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Development of a Spectrophotometric Method for the Quantitative Estimation of Zidovudine Concentration in Bulk and Pharmaceutical Dosage Forms

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Abstract

A simple, accurate, precise, sensitive and a highly selective spectrophotometric method was developed and validated for the estimation of Zidovudine in bulk and pharmaceutical dosage forms. The stock solutions were prepared as per procedure and were scanned at maximum absorbance of 266 nm. The linearity was found in the concentration range of 2-20 µg / mL. The Coefficient of determination (r^2) was 0.999. The regression equation was found to be Y = 0.0435. C + 0.0205 and % RSD was found to be 0.630. The developed method was validated according to ICH guidelines and was found to be accurate and precise. The validation parameters are linearity, accuracy, precision, limit of detection, limit of quantitation, robustness and ruggedness. Thus the proposed method can be successfully applied for the estimation of Zidovudine in bulk and pharmaceutical dosage forms.

Keywords: Zidovudine, Validation, ICH guidelines, Spectrophotometric method

1. Introduction

Zidovudine (INN) or azidothymidine (AZT) is a nucleoside analog reverse transcriptase inhibitor (NRTI), a type of antiretroviral drug [1-3]. It is a synthetic drug with pyrimidine nucleoside analogue active against HIV-1, AIDS and pre- AIDS. The chemical name of Zidovudine is 1- (3-azide-2, 3-di deoxy-β-D-ribofuranosyl)-5-methyl Pyrimidin-2, 4 (1H, 3H) – dione. Zidovudine also has been referred to as 3′azido-3′-deoxythymidine. It has a molecular formula of $C_{10}H_{13}N_5O_4$ and a molecular weight of 267.24 g/mol. It has the structural formula as shown in Figure 1. Zidovudine is a white to beige, odorless, crystalline solid and it is soluble in ethanol (95%), sparingly soluble in water. The drug is officially listed in United States of Pharmacopiea [4]. Several analytical methods that have been reported for the estimation of Zidovudine in biological fluids or pharmaceutical formulations include UV-Visible Spectrophotometry [5-6], High Performance Liquid Chromatography [7-12] and HPTLC [13-14]. The objective of the work is to develop a simple, accurate, precise and economic UV spectrophotometric method for the estimation of Zidovudine in bulk and pharmaceutical dosage forms. The method is simple, reproducible and statistically valid.

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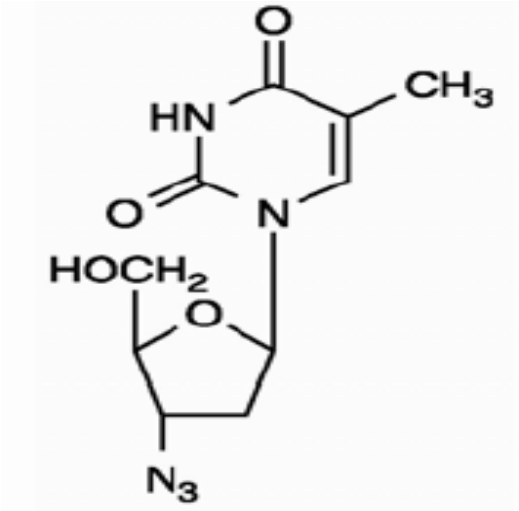

Figure 1 Structure of Zidovudine

2. Materials and Method

2.1 Materials

Zidovudine was obtained as a gift sample from Matrix Laboratories Ltd, Hyderabad. Methanol, Distilled water and other reagents were of analytical grade. UV-Vis Spectrophotometer Shimadzu UV-1800 with a fixed slit width (2 nm) and 10 millimeter quartz cell was used to obtain spectrum and absorbance measurement.

2.2 Method

2.2.1 Preparation of Stock solutions

Standard Zidovudine 100 mg was weighed and dissolved in 50 mL of methanol in a 100 mL volumetric flask. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000 µg / mL (stock solution I). From the stock solution I, pipette out 10 mL and placed into 100 mL volumetric flask. The volume was made up to mark with distilled water to give a stock solution containing 100 µg / mL (stock solution II).

2.2.2 Selection of analytical concentration ranges

From the standard stock solution II of Zidovudine, appropriate aliquots were pipetted out into 10 mL volumetric flasks and dilutions were made with distilled water to obtain working standard solutions of concentrations from 2 to 50 µg / mL. Absorbance for these solutions were measured at 266 nm and the spectra was shown in Figure 2. For the standard solution analytical concentration range were found to be 2-20 µg / mL and those values were reported in Table 1.

