavir in RP-HPLC

| INJECTION | AREA |
|---|---|
| Injection-1 | 2720665 |
| Injection-2 | 2732682 |
| Injection-3 | 2732214 |
| Injection-4 | 2723099 |
| Injection-5 | 2718033 |
| **AVERAGE** | **2725349** |
| **STANDARD DEVIATION** | **6721.2** |
| **%RSD** | **0.25** |

**Table 4:** Accuracy studies of Abacavir in RP-HPLC

| % CONCENTRATION (at specification Level) | AREA | AMOUNT ADDED (mg) | AMOUNT FOUND (mg) | % RECOVERY | MEAN |
|---|---|---|---|---|---|
| 50% | 1551884 | 5.23 | 5.29 | 101.3% | 99.9 % |
| 100% | 2842401 | 9.8 | 9.70 | 99.0% | |
| 150% | 3987551 | 15.0 | 14.9 | 99.5% | |

**Table 5:** Effect of variation of flow rate

| FLOW RATE | SYSTEM SUITABILITY RESULTS | |
|---|---|---|
| | USP PLATE COUNT | USP TAILING |
| 0.6 | 2264.7 | 1.7 |
| 0.8 | 2349.5 | 1.6 |
| 1.0 | 2087.4 | 1.5 |

**Table 6:** Effect of variation of organic phase

| FLOW RATE | SYSTEM SUITABILITY RESULTS | |
|---|---|---|
| | USP PLATE COUNT | USP TAILING |
| 0.6 | 2264.7 | 1.7 |
| 0.8 | 2349.5 | 1.6 |
| 1.0 | 2087.4 | 1.5 |
| | | |

## DISUSION

### RP-HPLC METHOD

The objective of the proposed work was to develop methods for the determination of Abacavir and to validate the methods according to ICH guidelines and applying the same for its estimation in pharmaceutical formulations. There is no official method for the estimation of Abacavir. The present developed HPLC method developed was found to be rapid, simple, precise, accurate and economic for routine estimation of Abacavir in commercial dosage forms.

In RP- HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to elute title ingredient. Mobile phase and flow rate selection was based

on peak parameters (height, capacity, theoretical plates, tailing or symmetry factor), run time, resolution. The average retention time for Abacavir was found to be 2.57 ± 0.002 min. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 20-60 µg $mL^{-1}$.

The low values of % R.S.D indicate the method is precise and accurate. The mean recoveries were found in the range of 98.5 – 102 %. Sample to sample precision and accuracy were evaluated using three samples of five different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using five concentrations analyzed on three trials over a period of three days. These results show the accuracy and reproducibility of the assay.

The proposed methods are accurate, simple, rapid and selective for the estimation of Abacavir in pharmaceutical formulations [6].

## CONCLUSION

### RP-HPLC method

For routine analytical purpose it is desirable to establish methods capable of analysing huge number of samples in a short time period with good robust, accuracy and precision without any prior separation step. HPLC method generate large amount of quality data which serve as highly powerful and convenient analytical tool.

The run time of the HPLC procedure is only 5 minutes. Good agreement was seen in the assay results of pharmaceutical formulation as well as in laboratory prepared mixtures by developed methods. We concluded that all the proposed methods are a good approach for obtaining reliable results and were found to be suitable for the routine estimation of Abacavir in pharmaceutical formulation.

**Declaration of interest**

The authors declare that there is no conflict of interest.

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