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A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF ABACAVIR IN BULK AND TABLET DOSAGE FORMS

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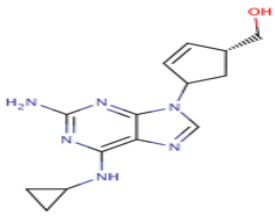
Abstract
A rapid, precise, accurate, specific and simple RP-HPLC method was developed for the estimation of Abacavir in bulk and in tablet dosage form. A High performance liquid chromatograph 10AT SHIMADZU- SPD10A, using Phenomenex - Luna RP-18(2),250X4.6mm, 5 µm column, with a mobile phase composed from water: Acetonitrile [80:20 %(v/v)] were used. The flow rate of 1.0 ml/min and the effluent was detected at 285 nm by using a UV detector. The retention time of Abacavir was 7.761 min. Linearity was observed over concentration range of 100-2800 ng ml^{-1}. The Limit of detection was found to be 21.04 ng ml^{-1}while quantification limit was 63.77 ng ml^{-1}. The accuracy of the proposed method was determined by recovery studies and found to be 98.23 to 100.61 %. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to stability studies and routine analysis of Abacavir in bulk and pharmaceutical formulations.
The proposed method was validated for various ICH parameters like linearity, limit of detection, limits of quantification, accuracy, precision, range and specificity.
***Keywords*:** Abacavir, RP-HPLC, Stability studies, Validation, ICH guidelines

1. Introduction

Abacavir is chemically [(1R)-4-[2-amino-6-(cyclopropylamino) purin-9-yl]-1-cyclopent-2-enyl] methanol (Fig. 1). It is a white crystalline powder used as antiretroviral agents, for the treatment of HIV infection. Abacavir belongs to a class of antiretroviral drugs known as nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1)[1]. Literature survey reveals that very few analytical methods has been established for the determination of abacavir viz. abacavir, lamivudine and zidovudine in Pharmaceutical Tablets, Human Serum and in Drug Dissolution Studies by HPLC[2], Hypersensitivity reaction to abacavir is strongly associated with the presence of the HLA-B*5701 allele[3], Simple and Reliable HPLC Method of Abacavir Determination in Pharmaceuticals, Human Serum and Drug Dissolution Studies from Tablets[4], Spectrophotometric determination of abacavir sulphate[5], RP-HPLC Method for the simultaneous Estimation of Lamivudine and Abacavir Sulphate in Tablet Dosage Form[6], Development and Validation of RP-HPLC Method for the Estimation of Abacavir , Lamivudine and Zidnovudine in Pharmaceutical Dosage Form[7], Spectrophotometric Estimation of Abacavir Sulphate in Bulk and Tablet Dosage Form[8], Visible spectrophotometric determination of Abacavir sulphate in Bulk Drug and Tablet Dosage Form[9].

Fig. 1: Chemical structure of Abacavir



The stability of a drug substance or drug product is defined as its capacity to remain within established specifications, i.e. to maintain its identity, strength, quality, and purity until the retest or expiry date[10]. Stability testing of an active substance or finished product provides evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light. Knowledge from stability studies is used in the development of manufacturing processes, selection of proper packaging and storage conditions, and determination of product shelf-life[11-12]. There was no reported stability-indicating analytical method for analysis of abacavir in the presence of its degradation products in bulk and pharmaceutical dosage forms. The objective of this work was to develop a new, simple, economic, rapid, precise, and accurate stability-indicating HPLC

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method for quantitative analysis of abacavir, and to validate the method in accordance with ICH guidelines[13] with shorter retention time, runtime, and economic mobile phase.

2. Experimental

2.1 Materials and Methods: Pure standard of Abacavir (Assigned purity 99.97%) was obtained as a gift sample from Ranbaxy labs Pvt. Ltd, Jammu (H.P). The gift samples were used as standard without further purification. HPLC grade water, CH_3CN (Acetonitrile) and CH_3OH were purchased from (Qualigens), HCl, NaOH, H_2O_2 were purchased from (S.D. fine chemicals, Mumbai, India), and were used throughout the experiment. Commercial pharmaceutical preparation which was received from (Ziagen) which was claimed to contain 300mg of Abacavir is used in the analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies.

