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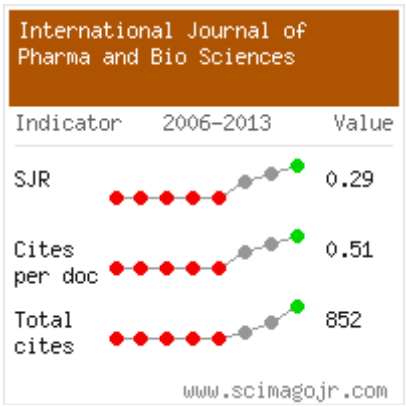

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A VALIDATED STABILITY INDICATING ASSAY METHOD OF ZIDOVUDINE BY UV-VISIBLE SPECTROPHOTOMETER

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ABSTRACT

The objective of the present work was to develop a simple, precise, cost effective and accurate stability indicating assay method for Zidovudine using UV-Vis spectrophotometer under different stress conditions (hydrolytic, oxidative, photolytic and thermal) recommended by the International Conference for Harmonisation. The stress study was performed by taking distilled water as solvent and absorbance was taken at absorption maxima (λ_{max}) of 267nm. After performing a hydrolytic study of the drug in different conditions of acidic, alkali and neutral medium, the drug was found to be degraded very slowly about 8.7 % in highly acidic condition, 19.5% in alkali media and 5 % in neutral medium. The drug was found to be oxidized by 25.0 % in 3%, H_2O_2 solution, whereas the drug was found to be almost stable in thermal condition but degraded by 15.8% in photolytic condition. A linear response was observed in the range of 5-50µg/ml with a regression coefficient of 0.999. The method was validated as per the ICH (International Conference for Harmonization) guidelines. The % COV value for intermediate precision studies was < 2 indicates the good precision of the method. The % of the recovery of the drug ranged from 100.40 - 102.55 indicates the accuracy of the method for estimation of degradants in different stress conditions. The method was specific to the drug and also selective to degradation products formed under different stressed conditions.

KEYWORDS: **Zidovudine, Stability Indicating Assay , Validation , UV- Vis Spectrophotometer.**

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INTRODUCTION

In recent times, there is an increase tendency towards the development of stability-indicating assay using the approach of stress testing as mentioned in the ICH guidelines (Q1A). It also recommends carrying out of stress testing on the drug substance to establish its inherent stability characteristics and to support the suitability of the proposed analytical procedure. The stress testing encompasses the influence of temperature, humidity, light, oxidising agent as well as susceptibility over a wide range of pH values[1]. The objective of the present work is to study the degradation of Zidovudine (ZDV) under different ICH recommended stress conditions and to established a validated stability-indicating assay method (SIAM). Chemically Zidovudine (ZDV) is 3'-Azido-3'-deoxythymidine[2] , it is a thymidine analogue, the prototype nucleoside reverse transcriptase inhibitors and is official in the United States Pharmacopoeia, British Pharmacopoeia and European Pharmacopoeia[3-5]. Zidovudine is used against human immunodeficiency virus (HIV-I and -II) and human T-cell lymphotropic virus (HTLV-I and -II) [6]. From the literature survey, it was revealed that various chromatographic and spectroscopic methods are available for simultaneous estimation of ZDV in single or combine dosage forms[7-12]. Further methods are also available for estimation of ZDV and its major metabolites in biological fluids[13-15]. But it was observed that no literature was found regarding the development of stability-indicating assay method for ZDV using UV- Spectroscopy, hence it was felt to develop a validated stability-indicating assay method for analysis that separates the drug from its degradants formed under ICH suggested stress conditions (hydrolysis, oxidation, photolysis and thermal stress).

EXPERIMENTAL

(i) Materials

Working standards of pharmaceutical grade ZDV were obtained as gift sample from M/s Hetero Labs Ltd., Hyderabad, India and was used without further purification. All the chemicals used were of analytical reagent grade. Distilled Water was taken as solvent for the preparation of different dilutions during study. Hydrogen peroxide (H2O2), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Merck (Mumbai, India).

(ii) Instrumentation

UV-Visible double beam spectrophotometer, Shimadzu model-1800 having spectral bandwidth 3 nm and of wavelength accuracy ±1nm, with 1cm quartz cells was used for absorbance measurement.Précision mentol heater (Biotech, Mumbai) with temperature regulator equippted with a reflux condencer was used for degradation study in acid, alkali and neutral conditions. Dry air oven was used to study the effect of dry heat. Photolytic study was carried out by exposing the drug to direct sunlight.

(iii) Preparation of stock solutions

100 mg of ZDV was accurately weighted and dissolved in 100 ml of distilled water to get solution of 1mg/ml. Stock solutions of 100µg/ml were prepared by further diluting 10 ml of above solutions to 100 ml with distilled water.

