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Research Article

METHOD DEVELOPMENT AND VALIDATION OF ZIDOVUDINE BY RP-HPLC

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ABSTRACT

Zidovudine is a reverse transcriptase inhibitor and it is used in HIV therapy. Chemical name of the drug is 1-[(2R, 4S, 5S)-4-azido-5-(hydroxymethyl) oxolan-2-yl]-5-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione. Pharmacokinetics profile of Zidovudine in preclinical animal models helps in further development of Zidovudine as an effective drug in treatment of HIV. Therefore, we have established a sensitive and accurate High performance liquid chromatographic method for determination of Zidovudine in tablet dosage form. Chromatography was performed with an analytical Inspire C_{18} column (250 mm x 2.0 mm, 5 µm), Shimadzu HPLC model with LC 10AD HPLC Pump and SPD 10A HPLC UV-Detector, and using methanol: water (80: 20 % v/v) as the mobile phase. The linearity of Zidovudine is 0.992 over a concentration range of 10 to 5000ng/ml. Interday and intraday variability was < 10%.

Keywords: Zidovudine, HIV, Reverse transcriptase, HPLC.

1. INTRODUCTION[1-3]

Human Immunodeficiency Virus (HIV) belongs to the family of retrovirus and it causes Acquired Immunodeficiency Syndrome (AIDS). In HIV infected patients as immunity is severely compromised they are vulnerable to different diseases.

Zidovudine, a structural analog of thymidine, inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate dGTP and by its incorporation into viral DNA. Rapid and nearly complete absorption from the gastrointestinal tract following oral administration however, this is due to the hepatic first pass metabolism, systemic bioavailability of Zidovudine capsules and solution is approximately 65% and effect of the multidrug resistance transporter P-glycoprotein, which is responsible for an efflux mechanism resulting in a reduced crossing of the intestinal barrier. Some analytical method has been developed and validated but this is accurate method to measure the concentration of Zidovudine. Therefore, in this study we report the development and validation of a sensitive HPLC assay to quantify Zidovudine in tablet samples.

2. EXPERIMENTAL METHODS

2.1. HPLC Method Development

2.1.1. Chemicals and reagents

Zidovudine was obtained as a gift sample from Aurobindo laboratories ltd (Hyderabad). Methanol, Acetonitrile, Water and Glacial acetic acid were of HPLC grade obtained from Sigma Aldrich chemicals Pvt. Ltd. (Maharashtra).

2.1.2 Instruments and Chromatographic Conditions[4]

The Shimadzu UV-1800 model was used to determine the absorption maximum (λ_{max}) of Zidovudine. Shimadzu HPLC model with

LC10AD Pump and SPD-10A UV-Detector. The column and HPLC instrument was maintained at room temperature. The reverse phase chromatography was performed with an analytical Inspire C_{18} column (250 mm x 4.5 mm, 5 µm), using Methanol: water (80:20v/v) was used as the mobile phase. The flow rate was set at 1 ml /min and the injection volume was 20 µL. The HPLC detector was set at a wavelength of 267 nm and AUFS at 0.01.

2.1.3. Standard solution

Primary stock solutions of Zidovudine was prepared by weighing 50 mg dissolved in 50 ml of mobile phase to give a concentration of 1 mg/ml and stored at -80^0 C until use. Primary stock solution of Zidovudine was firstly diluted with mobile phase to give working solutions with concentrations of 0.1, 0.25, 0.5, 1, 2.5 and 5 µg /ml. 20 µL of these samples were used for the determination of LLOD and LLOQ.

2.2. HPLC Method Validation[5-9]

2.2.1. Specificity and selectivity

The chromatographic interference from endogenous compounds was assessed by comparing chromatograms with that of the Zidovudine tablet samples.

2.2.2. Sensitivity

The lowest limit of quantification (LLOQ) was determined as the minimum concentration that could be accurately and precisely quantified with the relative standard deviation of < ± 10%. The lowest limit of detection (LLOD) was defined as the amount that could be detected with a signal-to-noise ratio of 4.