2.2 Instrumentation and Chromatographic conditions: High performance liquid chromatograph with Shimadzu pumpLC-10AT VP equipped with universal injector (Hamilton 25 µL) SPD10A and a UV-VIS detector SPD10A-10A VP (Shimadzu) was used. Isocratic elution of mobile phase composed of water and Acetonitrile in the ratio of 80:20 % (v/v) with flow rate of 1.0 ml/min was performed on C18 column (250x 4.6 mm, 5µm). The effluent was detected at 285 nm. The retention time of Abacavir was 7.761 min. The column temperature was maintained at ambient and the volume of injection was 20 µl. Prior to injection of the analyte, the column was equilibrated for 30- 40 min with mobile phase.

Different kinds of equipments viz Analytical weighing balance (Shimadzu AX 200), Sonicator (model SONICA 2200MH), Water purification system, Vacuum pump (model XI 5522050 of Millipore), Millipore filtration kit for solvents and sample filtration were used throughout the experiment. The Spinchrom CFR software was used for acquisition, evaluation and storage of chromatographic data.

2.3 Preparation of Mobile phase: The HPLC grade solvents of water and Acetonitrile were used for the preparation of mobile phase in the ratio of 80:20 % (v/v). The contents of the mobile phase were filtered before use through a 0.45µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 ml/min.

2.4 Preparation of Standard Solution: A stock solution of the drug was prepared by dissolving 100 mg of Pure Abacavir in a 100 ml volumetric flasks containing sufficient amount of methanol (HPLC grade) to dissolve the drug, sonicated for about 15 min and then made up to volume with mobile phase. Daily working standard solutions of Abacavir was prepared by suitable dilution of the stock solution with the mobile phase. Six sets of the drug solution were prepared in the mobile phase containing Abacavir at a concentration of 600-1600ng/ml. Each of these drug solutions (20µl) was injected six times into the column, the peak area and retention times were recorded.

2.4.1 Procedure for sample solution preparation (from Formulation): Twenty tablets were weighed accurately and powdered. An amount of the powder equivalent to 300 mg of Abacavir (content of one tablet) was dissolved in 50 ml of the mobile phase. The solution was stirred for 10 min using a magnetic stirrer and filtered into a 100 ml volumetric flask through 0.45 µm membrane filter. The residue was washed 3 times with 10 ml of mobile phase, and then the volume was completed to 100 ml with the same solvent. Further addition of the mobile phase was performed to obtain a stock solution of 10 µg/ml. An aliquot of this solution (1 ml) was transferred to a 10 ml volumetric flask and a sufficient volume of the mobile phase was added to give an expected concentration of 1 µg/ml. All determinations were conducted in triplicate.

2.5 Stability Studies

2.5.1 Thermal degradation at different temperature and different time interval: Expose about 2 to 3 g of drug sample (Bulk drug and Tablet dosage form) at different time intervals viz. 0, 90, and 180 days and at different temperatures viz.-20^{0C}, 25^{0C}, and 40^{0C}.The drug sample solution (Abacavir) in mobile phase was demonstrated by injecting the sample solution in HPLC, no degradants were observed in the chromatogram. However, after 180 days and 40^{0C} the chromatographic peak area of abacavir decreased insignificantly. Hence, the sample was stable at least for 180 days at 40^{0C} (The temp. can be maintained by using freezer and oven).

2.5.2 Photochemical Degradation: The photochemical stability of the abacavir was studied by exposing the methanolic stock solution to direct sunlight for 8 h (from 9 AM to 5 PM, 20±5^{o}C).

2.5.3 Thermal stress (test sample exposed to sunlight): Transfer about 2 to 3 g of drug

sample (Bulk drug and Tablet dosage form) into a clean dry watch glass and spread evenly. Expose to sunlight for 10 hours. After the sample got exposed to prescribed time, weigh accurately 25 mg of sample into a clean dry 50 ml volumetric flask, dissolve and dilute to the mark with mobile phase, finally make a concentration of 1000ng/ml with mobile phase and inject 20µl of this sample into HPLC, observe the degradation.

