A Validated RP-HPLC Method for Simultaneous Quantification of Abacavir and Zidovudine and their Impurities in Combined Dosage Forms

Vimla Soni*, Dr. Priyadarshani R. Kamble
Department of pharmaceutical chemistry
Bhupal Nobel's college of pharmacy, Udaipur, Rajasthan, India
Correspondence author: Vimla soni, Email id- vimlasoni1989@gmail.com, Bhupal Nobel's college of pharmacy, Udaipur, Rajasthan, India

Abstract: Develop a simple, accurate, and precise method for simultaneous analysis of Abacavir and Zidovudine, along with their related impurities, in combined pharmaceutical dosage forms. A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed using an LC-20 AT C18 column and a Water: Methanol (80:20) mobile phase. Detection was carried out at 240 nm. The method successfully separated Abacavir, Zidovudine, and their respective impurities with distinct retention times. Validation demonstrated good linearity, accuracy, and precision for both drugs and their impurities. Limits of detection (LOD) and quantification (LOQ) were determined and found to be suitable for analyzing impurities in commercial dosage forms. The developed RP-HPLC method offers a simple, accurate, and reliable tool for simultaneous quantification of Abacavir, Zidovudine, and their impurities in combined dosage forms, thereby contributing to quality control and ensuring drug safety.

Keywords

Abacavir, Zidovudine, Related Substance RP-HPLC Method, ICH Q2 (R1) guidelines

Abacavir and Zidovudine are two prominent antiretroviral medications used in the treatment of HIV infection. They belong to a class of drugs called nucleoside reverse transcriptase inhibitors (NRTIs), which work by interfering with the virus's ability to replicate within the body. By stopping this replication, the drugs can significantly reduce the amount of HIV in the bloodstream and slow down the progression of the disease.

Abacavir (ABC):

Available under the brand name Ziagen, it is a potent NRTI that blocks a key enzyme necessary for HIV replication.

Abacavir is particularly effective in suppressing HIV viral load and improving immune function.

However, some individuals experience hypersensitivity reactions to the drug, requiring careful monitoring and potential alternative options.

Zidovudine (AZT):

One of the earliest and most widely used antiretroviral drugs, marketed under the brand name Retrovir.

Zidovudine works by blocking the same enzyme as Abacavir, limiting the virus's ability to multiply.

It is well-tolerated in most people but can cause side effects like anemia and muscle pain.

Combined Therapy:

Abacavir and Zidovudine are often used together in combination with other antiretroviral medications as part of highly active antiretroviral therapy (HAART). This strategy helps to target the virus at multiple points in its lifecycle, making it more difficult for the virus to develop resistance.

Benefits of Combination Therapy:

Reduced viral load: HAART can effectively suppress HIV to undetectable levels, significantly reducing the risk of transmitting the virus to others and greatly improving health outcomes.

Improved immune function: By lowering viral load, HAART allows the immune system to recover and fight off infections more effectively.

Increased life expectancy: Individuals with HIV on HAART can expect to live nearly as long as those without the virus.

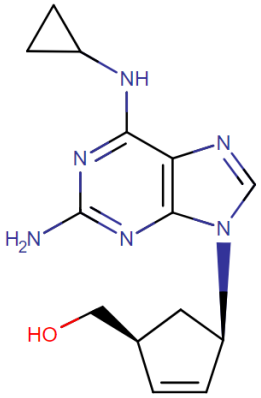
Overall, Abacavir and Zidovudine are crucial tools in the fight against HIV. Their combined use as part of HAART has revolutionized HIV treatment, offering hope and longevity to millions of people living with the virus.

Literature review reveals that numbers of individual analytical methods available for estimation of Abacavir and Zidovudine in their individual dosage forms and combined dosage form. But related impurities method has not been reported for simultaneous estimation of Abacavir and Zidovudine in combined pharmaceutical dosage form by RP-HPLC. So it is thought to develop related impurities method for the simultaneous estimation of Abacavir and Zidovudine in Combined Dosage Form using RP-HPLC. So Aim of present work is to develop simple, accurate, precise, rapid, specific, sensitive and selective related impurities RP-HPLC method for simultaneous estimation of Abacavir and Zidovudine in combined pharmaceutical dosage form.

Zidovudine is a dideoxynucleoside used in the treatment of HIV infection. Abacavir (ABC) is a powerful nucleoside analog reverse transcriptase inhibitor (NRTI) used to treat HIV and AIDS. Abacavir is indicated in combination with other antiretroviral agents for the prevention and treatment of HIV-1 infection**.** Zidovudine is a potent inhibitor of HIV replication, acting as a chain-terminator of viral DNA during reverse transcription. It improves immunologic function, partially reverses the HIV-induced neurological dysfunction, and improves certain other clinical abnormalities associated with AIDS. Analytical method development and validation plays an important role in drug discovery and manufacture of pharmaceuticals. These methods are used to ensure the identity, purity, potency and performance of drug products. Spectrophotometry and HPLC methods are considered to be most suitable for estimation of drugs present in pharmaceutical dosage form. The Literature survey reveals that these drugs have been analyzed individually and in combination by many analytical methods like HPLC, HPTLC and spectroscopic method, but related impurities method has not been reported for the estimation of Abacavir and Zidovudine in combined pharmaceutical dosage form BY RP-HPLC. So here attempt will be made to develop and validate related impurities RP-HPLC method for estimation of Abacavir and Zidovudine in its combined dosage form.

To develop related impurities RP-HPLC method for simultaneous estimation of Abacavir and Zidovudine in pharmaceutical dosage form. Applying the newly developed, validated analytical method for the estimation of Abacavir and Zidovudine in formulations.

