Research Article **CODEN: AJPAD7** **ISSN: 2321-0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

METHOD DEVELOPMENT AND VALIDATION OF ZIDOVIDINE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV SPECTROPHOTOMETER

B. Prathap*[1], Akalanka Dey[2], G. Srinivasa Rao[3], S. Jeganath[4], D. Kalyani[1]

[1*]Department of Pharmaceutical Analysis, Saastra College of Pharmaceutical Education and Research, Nellore, Andhra Pradesh, India.

[2]Faculty of Pharmacy, Annamali University, Annamalai Nagar, Tamilnadu, India.

[3]Department of Pharmacology, Saastra College of Pharmaceutical Education and Research, Nellore, Andhra Pradesh, India.

[4]Department of Pharmaceutics, Saastra College of Pharmaceutical Education and Research, Nellore, Andhra Pradesh, India.

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 265 nm. The linearity was found in the concentration range of 2.5-15 µg / mL. The Coefficient of determination (r2) was 0.999. The regression equation was found to be Y = 0.062X + 0.0205 and % RSD was found to be 0.052. The developed method was validated according to ICH guidelines and was found to be simple, 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 and Spectrophotometric method.

Author for Correspondence:
Prathap B,
Department of Pharmaceutical Analysis,
Saastra College of Pharmaceutical Education and Research, Nellore, Andhra Pradesh, India.
Email: prathapnila@gmail.com

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 No.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.

MATERIALS AND METHOD

MATERIALS

Zidovudine was obtained as a gift sample from Matrix Laboratories Ltd, Hyderabad. Distilled water and other reagents were of analytical grade. UV-Vis Spectrophotometer Analytical UV 2060 plus with a fixed slit width (2 nm) and 10 millimeter quartz cell was used to obtain spectrum and absorbance measurement.

METHOD

Preparation of Stock solutions

Standard Zidovudine 100 mg was weighed and dissolved in pH 9.2 buffer in a 100 mL volumetric flask. The flask was shaken and volume was made up to the mark with pH 9.2 buffer to give a solution containing 1000 µg / mL (stock solution I). From the stock solution I, pipette out 10mL 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).

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 2.5 to 15 µg / mL. Absorbance for these solutions were measured at 265 nm and the spectra was shown in Figure No.2. For the standard solution analytical concentration range were found to be 2.5-15 µg / mL and those values were reported in Table No.1.

Calibration curve for the Zidovudine

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 2.5, 5, 7.5, 10, 12.5, and 15 µg / mL. Absorbance spectra of each solution against pH 9.2 buffer as blank were measured at 265 nm and the graphs of absorbance against concentration were plotted and shown in Figure No.3. The regression equation and coefficient of determination was determined.

Sample preparation for determination of Zidovudine from dosage form

Twenty tablets 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 pH 9.2 buffer solution 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 solvent solution 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.

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.

RESULTS AND DISCUSSION

Maximum absorption for Zidovudine in UV spectrophotometric method was recorded at 265 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 No.2. The results of accuracy, precision and ruggedness studies were shown in Tables No.3-6, respectively.

Table No.1: Results of calibration curve at 265 nm for Zidovudine by UV spectroscopy

S.No	Concentration (µg/ml)	Absorbance at 265nm
1	2.5	0.156
2	5	0.313
3	7.5	0.471
4	10	0.631
5	12.5	0.778
6	15	0.939

Table No.2: Optical Characteristics of Zidovudine

S.No	PARAMETERS	RESULTS
1	Absorption maximum	265nm
2	Beer's law limit (µg / ml)	2.5 – 15
3	Correlation coefficient (r^2)	0.999
4	Regression equation (y = mx + c)	0.062x+0.0263
5	Slope (m)	0.062
6	Intercept (c)	0.0263
7	% RSD*	0.241
8	Limit of detection (µg / ml)	0.14
9	Limit of quantitation (µg / ml)	0.44

*Average of six determinations.

Table No.3: Accuracy results of Zidovudine at 265 nm

S.No	Amount of sample (µg / ml)	Amount of drug added (µg / ml)	Amount Recovered** (µg / ml)	% Recovery ± SD**
1	10	9.0	18.95	99.44±0.17
2	10	10	20.15	100.38±0.11

**Average of six determinations.

Table No.4: Precision results of Zidovudine at 265 nm

S.No	Conc. µg / ml	Inter-day Absorbance** ± SD	% RSD	Intra-day Absorbance** ± SD	% RSD
1	2.5	0.153±0.001	0.547	0.154±0.001	0.548
2	5	0.315±0.00057	0.254	0.313±0.00057	0.252
3	7.5	0.468±0.001	0.214	0.472±0.001	0.214
4	10	0.635±0.001	0.078	0.633±0.001	0.077
5	12.5	0.776±0.001	0.068	0.776±0.001	0.068
6	15	0.938±0.00057	0.054	0.936±0.00057	0.053

**Average of six determinations.

Table No.5: Ruggedness results of Analyst I of Zidovudine at 265 nm

S.No	Sample	Label claim (mg)	Amount found** (mg)	% Recovery ± S.D**
1	Tablet formulation	100	99.40	99.40±0.026