2.2.3 Calibration curve for the Zidovudine (2 – 20 µg / ml)

Appropriate volume of aliquots from standard Zidovudine stock solution II 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 were plotted and shown in Figure 3. The regression equation and coefficient of determination was determined.

2.2.4 Sample preparation for determination of Zidovudine from dosage form

Twenty tablets of two brands were weighed and finely powdered. The powder equivalent to 100 mg of Zidovudine was accurately weighed and transferred to 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 (stock solution I). The above solution was centrifuged at 2000 rpm for 10 minutes and carefully filtered through Whatmann filter paper (No. 41). From this solution, 10 mL was taken and diluted to 100 mL with distilled water to give a solution of 100 µg / mL (stock solution II) and used for the estimation of Zidovudine. To examine the absence of either positive or negative interference of excipients used in formulation, recovery studies were carried out.

2.2.5 Method validation

Accuracy was determined by recovery studies. The recovery studies were carried out by adding the known amount of standard Zidovudine drug to the sample solution of the tablets. Precision for assay were determined by repeatability, interday, intraday precision for drug (each in three replicate). Ruggedness studies were carried out by changing the analysts. LOD and LOQ were performed and those were values within the limits.

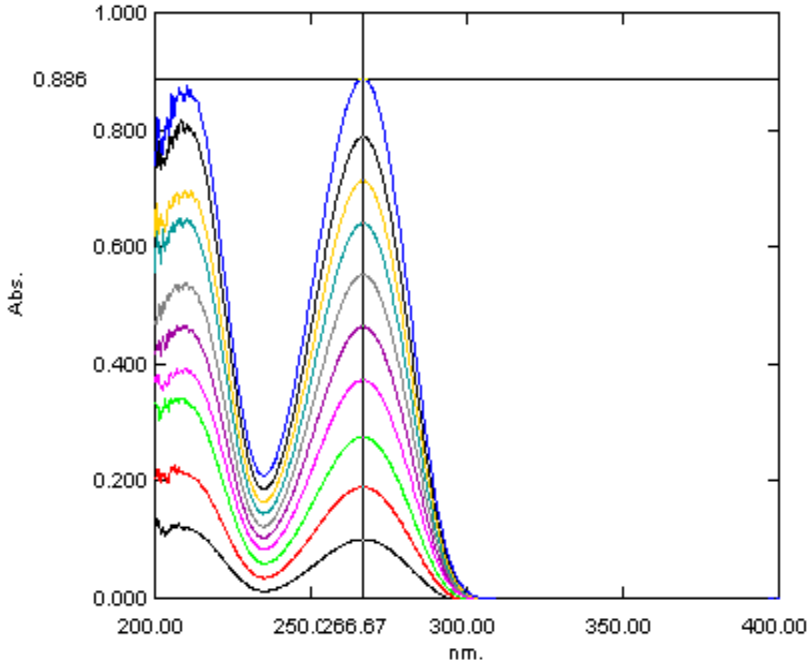

Figure 2 UV Spectra of Zidovudine at 266 nm

Table 1 Results of calibration curve at 266 nm for Zidovudine by UV spectroscopy

Sl. No.	Concentration (µg/ml)	Absorbance at 266 nm
1	2	0.099
2	4	0.190
3	6	0.278
4	8	0.374
5	10	0.465
6	12	0.553
7	14	0.641
8	16	0.714
9	18	0.791
10	20	0.886

