ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR THE DETERMINATION OF ZIDOVUDINE AND ITS RELATIVE SUBSTANCES IN BULK

Charitha Doppalapudi*, Rajini Kanth, Rama Rao Nadendla

Chalapathi institute of Pharmaceutical Sciences, Lam, Guntur, Andhra Pradesh, India

**Corresponding author: doppalapudi charitha 5@gmail.com*

ABSTRACT

A simple, specific, accurate and economic reverse phase liquid chromatographic method was developed for the estimation of Zidovudine and its Relative substances in bulk. The method has shown adequate separation of Zidovudine and its Relative substances. Separation was achieved on a Shimadzu RP-C18 ODS (250×4.6mm, 5µm) column at wavelength of 270 nm, using a mobile phase water and Methanol (70:30) in an isocratic elution mode at a flow rate of 1.0 ml/min. The retention time for Zidovudine and Relative substances is found to be 9.5, 11.03 and 3.5 min correspondingly. Quantitation was achieved with UV detection at 270 nm based on peak area with linear calibration curve at concentration range 5-25 µg/ml for Zidovudine, 1-5 µg/ml for Zidovudine impurity B and Zidovudine impurity C. The LOD's were 0.343µg/ml, 0.098 µg/ml and 0.776 µg/ml for Zidovudine and its Relative substances respectively. The LOQ's were found to be 1.04 µg/ml, 0.29µg/ml and 0.47 µg/m for Zidovudine and Relative substances. The proposed method is therefore suitable for purpose in quality-control laboratories for quantitative analysis of both the drugs individually and in combined dosage form, as it is simple and rapid with tremendous precision and accuracy.

Keywords: Zidovudine (AZT), impurities B and C, RP-HPLC, anti-viral, HIV.

1. INTRODUCTION

Zidovudine 1-[(2R, 4S, 5S)-4-azido-5-(hydroxyl methyl) oxolan-2-yl]-5-methylpyrimidine-2, 4-dione. It has a molecular formula of $C_{10}H_{13}N_5O_4$ and a molecular weight of 267.245 g/mol. generously soluble in water, methanol, ethanol and DMSO. It has the structural formula shown in (fig. 1) is a combination with other antiretroviral agents for the treatment of human immuno virus (HIV) infections. Zidovudine impurity B 1-(3-Chloro-2, 3-dideoxy-b-D-erythro-pentofuranosyl)-5-methylpyrimidine-2, 4(1H, 3H)-Dione. It has a molecular formula of $C_5H_6N_2O_2$ and a molecular weight of 260.67 g/mol. (fig. 2) and Zidovudine impurity C Methylpyrimidine-2,4(1H,3H)-dione. It has a molecular formula of $C_{10}H_{13}ClN_2O_4$ and a molecular weight of 126.11 g/mol. (fig. 3) [1-4]. Literature review reveals very few methods are reported for the assay of zidovudine (AZT) and related substances in Tablet dosage forms using RP-HPLC method [5-8]. The reported HPLC methods was having disadvantages like high flow rate, more organic phase and use of costly solvents. The RP-HPLC method utilizes economical solvent system and having advantages like better retention time, less flow rate, very sharp and symmetrical peak shapes. The aim of the study was to develop a simple, precise, economic and accurate RP-HPLC method for the estimation of Zidovudine and Relative substances.

2. MATERIAL AND METHODS

2.1. Materials

Zidovudine (AZT) and related substances were obtained from Indian Pharmacopeia Reference Standards (IP). HPLC grade acetonitrile Merck, HPLC grade water was obtained from Fischer scientific, methanol HPLC grade was obtained from Merck, all the above chemicals and solvents are from Ranchem.

2.2. Instrumentation

Liquid chromatographic Agilent (LC-1220) system was manufactured by Agilent and equipped with UV detector. Rheodyne injector with 20 µL loop volume and HPLC column- Agilent RP-C_{18} ODS (250 X 4.6mm), 5µm. Weighing was done on an Electronics Balance of Single pan balance of model AX-200-shimadzu. pH of buffer was

maintained by pH analyzer, digital electronics model-7007.