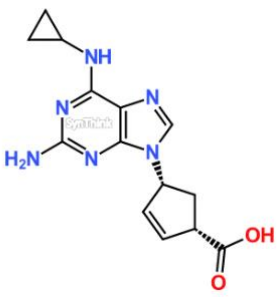
DRUG PROFILE OF ABACAVIR

Name	**Abacavir**
Official in	Not Official in any Pharmacopoeia
Description	Abacavir is a nucleoside reverse transcriptase inhibitor (NRTI) indicated for the treatment of HIV infection
Structure	
Chemical Formula	$C_{14}H_{18}N_6O$
Mol. Weight	286.33 g/mol
IUPAC Name	{(1S-cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]cyclopent-2-en-1-yl}methanol
Categories	Anti-HIV Agents
Solubility	with a solubility methanol, sparingly soluble in water
PHARMACOLOGY	
Classes	Nucleoside reverse transcriptase inhibitor
Mechanism of Action	Abacavir is an antiviral drug and an analogue of carbocyclic synthetic nucleosides. Cellular enzymes transform abacavir intracellularly into carbovir triphosphate, an active metabolite that is a counterpart of deoxyguanosine-5'-triphosphate (dGTP). HIV-1 reverse transcriptase (RT) activity is inhibited by carbovir triphosphate through its incorporation into viral DNA and competition with the natural substrate dGTP. Because the integrated nucleotide lacks the 3'-OH group required to

	construct the 5′ to 3′ phosphodiester linkage necessary for DNA chain elongation, viral DNA growth is stopped.
PROPERTIES	
State	Solid.
CAS NO.	136470-78-5
Melting point	274-276°C
pK_a	7.9
Water solubility	72 mg/mL
Log P	1.37

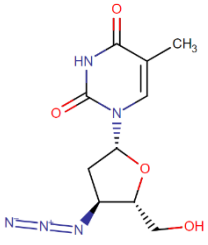
Table 1: Description of Abacavir

RELATED IMPURITIES OF ABACAVIR

Name	Structure	Chemical name
Abacavir related impurity		(1S,4R)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-carboxylic Acid

Name and Structure of Impurities of Abacavir

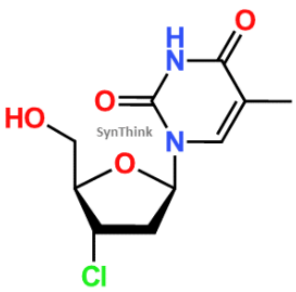
RUG PROFILE OF ZIDOVUDINE [28]

Name	**Zidovudine**
Official in	Not Official in any Pharmacopoeia
Description	Zidovudine is a type of nucleoside reverse transcriptase inhibitor (NRTI) that works to reduce the activity of the HIV-1 virus. Phosphorylation of zidovudine produces active metabolites that vie with one another to be incorporated into viral DNA. They function as a chain terminator of DNA synthesis and competitively inhibit the HIV reverse transcriptase enzyme.
Structure	
Chemical Formula	$C_{10}H_{13}N_5O_4$
Mol. Weight	267.24 g/mol
IUPAC Name	3'-deoxy-3'-azido-thymidine 1-[(2R,4S,5S)-4-Azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione
Categories	Anti-HIV Agents
Solubility	is soluble in methanol and slightly soluble in water
PHARMACOLOGY	
Classes	Nucleoside reverse transcriptase inhibitor (NRTI)
Mechanism of Action	Structurally similar to thymidine, zidovudine is a prodrug that needs to be phosphorylated in order to become zidovudine triphosphate (ZDV-TP), which is the active 5′-triphosphate metabolite. Following the inclusion of the nucleotide analogue, it inhibits the activity of HIV-1 reverse transcriptase (RT) by causing DNA chain termination. It integrates into viral DNA and competes

	with the natural substrate dGTP. Moreover, it has modest inhibitory effects on cellular DNA polymerase α and γ.
PROPERTIES	
State	Solid.
CAS NO.	30516-87-1
Melting point	109-110^{0}C
pK$_a$	9.4
Water solubility	26 mg/mL
Log P	0.45

Description of Zidovudine

RELATED IMPURITIES OF ZIDOVUDINE

Name	Structure	Chemical name
Zidovudine impurity 1		1-(3-Chloro-2,3-dideoxy-β-D-erythro-pentofuranosyl)-5-methylpyrimidine-2,4(1H,3H)-dione

Name and Structure of Impurities of Zidovudine

COMBINATION PRODUCT

Brand Name	Content	Marketed By	Dosage Form	Dose
Trizivir	Zidovudine + Abacavir + Lamivudine	GSK	Tablet	Zidovudine (300mg) and Abacavir Sodium (300mg)

Combination Brand Available in Market

Figure 1: Marketed Formulation of Zidovudine and Abacavir

Experimental Works

In present research work, an attempt was made for development and validation of Related Substance method for simultaneous estimation of Abacavir and Zidovudine pharmaceutical dosage form by RP-HPLC.

Instruments

Instruments Name	Manufacturer
HPLC	Shimadzu LC-20 AT
UV Visible spectrophotometer	Systronic 119
Electronic balance	Shimadzu ATX-224
Sonicator	Frontline Ultrasonic Cleaner
Hot air oven	ThermolabMumbai, India
pH meter	Analab Scientific Pvt Ltd

List of Instruments

Apparatus

Components	Description
Volumetric flasks	Borosilicate glass
Pipettes	Borosilicate glass
Measuring cylinder	Borosilicate glass
Beaker	Borosilicate glass
Whatman Filter	Filter Paper No.42

List of Apparatus

Reagents

Chemicals	Grade	Manufacturer
Acetonitrile	HPLC	Merck, Rankem
Potassium Dihydrogen Phosphate	AR	Merck, Rankem
Water	HPLC	HPLC Grade
Orthophosphoric Acid	AR	Merck, Rankem
Methanol	HPLC	Merck, Rankem
Abacavir and its Related Impurities	Yash Pharma	
Zidovudine and its Related Impurities	RPG Life Science	

List of Reagents

Drug Identification

The identification of standard API for experimental work had done for confirmation of its identity,standard,quality and purity. The identification was doneby taking IR and UV spectra, Solubility and Melting point determination.

