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Development and Validation of New UV Spectroscopic and HPLC Methods for the Estimation of Zidovudine in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

In the present work, two simple, sensitive and specific methods (UV Spectroscopy and RP-HPLC) have been developed for the quantitative estimation of Zidovudine in bulk and pharmaceutical formulations. A UV Spectroscopic method was developed and validated for the estimation of Zidovudine in pharmaceutical dosage forms. The working standard solution was prepared in methanol. The λmax was found to be 266 nm. The linearity was found in the concentration range of 2-20 µg / ml. The Correlation coefficient was 0.999. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and ruggedness. The limit of detection and limit of quantitation for estimation of Zidovudine was found to be 0.195 (µg / ml) and 0.585 (µg / ml), respectively. Recovery of Zidovudine was found to be in the range of 100.38-101.47 %. A simple, specific, accurate, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Zidovudine in pharmaceutical dosage forms. A Phenomenex Gemini C-18, 5-µm column having 250 x 4.6 mm i. d. in isocratic mode, with mobile phase containing Methanol: Water (20: 80, v/v) was used. The flow rate was 1 ml/min and effluents were monitored at 266 nm. Chromatogram showed the main peak at a retention time of 4.47 min. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and ruggedness. The limit of detection and limit of quantitation for estimation of Zidovudine was found to be 0.331 (µg / ml) and 1.005 (µg / ml), respectively. Recovery of Zidovudine was found to be in the range of 98.06-100.90 %. Proposed methods were successfully applied for the quantitative determination of Zidovudine in pharmaceutical dosage forms.

Keywords: Zidovudine, Method validation, UV Spectroscopy, RP-HPLC, ICH guidelines.

A R T I C L E I N F O

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1. Introduction

Zidovudine (INN) or azidothymidine (AZT) (also called ZDV) is a nucleoside analog reverse transcriptase inhibitor (NRTI), a type of antiretroviral drug used for the treatment of HIV/AIDS. It is an analog of thymidine. AZT was the first approved treatment for HIV, sold under the names Retrovir and Retrovis[1]. AZT use was a major breakthrough in AIDS therapy in the 1990s that significantly altered the course of the illness and helped destroy the notion that HIV/AIDS was a death sentence. Zidovudine is chemically 1- [(2R, 4S, 5S)-4azido-5(hydroxymethyl) tetrahydrofuran-2-yl]-5-methylprimidine-2, 4 (1H, 3H0-dione (Figure 1) and used as an antiretroviral activity[2]. The literature survey reveals that, several spectrophotometric method[3, 4] titrimetric and spectrophotometric method[5], HPLC method[6] have been reported for the estimation of zidovudine in pharmaceutical formulations. Hence, an attempt has been made to develop new UV and RP-HPLC methods for its estimation in pharmaceutical formulations with good accuracy, simplicity, precision and economy.

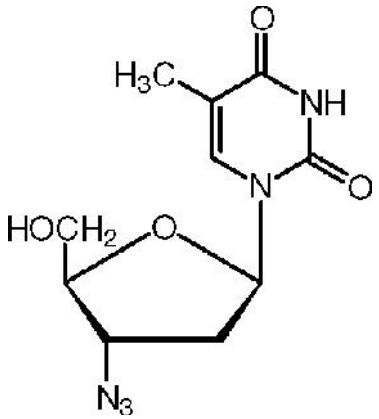

Figure 1: Chemical structure of Zidovudine

2. Materials and Methods

All absorbance measurements were made on a Spectronic 1001 plus spectrophotometer (Milton Roy Company, USA) with 1 cm matched quartz cells.

Chromatographic Conditions:

The liquid chromatographic system consisted of following components: Shimadzu HPLC model containing LC-20AT (VP series) pump, variable wavelength programmable UV / VIS detector SPD-20A (VP series) and Hamilton syringe (705 NR, 50 µL). Chromatographic analysis was performed using the Spinchrom software on a Phenomenex-Gemini C-18 column with 250 x 4.6 mm i.d. and 5 µm particle size. Chromatographic separation was achieved at ambient temperature on an RP- HPLC by using a mobile phase consisting of Methanol and Water in the ratio of 20:80 (v/v). The mobile phase was pumped at a flow rate of 1 ml / min. The detector wavelength was set at 266 nm.

Reagents:

The reference standard Zidovudine was kindly gifted by Matrix Laboratories Limited (Hyderabad, A.P and India). The standard drugs were used without further purification. Water, Methanol and other reagents of HPLC grade from Qualigens, Mumbai were used throughout the experiment. Commercial pharmaceutical preparations Retrovir (Brand name) from Matrix Laboratories Ltd (Hyderabad, A.P, India) which were claimed to contain 300 mg of Zidovudine was used in the analysis. The mobile phase consists of a mixture of Methanol and Water in the ratio of 20: 80 (v/v).