2.5.4 Forced degradation of Abacavir and tablets of Abacavir: In order to establish whether the analytical method and the assay were stability indicated, the tablets and pure active pharmaceutical ingredient of abacavir were stressed under various conditions to promote degradation. As this drug was freely soluble and stable in methanol and this solvent was used as solvent in all forced degradation studies. All solutions were prepared to use in forced degradation studies were prepared by dissolving Abacavir or drug product in small amount of methanol and later diluted with 3% hydrogen peroxide, 0.1N hydrochloric acid and 0.1 N sodium hydroxide to achieve concentration of 100µg/ml After the degradation, these solutions were diluted with mobile phase to get starting concentration of 10µg/ml with the objective of evaluating stability of abacavir. The degradants were observed in the chromatogram and showing good resolution from the abacavir.

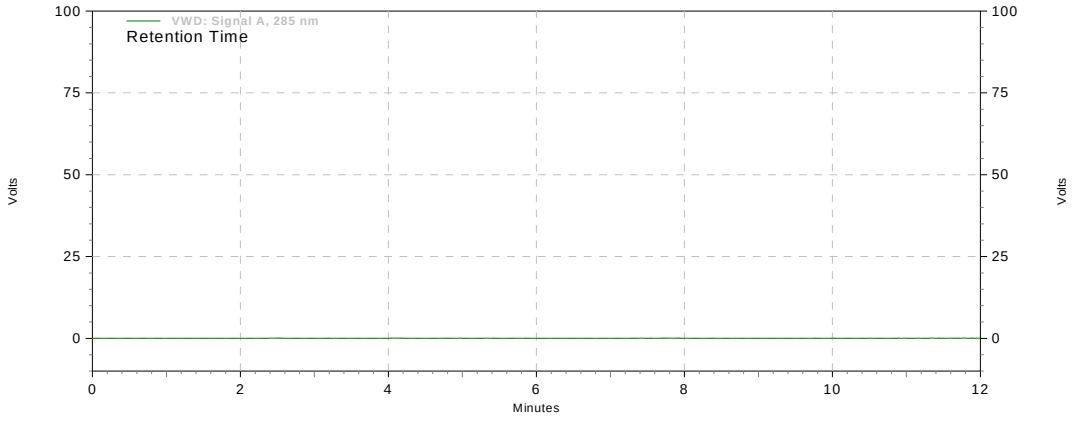
2.5.5 Hydrolysis (Acid and Alkali): Initially for hydrolytic degradation the abacavir was dissolved in known amount of methanol and diluted with 0.1N HCl or 0.1N NaOH to obtain a concentration of 100µg/ml. After completion of degradation process, both the solutions were neutralized with acid or base, as necessary and diluted with the mobile phase to achieve a concentration of 10µg/ml. The solutions for hydrolysis were prepared in methanol and 0.1 N HCl and 0.1N NaOH (60:40 v/v). The prepared acidic and alkali solutions were injected to the chromatographic system at 0 h (immediately after preparing the solution) and after reflux at 60 ^{0}c about 2 h. The respective chromatograms were recorded for the study of extent of degradation.

2.5.6 Peroxide Degradation: The solutions for peroxide degradation were prepared in methanol and 3% hydrogen peroxide (60:40 v/v). The prepared solution was refluxed at 60 ^{0}c about 2 h and injected into chromatographic system after 2 h. The respective chromatogram was recorded for the study of extent of degradation.

