INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
Available online http://www.irjponline.com
Research Article

METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF ZIDOVUDINE BY UV-SPECTROPHOTOMETER

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Article Received on: 14/11/10 Revised on: 30/11/10 Approved for publication: 09/12/10

ABSTRACT

This paper describes validated Spectrophotometry method for determination of zidovudine in pure powder and formulation. The solutions of standard and sample were prepared in methanol. Quantitative determination of the drugs was performed at 266 nm for zidovudine. Proposed method was evaluated for the different validation parameters. The specificity test showed that there was no interference from excipients commonly found in the commercial pharmaceutical formulations at analytical wavelength of zidovudine. Quantification was achieved over the concentration range 1-10 µg.mL-1 with mean recovery of 98.26 ± 0.84. This method is simple, precise, and sensitive and applicable for the determination of zidovudine in pure powder and formulation. The method was compared to high-performance liquid chromatography (HPLC) method, which was reported for the same drug. No significant difference was found between the methods for zidovudine quantitation.

KEYWORDS: Zidovudine, Determination, Spectrophotometry, Formulations

INTRODUCTON

Zidovudine is chemically 1-[(2R,4S,5S)-4azido-5- (hydroxymethyl)tetrahydrofuran-2-yl]-5-methylprimidine-2,4(1H,3H0-dione and used as an antiretroviral activity[1,2]. Zidovudine or azidothymidine (AZT) is an antiretroviral drug, the first approved for treatment of HIV. Zidovudine was the first drug approved for the treatment of AIDS and HIV infection. Like other reverse transcriptase inhibitors, AZT works by inhibiting the action of reverse transcriptase, the enzyme that HIV uses to make a DNA copy of its RNA. The viral double-stranded DNA is subsequently spliced into the DNA of a target cell, where it is called a provirus. Jerome Horwitz of Barbara Ann Karmanos Cancer AZT has been shown to reduce this risk to approximately 8% when given in a three-part regimen during pregnancy, delivery and to the infant for 6 weeks after birth. Use of appropriate combinations of antiretroviral medications and cesarean section when necessary can further reduce mother-child transmission of HIV to 1-2%. Common side effects of AZT include nausea, headache, changes in body fat, and discoloration of fingernails and toenails. More severe side effects include anaemia and bone marrow suppression. These unwanted side effects might be caused by the sensitivity of the γ-DNA polymerase in the cell mitochondria. AZT has been shown to work additively or synergistically with many anti-HIV agents; however, acyclovir and ribavirin decrease the antiviral effect of AZT.

Most of the analytical methods in the literature to determine AZT are aimed at either quantifying AZT in biological fluids, pharmaceutical formulation or and include analysis using high-performance liquid chromatography (LC), LC/tandem mass spectrometry (LC/MS), gas chromatography/MS (GC/MS)[5-11]. To the best of our knowledge, there is no reported spectrophotometric or pharmacopoeial method for determination of AZT in pharmaceutical formulations, previous to our work. Thus, efforts

were made to develop fast, selective and sensitive analytical method for the estimation of AZT in their dosage form using spectrophotometry method[3-5].

MATERIALS AND METHODS

Apparatus Absorbance was measured, and spectra were recorded over the wavelength range 200-400 nm in two matched quartz cells with a 1 cm light path using a double beam 1601 UV-Visible spectrophotometer.

Reagents and Materials AZT pure powder were procured as gratis samples from Torrent Pharmaceuticals. Analytical grade methanol was purchased from E. Merck (Mumbai, India). Membrane filters (nylon 0.45 µm - 47 mm) were purchased from Gelman Laboratory (Mumbai, India).

Choice of solvent Zidovudine is soluble in alcohol, sparingly soluble in denatured alcohol and insoluble in water. Methanol was found to be suitable solvent for the UV spectrophotometric method; its absorbance gave individual peak with maximum absorbance. Hence, methanol was selected and used for the entire experimental work.