1. Melting Point Determination

Melting point of Abacavir and Zidovudine have been determined using Capillary Method. Drug was taken in capillary and injected into melting point apparatus. Result of determination is shown in Table 6.1.

Solubility Study

The solubility of Abacavir and Zidovudine were practically determined as per Indian pharmacopoeia. Solubility was determined by taking 30mg of Abacavir and 15 mg of Zidovudine 100 ml volumetric flasks, adding required quantity of solvent at room temperature and shaken for few minutes. Solubility data for each study was observed and recorded in Table 6.2.

IR spectra and Structure Interpretation

IR spectra of drugs were taken for structure interpretation from % transmission at specified wave numbers. Diffuse reflectance method was used. Potassium Bromide (KBr) was preheated at 105 ºC for 1 hour. Then it was triturated to convert crystalline form into amorphous powder. Then a suitable amount was filled in sample holder and background spectra were taken. After the drug was mixed with KBr in a ratio of 1:100. It was triturated and spectra were taken as shown in figure 6.2 and 6.4.

Method Development

Selection and Detection of Wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. At 210 nm both drug give good response. So 210 nm was selected as detection wavelength for estimation of Abacavir and Zidovudine in tablet dosage form by RP-HPLC.

Selection of Chromatographic Condition

Proper selection of the HPLC method depends upon the nature of the sample (ionic or neutral molecules), its molecular weight, pK_a and solubility. RP-HPLC was selected for the initial separation based on literature survey and its simplicity and suitability. To optimize the chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. Finally the chromatographic condition was chosen that give the best resolution, symmetry and capacity factor for estimation of both drugs and its related impurities.

Selection of Column

For RP-HPLC method, various columns are available and pure drugs chromatogram was developed in different mobile phase, different columns (e.g. C_8, C_{18}, phenyl etc) with different dimensions. The retention time and tailing factor was calculated for each drugs and its related impurities and for each chromatogram. Sharp peak and good resolution was found in C_{18}. Finally BDS Hypersil C_{18} (250mm X 4.6mm, 5µm) column was chosen for method development.

Procedure for Solution Preparation

Preparation of Standard Stock Solution

- **Standard Stock Solution of Abacavir (5000 ppm):**

Take 500 mg of Abacavir into a 100ml volumetric flask and dissolve with methanol upto the mark to get 5000 µg/ml of Abacavir Standard Stock Solution.

- **Standard Stock Solution of Abacavir Impurity (50 ppm):**

Take 5 mg of Abacavir into a 100ml volumetric flask and dissolve with methanol upto the mark to get 50 µg/ml of Abacavir Impurity Standard Stock Solution.

- **Standard Stock Solution of Zidovudine (5000 ppm):**

Take 500 mg of Abacavir into a 100ml volumetric flask and dissolve with methanol upto the mark to get 5000 µg/ml of Zidovudine Standard Stock Solution.

- **Standard Stock Solution of Zidovudine Impurity (50 ppm):**

Take 5 mg of Abacavir into a 100ml volumetric flask and dissolve with methanol upto the mark to get 50 µg/ml of Zidovudine Impurity Standard Stock Solution.

Preparation of Working Standard Solution

- **Working Standard Solution of Abacavir (500 ppm):**

From above Abacavir Standard Stock solution 1 ml was taken in to 10 ml volumetric flask and was made up to the mark with the mobile phase to get 500 µg/ml of Abacavir Working Standard Solution.

- **Working Standard Solution of Abacavir Impurity (5 ppm):**

From above Abacavir Impurity Standard Stock solution 1 ml was taken in to 10 ml volumetric flask and was made up to the mark with the mobile phase to get 5 µg/ml of Abacavir Impurity Working Standard Solution.

- **Working Standard Solution of Zidovudine (500 ppm):**

From above Zidovudine Standard Stock solution 1 ml was taken in to 10 ml volumetric flask and was made up to the mark with the mobile phase to get 500 µg/ml of Zidovudine Working Standard Solution.

- **Working Standard Solution of Zidovudine Impurity (5 ppm):**

From above Zidovudine Impurity Standard Stock solution 1 ml was taken in to 10 ml volumetric flask and was made up to the mark with the mobile phase to get 5 µg/ml of Zidovudine Impurity Working Standard Solution.

- **Preparation of Mobile Phase**

Prepare 0.05M Potassium Dihydrogen Phosphate by dissolving 6.8gm of Potassium Dihydrogen Phosphate in 1000ml water; adjust pH 3 with o-Phosphoric acid (OPA). This solution was sonicated for

5 min for degassing and filtered through 0.45µ Millipore filter. Prepare the ratio of Buffer (pH 3.0): Acetonitrile.

Preparation of Test Solution

The average weight of 10 tablets was determined and was ground in a mortar. Test solution was prepared by dissolving tablet powder equivalent to 500mg of Abacavir and Zidovudine was transferred to 100ml volumetric flask. Then 60 ml mobile phase was added and sonicated for 15 mins to ensure complete solubilization of drug. After sonication, volume was made up to the mark with mobile phase. Filter the solution with 0.45 micron membrane filter and the final filtrate is collected as test solution.

5. Chromatographic Separation

Standard solutions of Abacavir and Zidovudine along with its related impurities were injected in column with 20 µl micro-syringe. The chromatogram was run for appropriate minutes with mobile phase.The detection was carried out at wavelength 210nm. The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc. were recorded using software.

Chromatographic Conditions

Components	**Description**
Column	C_{18} (25 cm × 0.46 cm) Hypersil BDS
Mobile Phase	Water: Methanol (80:20)
Flow Rate	1.0 ml/min
DetectionWavelength	210 nm
Runtime	15 min
Injectionvolume	20.0 µl

Chromatographic Conditions of HPLC

Validation Of Rp-HPLC Method

1. System Suitability Test

It is an integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System

suitability testing provides assurance that the method will provide accurate and precise data for its intended use.