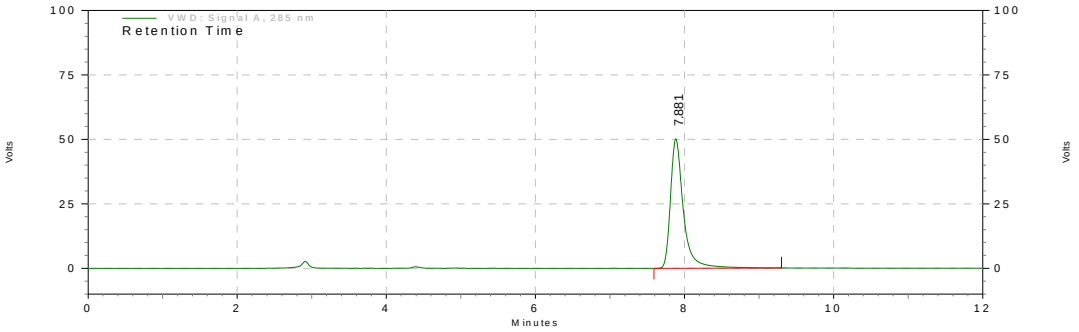
3. Results and Discussion

3.1 Stability Studies:

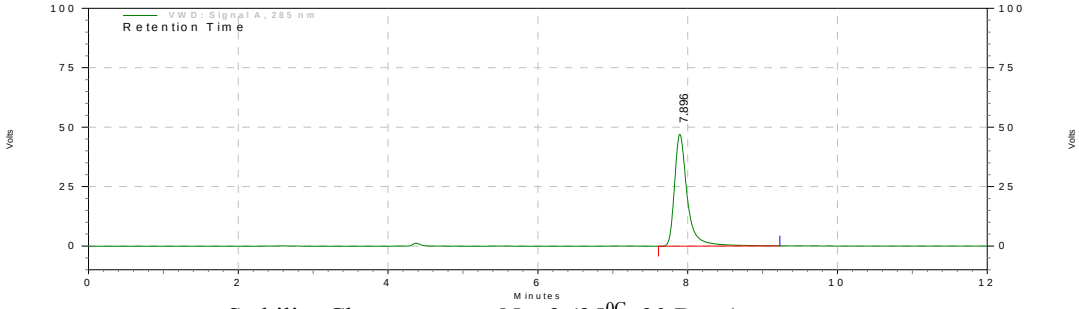
3.1.1 Thermal degradation at different temperature and different time intervals: Expose about 2 to 3 gm of drug sample (Bulk drug and Tablet dosage form) at different time intervals viz. 0, 90, and 180 days and at different temperatures viz.-20^0 C, 25^0 C, and 40^0 C.The drug sample solution (Abacavir) in mobile phase was demonstrated by injected the sample solution in HPLC, no degradants were observed in the chromatogram. However, after 180 days and 40^{0}C the chromatographic peak area of abacavir decreased insignificantly. Hence, the sample was stable at least for 180 days at 40^{0}C. (The temp. can be maintained by using freezer and oven). (Chromatogram no.1-8)



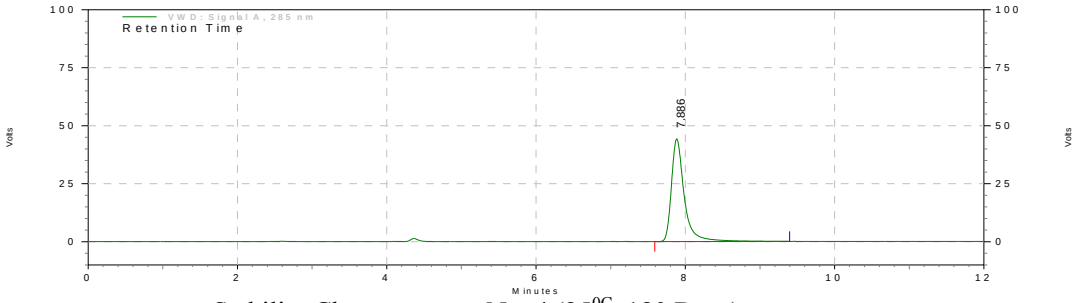
Stability Chromatogram No.1 (blank)