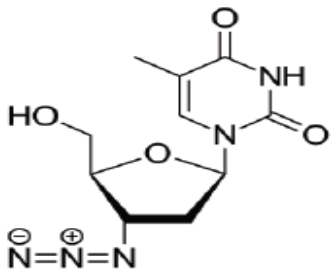

Fig. 1: Chemical structure of Zidovudine

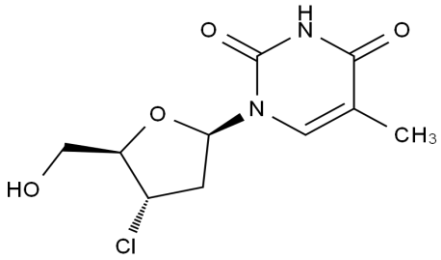

Fig. 2: Chemical structure of Zidovudine B

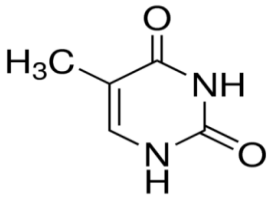

Fig. 3: Chemical structure of Zidovudine C

2.3. Preparation of solutions

2.3.1. Mobile phase

Water and Methanol were mixed in the ratio of 70:30 followed by filtration through 0.45m membrane filter paper.

Diluents: Mobile phase is used as diluents.

2.3.2. Preparation of standard solution

Solution A: Weighed exactly about 10 mg of zidovudine working standard into a 10 ml volumetric flask. Added 7 ml of diluent, sonicated to dissolve and diluted to volume with diluents. Transferred 1 mL of above solution to 10 ml with the diluent. Further diluted 1 mL of above solution to 10 ml with the diluents.

Solution B: Weighed exactly about 10 mg of zidovudine B working standard into a 10 ml volumetric flask. Added 7 ml of diluent, sonicated to dissolve and diluted to volume with diluent. Transferred 1 mL of above solution to 10 ml with the diluents. Further diluted 1 mL of above solution to 10 ml with the diluents.

Solution C: Weighed exactly about 10 mg of zidovudine C working standard into a 10 ml volumetric flask. Added 7 ml of diluents, sonicated to dissolve and diluted to volume with diluents. Transfers 1 mL of above solution to 10 ml with the diluents. Further diluted 1 mL of above solution to 10 ml with the diluents.

2.4. Chromatographic conditions

HPLC experiment was carried out on a Agilent (LC-1220) separation module, with photodiode array detector using Auto sampler. Data gathering and processing was prepared using lab- solution DB software. The analytical column used for the separation was Agilent RP-C_{18} ODS (250 X 4.6mm), 5µm, Other equipments used were ultra-Sonicator (Remi), Analytical balance Single pan balance of model AX-200-shimadzu. Characteristic chromatogram of zidovudine and related substances were as shown in Fig 4 and optimized chromatographic conditions were as shown in the Table 1.

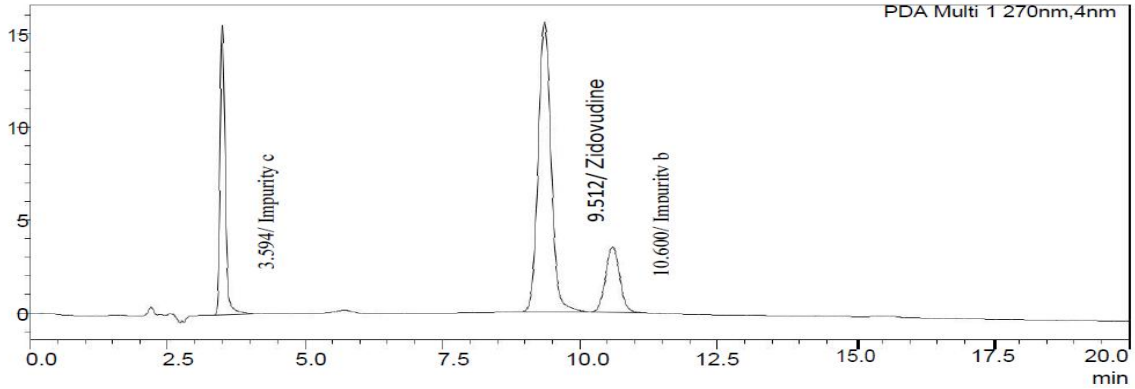

Fig. 4: A typical chromatogram Zidovudine, Zidovudine B and C.

