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UV Spectroscopic Method Development and Validation on Anti-HIV Drug Abacavir

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ABSTRACT

The aim of this work was analytical method development and validation for abacavir in bulk dosage form using the UV spectrometric method as per ICH guidelines. It describes sensitive and robust method development of abacavir by UV spectroscopic method for estimation of abacavir in bulk and pharmaceutical dosage forms using UV-Jasco (V-730). This method was developed using methanol as solvent and absorbance were measured at 286nm. Beers law was obeyed with a linearity range of 0-10 μ g/ml. The line equation $y= 0.0415x+ 0.0063$ with correlation coefficient of 0.9989 was obtained. The method was validated for precision, linearity, and robustness to check its consistency. The results obtained that the developed analytical method was sensitive, and accurate, with low variance values for all validation parameters, and could be used in the regular analysis of abacavir in pharmaceutical formulations.

Keywords: Abacavir, UV Spectroscopic method, Linearity, Validation parameters

INTRODUCTION

Analytical chemistry could be a science that is directed towards creating new knowledge so that chemical analysis can be improved to respond to increasing or new demands. Analytical chemistry studies and uses instruments and methods to separate, identify, and quantify matter. In practice, separation, identification, or quantification may constitute the complete analysis or be combined with another method. Qualitative analysis identifies analytes, while quantitative analysis determines the numerical amount or concentration. Many of the methods can be used for both qualitative, and quantitative analysis. The major categories of instrumental methods are spectral, electroanalytical, and separatory. Compared to simple laboratory tests, instrumental methods of analysis may give improved: speed, accuracy, and sensitivity.

UV Spectroscopy

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry refers to absorption spectroscopy or reflectance spectroscopy partly of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the spectrum, atoms and molecules undergo electronic transitions. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a set path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution.

Principle:

The Principle of UV-Visible Spectroscopy relies on the absorption of ultraviolet light or visible light by chemical compounds, which ends in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter. When matter absorbs ultraviolet light, the electrons present in it undergo excitation. This causes them to jump from a ground state to an excited state.

Method validation parameters performed:

- **Selectivity/specificity:** Selectivity of an analytical method is its ability to measure accurately an analyte in presence of interferences that may be expected to be present in the sample matrix.
- **Precision:** The precision of a technique is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. It is measured by injecting a series of standards or analyzing a series of samples from multiple samplings from a homogeneous lot.



- **Linearity:** The linearity of an analytical method is its capability to elicit check consequences which might be at once. Linearity is set by injecting a series of standards of stock solution using the solvent/mobile phase, at a minimum of five different concentrations.
- **Robustness:** Robustness could be a measure of the assay capacity to remain unaffected by small but deliberate changes in test conditions. Robustness measures the effect of deliberate changes that can be controlled through specifications in the assay protocol.
- **Limit of detection and limit of quantitation:** The term LOD is defined as the lowest concentration at which the instrument can detect but not quantify and the noise to signal ratio for LOD should be 1:3. The term LOQ is defined as the lowest concentration at which the instrument can detect and quantify. The noise-to-signal ratio for LOQ should be 1:10.

RECENT LITERATURE REVIEWS ON ABACAVIR

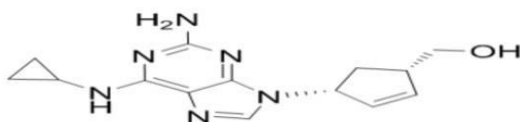
- Investigation involves development and validation of UV spectroscopic method for abacavirsulfate as per ICH guidelines also describes sensitive and robust method development of abacavirsulfate by UV spectroscopic method for estimation of abacavirsulfate in bulk and pharmaceutical dosage forms. Methanol and 1% orthophosphoric acid in the ratio of 75:25 was used as a solvent and various validation parameters were performed. Results obtained in this study demonstrate that the UV method described in the protocol was valid for the determination and assay of Abacavirsulfate. [1]
- Simple, sensitive, precise Spectrophotometric method of analysis for abacavirsulfate both as bulk, and tablet formulation was developed and validated. The method was developed with 15 % phosphoric acid and distilled water as solvent. The strategy has been successfully applied in the analysis of marketed formulations. [2]
- An easy, sensitive spectrophotometric method has been developed for the determination of abacavirsulfate in pharmaceutical dosage forms. Methanol was used as a solvent. The authors attempted to design a precise, inexpensive colorimetric method for estimation, which can be applied to analyze abacavirsulfate in a pharmaceutical dosage form. [3]
- Two rapid and sensitive methods A and B for the spectrophotometric determination of abacavirsulfate in either the pure form or in pharmaceutical formulations were proposed. These methods are based on the formation of purple color and red-violet colored chromogens obtained when the drug was diazotized with nitrous acid. [4]
- A new, fully validated, simple, rapid, and sensitive HPLC method with UV detection for the direct determination of abacavir in pharmaceutical dosage forms, raw materials, spiked human serum, and drug dissolution studies without any time-consuming extraction or evaporation steps before drug assay. The mobile phase employed was methanol:acetonitrile. [5]
- A simple, selective, precise, and accurate RP-HPLC method was developed and validated for rapid assay of Abacavir in a tablet dosage form. The mobile phase consisted of 0.1% orthophosphoric acid: acetonitrile: methanol. The method was successfully applied for routine analysis of Abacavir in tablet dosage form [6]
- RP-HPLC method was developed for the estimation of abacavirsulfate in a bulk and pharmaceutical dosage form. The mobile phase consists of a mixture of 10mM phosphate buffer: ACN (60:40 v/v %) PH: 4.0 with detection of 287 nm. Validation parameters according to ICH guidelines were performed. [7]
- Accurate isocratic HPLC method was developed and validated for the assay of abacavirsulfate in bulk dosage forms. The assay involved an isocratic elution of abacavirsulfate using a mobile phase composition consisting of methanol and potassium dihydrogen orthophosphate. The results showed that this method can be used for the rapid determination of abacavirsulfate in pharmaceutical tablets. [8]
- LC-MS method is developed and validated for the quantification of abacavir, a nucleoside polymerase inhibitor. Ester and dichloromethane (90:10, v/v) were used as a solvent. Different validation parameters were performed and results were obtained. [9]