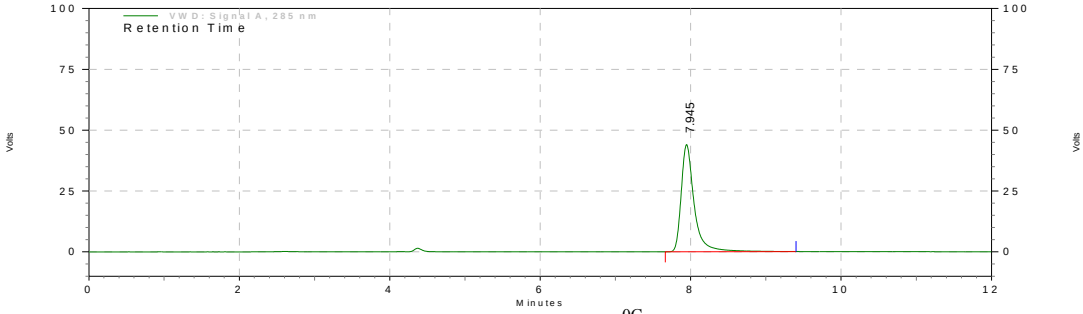
Stability Chromatogram No. 2 (Initial)



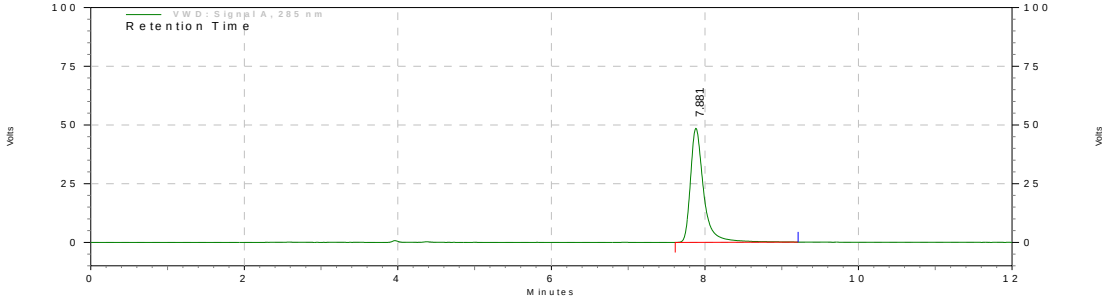
Stability Chromatogram No. 3 (25^{0C}, 90 Days)



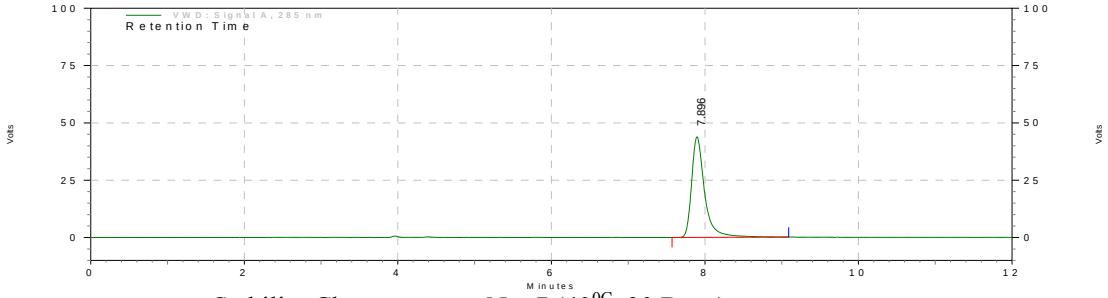
Stability Chromatogram No. 4 (25^{0C}, 180 Days)



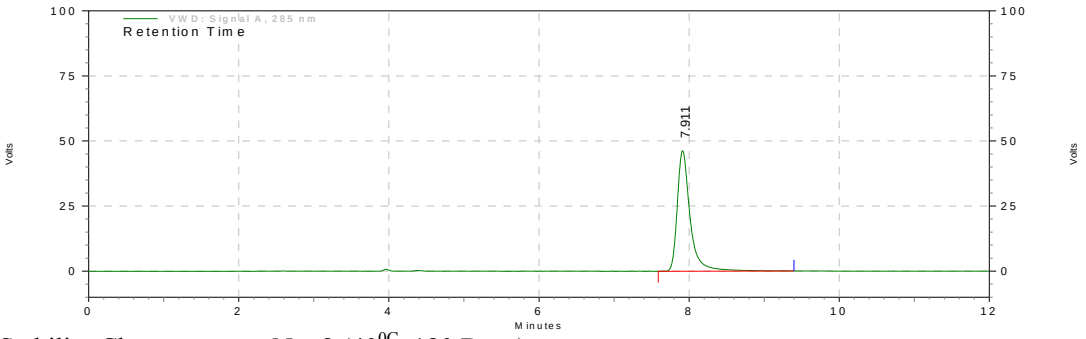
Stability Chromatogram No. 5 (-20^{0C}, 90 Days)