Table 1: Optimized Chromatographic conditions

Column	:	Shimadzu 250×4.6mm, 5µm
Mobile phase	:	Methanol: HPLC water (30:70)
Flow rate	:	1ml/min
Detector wave length	:	270nm
Column temperature	:	Ambient temperature
Injection volume	:	20µL
Run time	:	20min
Retention time	:	9.5(ZIDO), 10.6(ZIDO B) and 3.5(ZIDO C) min

2.5. Method development

To saturate the column, mobile phase was pumped for about 30 min thus to get the base line corrected. Standard calibration lines were constructed for each drug. A series of aliquots were prepared from the above stock solutions using diluents to get the concentrations 5-25µg/ml for zidovudine, 1-5 µg/ml for zidovudine B and C. Inject each concentration 6 times in to the chromatographic system. Every time peak area and retention time were recorded separately for both the drugs. Calibration curves are constructing as by taking average peak area on Y-axis and concentration on X-axis individually for both drugs. Regression equations were calculated from the calibration curves, these regression equations is used to calculate drug substance in formulation.

2.6. Estimation of Zidovudine in tablet dosage forms

Weighed 20 tablets and crushed to powder then taken the powder equivalent 5.5 mg Zidovudine Sofosbuvir into a 100 ml volumetric flask. Added 70 ml of diluent, sonicated to dissolve and diluted to volume diluents. Filtered through 0.45m Nylon syringe filter. Further diluteed 1 ml-10 ml with the diluents. This solution was estimated by above developed method.

Table 2: Results of Marketed formulation analysis

Tablet Sample	Label Claim (mg)	Amount Present (mg)	Assay %
Zidovudine	300	286	95.33

The assay procedure was repeated 6 times (n ¼ 6) the drug content was estimated using above calculated regression equation; the results of tablet dosage form shown in Table 2.

2.7. Method validation

The described method has been validated for linearity, accuracy, limit of detection limit of quantification, precision and robustness as per the ICH guidelines.

2.7.1. Linearity

The linearity of the method was determined by preparing six different concentrations of Zidovudine, Zidovudine B and C in the concentration range of 5-25 µg/ml and 1-5 µg/ml. Each solution was prepared in triplicate. The calibration curves were obtained by plotting peak area versus concentration. Linearity was check over the same concentration range on three successive days and the results obtained. The results were shown in Table 3 obtained graphs were shown in Fig. 5a-c.

Table 3: Linearity table for Zidovudine and related substances

Zidovudine		Impurity B		Impurity C	
Conc. µg/ml	Peak area	Conc. µg/ml	Peak area	Conc. µg/ml	Peak area
5	255957	1	63026	1	101354
10	551910	2	132826	2	252870
15	822334	3	190062	3	368315
20	1106549	4	252198	4	496267
25	1337389	5	310576	5	607764
R^2= 0.999		R^2= 0.999		R^2= 0.998	