METHODOLOGY

Work planned to perform method development and validation parameter of a drug (Abacavir). The active pharmaceutical ingredient of the actual drug was searched in various industries and collected. Then the anti-HIV drug Abacavir was chosen for my investigation. UV Visible Spectrometer instrument was used for the detection of all the method development and validation parameters. The UV Absorption maxima of Abacavir were analyzed. Different validation parameters were performed like selectivity/specificity, linearity, precision, robustness, stability, the limit of detection (LOD), and limit of quantitation (LOQ).

Drug Profile:

- Name- Abacavir
- Molecular Formula- C₁₄H₁₈N₆O
- Molecular Weight- 286.33



- Structure-
- Description- Abacavir is a nucleoside analogue and reverse transcriptase inhibitor used as prescription medicine approved by the U.S.FDA for the treatment of HIV infection in adults and youngsters 3 months of age and older. Abacavir is usually used in combination with other HIV medicines.
- Marketed Products- Ziagen, Abamune

Materials and Instrument:

- Active Pharmaceutical Ingredient(API) - Abacavir is employed. Abacavir belongs to a category of drugs known as nucleoside reverse transcriptase inhibitors-NRTI. Helps to decrease the quantity of HIV in your body so your immune system can work better. This lowers your chance of getting HIV complications.
- Solvent- Methanol is employed.
- Instrument- Ultraviolet-Visible Spectrophotometer used. (Jasco UV)

Model Name- V-730, Serial No- C203061798

- Description- UV spectroscopy or UV-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy partially of the ultraviolet and the full, adjacent visible regions of the spectrum. Being relatively inexpensive and simply implemented, this system is widely used in diverse fundamental applications.

Experimental:

➤ Solubility

- Soluble in – Methanol, Acetonitrile, and water.
- The measured amount of drug(10mg) is added to the volumetric flask of 10ml and therefore the volume is made up.
- Solutions- 0.1N NaOH and 0.1N HCL used for stability.

Preparation of NaOH:0.4gm of NaOH pellets dissolved in 100ml water.

Preparation of HCL:0.86ml of concentrated HCL added to 100ml water.

➤ UV spectroscopic method development of Abacavir

Abacavir shows maximum absorbance at 286nm. The literature informs that there are very few methods reported for abacavir estimation by the UV method directly. Therefore with this rationale, this study was undertaken for the development and validation of the spectroscopic method of abacavir for system suitability parameters followed by validation for, linearity, precision, and robustness for the proposed drug.

- **Preparation of Abacavir stock solution (1000µg/ml):** Weigh accurately about 10 mg of Abacavir API and transfer to a 10 ml volumetric flask. Add methanol, shake for five minutes to dissolve, and make up the volume up to 10ml.
- **Preparation of Abacavir stock solution (100 µg/ml):** Pipette out 1ml from 1000 µg/ml Abacavir stock solution and transfer to a 10 ml volumetric flask. Add methanol and structure the volume up to 10ml.
- **Preparation of Abacavir stock solution (10 µg/ml):** Pipette out 1ml from 100 µg/ml Abacavir stock solution and transfer to a 10 ml volumetric flask. Add methanol and structure the volume up to 10ml.
- **Solvent Composition:** Methanol is employed.
- **Determination of absorption maxima of Abacavir in the selected solvent blend:** Standard stock solutions were scanned on a UV spectrophotometer within the UV range of 200-400 nm and find out the absorption maxima.

