International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 14, Issue 5, 2022

Original Article

# DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF ABACAVIR IN TABLET DOSAGE FORM

**AKHILESH S. TOKEY*, MUGDHA R. SURYAWANSHI, PRANAV P. TAMBE**

**Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be) University, Erandwane, Pune (MH), India 411038**
**Email: akhilesh.tokey@gmail.com**

*Received: 15 Jun 2022, Revised and Accepted: 22 Jul 2022*

**ABSTRACT**

**Objective:** To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Abacavir in tablet dosage form.

**Methods:** The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on the melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption, which reflect in linearity. The UV spectroscopic method was developed for estimation of Abacavir in tablet dosage form and also validated as per ICH guidelines.

**Results:** The drug is freely soluble in analytical grade methanol, slightly soluble in water and practically insoluble in ethanol. So, the analytical grade methanol is used as a diluent in method. The melting point of Abacavir was found to be 164-165 ˚C (uncorrected). It showed absorption maxima 256 nm in analytical grade methanol. On the basis of absorption spectrum the working concentration was set on 15µg/ml (PPM). The linearity was observed between 5-25 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 101 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.32% and for Ruggedness is 0.46%

**Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Abacavir in tablet dosage form. The method could be considered for the determination of Abacavir in quality control laboratories.

**Keywords:** Abacavir, UV Spectrophotometer, Melting point, Assay method, Validation, Accuracy, Linearity, Ruggedness, Precision


DOI: https://dx.doi.org/10.22159/ijcpr.2022v14i5.2024 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

## INTRODUCTION

Abacavir is a 2,6-diaminopurine that is (1S)-cyclopent-2-en-1-ylmethanol in which the pro-R hydrogen at the 4-position is substituted by a 2-amino-6-(cyclopropylamino)-9H-purin-9-yl group [1]. Abacavir is a nucleoside reverse transcriptase inhibitor that inhibits viral replication. It is a guanosine analogue that is phosphorylated to carbovir triphosphate (CBV-TP). CBV-TP competes with the viral molecules and is incorporated into the viral DNA [2]. Abacavir is used along with other medications to treat human immunodeficiency virus (HIV) infection. Abacavir is in a class of medications called nucleoside reverse transcriptase inhibitors (NRTIs). It works by decreasing the amount of HIV in the blood. Abacavir is rapidly absorbed after oral administration, with peak concentrations occurring 0.63-1 hour after dosing. The absolute bioavailability of abacavir is approximately 83% [3]. Abacavir pharmacokinetics are linear and dose-proportional over the range of 300-1200 mg/day. The most common side effects of abacavir are hypersensitivity (allergic) reaction (see the previous section), feeling sick, headache, being sick, diarrhoea, loss of appetite, tiredness, lack of energy, fever (high temperature) [4].

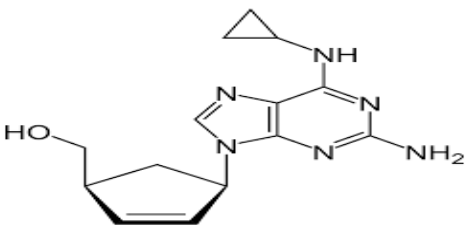


**Fig. 1: Chemical structure of abacavir**

Literature survey revealed that a limited number of Spectrometric method found in combination with lamivudine [5-7], one spectroscopic method through oxidative coupling [8], RP-HPLC [9, 10] methods were reported for the assay of Abacavir alone and in combination with other drugs. Also some method were reported for Quantitative determination of abacavir in Human urine, CSF, Human Plasma and rat tissue [11-14]. Some of these methods lack adequate sensitivity, and some are expensive and time-consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Abacavir alone in tablet dosage form.

## MATERIALS AND METHODS

### Instruments

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance. Melting point apparatus of Equiptronics were used.

### Materials

Abacavir was obtained as a gift sample. Abacavir tablets were procured from the local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

### Method development

### Determination of λ max (15 PPM) [15, 16]

50 mg weighed amount of Abacavir was dissolved into 100 ml of the volumetric flask with analytical grade methanol. Pipette out 1.5 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and the absorption maximum was determined.

