EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 2
640-644
Year: 2018

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ZIDOVUDINE BY RP-HPLC METHOD

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Article Received on 17/12/2017 **Article Revised on 06/01/2018** **Article Accepted on 27/01/2018**

ABSTRACT
A simple, precise, rapid, selective, and economic Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed for the estimation of Zidovudine in bulk dosage. Chromatographic separation was achieved isocratically for the combination was done with a SHISEIDO (250 x 4.6mm, 5µm utilizing mobile phase of composition Acetonitrile, methanol and at pH 7 phosphate buffer in the ratio of 50:25:25 %v/v, the flow rate was 1ml/min and the eluates was monitored at 275nm. Zidovudine was eluted with retention time of 3.7min. The method was found to be linear over a range of 10-35 µg/ml for Zidovudine. The method was validated according to the guidelines of International Conference on Harmonization (ICH) and was successfully employed in the estimation of commercial formulations.

KEYWORDS: Zidovudine, RP-HPLC, ICH, Method validation, acetonitrile, methanol, and pH7 buffer.

INTRODUCTION
Reverse transcriptase inhibitors like Zidovudine have been used in combination to treat patients with HIV. The treatment is used to prevent or prolong the onset of acquired immune deficiency syndrome (AIDS), which can lead to a variety of fatal complications. Zidovudine is used along with other antiviral medications to treat patients with HIV. Zidovudine is also used in pregnant women to reduce the risk of HIV transmission from a mother to an unborn child. Several analytical methods were developed based on HPLC, was reported for the determination of Zidovudine and combinatioon with other drugs.[1-13]

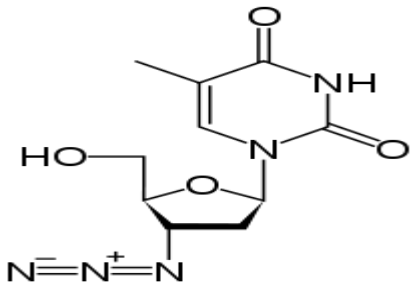

Figure 1: structure of zidovudine.

MATERIALS AND METHODS
Equipment
Isocratic HPLC system (CYBER LAB 22 HPLC Model-LC-100 with Chrom work station Software) containing C_{18} (SHISEIDO 250 x4.6 mm, 5µm) column with UV detection.

Reagents and Chemicals
Pharmaceutical grade pure Zidovudine gift samples were procured from Hetero Laboratories Pvt.Ltd.Hyderabad. HPLC grade Acetonitrile and HPLC grade Water were procured from Merck specialties private limited, Mumbai.

Preparation of Standard Solutions
Stock solutions (0.1mg/ml) of zidovudine was prepared by dissolving 10 mg of each in 100 ml volumetric flasks containing 100 ml of diluents and sonicated for about 15 min. Subsequent dilutions of this solution was made with diluents to get concentration of 10 - 35µg/ml.

Chromatographic Conditions
Separation was performed on reverse phase SHISEIDO (250 x 4.6, 5µm particle size) column. Mobile phase consists of mixture of composition Acetonitrile, methanol and pH7 phosphate buffer (50:25:25). Injection volume of 20 µl was used. Mobile phase was filtered through 0. 45 µm membranes filter and degassed with nitrogen purge for 30 min. The components of the mobile phase were pumped from solvent reservoir to the column at flow rate 1.0 ml/min and wavelength was set to 275 nm. The column temperature was set at ambient temperature.

Validation of the RP-HPLC Method

Validation of the optimized method was performed according to the ICH Q2R1 guidelines.[14]

System Suitability

System suitability was carried out with six injections of solution of 100% concentration having 100µg/ml of Zidovudine in to the chromatographic system.

Linearity

The standard calibration curve was constructed for different volumes of stock solutions of each were accurately transferred in to 10ml volumetric flasks and diluted to mark to yield a concentration range of 10- 35 µg/ml solutions of zidovudine. The calibration line was obtained by plotting the peak area against concentration of drug. Each solution was injected in triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients.

Precision

The reproducibility of the method was verified by calculating the % RSD of three replicate injections of 10 µg/ml, 20 µg/ml and 30 µg/ml of Zidovudine on the same day(interday) and for intraday precision % RSD was calculated from repeated studies.