Determination of λ_{max}: λ_{max} is the wavelength of an absorption maximum. The standard drug was dissolved in methanol to obtain 1 µg/ml solution. The solution was scanned between 230-350 nm and found that the peak at 266nm showed maximum absorbance. Further 1 µg/ml to 50µg/ml concentrations were also scanned between 230-350nm.

Determination of molar absorptivity

Absorptivity constant a is the ratio of the absorbance of the sample to the product of the thickness of the medium and concentration of the sample. As the thickness of the medium for various determinations is the same, absorptivity depends upon the absorbance and the concentration of the sample.

Due to increase or decrease in the concentration of the sample, the absorbance also will increase or decrease respectively, which is always a constant.

From the stock solution, Zidovudine 1mcg/ml to 10mcg/ml of standard solutions were prepared. The absorbance of different concentrations was noted at 266nm, the absorptivity was determined by using the formula.

$$a=A/bc$$

where a=absorptivity, A=absorbance, b=pathlength (1cm) and c=concentration.

Effect of time on stability of absorbance:

The stability of the solution was checked by measuring the absorbance at regular intervals of time. It was observed that the absorbance remained stable for a period of 180 minutes and then the absorbance decreased with increase in time.

Preparation of standard solutions:

About 100mg of zidovudine was accurately weighed and transferred to a clean dry 100ml calibrated standard flask and dissolved in methanol.

It was shaken for few minutes and the solution was diluted to 100ml with same. 10ml of this solution was diluted to 100ml clean dry calibrated 100ml volumetric flask and the volume was made up with methanol. Which consist of 100mcg/ml concentration from this pipetted out 10ml and transferred to another clean dry calibrated 100ml volumetric flask and the volume was made up to 100ml (10mcg/ml) with methanol. Further resulting solution 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml, and 10ml were diluted to 10ml with methanol in 10ml standard flasks. The absorbance of resulting solutions was measured at 266nm against blank. The absorbance obtained was ploted against the concentration of the solution and standard graph was obtained.

Preparation of sample solution:

Twenty tablets were accurately weighed and average weight was taken, weight of the sample equivalent to 100mg of zidovudine was taken in 100ml standard flask and the sample is dissolved in methanol and made up to 100ml with the same. The solution was then filtered through whatmann filter paper no.1. Then the aliquots of 2ml, 4ml and 6ml were transferred into a 10ml dry, clean calibrated volumetric flask. The solutions were scanned at 266nm against blank. The above procedure was followed for two marked

samples and the absorbance values were recorded. The amount of zidovudine per tablet was calculated by comparing absorbance values of standards and samples at 266nm.

VALIDATION METHOD

Tablet brand used

S.No	Brand Name	Company	Mfg.Date	Batch No
1	Zidovir	Cipla	June 2007	X 70505
2	Retrovir	GSK	Sep 2007	TJ 7275

Accuracy

Accurately weighed formulation sample equivalent to 50 mg of sample was mixed with 50 mg Zidovudine pure drug. The quantity of mixture corresponding to 100 mg, from this 50 mg equivalent weight of sample was taken and dissolved in methanol and made up with the same. The solutions were analysed according to the procedure described under preparation of standard absorbance curve. The above procedure was also followed for the recovery of other formulations.

Precision

Standard drug solution was prepared as per procedure given under preparation of standard absorbance curve. This parameter was validated by assaying number of aliquots samples of Zidovudine and estimating its validity using parameters such as standard deviation (SD) and Relative standard deviation (RSD) which was shown in table no 1.

Recovery studies

Accurately weighed 50 mg pure drug was mixed with 60 mg of tablet powder (equivalent to 50 mg of pure drug) is diluted with 100ml of methanol (1000µg/ml). From the above solution pipetted out 10ml and diluted to 100ml to get 100µg/ml concentration repeated the dilutions as mentioned above, till to get 10µg/ml. Different types of concentrations like 2, 4, 6, 8, 10µg/ml were taken and absorbance was recorded for above samples, which was shown in table no 2 & 3.