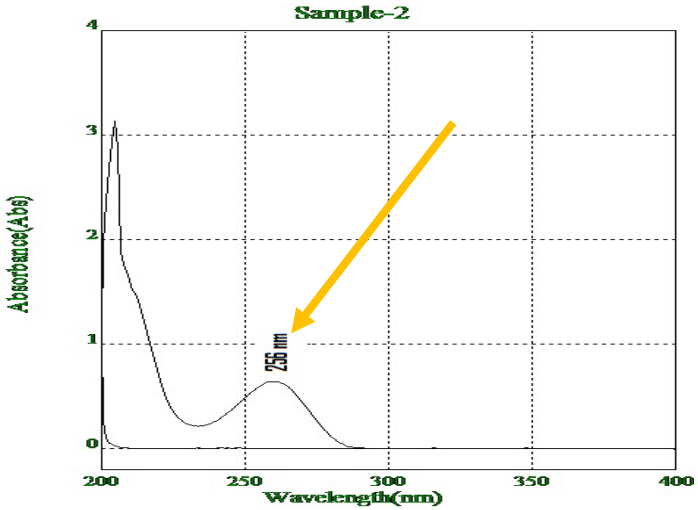


**Fig. 2: Calibration curve**

**Preparation of working concentration**

**Preparation of Standard stock solution**

Standard stock was prepared by dissolving 50 mg of Abacavir in 100 ml of analytical grade methanol to get the concentration of 500 µg/ml (PPM).

**Preparation of standard solution**

Pipette out 1.5 ml from standard stock solution and diluted up to 50 ml with analytical grade methanol to get the concentration of 15 µg/ml (PPM).

**Procedure for UV reading**

**Blank solution:** (For Auto zero)

Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly, then placed inside the chamber. Note down the reading.

**Standard solution**

Fill the cuvette with the standard solution. Wipe it with tissue paper properly, then placed it inside the chamber. Note down the reading.

**Sample solution**

Fill the cuvette with a sample solution. Wipe it with tissue paper properly, then placed it inside the chamber. Note down the reading.

**Procedure for sample preparations [17-19]**

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Abacavir was accurately weighed and transferred into the 100 ml of volumetric flask, added 70 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication, cool the flask and diluted upto 100 ml with analytical grade methanol. Filtered the solution through nylon syringe filter 0.45 µ. Pipette out 1.5 ml of the filtered solution and diluted up to 50 ml with analytical grade methanol. The absorbance was measured at 256 nm. The absorbance was recorded.

**Table 1: Absorbance of dosage form**

| Cipla pharma Pvt. Ltd. (Abacavir sulfate 300 mg tablets) | | |
|---|---|---|
| **S. No.** | **Sample** | **Absorbance** |
| 1 | Blank | 0.0000s |
| 2 | Standard | 0.6584 |
| 3 | Sample | 0.6514 |

**Table 2: Dosage form specifications**

| Type | Brand/Company | M.D. | E.D. | Batch No. | Avg wt (g) | Assay (%) |
|---|---|---|---|---|---|---|
| 1 | ABAMUNE-300 Cipla Pharma Pvt LTD (300 mg) | 08/2021 | 07/2023 | SHD25487 | 0.4101 | 98.94 |

**Method of validation [18, 20, 21]**

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

**Linearity**

The linearity of the proposed assay was studied in the concentration range 5-25 PPM at 256 nm. The calibration data showed a linear relationship between concentrations.

**Accuracy**

To ensure the accuracy of the method, a recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100 ml of the volumetric flask with analytical grade methanol and measuring the absorbance at 256 nm.

**Table 3: Linearity studies**

| S. No. | Sample concentration | Absorbance |
|---|---|---|
| 1 | 5 PPM | 0.2284 |
| 2 | 10 PPM | 0.4212 |
| 3 | 15 PPM | 0.6564 |
| 4 | 20 PPM | 0.8654 |
| 5 | 25 PPM | 1.0895 |
| Correlation coefficient | | 0.9993 ~ 0.999 |

**Table 4: Accuracy studies**

| Spectrophotometric method | | | |
|---|---|---|---|
| **Accuracy (%)** | **Qty weighed (mg)** | **Qty found (mg)** | **Recovery (98-102%)** |
| 80 | 0.8 | 0.79 | 98.75 |
| 100 | 1 | 1.01 | 101.00 |
| 120 | 1.2 | 1.19 | 99.17 |

**Precision**

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

**Ruggedness**

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating conditions from instrument to instrument and from analyst to analyst.