Stability Chromatogram No. 6 (-20^{0C}, 180 Days)



Stability Chromatogram No. 7 (40^{0C}, 90 Days)



Stability Chromatogram No. 8 (40^{0C}, 180 Days)

The result obtained by thermal degradation at different temperature and different time interval viz. Initial, 25^{0}C at 90days, 25^{0}C at 180days, -20^{0}C at 90days, -20^{0}C at 180days, 40^{0}C at 90days, 40^{0}C at 180days was found to be 99.62%, 99.46%, 99.02%, 99.54%, 99.39%, 98.06%, 97.23% respectively. Further study was carried out by employing the following tests: hydrolysis (neutral, acidic and basic), photolysis and thermolysis. No decomposition was observed when the abacavir was exposed to sunlight, temperature, UV. A significant change was observed as a decrease of assay of about 20 to 25 % when the sample was treated with 0.1 N NaOH and 0.1 N HCl. The sample which was treated with 3 % H_2O_2 was almost completely degraded.

3.2 Validation of analytical Method: Analytical validation is the corner stone of process validation without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purports to do. All new developed methods should be validated by using these parameters (According to ICH guidelines).

1. Linearity
2. Accuracy
3. Precision
4. Specificity
5. Limit of detection
6. Limit of quantification
7. Range
8. Ruggedness
9. Robustness
10. System suitability
11. Solution stability

3.2.1 Linearity

***Acceptance criteria*:** For linearity the Coefficient of correlation value (r^2) should be greater than 0.998 (Regression value in linear plot).

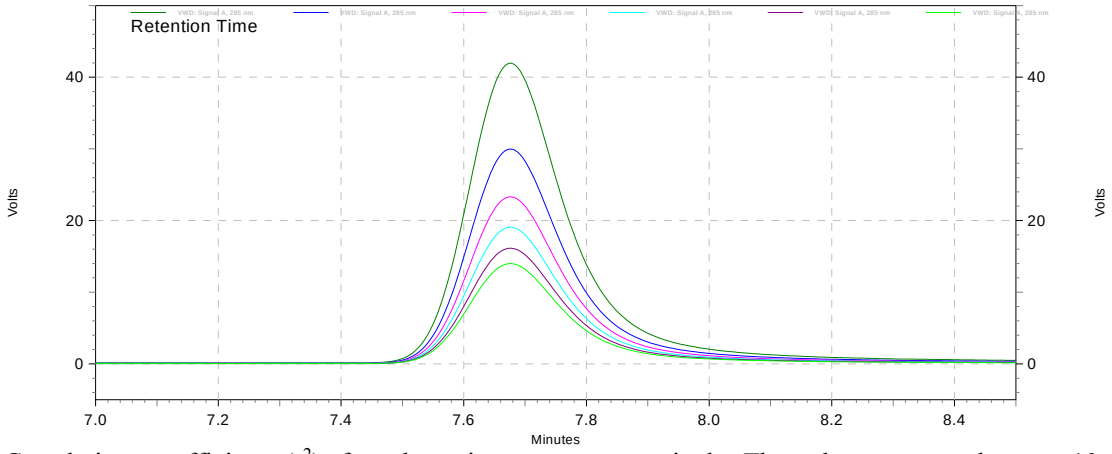
Procedure: A stock solution of drug was prepared by dissolving 100 mg of pure Abacavir in a 100 ml volumetric flasks containing sufficient amount of methanol (HPLC grade) to dissolve the drug, sonicated for about 15 min and then made up to volume with mobile phase. Daily working standard solutions of Abacavir was prepared by suitable dilution of the stock solution with the mobile phase. Six sets of the drug solution were prepared in the mobile phase containing abacavir at a concentration of 600-1600ng/ml. Each of these drug solutions (20µl) was injected in six concentrations in three replicates times into the column, the peak area and retention times were recorded. (Table No. 1 and chromatogram No. 9)