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified placebo preparation at 3 different concentration levels 50, 100 and 150 %, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae

$$LOD = 3.3\ \sigma/s$$
$$LOQ = 10\ \sigma/s$$

Robustness

Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, flow rate, detection wave length, etc. and the % RSD should be reported. Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of ± 2 nm in the detection wave length and ± 0.2 ml/min in the flow rate, and mobile phase composition were tried individually. Solutions of 100% test concentration with the specified changes in the operational conditions were injected to the instrument in triplicate.

RESULTS AND DISCUSSION

A typical chromatogram was shown in Figure 2. A well resolved peak was produced a retention times of 3.72 min using water, acetonitrile, methanol, and pH7 phosphate buffer (50:25:25) as mobile phase, flow rate of 1.0 ml/min and UV detection was set at 275 nm

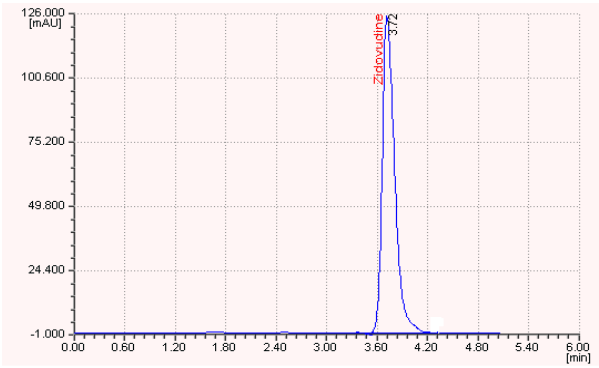

Figure 2: Typical chromatogram of Zidovudine.

Validation Parameters

System Suitability

System suitability was carried out with six injections of solution of 100% concentration having 100µg/ml of Zidovudine in to the chromatographic system. Number of theoretical plates, HETP and resolution were satisfactory. The chromatograms confirm the presence of Zidovudine at 3.7 min respectively without any interference. Number of theoretical plates (N) obtained and calculated tailing factor (T) was reported in table 1.

Table 1: System Suitability of Zidovudine.

Injection (20µg/ml)	HETP	Tailing factor	Retention time (min)
1	1960.5	1.845	3.7 min
2	1855	1.57	
3	1941.5	1.96	
4	1911.5	1.73	
5	1840	1.86	
6	1977.5	1.735	
Average±S.D	1914.33±56.41	1.783± 0.135	

Linearity

The standard calibration curve was constructed for different volumes of stock solutions of each were accurately transferred in to 10ml volumetric flasks and diluted to mark to yield a concentration range of 10- 35 µg/ml solutions of zidovudine. Concentration range of 10-35µg/ml for Zidovudine was found to be linear with correlation coefficients 0.9998 for Zidovudine respectively. The parameters were given in table 2& Figure 3.

Table 1: Calibration data of Zidovudine.

Conc.	Peak Average	%RSD	
10µg/ml	82737.5	0.88626	Y= 8637.x-4137 Intercept= 4137 Slope= 8637
15µg/ml	121087.5	0.612578	
20µg/ml	163404	0.918264	
25µg/ml	218526	0.620627	
30µg/ml	253324.5	0.319047	
35µg/ml	303568	0.982973	R^2= 0.998

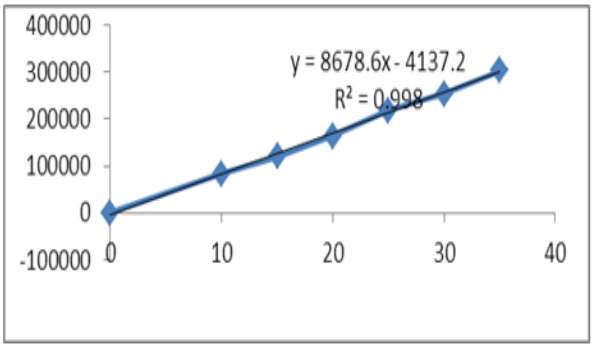

Figure 3: Calibration curve of Zidovudine.