- **Selection of optimum pH for max absorbance:** Absorbance of different concentrations of standard solutions was recorded at different pH ranges and pH where maximum absorbance is observed is selected for the method.

➤ **Validation of proposed UV method of abacavir:**

- **Determination of linearity range and calibration curve for Abacavir**

Standard solutions of Abacavir within the concentration range of 1-1000 $\mu\text{g} / \text{ml}$ were prepared and absorbance was measured at 286 nm taking solvent blend as a blank to determine the linearity range. Appropriate aliquots from standard Abacavir stock solutions were transferred to a series of 10 ml volumetric flasks. The quantity was adjusted to the mark with the solvent blend to obtain concentrations of 1, 4, 6, 8, and 10 ppm. Absorbance spectra of every solution against the respective solvent blend as blank were measured at 286 nm and concentration vs absorbance was plotted and interpreted statistically.

- **Precision:** The precision was evaluated by assaying 3 determinations at the identical concentration (100 $\mu\text{g}/\text{ml}$), during the identical day, under identical experimental conditions. Intermediate precision was analyzed by comparing the assays in 3 determinations at the identical concentration (100 $\mu\text{g}/\text{ml}$) over 3 days. Precision was expressed like relative standard deviation (RSD).

Preparation of Abacavir sample solution: In 3 different volumetric flask pipettes out 3 different concentrations like 0.4, 0.6, and 0.8 ml of the 100 $\mu\text{g}/\text{ml}$ of stock solution of abacavir. Add methanol and structure the volume up to 10ml in all three volumetric flasks. The % RSD values were calculated and it should be less than 2%.

- **Robustness:** The robustness of the proposed methods is decided by the analysis of samples and standard solutions (100 $\mu\text{g}/\text{ml}$) at different wavelengths (284, 286, 288nm), at different ratios of the solvent blend. Appropriate concentrations of Abacavir from bulk and formulations were prepared in respective solvent blends. Analysis was administered at a different wavelength (284, 286, 288nm).

- **Limit of detection and limit of quantitation:**

Determination of Limit of Detection (LOD) and Limit of Quantitation (LOQ) from Detector Linearity experiments (applicable to only instrument sensitivity). LOD and LOQ values are calculated manually by taking the Noise to signal ratio of the lowest/known concentration of linearity samples and it'll be expressed in $\mu\text{g}/\text{ml}$ or ppm. To calculate during which, values of LOD and LOQ are going to be multiplied by 100/lowest or known concentration of test item (mg/L) taken for analysis of that particularly or impurity analysis.

The formula of LOD and LOQ values for instrument sensitivity: $Y = mx + c$ where $m = \text{slope}$, $c = \text{intercept}$, $N = \text{number of test sample}$

SE of intercept = By excel—data analysis—Regression

SD of Intercept = SE intercept * \sqrt{N} , LOD = $3.3 * \text{SD of Intercept} / \text{slope}$

RESULT AND DISCUSSION

Simple, rapid, and reproducible UV spectroscopic method was developed and validated as per ICH guidelines for an antiretroviral drug viz., Abacavir. The developed method was further validated for, precision, specificity, and robustness, with statistical data. The λ_{max} with characteristic peak for Abacavir in solvent phase methanol at 286 nm was observed and therefore the results are shown in figure 1.

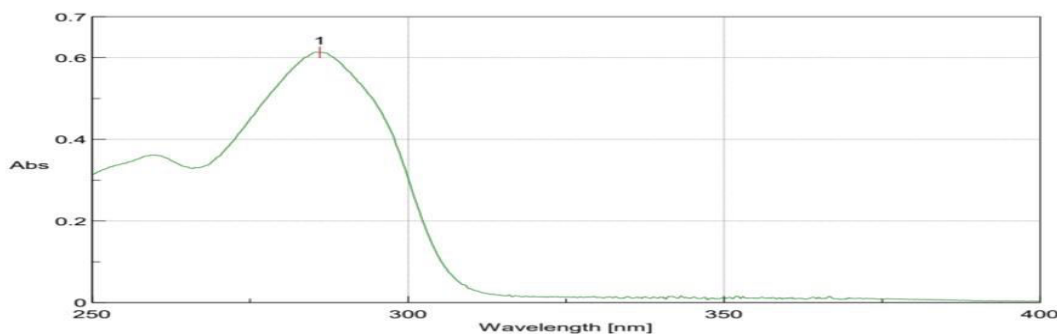


Fig 1:UV Absorption maxima of Abacavir.