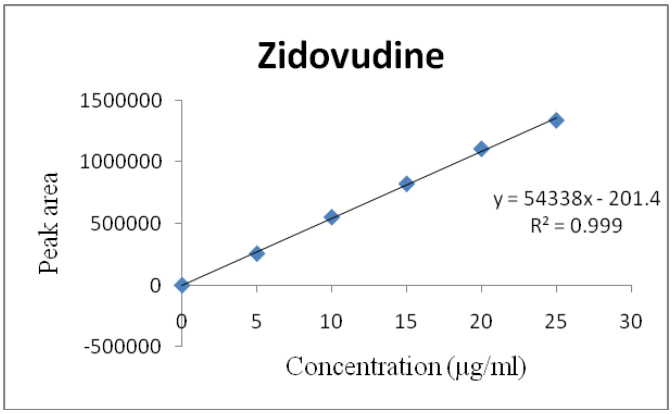

Fig. 5(a): Linearity plot for Zidovudine

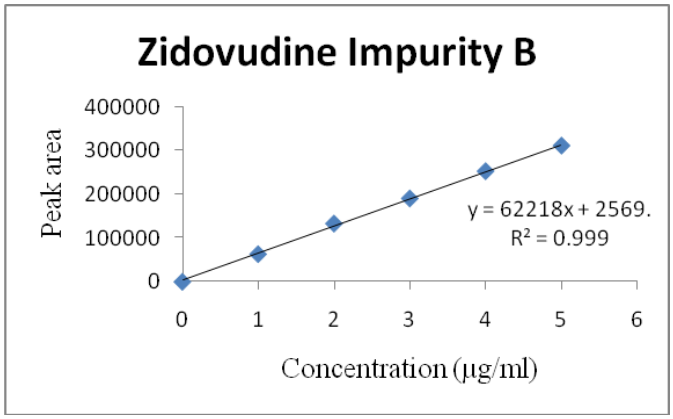

Fig. 5 (b): Linearity plot for Zidovudine B

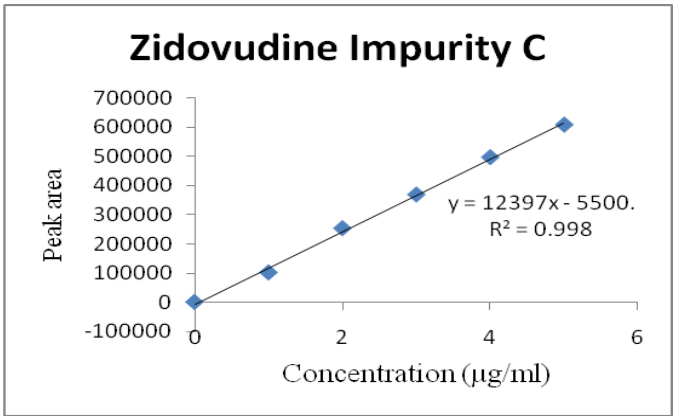

Fig. 5 (c): Linearity plot for Zidovudine C

2.7.2. Accuracy, as recovery

The accuracy of the method was determined at three different concentration levels 50%, 100%, and150% by spike known quantities of the drug analyte and % of recovery were calculated and the results were shown in Table 4.

Table 4: Accuracy table of Zidovudine

%Conc. (at specification Level)	Area		Amount added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
	Sample Area	Average				
50%	725748	725178.3	6.00	6.25	104.16	101.17
	724644					
	725143					
100%	1112710	1110252	8.00	8.34	104.25	
	1105140					
	1112907					
150%	1358936	1358557	10.0	9.51	95.1	
	1359345					
	1357389					

2.7.3. Precision

2.7.3.1. Method precision (repeatability)

The method precision is determined by inject six working standard solutions and six sample injections. The areas of all the injections were taken and standard deviation, % relative standard deviation (RSD), % assay were calculated.

2.7.3.2. Intermediate precision

The intermediate precision was determined by inject six working standard solutions and six sample injections on different days by different operator or by different instruments. The areas of all the injections were taken and standard deviation, % relative standard deviation (RSD), % assay was calculated.

2.7.3.3. Intra-day

The intra-day precision was determined by inject six working standard solutions and six sample injections by the same operating condition over a short interval of time. The areas of all the injections were taken and standard deviation, % relative standard deviation (RSD), % assay was calculated. And the results were shown in Table 5a , 5b and 5c.

2.7.4. LOD and LOQ

2.7.4.1. LOD

It is lowest amount of analyte in a sample that can be detected but not necessarily quantized as an exact value under the stated experimental conclusions. The detection limit is usually expressed as the concentration of analyte. The standard deviation and response of the slope and the results obtained.

LOD ¼ 3.3*S/N

Table 5(a): Method precision studies of zidovudine and related substances

S.No	Zidovudine	Impurities B	Impurities C
1	555962	149698	252797
2	556910	152223	260328
3	556315	151248	252969
4	568826	156798	253644
5	556843	149891	263254
6	565922	149299	253143
Mean	560129.7	151526.2	256022.5
S.D	5696.787	2804.689	4571.836
%RSD	1.01%	1.85%	1.78%

Table 5(b): System precision studies of zidovudine and related substances

S.No	Zidovudine	Impurities B	Impurities C
1	556315	150248	252969
2	567826	147290	256441
3	563157	149278	257254
4	556317	151908	260268
5	562319	151781	253950
6	569826	147798	260644
Mean	562626.7	149717.2	256921
S.D	5637.17	1954.899	3157.517
%RSD	1.00%	1.30%	1.22%

Table 5(c): Inter-day precision studies of zidovudine and related substances

S.No	Zidovudine	Impurities B	Impurities C
1	556843	149891	253254
2	568922	149299	253143
3	556315	150248	252969
4	567826	147290	256441
5	562157	149278	257254
6	567317	152908	260268
Mean	563230	149819	255554.8
S.D	5656.642	1827.023	2955.798
%RSD	1.00%	1.19%	1.15%

2.7.4.2. LOQ

The Quantitation limit of an analytical procedure is the lowest amount of an analyte of a sample which can be quantitatively determined with suitable precision and accuracy. The standard deviation and response of the slope and the results obtained. And the results were shown in Table 6.