(iv) Selection of appropriate wavelength

From standard stock solutions of 100µg/ml appropriate dilution of 30 µg/ml was prepared and scanned over the range of 400-200nm in the spectrum mode maintained at slow scan speed. The spectra show that the drug has a λ_{max} at 267nm. The SIAM study of ZDV for all the stressed conditions were studied at 267nm wavelength.

(v) Forced Degradation study

Degradation study of ZDV under different stress conditions mentioned as per ICH guideline using UV-Spectroscopy was carried out by using distiller water as solvent. Drug at a concentration of
1mg ml^{-1} was used in all degradation studies.

(vi) Hydrolysis degradation study

Hydrolysis degradation of drug was carried out in acidic, alkali and neutral mediums.

(vii) Acidic conditions

Initially a solution of concentration 1mg ml^{-1} of ZDV was prepared in 0.01 N HCl and refluxed it for 6h at 60^0C. Regular sampling was carried out in an interval of 1h. After 6h of reflux the solutions were further diluted to with water to a concentration of 30 µg/ml and the absorbance was measured at the $\lambda_{max.}$. From the absorbance it was observed that the drug was not degraded. As no degradation found hence the same procedure was followed for drug dilution having same concentration prepared in 0.1 N HCl and refluxed for 6h and subsequently the studied was also carried out in 1.0N HCl and refluxed for 6h at 60^0C. In all the cases no extra peaks appeared for any degradent, so the degradation was conformed by compairing the absorbance of ZDV in all the cases. As the absorbance value of the drug decreased very slightly suggests that the drug was degraded to a product very slightly.

(viii) Alkali conditions

A stock solution of concentration 1mg ml^{-1} was prepared in 0.01 N NaOH, then the solution was refluxed for 6h at 60^0C. After refluxed for 6h, the solution was further diluted to with water to a concentration of 30 µg/ml and the absorbance was measured at the $\lambda_{max.}$. From the absorbance it was observed that the drug was not degraded. As no degradation found hence the same procedure was followed for drug dilution having the same concentration prepared in 0.1 N NaOH and refluxed for 6h and subsequently the studied was also carried out in 1.0N NaOH and refluxed for 6h at 60^0C. In all the cases no extra peaks appeared for any degradent, so the degradation was conformed by compairing the absorbance of ZDV in all the cases. As the absorbance value of the drug in 1.0N NaOH decrease suggests that the drug was degraded to a degradent.

(ix) Neutral conditions

In neutral condition, 1mg ml^{-1} solution of the drug was prepared in water and refluxed for 6h at 60^0C. The solution was further diluted with water to a concentration of 30 µg/ml and the absorbance was measured at the $\lambda_{max.}$. The percentage of degradation was confirmed by measuring the concentration of ZDV in above prepared solutions.

(x) Oxidative degradation study

Oxidative degradation study of ZDV was carried out by preparing 1mg ml^{-1} concentration solution in 3% H_2O_2 and kept at room temperature for 6h. After 6h, the solution was further diluted with water to a concentration of 30 µg/ml and the absorbance was measured at the λ_{max}. The percentage of degradation was confirmed by measuring the concentration of ZDV in above prepared solutions.

(xi) Thermal degradation study

Thermal degradation study of drug was also carried out in the dry form of the drug. Here the drug exposed to 80^0C for 6h in a hot air oven. Regular sampling was done to check the degradation behavior of drug. After 6h of exposer, 30 µg ml^{-1} solution was prepared by diluting with water and the absorbance was measured at the λ_{max} and concentration of drug was calculated.

(xii) Photolytic studies

Photolytic study was done by exposing the dry drug to direct sunlight for 4h[16]. Around 200mg of pure drug was taken in a clean petridish and was placed under direct sun light for 4 hours. Sampling was done at regular intervals of 1 hr, from the samples accurately weighed drug was dissolved in distilled water to prepare 30 µg ml^{-1} solution.. The absorbances were measured at the specified λmax and concentration were calculated.

(xiii) Validation of the method

Validation of the developed method was done with respect to the parameters mentioned in ICH guideline[17].

(xiv) Linearity and range

To check the linearity of the above method various concentration of the drug were prepared from its stock solution by diluting with distilled water. Then the absorbance of the prepared dilutions were observed at its λmax and recorded against distilled water as blank. Calibration curve was plotted between concentration (X-axis) and

absorbance (Y-axis). Beer-Lambert's law was found to be obeyed in the concentration range of 5-50µg/ml. The result was reported in Table No. 1.

(xv) Accuracy

To further ascertain the accuracy and reliability of the proposed method recovery study was carried out via standard addition method. Fortifying a mixture of decomposed reaction solutions with three concentration of (10,20,30 µg/ml) the drug and determining the percentage of recovery of added drug. Each determination was repeated three times. The percent recovery of pure ZDV added was within the permissible limits indicating the accuracy of the method for estimation of drug. The results are as illustrated in Table No. 2.