Acceptance criteria

- Theoretical Plates for the analyte peak should not be less than 2000.
- Tailing factor for the analyte peak should not be more than 2.0.

Linearity and Range

The linearity for Abacavir impurity and Zidovudine impurity

were assessed by analysis of combined standard solution in range of 2.5-7.5µg/ml and 2.5-7.5µg/ml respectively, 0.5, 0.75,1.0, 1.25,1.5 ml solutions were pipette out from the Stock solution of Abacavir impurity (5 µg/ml) and Zidovudine impurity(5 µg/ml) and transfer to 10 ml volumetric flask and make up with mobile phase to obtain 2.5, 3.75, 5.0, 6.25 and 7.5 µg/ml for Abacavir impurity and Zidovudine impurity respectively. In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Acceptance criteria: Value of r^2 should be nearer to 1 or equal to 1.

Precision

- **Repeatability**

Standard solution containing Abacavir impurity (5µg/ml) and Zidovudine impurity (5 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.

Acceptance criteria: % RSD of Area should not be more than 5.0%

- **Intraday Precision**

Standard solution containing (2.5, 5, 7.5µg/ml) of Abacavir impurity and (2.5, 5, 7.5µg/ml) of Zidovudine impurity were analyzed three times on the same day and % R.S.D was calculated.

Acceptance criteria: % RSD of Area should not be more than 5.0%

- **Interday Precision**

Standard solution containing (2.5, 5, 7.5µg/ml) of Abacavir impurity and (2.5, 5, 7.5µg/ml) of Zidovudine impurity were analyzed three times on the same day and % R.S.D was calculated.

Acceptance criteria: % RSD of Area should not be more than 5.0%

4. Accuracy

For Abacavir

12.25 µg/ml drug solutions was taken in three different flask label A, B and C. Spiked LOQ, 80%, 100% and 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 210 nm. The amount of Abacavir was calculated at each level and % recoveries were computed.

For Zidovudine

5 µg/ml drug solutions was taken in three different flask label A, B and C. Spiked LOQ, 80%, 100% and 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 210 nm. The amount of Zidovudine was calculated at each level and % recoveries were computed.

Acceptance criteria

% Recovery (individual) at each level should be between 95.00% and 105.00%

5. Limit of Detection and Limit of Quantitation

The LOD was estimated from the set of 3 calibration curves used to determination method linearity. The LOD may be calculated as,

LOD = 3.3 × (SD/Slope)

Where, SD= Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method linearity. The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

6. Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.

2.pH of Mobile phase was changed (± 0.2) 3.2 and 2.8

3.Ratio of Mobile phase was changed(±2) Water: Methanol (78:22) and Water: Methanol (82:18).

Acceptance criteria

- Number of theoretical plates for the analyte peak should not be less than2000.
- Asymmetry value for the analyte peak should not be more than 2.0
- % RSD for the analyte peak should not be more than 5.0%

7. Calculation of Known Impurities of Abacavir and Zidovudine

Analyzed test solution for three times and calculate % of each known impurities in comparison with standard preparations of Abacavir and Zidovudine impurities.

The amount of known related impurities present in the formulation of Abacavir and Zidovudine is calculated by using the formula given below.

For each known impurities of Abacavir and Zidovudine:

% of each known impurities = (Cu/Cs) X (Ru/Rs) X 100

Where,

Cu= Concentration of each impurity in standard preparation

Cs= Concentration of each impurity in test preparation

Ru= Area of each impurity in test preparation

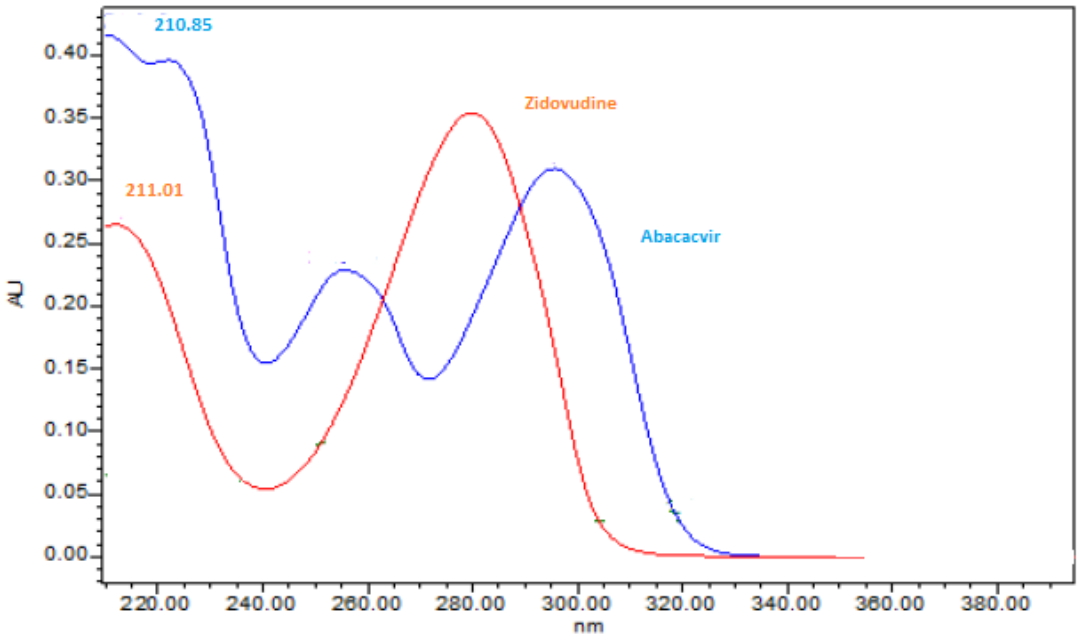
Rs= Area of each impurity in standard preparation

Result and Discussion

HPLC Method Development

Wavelength Determination

UV spectra of Abacavir and Zidovudine were taken in Methanol and λmax was observed using Systronic 119



Overlay UV Spectrum of Abacavir and Zidovudine showing Selection of Wavelength Detection.