Percentage Recovery was calculated by using the formula

$$\% \text{ Recovery} = \frac{\text{Amount of drug found in sample after addition of drug} - \text{Amount of drug found in sample}}{\text{Amount of standard drug added}} \times 100$$

RESULTS AND DISCUSSION

Determination of λmax

The Zidovudine standard drug was dissolved in methanol to obtain 1µg/ml solution. The solution was scanned between 230-250 nm and found that the peak at 266 nm showed maximum absorbance. Further 2µg/ml to 10µg/ml concentrations were also scanned between 230-250nm.The λmax of the Zidovudine was found to be 266nm which was shown in figure 1.

Determination of Overlay

The Zidovudine standard drug was dissolved in methanol to obtain 1µg/ml solution. The solution was scanned between 230-350 nm and found that the peak at 266nm showed maximum absorbance. Further 2µg/ml to 10µg/ml concentrations were also scanned between 230-250nm in the overlay mode. The overlay of the Zidovudine was found to be 266nm, the obtained spectrum was shown in fig no 2.

Determination of standard absorbance

The standard drug absorbance was observed at 266nm in the concentration of 1 to 2µg/ml solutions were found to obey Beer's law with the correlation coefficient (r) of 0.9902.

Determination of molar absorptivity

Absorptivity constant 'a' is the ratio of the absorbance of the sample to the product of the thickness of the medium and concentration of the sample. Due to increase or decrease in the concentration of the sample, the absorbance also will increase or decrease respectively, which is always a constant.

From the stock solution, Zidovudine 1µg/ml to 20µg/ml of standard solutions was prepared. The Absorbance of different concentrations was noted at 266nm and the molar absorptivity was determined using the formula a=A/bc

Where a = Absorbtivity, A = Absorbance, b = Pathlength, c = Concentration, the results were shown in table no 4.

Effect of time on stability of absorbance

The stability of the solution was checked by measuring the absorbance at regular intervals of time.

It was observed that the absorbance remained stable for a period of 180min and then the absorbance decreased with increase in time which was shown in table no 5.

CONCLUSION

The thesis deals with involved the development of new, simple spectrophotometric method for the estimation of Zidovudine in the pure form and its formulation. The literature survey revealed that only HPLC methods have been done and reported. The method is based on the absorbance in the uv region. It showed maximum absorbance at 266 nm in methanol. The solution was stable upto 3 hrs. The Beer's law was obeyed over a range of 1-20µg/ml. The quantitative reproducibility, precision and accuracy of the method were carried out. The results confirm the reproducibility, precision and accuracy of the method.

The marked formulations were analyzed by the proposed method and found that there was no interference with the excipients incorporated in the tablet formulation as seen from recovery studies. The method can be adopted for confirmation of Zidovudine in pure as well for its formulation. The results obtained all in close declaration and found to be satisfactory. Hence it was concluded that the above proposed method can be used for the estimation of tablet formulations due to simple, accurate and economic for the routine analysis.

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Table 1: Precision

S.No	Volume solution pipette out (ml)	Concentration of drug (µg)	Absorbance at 266 (nm)			Mean absorbance
1	1	10	0.3945	0.3944	0.3946	0.3945
2	2	20	0.8217	0.8216	0.8214	0.4915
3	3	30	1.2041	1.2040	1.2039	1.2040

Table 2: Analysis of formulation

Sample 1

Label claim = 150 mg
Weight of five tablets = 1.815 g
Weight of one tablet = 0.363
Weight of one tablet equivalent to 100mg = 120 mg

S.No	Volume of solution Pipetted out (ml)	Absorbance (nm)	Amount found (mg/tab)	Percentage of purity
1	2	0.0739	146.04	97.36
2	4	0.1644	148.19	98.79
3	6	0.2764	148.92	99.28

Recovery studies

Amount of pure drug added = 50 mg
Weight of drug equivalent to drug = 60.2 mg
Weight of mixture equivalent to50 mg = 55.1 mg

S.No	Volume of solution Pipetted out	% Recovery
1	2	97.64
2	4	97.84
3	6	98.09

Table 3: Analysis of Formulation

Sample 2

Label claim = 300 mg
Weight of five tablets = 1.560 g
Weight of one tablet = 0.312 g
Weight of one tablet equivalent to 100mg = 104 mg