2.2.3. Linearity

Calibration curve was plotted by taking six concentrations of Zidovudine ranging from 0.1 to 5 µg/ml. Blank samples were analyzed to confirm the absence of interferences. Calibration curves were plotted by taking Peak area of Zidovudine on Y-axis and Concentration of corresponding values on X-axis. The minimally acceptable correlation coefficient (r^2) for the calibration curve was 0.99 or greater.

2.2.4 Precision and accuracy

In order to assess the intra - and inter-day precision and accuracy for the method, Zidovudine samples at low, medium and high concentrations were prepared as described above. The intra-day precision of the method was assessed by calculating the coefficient of variation (CV) for the analysis of samples in three replicates. And inter-day precision was determined by the analysis of samples on three consecutive days. Accuracy was calculated by comparing the measured values to the true values and was expressed in percent. The precision was accepted when the coefficient of variance for each concentration doesn't exceed ± 10, and accuracy was accepted when the average values are > 95% of true concentration except for the LLOQ where the limit was > 92%.

2.3 Assay of Zidovudine in Marketed drug[10]

Tablet rand of Zidovudine, 10 tablets each were purchased from local pharmacist and these 10 tablets were powdered using motor and pestle and the powder weight was measured by using Shimadzu analytical balance. Weight of each tablet was measured by taking the average of powder weight. 100 mg equivalent of Zidovudine was weighed and dissolved in mobile phase; the undissolved materials were removed by filtration using syringe filters. The filtrate is further diluted to produce the concentrations of 0.3, 1.5, 3 µg /ml in three replicates and their chromatograms were recorded using HPLC. By using the standard graph the concentration of the diluted sample was calculated and by multiplying it with the dilution factor we have calculated the exact amount of Zidovudine present in 100 mg equivalent of the drug by using the formula. Results were shown in table 4.

3. RESULTS AND DISCUSSION

3.1. Method development

The UV-Vis absorbance of Zidovudine was scanned from wavelength of 200-600 nm on a Shimadzu UV-Vis spectrophotometer (UV 1800). And maximum absorbance was at wavelength of 267 nm in methanol (Fig-1) therefore, wavelength of 267 nm was chosen for HPLC-UV detection in this method. The mobile phase used for the method was very simple and achieved optimal separation of Zidovudine without interference from the other components.
The flow rate was selected as 1 ml/min.

3.2. HPLC method validation

3.2.1. Specificity and Selectivity

Fig. 3 and 4 represents chromatograms of Zidovudine of tablet samples after extraction. No interference of endogenous peaks with Zidovudine was observed with a retention time of Zidovudine t_R = 10.2 min (fig-2).

3.2.2. Sensitivity

The LLOQ of Zidovudine was found to be 0.025µg/ml. The mean percent accuracy value for samples was 91.37 % and coefficient of variation was below 7.03 % at the LLOQ.

3.2.3 Linearity of calibration curve

The calibration curves of Zidovudine were linear over the different concentration range in mobile phase. The correlation coefficient was found to 0.991 (fig1).

3.2.4. Precision and Accuracy

Table 2 shows a summary of intra- and inter-day precision and accuracy. Intra- day accuracy of 40, 400 and 4000 ng/ml was found to be 91.76, 97.58 and 98.79 respectively and inter- day accuracy was found to be 93.26, 95.9, and 95.09 respectively. Therefore, the intra- and inter- day accuracies (% deviation) were within < ± 10% for the LLOQ. The intra- and inter-day assay precision (CV) ranged from 7.254 to 1.323 and 7.83 to 1.30 % respectively. These results indicated that the present method has very good accuracy and precision.

4. CONCLUSION

A simple, sensitive, accurate and precise HPLC method was developed and validated to quantify Zidovudine in tablet samples. The sample preparation method and the chromatographic condition in the present method will likely facilitate the quantification of Zidovudine in other biological matrices in the future studies.