Table No.6: Ruggedness results of Analyst II of Zidovudine at 265 nm

S.No	Sample	Label claim (mg)	Amount found** (mg)	% Recovery ± S.D**
1	Tablet formulation	100	99.68	99.68±0.029

**Average of six determinations.

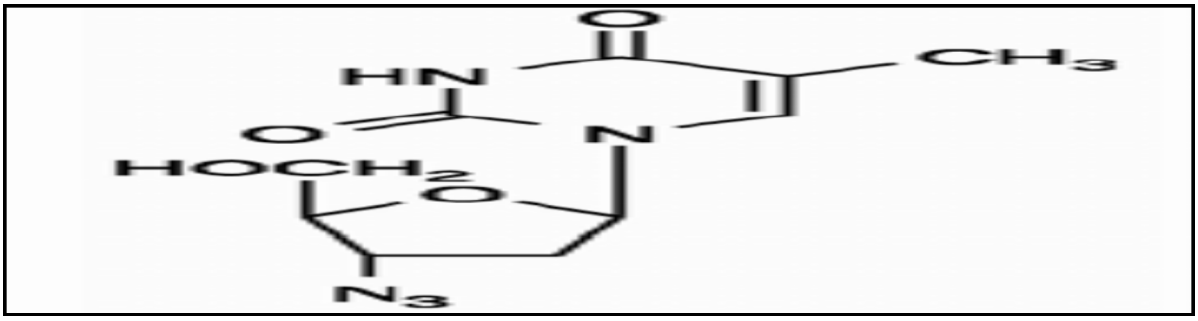

Figure No.1: Structure of Zidovudine

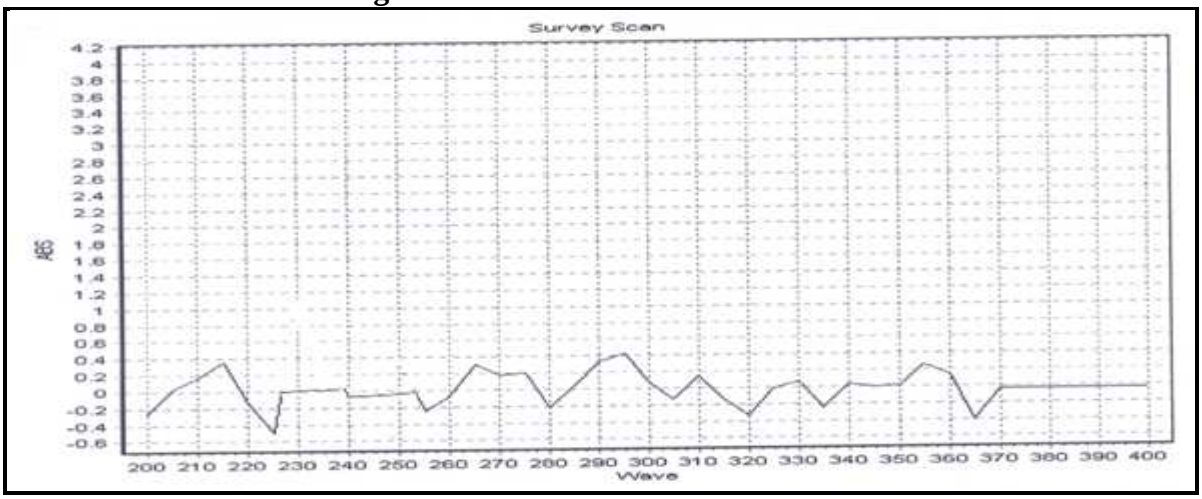

Figure No.2: UV Spectra of Zidovudine at 265 nm

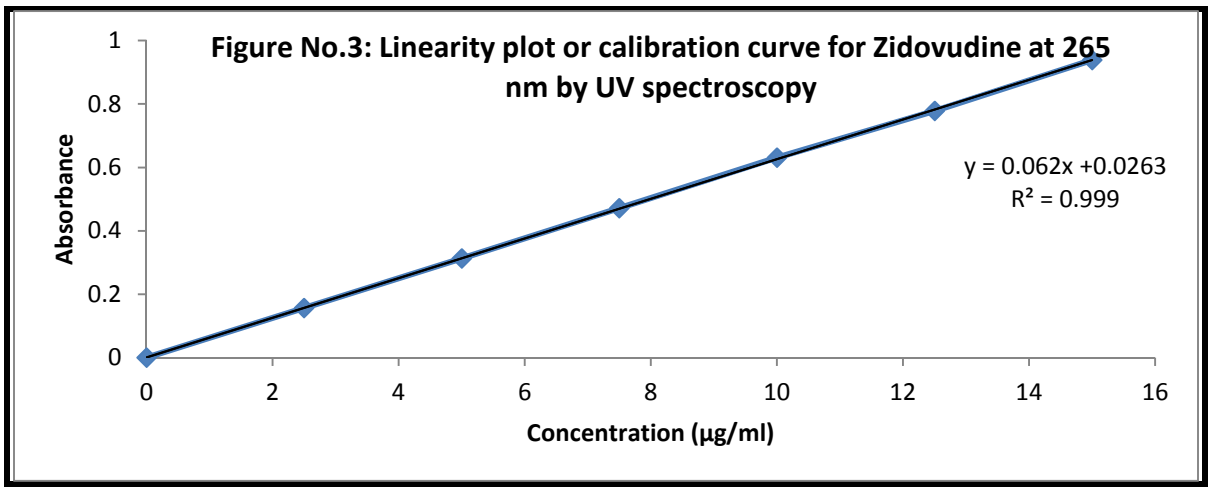

Figure No.3: Linearity plot or calibration curve for Zidovudine at 265 nm by UV spectroscopy

CONCLUSION

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.