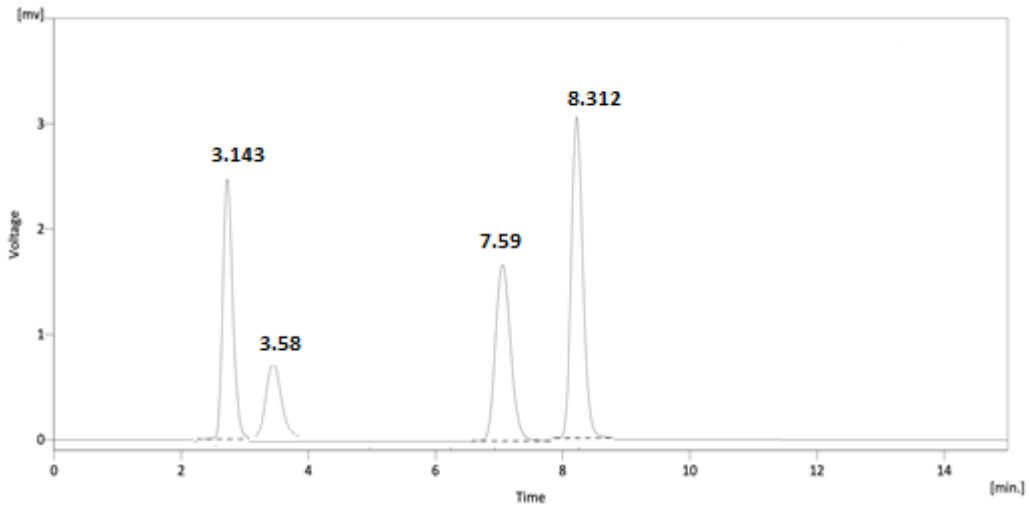
Observation

Abacavir and Zidovudine both drug give higher absorbance at 211.01 and 210.85nm.

So 210 nm has been selected as detection wavelength.

Note: All the chromatograms are shown at wavelength of 210 nm. So, 210 nm is shown in final optimized method.

After considering the varying combinations of various mobile phases, Water: Methanol (80:20), was finalized as it was showing good peak shapes and a significant amount of resolution.



Chromatogram of Abacavir and Zidovudine along with its Related Impurities in Water: Methanol (80: 20 v/v). Final

The mobile phase Water: Methanol (80: 20 v/v). was selected because it was found to ideally resolve the peaks with retention time (RT) 3.14 and 8.31 min for Abacavir and Zidovudine and the retention time of Abacavir impurity and Zidovudine impurity were found to be 3.58 min and 7.59 min respectively respectively and the same is shown in fig 6.21.

Final Chromatographic Condition for Abacavir and Zidovudine

- **Stationary Phase :** BDS Hypersil C18 (250 mm×4.6 mm, 5 µm particle size)
- **Mobile Phase :** Water: Methanol (80: 20 v/v).
- **Flow Rate :** 1 ml/min
- **Detection Wavelength :** 224 nm
- **Run Time :** 10 min

- **Injection Volume :** 20 µl

Observed values for System Suitability Test

1. Retention Time (Rt): Retention Time was observed depicted in Table 6.6.

2. Column efficiency (N): Number of plates observed for Abacavir and Zidovudine was observed depicted in Table 6.6.

3. Symmetry factor (S): Tailing factor observed for Abacavir and Zidovudine was observed depicted in Table 6.6

Parameters	**Abacavir**	**Zidovudine**	**Abacavir Impurity**	**Zidovudine Impurity**
Retention Time	3.14	8.31	3.58	7.59
Theoretical plates per column	8654	7546	7245	9452
Tailing factor	1.645	1.945	1.852	1.524

Results for System Suitability Test.

Method Validation

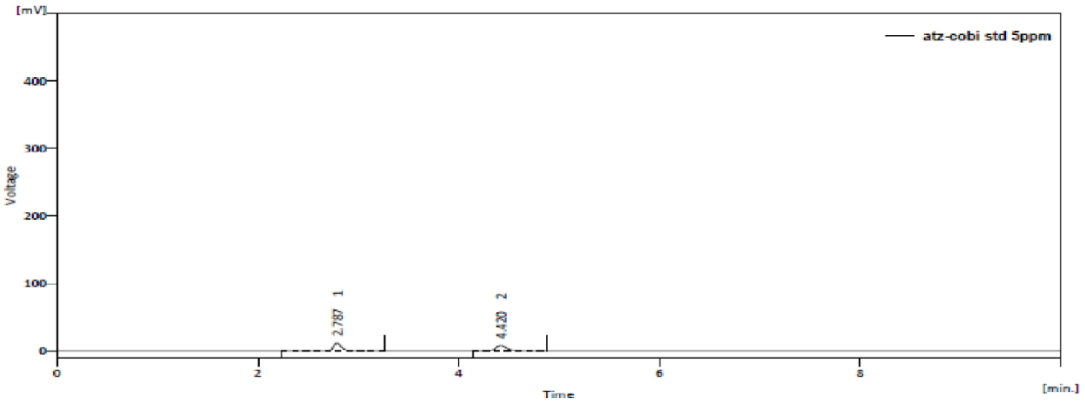
System Suitability Parameters

System suitability tests are used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters used in this test were the chromatographic peak, retention time, resolution, theoretical plate number and tailing factor.