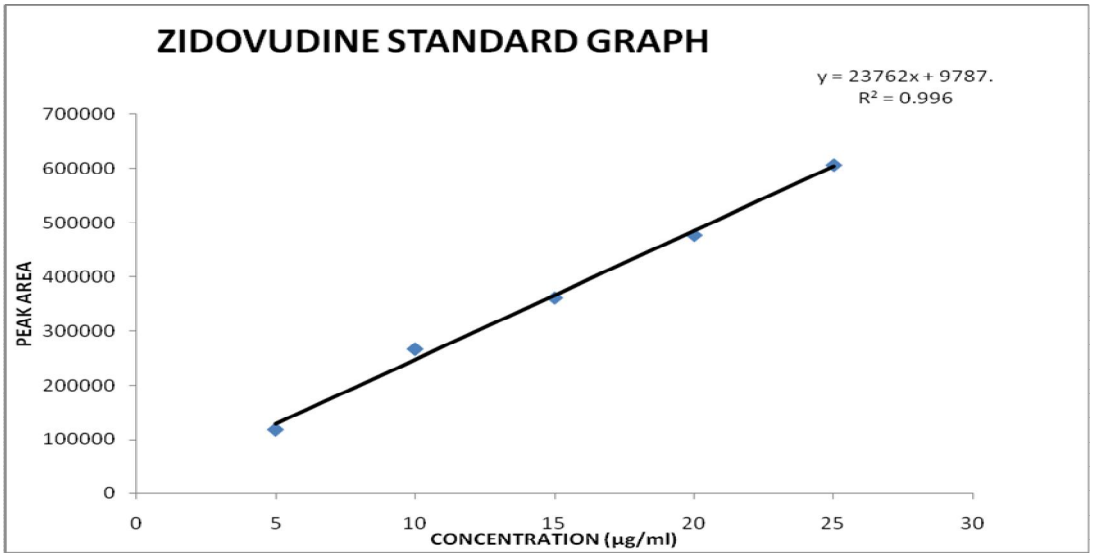

Fig. 1: Standard graph of Zidovudine

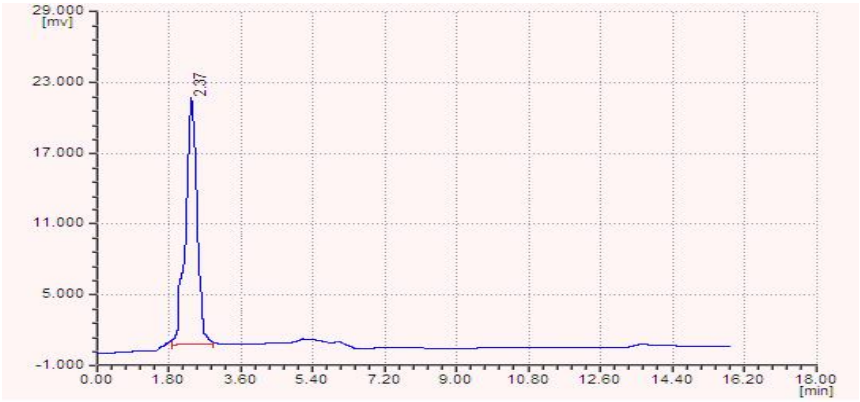

Fig. 2: Chromatogram of Zidovudine tablet sample

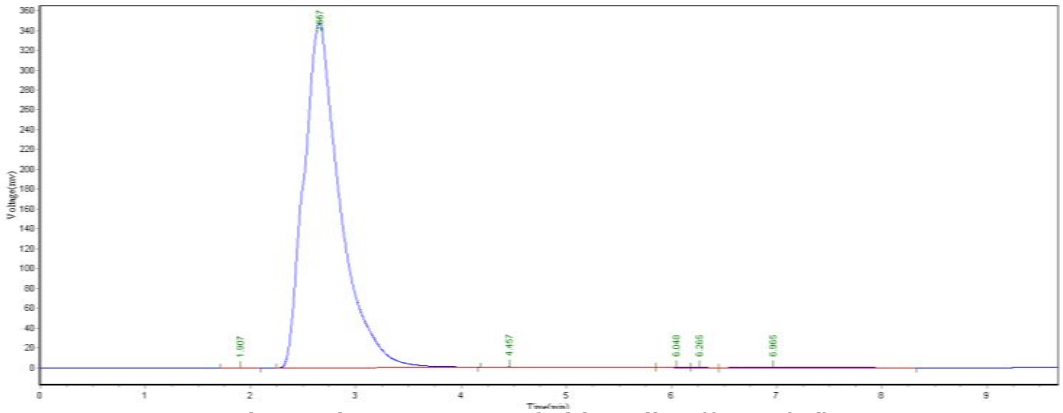

Fig. 3: Chromatogram of Zidovudine (0.5 µg/ml)

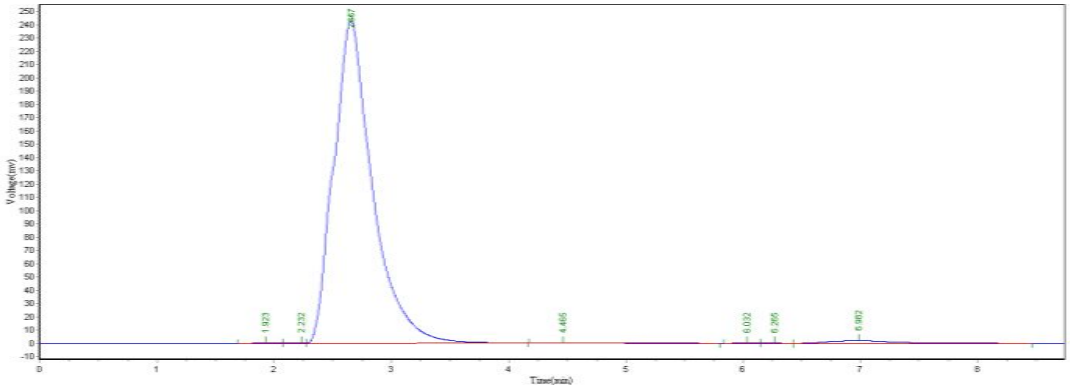

Fig. 4: Chromatogram of Zidovudine (5 µg/ml)

Table1. Representing drug conc. Vs peak area of Zidovudine

Drug Concentration (µg/ml)	Peak Area of Zidovudine
0.1	152752.90
0.25	348026.00
0.5	793251.13
1	1490232.80
2.5	2521322.80
5	5114290.50

Table 2: Showing inter- and intra-day variation of tablet samples

	Trail 1	Trail 2	Trail 3	Mean	SD	Accuracy %		CV %
						Mean	SD	
Intra day								
40	37.92	38.54	33.65	36.70	2.66	91.76	6.66	7.254
400	397.90	392.00	401.00	396.97	10.19	97.58	1.99	2.043
4000	3984.0	3979.19	4891.12	3951.44	52.29	98.79	1.31	1.323
Inter day								
40	34.56	35.54	39.95	36.68	2.87	91.71	7.18	7.83
400	382.99	391.54	379.44	384.66	6.22	96.16	1.55	1.62
4000	3987.54	3892.54	3907.2	3929.09	51.14	98.23	1.28	1.30

Table 3: Showing recovery of Zidovudine from the tablet samples

	Replicate 1	Replicate 2	Replicate 3	Mean	S.D
Intra day					
40	94.8	85.2	98.48	92.827	6.856
400	95.28	98.56	91.92	95.253	3.320
4000	97.184	95.378	97.832	96.798	1.272
Inter day					
40	93.60	85.80	100.40	93.27	7.31
400	94.50	99.64	93.56	95.90	3.27
4000	96.16	93.16	95.96	95.09	1.67

Table 4: Assay of Zidovudine tablet

Drug concentration (µg/ml)	Trial 1	Trial 2	Trial 3	Average	SD	% Purity
	Conc.	Conc.	Conc.			
0.3	0.289	0.315	0.25	0.285	0.033	94.88
1.5	1.298	1.52	1.4	1.406	0.111	93.73
3.0	2.98	2.76	3.01	2.917	0.137	97.22

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