(xvi) Precision

Precision of the method was verified by performing inter and intra day precision study. To check the intraday and interday precision of the developed method three replicates of three concentrations 10, 20 and 30 µg ml^{-1} were prepared and the % of drug content were calculated on the same day at an interval of 1hr for intra day and for 3 consecutive days in inter day study. The results of the precision study are reported in Table No.3.

RESULTS AND DISCUSSION

In the present work stability indicating assay method for ZDV by UV-Spectroscopy was performed by taking distilled water as solvent. By performing stability-indicating assay method in different stressed conditions mention in ICH guideline, one can find various degraded products formed and can suggest the degradation path. The developed method was validated by performing linearity, accuracy and precision study.

(i) Degradation study

As per ICH guideline degradation study of ZDV was performed at various stressed conditions for hydrolysis, oxidation, thermal and photolytic stability study. The percentage of degradation of ZDV was determined by taking the absorbance of the solutions at its λmax (267nm).

(ii) Hydrolysis

Hydrolysis of the drug was performed in various conditions like acidic, basic and neutral medium. The drug was found to be slowly degraded by 8.7 % after refluxed for 6 hours in strong acidic conditions. In alkali condition the drug was degraded about 19.5% after reflux for 6 hours. In neutral medium ZDV was degraded about 5 % after reflux for 6hours at 60^0C in distilled water.

(iii) Oxidation study

Oxidative degradation study of ZDV was carried out by diluting the drug in 3% solution of H_2O_2. ZDV was found to be oxidized by 25.0 % of its initial content after kept at room temperature for 6h in 3% H_2O_2 solution.

(iv) Thermal stability

The drug was found to be almost stable at stressed thermal conditions. Even after storage on 80^0C for 6 hours the drug was found to be degraded to very negligible amount about (~5%).

(v) Photolytic Study

After exposing the dry form of the drug to sunlight for 4 hours on a hot sunny day, the drug was found to be degraded to a concentration of 15.80%. The result of the degradation study of ZDV in various stress conditions was given in Table No.4.

(vi) Validation of method

The developed stability indicating assay method was validated for its linearity, accuracy and precision.

(vii) Linearity

The calibration curve of the drug was linear in the concentration range of 5-50 µg ml^{-1} given in Figure No. 3. The mean values of slope, intercept and correlation coefficent were 0.019, -0.054 and 0.9997 (r^2) respectively. From the result it was cleared there was an excellent correlation existed between the absorbance and concentration of the analyte.

(viii) Accuracy

Accuracy of the method was validated by performing recovery study. Recovery study was carried out by doing replicate study. The percentage of drug recovery was found to be with in the range of 100.40- 102.55, indicate that the developed method was accurate for estimation of degradants formed under different stressed conditions mention in ICH guideline.

(ix) Precision

Precision of the method was performed by doing intermediate precision study. Intermediate precision study was performed by doing intra and inter-day precision study. In case of intra-day precision study the percentage of drug content ranges from 102.8-100.22, where as in case of inter-day precision study the drug content ranges from 102.80-95.15. The developed method was found to be precise as the % COV value for intermediate precision studies was < 2 indicates that the developed method was suitable for estimation of degradants.