**Table 5: Precision studies**

| S. No. | Sample solution | Absorbance |
|---|---|---|
| 1 | Sample Solution 1 | 0.6552 |
| 2 | Sample Solution 2 | 0.6554 |
| 3 | Sample Solution 3 | 0.6511 |
| 4 | Sample Solution 4 | 0.6557 |
| 5 | Sample Solution 5 | 0.6525 |
| MEAN | | 0.6540 |
| SD | | 0.0021 |
| % RSD | | 0.3147 |

**Table 6: Results for ruggedness studies**

| S. No. | Analyst | Results | Mean | % Assay | % RSD |
|---|---|---|---|---|---|
| 1 | Analyst 1 | 0.6519<br>0.6526 | 0.6523 | 99.07 | 0.4553 |
| 2 | Analyst 2 | 0.6545<br>0.6584 | 0.6565 | 99.71 | |

**RESULTS**

**Solubility of abacavir**

Solubility test was passed as per the criteria.

**Table 7: Results for solubility studies**

| S. No. | Title | Result |
|---|---|---|
| 1 | Analytical grade Methanol | Freely Soluble |
| 2 | Water | Slightly soluble |
| 3 | Ethanol | Practically insoluble |

**Melting point of abacavir**

The melting point of Abacavir was found to be 164-165°C (uncorrected).

**Results for linearity for assay method of Abacavir**

The linearity of the method was determined at concentration level ranging from 5 to 25 µg/ml (PPM). The correlation coefficient value was found to be ($R^2$) 0.9993 ~ 0.999.

**Results for accuracy for assay method of Abacavir**

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery was calculated and represented in table 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

**Results for precision for assay method of abacavir**

The % RSD for a different sample of precision was found to be 0.3147 ~ 0.32 and it is within the acceptance criteria represented in table 5.

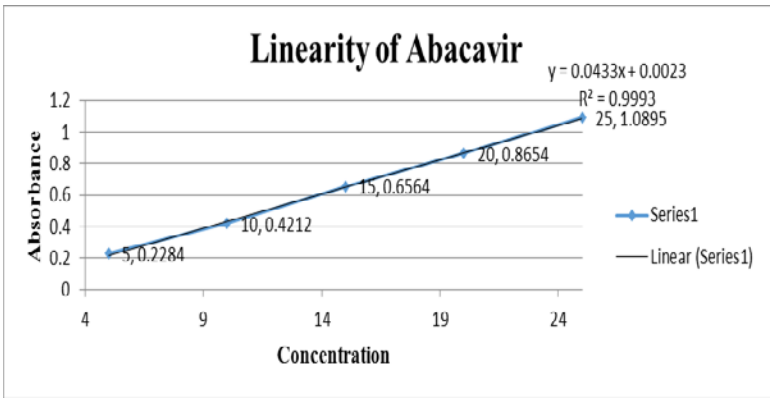


**Fig. 3: Abacavir standard curve**

**Results for ruggedness for assay method of abacavir**

The %RSD for a different sample of ruggedness was found to be 0.4553 ~ 0.46 and it is within the acceptance criteria represented in table 6.

## CONCLUSION

A method for the estimation of Abacavir in tablet form has been developed. From the spectrum of Abacavir, it was found that the maximum absorbance was 256 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 5-25 µg/ml (PPM). The high percentage of recovery indicates the high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Abacavir in solid dosage forms. Hence, the method could be considered for the determination of Abacavir in quality control laboratories.

## ABBREVIATIONS

PPM-Parts per Million, nm-Nanometer, HPLC-High Performance Liquid Chromatography, UV-Ultra violet, MS-Mass Spectroscopy, LC-Liquid Chromatography, ICH-International Council for Harmonization, RSD-Relative Standard Deviation, SD-Standard Deviation, Qty-Quantity, °C-Degree Celsius, M. D.-Manufacturing Date, E. D.-Expiry Date, µg/ml-Microgram per milliliter, Avg-Average, Wt-Weight, g-gm, CBV-TP-Carbovir Triphosphate, HIV-Human Immunodeficiency Virus, NRTIs-Nucleoside Reverse Transcriptase Inhibitors, CSF-Cerebro Spinal Fluid.