Preparation of stock solutions for UV method:

Standard Zidovudine 100 mg was weighed and transferred to a 100 ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000 µg / ml. From this stock solution, pipetted out 10 ml and placed into a 100ml volumetric flask. The volume was made up to mark with distilled water to give a solution containing 100 µg / ml.

Sample preparation for determination of Zidovudine from dosage form for UV method:

Twenty tablets were weighed and finely powdered. The powder equivalent to 100 mg of Zidovudine was accurately weighed and transferred to the volumetric flask of 100 ml capacity containing 25 ml of the methanol and sonicated for 5 min. The flask was shaken and volume was made up to the mark with methanol to give a solution of 1000 µg / ml. The above solution was centrifuged at 2000 rpm for 10 minutes and carefully filtered through Whatmann filter paper (No. 41). From this solution, 10ml was taken and diluted to 100 ml with distilled water to give a solution of 100 µg / ml and used for the estimation of Zidovudine.

Preparation of working stock solution of Zidovudine for HPLC method:

About 50 mg of Zidovudine was weighed accurately and dissolved in 100 ml of Methanol in a 100 ml volumetric flask and diluted up to the mark with mobile phase to get the concentration of 500 µg / ml.

Assay procedure for HPLC method:

Working standard solutions containing 25 to 75 µg / ml of Zidovudine were prepared by appropriate dilution of the stock solution with the mobile phase. Twenty µl aliquot of each solution was injected into the column for five times and the chromatograms were recorded and are presented in Fig: 5.7. The retention time was found to be 4.47 min. Calibration graph was constructed by plotting the mean peak area as a function of Zidovudine concentration.

Analysis of formulation by HPLC method:

Twenty tablets each of two different brands of Zidovudine (each containing 300 mg) were accurately weighed; average

weight was determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 50 mg of Zidovudine was transferred into 100 ml volumetric flask and dissolved in 25 ml of Methanol and sonicated for 5 minutes. The solution was filtered through Whatmann filter paper (no. 41). The residue was washed with 5 ml portions of Methanol for three times and the final volume of the filtrate was made up to 100 ml with mobile phase (500 µg / ml). This solution was further diluted stepwise with mobile phase in such a way to get various concentrations of aliquots range from 25 to 75 µg / ml. After dilution, the solutions were then analyzed by RP-HPLC method. All determinations were conducted for five times.

Validation of Analytical Methods:

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

3. Results and Discussion

Selection of analytical wavelength:

Appropriate dilutions were prepared for the drug from the standard stock solution and the solutions were scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained were derivative from Zero to Second order. The first and second order derivative spectrum was selected for the analysis of the drugs. The λmax was found to be 266 nm. Figure 2 shows the λmax curve.

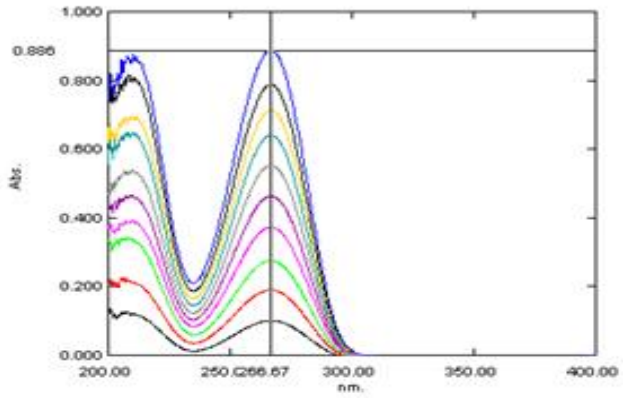

Figure 2: UV spectra of Zidovudine at 266 nm

Calibration curve for the Zidovudine by UV method (2 - 20 µg / ml): The appropriate volume of aliquots from standard Zidovudine stock solutions were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with distilled water to obtain concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg / ml. Absorbance spectra of each solution against distilled water as blank were measured at 266 nm and the graphs of absorbance against concentration was plotted and are shown in Figure 3.

Validation of UV spectrophotometric method:

The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and ruggedness. The summary of the results were presented in table 1.

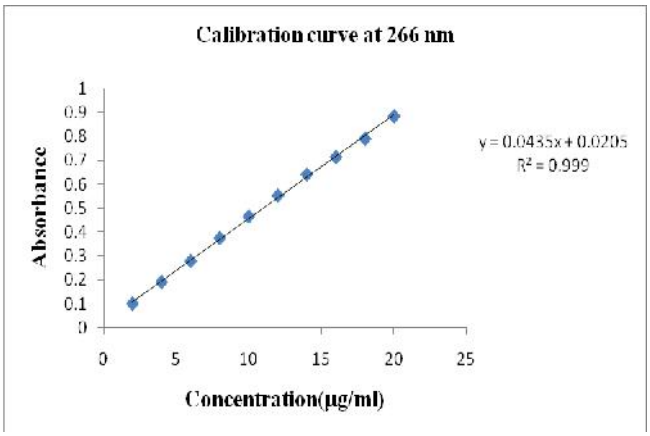

Figure 3: Linearity curve for Zidovudine at 266 nm by UV spectroscopy

Table 1: Optimum conditions, Optical characteristics and Statistical data of the Regression equation in UV method