Table no. 1: Peak Area* of Abacavir:

Replicate	Dilution I	Dilution II	Dilution III	Dilution IV	Dilution V	Dilution VI
1	8496377	9445587	10629587	11752141	12762178	13826313
2	8501452	9582471	10596328	11786254	12871521	13925411
3	8482517	9571968	10632596	11769851	12763652	13852137
Average	**8493449**	**9533342**	**10619504**	**11769415**	**12799117**	**13867954**
SD	**9801.271**	**76179.284**	**20127.026**	**17060.673**	**62708.034**	**51407.476**
RSD%	**0.12**	**0.80**	**0.19**	**0.14**	**0.49**	**0.37**

***Average of five readings

Chromatogram No. 9 (Overlay spectra for Linearity)



Correlation coefficient (r^2) for abacavir was found to be 0.9998, indicating that the linearity and the method is linear between the concentrations of 100-2800ng mL^{-1}.

3.2.2 Accuracy: The accuracy is the closeness of the measured value to the true value for the sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 0.2 ml, 0.4ml and 0.8 ml of sample drug (Abacavir) solution of 10µg/ml were pipetted into each of three volumetric flasks. To this 0.4 ml of standard drug (Abacavir) solution of 10 µg/ml was added to each volumetric flask respectively. The volume was made up to 10 ml with mobile phase. 20 µl of each solution was injected and chromatograms were recorded. The range was found between 98.234 to 100.615 % respectively The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients.

The recovery data was generated for Abacavir are presented in the Table No.2

Table No. 2:

Conc. taken in ng/ml (A)	**Std addition in ng/ml (B)**	**Total drug conc. in ng/ml (A+B)**	**Peak Area***	**% Recovery**
200	400	600	8545711	100.615
400	400	800	9562549	100.306
800	400	1200	11561673	98.234

***Average of three readings**

The percentage recovery by the proposed method was ranging from 98.23 to 100.61 % indicating no interference of the tablet excipients with drug under analysis.

3.2.3 Precision Precision is measure of repeatability or reproducibility and it was determined by injecting 5 times the expected operating range concentration. The chromatograms were recorded to determine mean standard deviation and relative standard deviation.

Acceptance criteria: For precision the Relative standard deviation (RSD) should be less than

2.0% (RSD<2.0%) for peak area and retention time.

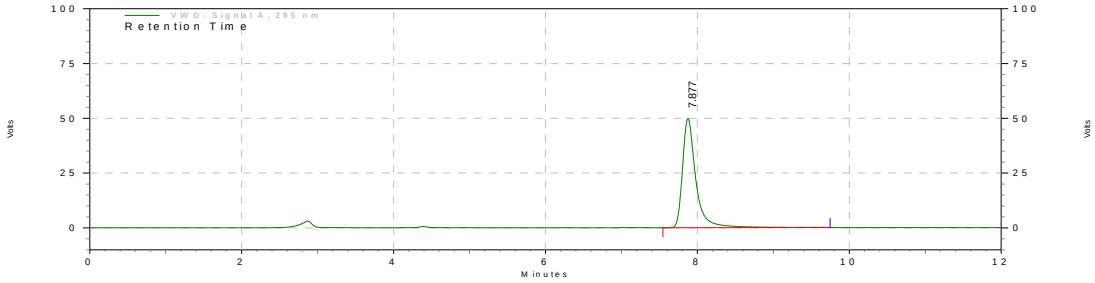
Table No.3 (Precision of Abacavir)

S.No.	Area Response
1.	9749901
2.	9693755
3.	9683955
4.	9650582
5.	9652257
Average	9686090
S.D	40437.124
R.S.D	0.42

From the above analytical data it is observed that RSD value for the assay is 0.42% which indicates that the method is precise and reproducible (According to ICH guidelines).

3.2.3 Specificity: Specificity is the ability to assess the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004). For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (chromatogram No. 10).

Chromatogram of Abacavir showing Specificity (chromatogram No. 10)



The excipients used in different formulation products did not interfere with the drug peak and thus, the method is specific for abacavir.