## FUNDING

Nil

## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICT OF INTERESTS

Declared none

## REFERENCES

1. en.wikepedia.org/wiki/abacavir. [Last accessed on 22 Mar 2022]
2. https://go.drug bankDrugbank.com/salts/DBSALT000871. [Last accessed on 22 Mar 2012]
3. Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J. HLA-b*5701 screening for hypersensitivity to abacavir. N Engl J Med. 2008;358(6):568-79. doi: 10.1056/NEJMoa0706135, PMID 18256392.
4. Rauch A, Nolan D, Martin A, McKinnon E, Almeida C, Mallal S. Prospective genetic screening decreases the incidence of abacavir hypersensitivity reactions in the Western Australian HIV cohort study. Clin Infect Dis. 2006;43(1):99-102. doi: 10.1086/504874, PMID 16758424.
5. Basavaiah K, Somashekar BC, Ramakrishna V. Rapid titrimetric and spectrophotometric assay methods for the determination of lamivudine in pharmaceuticals using iodate and two dyes. J Anal Chem. 2007;62(6):542-8. doi: 10.1134/S1061934807060081.
6. Venkatamahesh R, Dhachinamoorthi D. Visible spectrophotometric determination abacavir sulphate bulk drug tablet dosage form. International Journal of Pharm Tech Research. 2011;3(1):356-9.
7. Devmurari VP, Patel RB. Simultaneous spectrophotometric determination of lamivudine and abacavir in the mixture. Int J Pharm Sci Res. 2010;1:82-6.
8. Pant P, Saradhi SV, Felice CS, Gurung B, Divya VG, Rao NM. Spectrophotometric determination of acyclovir through oxidative coupling of with 2,2-bipyridine by hors radish peroxidase (HRP). J Appl Chem Res. 2009;10:7-12.
9. Anantha Kumar D, Rao SG, Seshagiri Rao JVLN. Simultaneous determination of lamivudine, zidovudine and abacavir in tablet dosage form by RPHPLC method. E-Journal of Chemistry 2010;7(1):180-4.
10. Sudha T, kumar RVR, Hemalatha PV. RP-HPLC method for simultaneous Estimation of lamivudine and abacavir sulfate in tablet form. International Journal on Pharmaceutical Biomedical Res. 2008;1(4):108-13.
11. Ravitch JR, Moseley CG. High-performance liquid chromatographic assay for abacavir and its two major metabolites in human urine and cerebrospinal fluid. J Chromatogr B Biomed Sci Appl. 2001;762(2):165-73. doi: 10.1016/s0378-4347(01)00361-9, PMID 11678376.
12. Veldkamp AI, Sparidans RW, Hoetelmans RM, Beijnen JH. Quantitative determination of abacavir (1592U89), a novel nucleoside reverse transcriptase inhibitor, in human plasma using isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Sci Appl. 1999;736(1-2):123-8. doi: 10.1016/s0378-4347(99)00457-0. PMID 10676991.
13. Sparidans RW, Hoetelmans RM, Beijnen JH. Liquid chromatographic assay for simultaneous determination of abacavir and mycophenolic acid in human plasma using dual spectrophotometric detection. J Chromatogr B Biomed Sci Appl. 2001;750(1):155-61. doi: 10.1016/s0378-4347(00)00439-4, PMID 11204216.
14. Lewis SR, White CA, Bartlett MG. Simultaneous determination of abacavir and zidovudine from rat tissue using HPLC with UV detection. J Chromatogr B. 2007;85:45-52.
15. ICH draft guidelines on validation of analytical procedures: definitions and terminology, federal register. Switzerland: International Federation of Pharmaceutical Manufacturers Associations 1995;60:1256.
16. Beckeet AH, Stenlak JB. Practical pharmaceutical chemistry edn 4th. CBS Publisher and New Delhi: Distribution; 2004. p. 275-337.
17. United States pharmacopeia. In: Validation of compendial methods. 26th ed: Pharmacopoeial Convention Inc, Rockville; 2003. p. 2439-42.
18. Indian pharmacopoeia. Ministry of Health and Family Welfare Government of India. Volume II. Ghaziabad: Indian Pharmacopoeia Commission; 2007. p. 692-3.