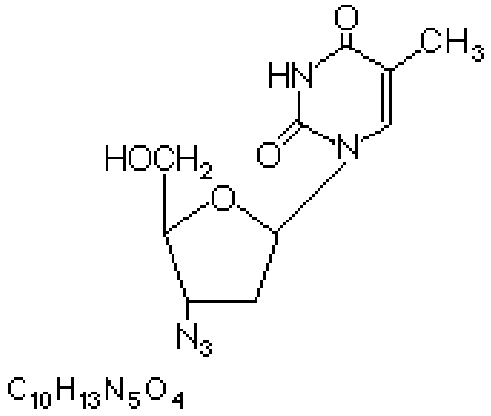

Figure 01
Chemical Structure of Zidovudin

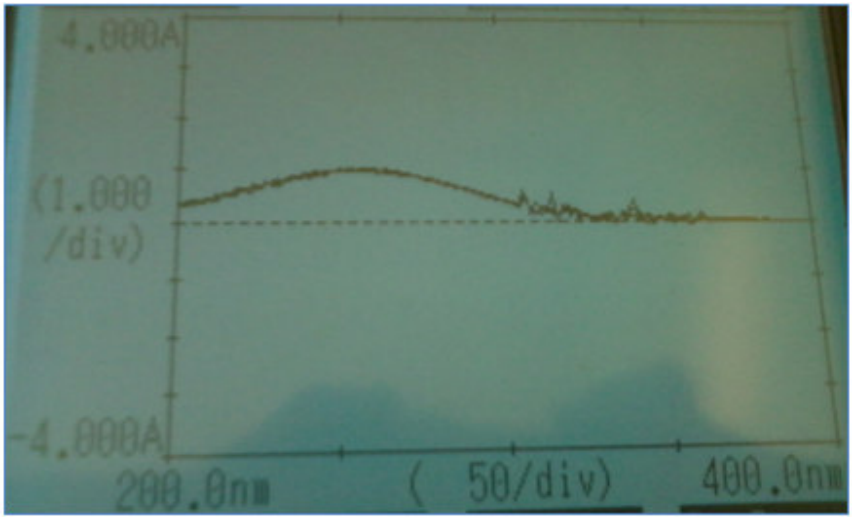

Figure 02
Overlay Spectra of ZDV in different Stressed Conditions

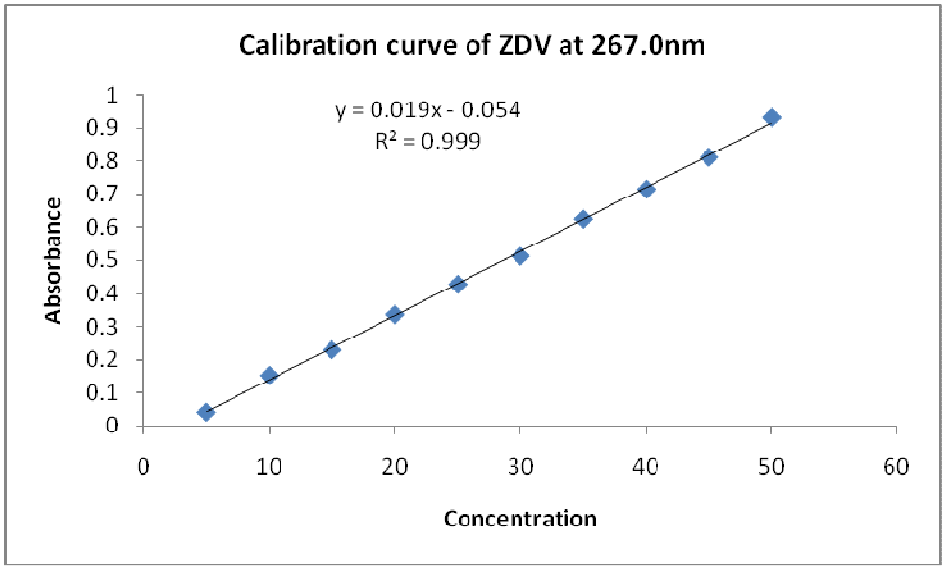

Figure 03
Linearity Curve of ZDV at λ_{max} 267 nm

Table 1
Absorbance value for the calibration curve of Zidovudine

S.No	Concentration of drug (µg ml^{-1})	Absorbance value at 267nm
1	5	0.042
2	10	0.153
3	15	0.230
4	20	0.337
5	25	0.428
6	30	0.513
7	35	0.625
8	40	0.715
9	45	0.811
10	50	0.931

Table 2
Result of Recovery Study

Replicate No.	Conc. Added (µg ml^{-1})	Conc. found µg ml^{-1}	% of Recovery±S.D
1		10.13	101.30±0.52
2	10	10.11	101.10±0.74
3		10.08	100.80±0.42
1		20.51	102.55±0.45
2	20	20.42	102.10±0.86
3		20.38	101.90±0.35
1		30.35	101.16±0.29
2	30	30.12	100.40±0.74
3		30.28	100.93±0.69

Table 3
Result of Precision Study

Actual concentration (µg ml^{-1})	Measured concentration ± S.D. (µg ml^{-1})			
	Intra day precision (n=3)	% COV	Inter day precision (n=3)	% COV
10	10.056±0.203	0.677	09.661±0.301	1.016
20	20.144±0.417	0.832	19.713±0.191	0.384
30	30.980±0.434	0.543	29.348±0.249	0.313

Table 4
Result of Forced Degradation Study of ZDV

S.no	Different stressed conditions	Concentration (µg/ml)		% of degradation
		Before	After	
1	Acidic condition	30	27.40	8.7
2	Alkali condition	30	24.15	19.5
3	Neutral condition	30	28.50	5
4	Oxidation	30	22.5	25.0
5	Thermal	30	28.61	~5
6	Photolytic Study	30	25.26	15.80

CONCLUSION

In the present study, a simple, sensitive and stability indicating UV-spectrophotometric method has been developed and validated. Stability-indicating assay method for Zidovudine was established by following the ICH recommended stress conditions. The drug was found to be degraded extensively in alkaline medium, oxidative stress and in the presence of sun light where as mild degradation of drug occured in higher acidic and neutral condition. But the drug was stable in thermal stress condition. The proposed method is highly sensitive and rapid, and requires no organic solvents or any additional reagents. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method can be used to determine the purity of the drug available from various sources and in stability studies.

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