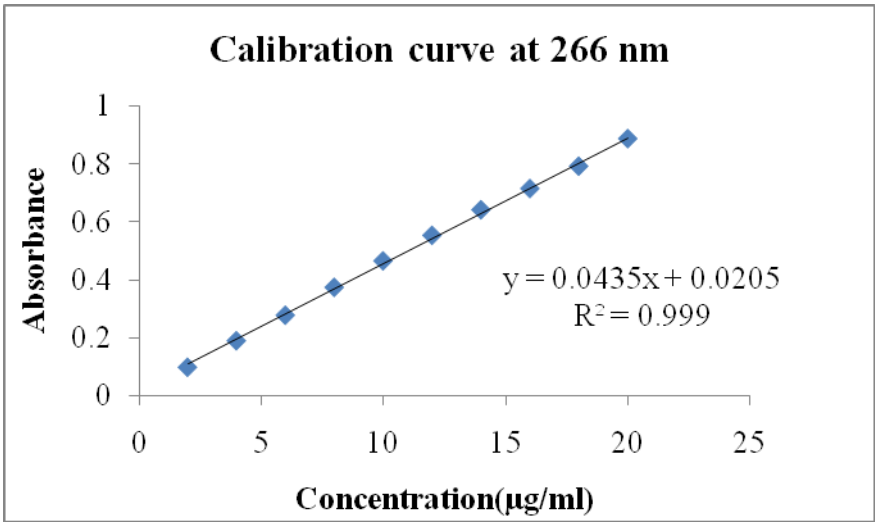

Figure 3 Linearity plot or calibration curve for Zidovudine at 266 nm by UV spectroscopy

3. Results and Discussion

Maximum absorption for Zidovudine in UV spectrophotometric method was recorded at 266 nm. The method was validated according to ICH guide lines [15-18]. The optical characteristics such as Beer's law limit, molar absorptivity and other parameters are summarized in Table 2. The results of accuracy, precision and ruggedness studies were shown in Tables 3-6, respectively.

Table 2 Optical Characteristics of Zidovudine

PARAMETERS	RESULTS
Absorption maximum	266 nm
Beer's law limit (µg / ml)	2-20 µg / ml
Correlation coefficient (r^2)	0.999
Molar absorptivity (mol^{-1} cm^{-1})	0.0465 X 10^4
Sandell's sensitivity (µg / cm^2-0.001 absorbance units)	0.0215
Regression equation (y = mx + c)	Y = 0.0435 X + 0.0205
Slope (m)	0.0435
Intercept (c)	0.0205
% RSD*	0.630
Limit of detection (µg / ml)	0.6
Limit of quantitation (µg / ml)	1.8

*Average of six determinations.

Table 3 Accuracy results of Zidovudine at 266 nm

Amount of sample (µg / ml)	Amount of drug added (µg / ml)	Amount Recovered** (µg / ml)	% Recovery ± SD**
		Tablet 1	
10	9.0	18.95	99.44 ± 0.17
10	10	20.15	100.38 ± 0.11
		Tablet 2	
10	9.0	19.08	100.08 ± 0.07
10	10	19.98	99.80 ± 0.17

**Average of six determinations.

Table 4 Precision results of Zidovudine at 266 nm

Conc. µg / ml	Inter-day Absorbance** ± SD	% RSD	Intra-day Absorbance**± SD	%RSD
2	0.099 ± 0.000577	0.582	0.099 ± 0.000577	0.582
4	0.190 ± 0.001	0.526	0.191 ± 0.001	0.523
6	0.277 ± 0.001	0.361	0.277 ± 0.001	0.361
8	0.374 ± 0.001	0.267	0.374 ± 0.001	0.267
10	0.466 ± 0.001	0.214	0.465 ± 0.001	0.215
12	0.553 ± 0.000577	0.104	0.553 ± 0.000577	0.104
14	0.642 ± 0.000577	0.089	0.641 ± 0.000577	0.090
16	0.713 ± 0.001	0.140	0.713 ± 0.001	0.140
18	0.795 ± 0.000577	0.072	0.790 ± 0.000577	0.073
20	0.880 ± 0.000577	0.065	0.886 ± 0.000577	0.065

**Average of six determinations.

Table 5 Ruggedness results of Analyst I of Zidovudine at 266 nm

Sample	Label claim (mg)	Amount found**(mg)	% Recovery ± SD**
Tab 1	100	99.81	99.81 ± 0.15
Tab 2	300	299.63	99.87 ± 0.09

Table 6 Ruggedness results of Analyst II of Zidovudine at 266 nm

Sample	Label claim (mg)	Amount found** (mg)	% Recovery ± SD**
Tab 1	100	99.86	99.86 ± 0.10
Tab 2	300	299.85	99.95 ± 0.16

**Average of six determinations

4. Conclusions

From the results, it can be concluded that the proposed method for the estimation of Zidovudine is simple, convenient, accurate, sensitive and reproducible. It can be successfully used for routine analysis of the Zidovudine in bulk and pharmaceutical dosage forms.

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