• **Linearity:**

Table 1: UV Linearity studies of Abacavir

TUBE	CONCENTRATION (in ppm)	ABSORBANCE
1	0	0
2	2	0.09
3	4	0.179
4	6	0.26
5	8	0.339
6	10	0.416

The linearity range for Abacavir was studied at respective absorption maxima with the coefficient of correlation of 0.9989, slope (0.0415), and intercept (0.0063). The linearity range data and graph result showed in table 1.

- **Calibration:**The calibration curve for Abacavir was analyzed. Best fit value slope was found to be $y = 0.0415x + 0.0063$. The results are shown in fig 2 and table 2.

Table 2: UV Statistical data of calibration curve for Abacavir

Parameter	Results
λ_{max} (nm)	286
Beer's Law Limit ($\mu\text{g/ml}$)	0-10
Correlation coefficient (r)	0.9989
Regression Equation ($y=mx+c$)	$Y=0.0415x + 0.0063$
Slope (m)	0.0415
Intercept (c)	0.0063
LOD ($\mu\text{g/ml}$)	0.0188 $\mu\text{g/ml}$
LOQ ($\mu\text{g/ml}$)	0.0569 $\mu\text{g/ml}$
Standard error of mean of Regression line	0.0004

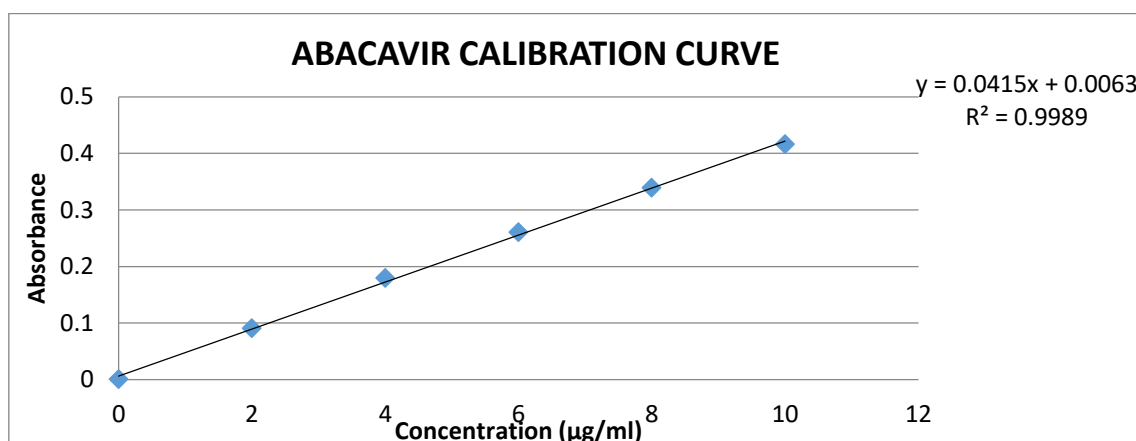


Fig 2: Calibration curve for Abacavir.

• **Precision:**

3 different concentrations of 0.4, 0.6, and 0.8 ml resulted in the subsequent precision value along with its mean, standard deviation, and %RSD results are shown in Table 3.

Table 3: Precision studies of Abacavir

Concentration(ml)	ABSORBANCE			Mean	SD	% RSD
0.4	0.0935	0.0925	0.0915	0.0925	0.001	1.081
0.6	0.017	0.0175	0.017	0.0171	0.0002	1.681
0.8	0.1885	0.1865	0.1872	0.1874	0.001	0.541

- Robustness:** Robustness of the proposed methods decided by the analysis of samples and standard solutions (100µg/ml) at a different wavelength (284,286, 288nm), at different ratios of the solvent result are shown in table 4.

Table 4: Robustness studies of Abacavir

CONCENTRATION (ml)	Change in Wavelengths			Mean	SD	%RSD
	284	286	288			
0.2	0.0615	0.0627	0.0609	0.0617	0.0009	1.485
0.4	0.1037	0.1038	0.1072	0.1049	0.0019	1.899
0.6	0.0211	0.0205	0.0204	0.0206	0.0003	1.831

- Limit of detection and limit of quantitation:** LOD and LOQ values for instrument sensitivity was found to be: SD of Intercept= 0.0004, LOD= 0.0188, and LOQ = 0.0569 respectively shown in Table 2.

CONCLUSION

The results obtained during this study informs that the UV method described was valid for the determination and assay of Abacavir. Therefore the method is suitable for its intended use. The study is an highly sensitive, accurate, and reproducible UV method for the determination of Abacavir. This proposed method involves a simple, rapid and cheap sample preparation method. The %RSD was found to be within the bounds. The very low quantification limit obtained with a UV detector helped to avoid the use of fluorimetric detection, which demands costlier equipment. The proposed method is often effectively applied for quality control analysis of Abacavir in bulk dosage forms.

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