Parameters	UV Method
λ_{max} (nm)	266
Beer's law limits (µg/ml)	2-20
Molar extinction coefficient (mol^{-1} cm^{-1})	0.0465 X 10^4
Sandell's sensitivity (µg/cm^2-0.001 absorbance units)	0.0215
Regression equation (Y*)	Y = 0.0435 C + 0.0205
Slope (b)	0.0435
Intercept (a)	0.0205
Correlation coefficient(r^2)	0.999
% RSD**	0.630
Limit of detection (µg/ml)	0.6
Limit of quantitation (µg/ml)	1.8

*Y= b C + a where C is the concentration of Zidovudine in µg/ml and Y is the absorbance at the respective $\lambda_{max.}$

**Average of six determinations.

Validation of RP-HPLC Method:

RT of the zidovudine was found to be 4.4 min. Figure 4 shows the chromatogram of zidovudine. RP-HPLC method was validated as per ICH guidelines for linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and ruggedness

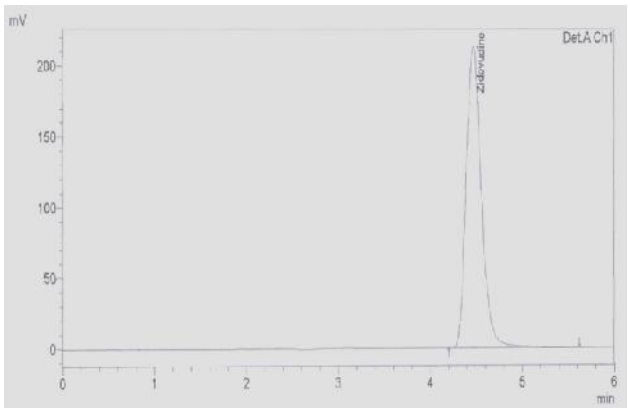

Figure 4: Chromatogram of Zidovudine

Accuracy:

The accuracy of a method was inferred by establishing the precision and linearity of the standard. The concentration range of 25-75 µg / ml was taken and % accuracy was calculated for both bulk and dosage form and those values were given in Table 2.

Table 2: Accuracy results for Zidovudine

Av. Area**	25µg/ml	37.5µg/ml	50µg/ml	62.5µg/ml	75µg/ml
	1227490	1799649	2318687	2775062	3322328
% Assay	49.10	75.21	99.12	124.60	148.78
Theoretical %	50	75	100	125	150
% Accuracy	98.20	100.28	99.12	99.68	99.19

Precision: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as standard deviation or the relative standard deviation. The results were given in table 3.

Table: 3. Precision results for Zidovudine

Sl. No.	Peak Area**	% Assay**	R.T**
1	2349937	99.42	4.499
% RSD	1.69	0.23	0.28

Linearity: The linearity of the method was demonstrated over the concentration range of 25-75 µg / ml of the target concentration. A calibration curve is produced by analyzing different concentrations of the pure drug from the chromatogram. The correlation coefficient for the average area at each level versus concentration of analyte was calculated and reported in figure 5.

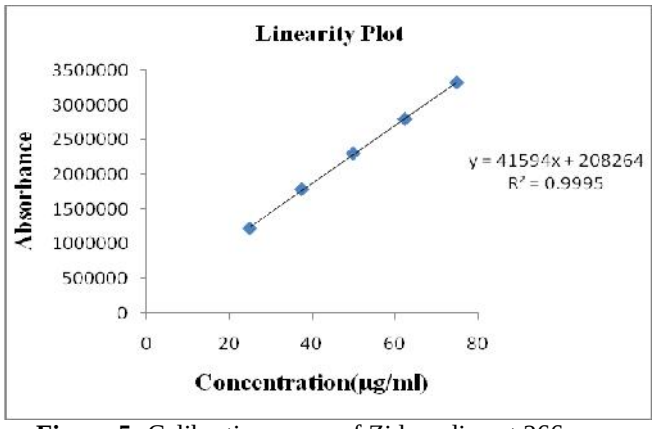

Figure 5: Calibration curve of Zidovudine at 266 nm

4. Conclusion

In the present investigation, simple and sensitive UV spectrophotometric and HPLC methods were developed for the quantitative estimation of Zidovudine in bulk drug and pharmaceutical formulations. In addition to positive requirements of these analytical methods, the striking advantage of all the presently developed methods was that they were economical.

5. Acknowledgement

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