S.No	Volume of solution Pipetted out (ml)	Absorbance (nm)	Amount found (mg/tab)	Percentage of purity
1	2	0.0849	293.07	97.69
2	4	0.1744	296.60	98.86
3	6	0.2874	297.90	99.30

Recovery Studies

Amount of pure drug added = 50 mg
Weight of drug equivalent to drug = 62 mg
Weight of mixture equivalent to50 mg = 56 mg

S.No	Volume of solution Pipetted out	% Recovery
1	2	99.02
2	4	98.90
3	6	98.09

Table 4: Molar absorbtivity of Zidovudine

Concentration (mg/ml)	Absorbance	a=A/bc
1	0.0262	0.0262
2	0.0739	0.0369
3	0.1085	0.0370
4	0.1644	0.0411
5	0.1924	0.0384
6	0.2764	0.0460
7	0.2834	0.0404
8	0.3192	0.0399
9	0.3772	0.0419
10	0.4203	0.0420

Table 5: Stability data of Zidovudine

S.No	Time (min)	Absorbance at 266 mm
1	30	0.4203
2	60	0.4203
3	90	0.4203
4	120	0.4204
5	150	0.4204
6	180	0.4204
7	210	0.4209
8	240	0.4209

Table 6: Qunatitative estimation statistical parameters of Zidovudine by UV spectrophotometric method

Drug code	Label claim (mg)	Mean amount found (mg/tablet)	PP	PD	SD	RSD	COA	SEM
Sample-1	150	148.82	98.80	1.20	0.1568	0.0193	1.9315	0.1095
Sample-2	300	297.60	99.60	0.40	0.3678	0.0370	0.7001	0.2123

PP-Percentage purity
PD-Percentage deviation
SD-Standard deviation
RSD-Related standard deviation
COA-Co efficient of variation
SEM-Standard error of mean