3.2.4 Solution Stability: The solution stability of the standard and sample that prepared in mobile phase was studied for 5 days at bench top. The solution under study was compared with freshly prepared standard solution. The samples were found to be stable for period of more than 72 hours.

3.2.5 Range: The range was calculated from the linearity graph. The specific range can be obtained from linearity graph. The range which is linear, accurate and precise between lower and higher concentration is the beer's range of the method

The range for abacavir was found to be 100-2800ng/ml.

3.2.6 Limits of detection and quantification: The limit of detection (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantized under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 2:1 or 3:1. The lower limit of detection for abacavir is 21.04 ng/ml in reference material and formulation. Limit of Quantification (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10:1 can be taken as LOQ of the method.

The LOQ values were found to be 63.77 ng/ml for raw material and formulations.

3.2.7 System suitability: A stock solution of abacavir (Approx. 800ng mL^{-1}) was prepared in the mobile phase and injected in the system in five replicates, after obtaining the chromatogram the system suitability parameters were calculated (Chromatogram No. 11).

3.2..8 Theoretical plates per column: Number of theoretical plates in the column were calculated from the data obtained from the peak.

$$n = (5.54Vr^2)/W_h^2$$

Where, 'n' is number of theoretical plates per length, 'Vr' is the distance along the base line between the point of injection and a perpendicular dropped from the maximum of the peak of interest (retention time) and 'W_h' is the width of the peak of interest at half peak height.

3.2.9 Tailing Factor (USP Method): A measure of the symmetry of a peak, given by the following equation, where $W_{0.05}$ is the peak width at 5% height and f is the distance from peak front to apex point at 5% height. Ideally, peaks should be Gaussian in shape or totally symmetrical.

$T = W_{0.05} / 2f$

The accuracy of quantization decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak ends and as a result the calculation of the area under the peak.

Integrator variables are preset by the analyst for optimum calculation of the area for the peak of interest.

Recommendations:

T of ≤ 2

T= (a+b) / 2a

Where: T = tailing factor (measured at 5% of peak height)

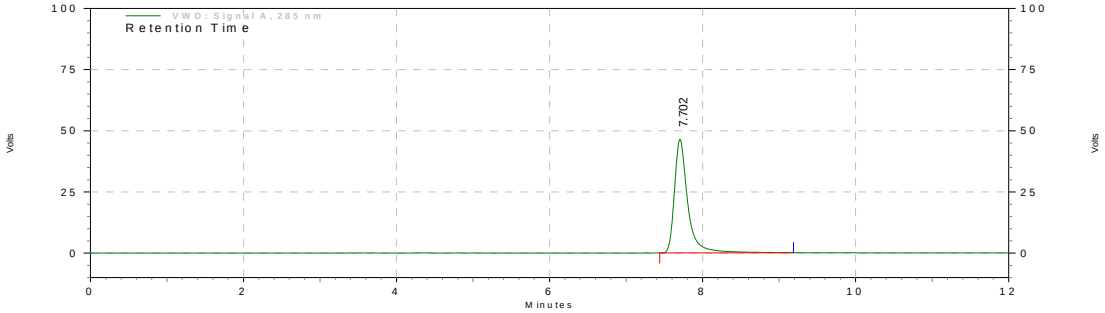
b = distance from the point at peak midpoint to the trailing edge

a = distance from the leading edge of the peak to the midpoint

Table4. Resultss of system suitability parameters

Parameters	Data obtained
	Abacavir
Theoretical plates per column	4632
Symmetry factor/Tailing factor	1.12

Chromatogram No. 11(showing system suitability)



Conclusion

The proposed RP-HPLC method is found to be accurate, precise, linear, stable, specific, and simple, for quantitative estimation of Abacavir in raw material and pharmaceutical formulations. Hence the present RP-HPLC method is suitable for routine assay of abacavir in raw materials and in pharmaceutical formulations in the quality control laboratories.

Acknowledgement

The authors thank Ranbaxy labs Pvt. Ltd, Jammu (H.P), India, for providing a sample of Abacavir as a gift.

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