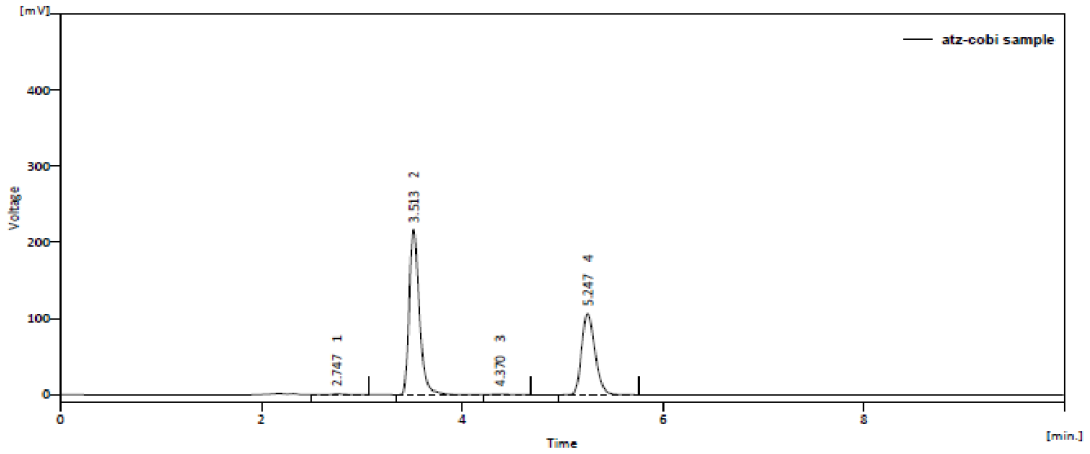
Parameters	**Abacavir**	**Zidovudine**	**Abacavir Impurity**	**Zidovudine Impurity**
Retention Time	3.14	8.31	3.58	7.59
Theoretical plates per column	8654	7546	7245	9452
Tailing factor	1.645	1.945	1.852	1.524

System Suitability Parameters

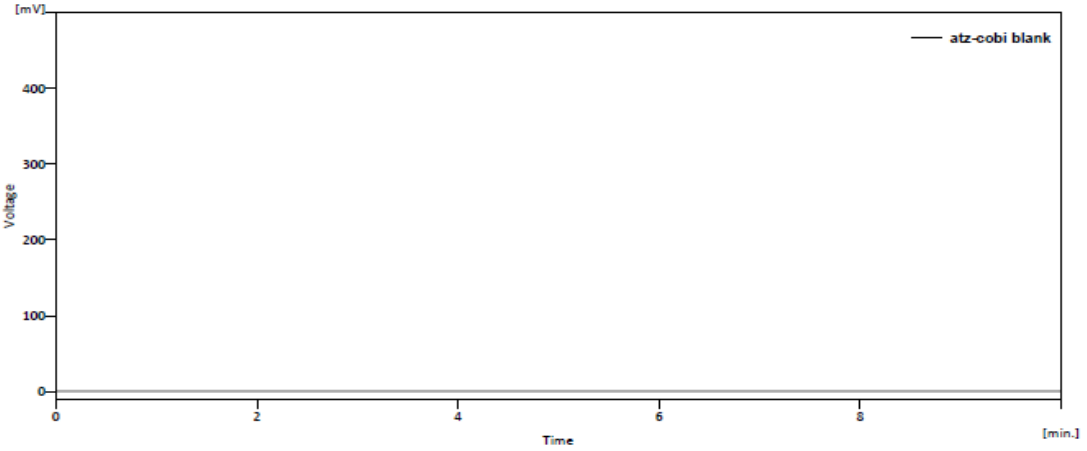
Specificity



gure chromatogram of Abacavir Impurity and Zidovudine Impurity Standard



Chromatogram of Abacavir and Zidovudine Sample



Chromatogram of Abacavir and Zidovudine Blank

The Chromatograms of Abacavir and Zidovudine Impurity standards and Abacavir and Zidovudine sample show no interference with the Chromatogram of Abacavir and Zidovudine Blank, so the Developed method is Specific.

Linearity and Range

The linearity for Abacavir Impurity and Zidovudine Impurity were assessed by analysis of combined standard solution in range of 2.5-7.5µg/ml and 2.5-7.5µg/ml respectively. Correlation co-efficient for calibration curve Abacavir Impurity and Zidovudine Impurity was found to be 0.9987 and 0.9945 respectively.

The regression line equation for Abacavir and Zidovudine are as following:

For Abacavir Impurity: y = 12.936x + 9.454 and

For Zidovudine Impurity: y = 13.225x + 14.005

Sr. No	Concentration (µg/ml)	Area
1	2.5	41.256
2	3.75	58.647
3	5	73.548
4	6.25	91.564
5	7.5	105.645

Linearity data for Abacavir impurity

Sr. No	Concentration (µg/ml)	Area
1	2.5	48.645
2	3.75	61.345
3	5	81.356
4	6.25	94.658
5	7.5	114.645