Precision

The reproducibility of the method was verified by calculating the % RSD of three replicate injections of 10 µg/ml, 20 µg/ml and 30 µg/ml of Zidovudine on the same day(interday) and for intraday precision % RSD was calculated from repeated studiesand the results were within the limit. %RSD was reported in table 3&4.

Table 3 Inter day Precision of Zidovudine.

Conc.	Peak area	%RSD
10µg/ml	81599	0.643
20µg/ml	162596.5	0.073
30µg/ml	254200.8	0.980

Table 4: Inter day Precision of Zidovudine.

Conc (µg/ml)	Day	Peak area	%RSD
10mg/ml	1	81433	0.928
20mg/ml		162596.5	0.0735
30mg/ml		254232.2	0.971
10mg/ml	2	82437.3	0.915
20mg/ml		162778.8	0.0912
30mg/ml		252841.8	0.835
10mg/ml	3	81926.6	0.661
20mg/ml		162758.7	0.0740
30mg/ml		252130	0.743

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified placebo preparation at 3 different concentration levels 50, 100 and 150 %, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured. Sample recovery for each concentratioon was within the limit. Results of recovery were presented in the Table 5.

Table 5: Accuracy of the Zidovudine.

S.NO	Spiked level of drug (%)	Amount of drug added (µg/ml)	Peak area Avg	% Recovery
1.	50	10	142984	99.35
2.	100	20	301111	100.49
3.	150	30	441272	100.04

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected and no interferences were found because of the presence of excipients. Hence the method was specific.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae. LOD and LOQ were found to be 0.000954 µg/ml and 0.002892 µg/ml respectively.

Robustness

Robustness of the method was verified by altering ± 2 nm in the detection wave length, mobile phase composition and ± 0.2 ml/min in the flow rate. By chnaging the parameters there was no change in no change in system suitabilty conditions. Hence this method was robust. Results were shown in table 6,7 & 8.

Table 6: Robustness data for Zidovudine.

Flow rate	Conc (µg/ml)	Average area	% RSD
0.8 ml/min	10µg/ml	82876	0.959
	20µg/ml	162666	0.088
	30µg/ml	257366.5	0.944
1 ml/min	10µg/ml	82737.5	0.886
	20µg/ml	163404	0.918
	30µg/ml	253324.5	0.319
1.2 ml/min	10µg/ml	82647	0.087
	20µg/ml	162537	0.043
	30µg/ml	257774	0.655

Table 7: Robustness data for Zidovudine.

Wave length	Conc (µg/ml)	Average area	% RSD
l= 273nm	10µg/ml	82508	0.068
	20µg/ml	162556.5	0.009
	30µg/ml	254012.5	0.308
l= 275nm	10µg/ml	82737.5	0.886
	20µg/ml	163404	0.918
	30µg/ml	253324.5	0.319
l= 277nm	10µg/ml	82002	0.782
	20µg/ml	162631.5	0.036
	30µg/ml	245013.5	0.257

Table 8: Robustness data for Zidovudine.

Mobile Phase	Conc (µg/ml)	Average area	% RSD
48:25:27	10µg/ml	82508	0.068
	20µg/ml	162556.5	0.009
	30µg/ml	254012.5	0.308
50:25:25	10µg/ml	82737.5	0.886
	20µg/ml	163404	0.918
	30µg/ml	253324.5	0.319
52:23:25	10µg/ml	82002	0.782
	20µg/ml	162631.5	0.036
	30µg/ml	245013.5	0.257

CONCLUSION

The RP-HPLC method developed and validated allows a simple and fast quantitative determination of Zidovudine from their formulations. The low solvent consumption (1ml/min), along with short analytical run time of less than 10.0 minutes lead to an environmental friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. All the validation parameters were found to be within the limits according to ICH guidelines. The proposed methods were found to be specific for the drugs of interest irrespective of the excipients present and the methods were found to be simple, accurate, precise, rugged and robust and can be involved in the routine analysis of the marketed formulations.

ACKNOWLEDGMENTS

We are thankful to Pulla Reddy Institute of Pharmacy and to the Department of Pharmaceutical Analysis Quality Assurance for providing the facilities such as lab, equipment and chemicals to carry out the work. We express our sincere gratitude to Hetero Laboratories, Hyderabad for providing the gift samples of Zidovudine, API.

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