LOQ ¼ 10* S/N

Table 6: LOD and LOQ of zidovudine and related substances

	Zidovudine	Impurities B	Impurities C
LOD	0.343µg/ml	0.098 µg/ml	0.776 µg/ml
LOQ	1.04 µg/ml	0.29µg/ml	2.35 µg/ml

2.7.5. System suitability parameters

For assessing system suitability, six replicates of working stan- dards samples of Ledipasvir and Sofosbuvir were injected and studied the parameters like plate number(N), tailing factor(K),re- solution, relative retention time and peak asymmetry of samples. The results were tabulated in Table 7.

Table 7: System suitability parameters for Zidovudine and related substances

Parameter	Zidovudine	Impurities B	Impurities c	Acceptance Criteria
Retention time	9.545	10.616	3.566	-----
Plate count	31797	42629	28142	NLT 2000
Tailing	1.153	1.138	1.2633	NMT 2
Resolution	16.401	2.346	------	NLT 1.5

2.7.6. Robustness

The robustness of the assay method was established by introducing small changes in the chromatographic condition which included wavelength (265 nme275 nm), flow rate (0.8 and 1.2 mL/ min) and organic phase (5% to 5%). The results were tabulated in Table 8.

Table 8: Robustness studies of zidovudine and related substance

Method Parameters	Conditions	Retention Time(R_t)			Theoretical plate count		
		Zidovudine	Impurities B	Impurities c	Zidovudine	Impurities B	Impurities c
Flow(+)	+ 0.2	9.781	11.105	3.569	34198	43938	35954
Flow(-)	-0.2	9.841	11.178	3.578	33398	42543	34390
Organic phase(+)	+5%	9.901	11.252	3.583	33590	43670	37589
Organic phase (-)	- 5%	9.966	11.33	3.582	34017	43289	35139
Wavelength (+)	+5nm	9.59	10.918	3.513	34013	43614	35581
Wavelength (-)	-5 nm	9.60	11.105	3.569	33693	42911	34832

3. CONCLUSION

A simple, fast, accurate and precise stability-indicating HPLC analytical method has been developed and validated for the quantitative analysis of zidovudine and its related substances in bulk drugs. The estimation of zidovudine and related substances was done by Reverse Phase HPLC. The mobile phase used consists of water and Methanol (70:30) (v/v). A Shimadzu (250×4.6mm, 5µm) was used as the stationary phase. The detection was carried out using PDA detector set at 270nm. The solutions are chromatographer at a constant flow rate of 1.0 ml/ min. Retention time for zidovudine, zidovudine B and C impurity was found to be 9.5, 11.3 and 3.5 min.

4. ACKNOWLEDGEMENT

Authors are very thankful to principal, Chalapathi Institute of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur, for providing the library facilities for literature survey to carryout entire study.

5. BIBILOGRAPHY

1. Sonar KV, Sapkale P, Jadhav A, Deshmukh T, Patil S, Murkute P. *International Journal of Current Pharmaceutical Research*, 2017; **9(6):**86-89.
2. Shaik K, Subramaniam S, Muthuraman MS, Sivasubramanian A. *IJPRIF*, 2013; **5(3):**1321-1331.
3. Dos Santos JV, de Carvalho La, Pina Me. *Anal Sci*., 2011; **27(3):**283-289.
4. Santoro Maria Ines RM, Andreia MT, Singh AK, Erika RM, Kedor-Hackmann, *Quim. Nova,* 2006; 29(2).
5. Mounika A, Yeshwanth Reddy M, Raghuram Reddy A. *IJRPC*, 2014; **4(3):**606-610.
6. Lakshmi KS, Yadav KKS, Nagaraju G, Kuchi R. *IJRPC,* 2011;**1(3):**677-680.
7. Raja T, Lakshmana RA. *JPRIF*, 2011; 3(2):852-857.
8. Karishma S, Subramanian S, Muthuraman MS and Sivasubramanian A. *IJPRIF*, 2013; **5(3):**1321-1331.