Linearity Data for Zidovudine Impurity

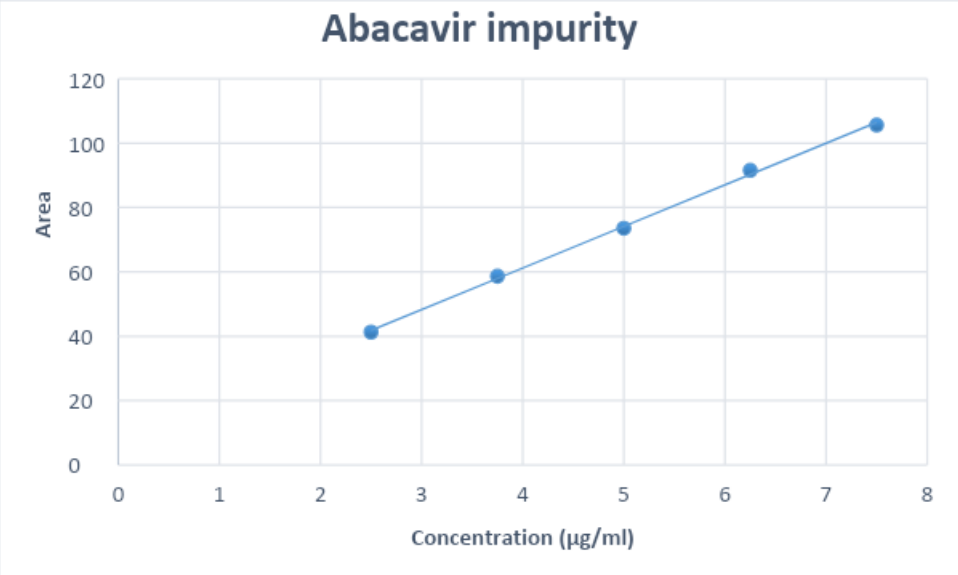


Figure 40 Calibration Curve of Abacavir Impurity

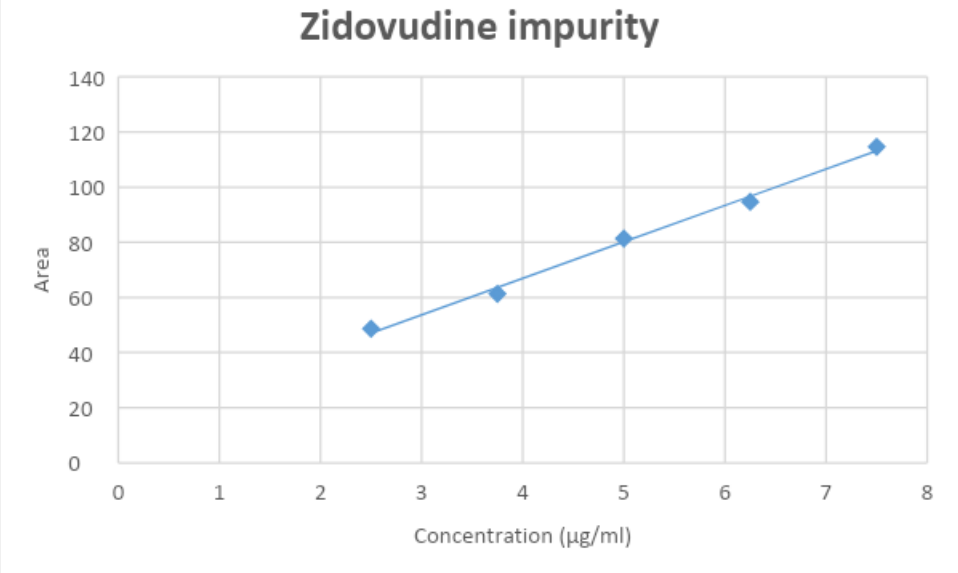


Figure 41 Calibration Curve of Zidovudine Impurity

Precision

Repeatability

The data for repeatability of peak area measurement for Abacavir and Zidovudine Impurity, based on six measurements of same solution of Abacavir and Zidovudine Impurity are depicted in table 6.14 and 6.15. The % RSD for Abacavir Impurity and Zidovudine Impurity was found to be 3.683 and 2.865 respectively.

Abacavir Impurity				
Sr. No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1	5	76.586 74.658 79.452 71.854 72.584 74.987	75.020±2.763	3.683

Repeatability Data for Abacavir Impurity.

Zidovudine Impurity				
Sr No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1	5	86.954 87.654 89.654 91.326 85.645 84.568	87.633±2.511	2.865

Repeatability data for Zidovudine Impurity

Intraday precision

The data for intraday precision for Abacavir and Zidovudine Impurity is shown in table. The % R.S.D. for Intraday precision was found to be 0.96-1.72 for Abacavir Impurity and 0.89-2.5 for Zidovudine Impurity.

Sr. No.	Abacavir Impurity			Zidovudine Impurity		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	LOQ	7.324 ± 0.123	1.67	LOQ	6.865± 0.172	2.5
2	5	76.854± 1.324	1.72	5	82.457±1.314	1.59
3	7.5	106.325± 1.023	0.96	7.5	116.457±1.045	0.89

Intraday precision data for Estimation of Abacavir and Zidovudine Impurity.

Interday precision

The data for intraday precision for Abacavir and Zidovudine Impurity is shown in table . The % R.S.D. for interday precision was found to be 1.43-2.66 for Abacavir Impurity and 1.55-3.24 for Zidovudine Impurity.

Sr. No.	Abacavir Impurity			Zidovudine Impurity		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	LOQ	7.564 ± 0.189	2.50	LOQ	6.412± 0.208	3.24
2	5	74.652± 1.986	2.66	5	76.214±1.421	1.86
3	7.5	101.235± 1.451	1.43	7.5	108.324±1.684	1.55

Interday Precision data for Estimation of Abacavir and Zidovudine Impurity.

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in table Percentage recovery for Abacavir Impurity was 99.10-105.05%, while for Zidovudine Impurity, it was found to be in range of 99.10-106.15 %.

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% R.S.D
1	LOQ	2.5	0.25	0.253	101.20	0.828
2		2.5	0.25	0.249	99.60	
3		2.5	0.25	0.252	100.80	
4	80%	2.5	2	1.991	99.55	2.87
5		2.5	2	2.012	100.60	
6		2.5	2	2.101	105.05	
7	100%	2.5	2.5	2.501	100.04	0.629
8		2.5	2.5	2.481	99.24	
9		2.5	2.5	2.512	100.48	
10	120%	2.5	3	2.992	99.73	0.668
11		2.5	3	3.013	100.43	
12		2.5	3	2.973	99.10	

Recovery Data for Abacavir Impurity.