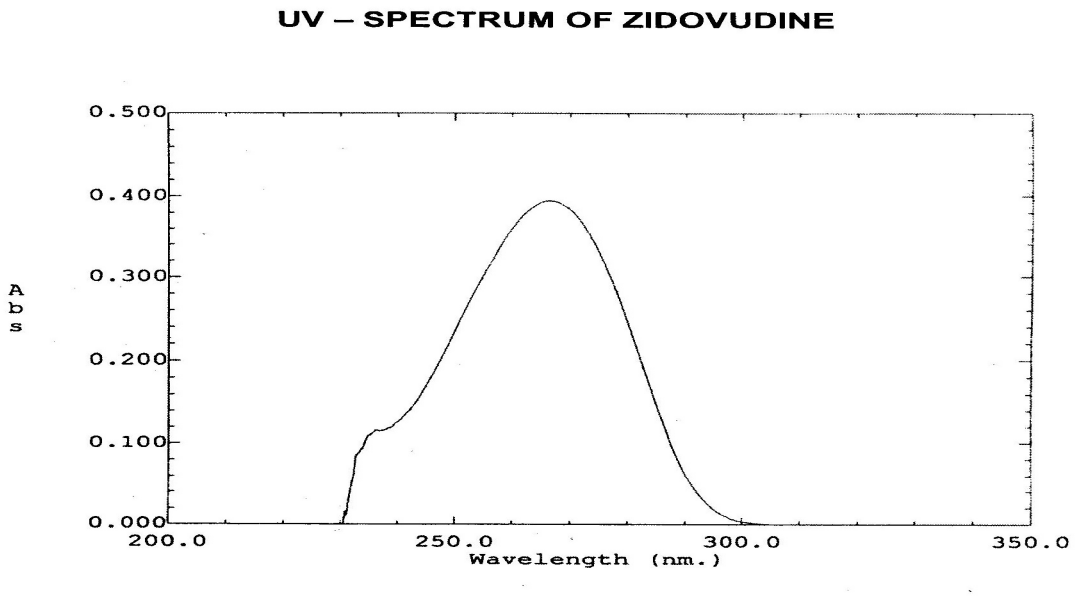

Figure 1: UV Spectrum of Zidovudine in methanol

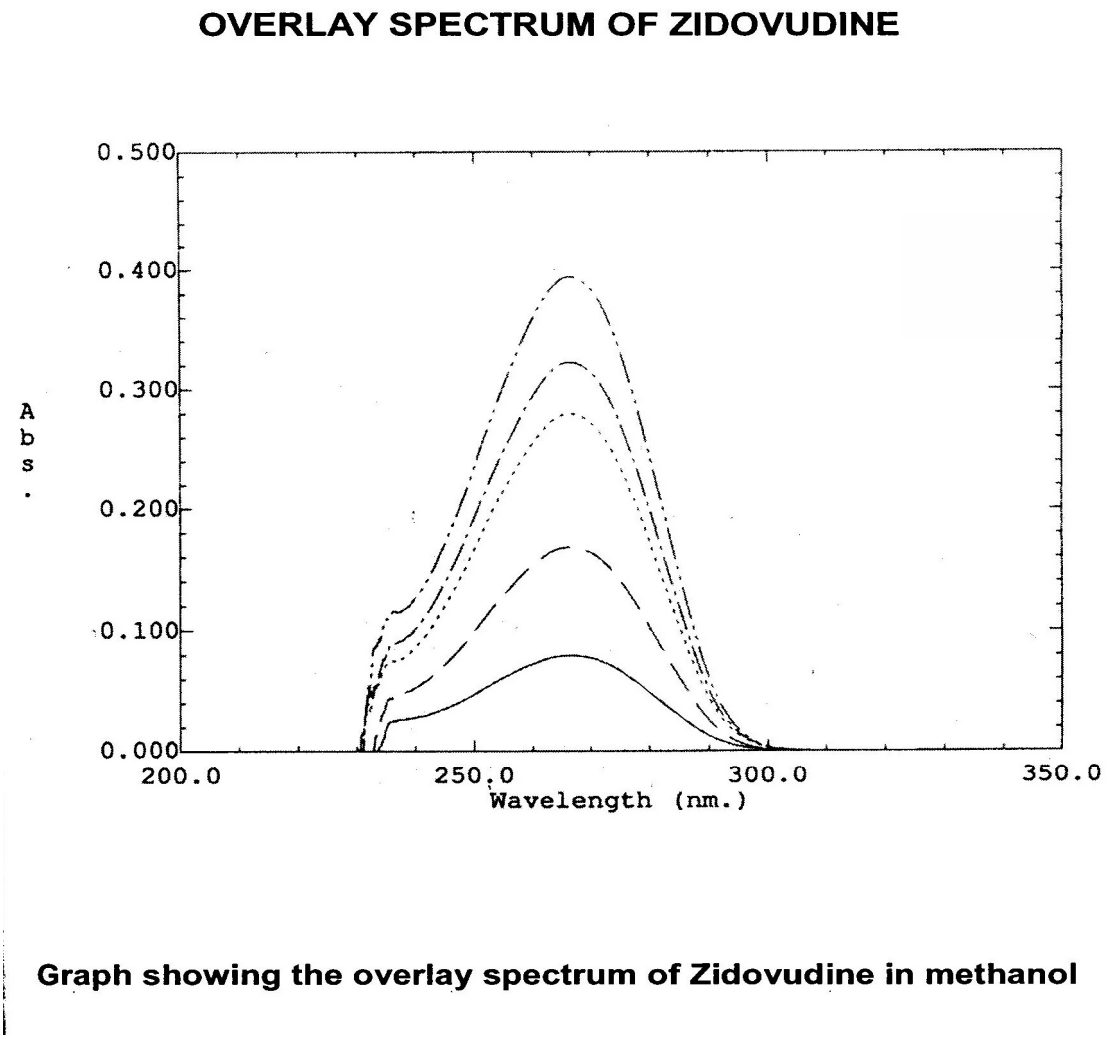

Figure 2: Overlay spectrum of Zidovudine in Methanol

Source of support: Nil, Conflict of interest: None Declared