ACKNOWLEDGEMENT

All authors very thankful to Saastra College of Pharmaceutical Education and Research, Nellore, Andhra Pradesh, India. We would also like to thank the Management, for provided the necessary facilities to carry out this work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. http://www.rxlist.com/retrovir-drug.htm
2. http://en.wikipedia.org/wiki/Zidovudine
3. Cimons, Marlene. U.S. Approves Sale of AZT to AIDS Patients, *Los Angeles Times*, 1987, 1.
4. United States Pharmacopoeia (USP-NF XXIV). Rockville MD 20852. United States Pharmacopoeial Convention Inc, 1985, 3489.
5. Bengi Uslu and Sibel A Ozkan. Determination of Lamivudine and Zidovudine in binary mixtures using First Derivative Spectrophotometric, first derivative of the ratio spectra and HPLC-UV methods, *Analytical Chemica Acta*, 466(1), 2002, 175-185.
6. Basavaiah K and Anil Kumar U R. Simple Spectrophotometric methods for the determination of Zidovudine in pharmaceuticals using Chloramine-T, Methylene Blue and Rhodamine-B reagents, *E-Journal of Chemistry*, 3(12), 2006, 173-181.
7. Hari krishanan N. Simultaneous estimation of Lamivudine, Zidovudine and Nevirapine by RP HPLC in pure and pharmaceutical dosage form, *Asian Journal of Chemistry*, 20(4), 2008, 2551-2556.
8. Arlene S. Pereira, Kathryn B. Kenney, Myron S. Cohen, James E. Hall, Joseph J. Eron, Richard R. Tidwell and John A. Dunn. Simultaneous determination of Lamivudine and Zidovudine concentrations in human seminal plasma using High Performance Liquid Chromatography and Tandem Mass spectrometry, *Journal of Chromatography B*, 742(1), 2000, 173-183.
9. Emilia Marchei, Luisa Valvo, Roberta Pacifici, Manuela Pellegrini, Gianna Tossini and Piergiorgio Zuccaro. Simultaneous determination of Zidovudine and Nevirapine in human plasma by Reverse Phase-Liquid

Chromatography, *Journal of Pharmaceutical Biomedical Analysis*, 29(6), 2002, 1081-1088.

10. Bin Fan and James T Stewart. Determination of Zidovudine / Lamivudine / Nevirapine in human plasma using ion-pair High Performance Liquid Chromatography, *Journal of Pharmaceutical Biomedical Analysis*, 28(5), 2002, 903-908.
11. Geetha Ramachandran, Hemanthkumar A K, Kumaraswami V and Soumya Swaminathan. A simple and rapid Chromatographic method for simultaneous determination of Zidovudine and Nevirapine in plasma, *Journal of Chromatography B*, 843(2), 2006, 339-344.
12. Ashenafi Dunge, Nishi Sharda, Baljinder Singh and Saranjit Singh. Validated specific High Performance Liquid Chromatography method for determination of Zidovudine during stability studies, *Journal of Pharmaceutica Bio Anal*, 37(5), 2005, 1109-1114.
13. Girum and Habte. Simultaneous determination of Lamivudine and Zidovudine in pharmaceutical formulations by High Performance Thin Layer chromatography (HPTLC) - Densitometric method, *Journal of Chromatography B*, 782(1), 2001, 130-141.
14. Neeraj Kaul. Stability indicating HPTLC determination of Zidovudine as the bulk drug and in pharmaceutical dosage form, *Journal of Planar Chromatography-Modern TLC*, 17(1), 2004, 264-274.
15. Robert A Nash and Alfred H Wachter. Pharmaceutical Process Validation, James Swarbrick, North Carolina, *Marcel Dekker, Inc., New York*, An international, *Revised and Expanded*, 129, 3rd edition, 507-522.
16. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human use, Validation of Analytical procedures: Methodology, ICHQ2B, Geneva, (CPMP/ICH/281/95), 1996.
17. ICH. Harmonized Tripartite guideline. Recommended for adaptation at step-4 of the ICH process, By ICH steering committee, 1996.
18. Green J M. A practical guide to analytical method validation, *Anal Chem*, News Feat 305A/309A, 199.

Please cite this article in press as: Prathap B. *et al.*, Method development and validation of zidovidine in bulk and pharmaceutical dosage form by UV spectrophotometer, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 1(1), 2013, 54 - 59.