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% R.S.D
1	LOQ	2.5	0.25	0.248	99.20	1.209
2		2.5	0.25	0.251	100.40	
3		2.5	0.25	0.245	98.00	
4	80%	2.5	2	1.983	99.15	3.613
5		2.5	2	2.123	106.15	
6		2.5	2	2.013	100.65	
7	100%	2.5	2.5	2.468	98.72	1.062

8		2.5	2.5	2.494	99.76	
9		2.5	2.5	2.521	100.84	
10		2.5	3	2.973	99.10	
11	120%	2.5	3	3.082	102.73	1.8
12		2.5	3	3.019	100.63	

Recovery Data for Zidovudine Impurity.

LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Limit of Detection

Abacavir Impurity	**Zidovudine Impuity.**
LOD = 3.3 x (SD / Slope) = 3.3 x (0.313/15.321) = 0.067 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (0.481/21.568) = 0.073 µg/ml

Limit of Detection Data for Abacavir Impurity and Zidovudine Impurity.

Limit of Quantitation

Abacavir Impurity	**Zidovudine Impurity**
LOQ = 10 x (SD / Slope) = 10 x (0.313/15.321) = 0.204 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (0.481/21.568) = 0.223 µg/ml

Limit of Quantitation Data for Abacavir Impurity and Zidovudine Impurity.

Robustness

The effect of changes was found to be within the acceptance criteria as shown in table**.** The % RSD should be less than 5%.

Sr No.	Area at Flow rate (+ 0.2 ml/min)	Area at Flow rate (- 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	64.352	78.651	69.421	61.231	71.324	64.857
2	68.263	81.321	66.325	60.861	72.548	62.751
3	67.841	77.521	68.751	64.321	75.684	67.845
% R.S.D	3.213	2.465	2.390	3.057	3.073	3.929

Robustness data for Abacavir Impurity.

Sr No.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	71.564	68.321	62.351	68.954	70.458	60.586
2	74.852	67.985	62.845	67.852	67.521	59.756
3	76.845	70.542	60.523	69.751	68.421	62.512
% R.S.D	3.583	2.015	1.976	1.385	2.187	2.320

Robustness data for Zidovudine Impurity

Calculation of Known Impurities of Abacavir and Zidovudine

Applicability of the proposed method was tested by analyzing the commercially available Tablet formulation Evotaz .The results are shown in table.

Impurity	Conc (µg/ml)	Area	% Impurity	% R.S.D
Abacavir	5	13.653	0.169	1.7
		12.869	0.167	
		15.754	0.172	
Zidovudine	5	11.856	0.148	1.35
		10.568	0.146	
		13.8547	0.15	

Calculation of Known Impurities of Abacavir and Zidovudine

The results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

Method Validation Summary

Sr. No.	Parameter	Abacavir		Zidovudine
1	Specificity	Specific		
2	Linearity & Range	2.5-7.5		2.5-7.5
3	Regression equation	y = 12.936x + 9.454		y = 13.225x + 14.005
4	Correlation co-efficient (r^2)	0.994		0.998
5	Precision	Repeatability	3.683	2.865
	(% RSD)	Interday	0.96-1.72	0.89-2.5
		Intraday	1.43-2.66	1.55-3.24
6	Accuracy (% recovery)	99.10-105.05		99.10-106.15
7	Limit of Detection(LOD)	0.067 µg/ml		0.073 µg/ml
8	Limit of Quantification(LOQ)	0.204 µg/ml		0.223 µg/ml

9	Robustness (% RSD)	The system suitability parameters were found well within the acceptance criteria as per system suitability

Summary of Validation Parameters for Abacavir and Zidovudine Related Impurities

Results and Discussion

- There is no analytical work has been available regarding Related Impurities RP-HPLC method for Abacavir and Zidovudine in a literature. Data regarding behaviour of drug and its related impurities in chromatographic conditions and other relevant analytical properties are not available.
- A novel attempt in a field of research has been made to develop and validate Related Impurities method via RP- HPLC.
- Abacavir is an antiretroviral drug of the protease inhibitor class used in combination with Zidovudine which is used to treat infection of human immunodeficiency virus (HIV).
- RP-HPLC method was developed for simultaneous estimation Abacavir and Zidovudine. In RP-HPLC method, good resolution and separation of two drugs and its related impurities was achieved. 0.05 M Potassium Dihydrogen phosphate (pH 3.0): Acetonitrile (75:25 v/v) was used as mobile phase.
- Retention time of Abacavir and Zidovudine were found to be 3.14 min and 5.77 min respectively and the retention time of Abacavir impurity and Zidovudine impurity were found to be 3.58 min and 6.14 min respectively The proposed method was accurate and precise. Therefore proposed method can be used for routine analysis of Abacavir and Zidovudine in tablets.
- The suitability, performance and applicability of developed method has been validated as per ICH guideline by applying various validation parameters like specificity, linearity and range, accuracy precision and robustness.
- The RP-HPLC method developed for the determination of related impurities of Abacavir and Zidovudine is found to be specific, linear, sensitive, precise, accurate and robust in nature.

- The method was successfully validated in terms of specificity, precision, linearity, accuracy and robustness as per ICH guidelines.
- It can be concluded that the proposed method can be used for routine analysis for estimation of related impurities of Abacavir and Zidovudine in combined dosage form by RP-HPLC.

Discussion

A new Related Impurities RP-HPLC method has been developed for estimation of Abacavir and Zidovudine Impurity in tablet dosage form was rapid, accurate, precise, economic and easy to perform. The linearity was investigated in the range of 2.5-7.5µg/mL (r^2 = 0.994) for Abacavir Impurity and 2.5-7.5 µg/ml (r^2 = 0.998) for Zidovudine Impurity. The LOD were 0.067µg/ml and 0.073µg/ml for Abacavir and Zidovudine Related Impurities, respectively. The LOQ were 0.204µg/mL and 0.223µg/mL for Abacavir and Zidovudine Related Impurities, respectively. This method was found to be simple, accurate, robust and reproducible.