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Spectrophotometric Determination And Validation Of Acyclovir In Tablet Dosage Form

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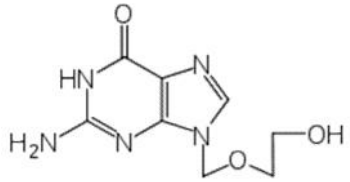
Abstract: Two simple, precise, accurate and economical UV spectrophotometric methods have been developed and validated for the routine estimation of acyclovir in bulk drug and pharmaceutical preparations. The drug shows maximum absorption at 252nm with molar absorptivity of 1.5899 $\times 10^4$ l/mol×cm and obeyed Beer-Lambert's law in the concentration range of 1-30µg/ml.. The same spectrum was derivatised into first order derivative the amplitude of trough at 238 nm, crest at 259nm and 288nm for D_1 were measured. In D_1 method the drug showed linearity in the concentration range of 1-30µg/ml. The linear regression equations were calculated to be y=0.0595x+0.0317 (R^2=0.9971) for D_0 at 252nm, y=-0.0025x-0.0005 (R^2=0.9981) for D_1 at 238 nm , y=0.0019x+0.0006 (R^2=0.9969) for D_1 at 259nm and y= -0.0025x-0.0014(R^2=0.9951) for D_1 at 288nm The results of estimation of marketed tablet formulations were found to be 99.386±0.1290-100.345±0.1546 with their SD less than 2. Recovery studies were carried out by addition of known amount of standard drug (80,100 and 120% of labeled claim of a tablet) to the preanalysed tablet solution. The % recovery was found to be 98.458-101.984, which indicates accuracy and reliability of the validated method as well as noninterference from excipients to the developed method. The intraday and inter day assay was within 2%. The methods were then validated statistically as per the ICH guidelines which yielded good results concerning range, precision, accuracy, specificity and repeatability.

Keywords: Acyclovir, Spectrophotometry, Molar absortivity, Validation.

INTRODUCTION

Acyclovir, chemically known as 9-[(2-hydroxyethoxy)methyl] guanine is a purine nucleoside analogue, active against herpes simplex virus type 1 and 2 and against viricella zoster virus. It inhibits enzyme thymidine kinase and interferes with DNA synthesis[1,2]. It is official in USP and BP.

Structure:



Survey of literature shows different UV-Vis Spectrophotometric method has been developed to determine different antiviral drug i.e. valacyclovir, gancyclovir, famcyclovir [3,4,5] . Several methods have been developed for spectrophotometric evaluation of acyclovir. The reported techniques for its estimation include solid phase extraction and HPLC[6] and electroimmunoassay[7] in serum and cerebrospinal fluid[8].

MATERIALS AND METHODS

Materials

UV-visible double beam spectrophotometer, JASCO-V630 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells were used. The commercially available tablet, Acivir 200DT

(Label claim: Acyclovir 200 mg) was procured from local market.

Selection of solvent

After assessing the solubility of drugs in different solvents distilled water has been selected as solvent for developing spectral characteristics.

Preparation of standard stock and calibration curve

The standard stock solutions of acyclovir was prepared by dissolving 25 mg of drug in 10mL distilled water in 100mL volumetric flask, final volume was adjusted with distilled water and sonicated for about 10 min to get 250 µg/mL. Working standard solutions of 10 µg/mL were scanned in the entire UV range of 400-200 nm to obtain the absorbance spectra. (Figure No.:-1) The drug shows maximum absorption at 252 nm. Eight working standard solutions for drug having concentration 1, 2, 5, 10, 15, 20, 25 and 30 µg/mL were prepared in distilled water from stock solution. The absorbance of resulting solutions were measured at respective λ max and plotted a calibration curve against concentration to get the linearity and regression equation. The same spectrum was derivatised into first order derivative, the amplitude of trough at 238nm, crest at 259nm and 288nm for D_1 (Figure No.:-2) were measured. In Do drug shows linearity in the range of 1-30µg/ml at 252nm while in D_1 1-30µg/ml at 238nm, 259nm and 288nm. The linear regression equations were calculated to be y=0.0595x+0.0317 (R^2=0.9971) , for D_0 at 252nm(Figure No.:-3) , y=-0.0025x-0.0005 (R^2=0.9981) for D_1 at 238 nm (Figure No.:-4), y=0.0019x+0.0006 (R^2=0.9969) for D_1 at 259nm(Figure No.:-5) and y= -0.0025x-0.0014(R^2=0.9951) for D_1 at 288nm (Figure No.:-6).

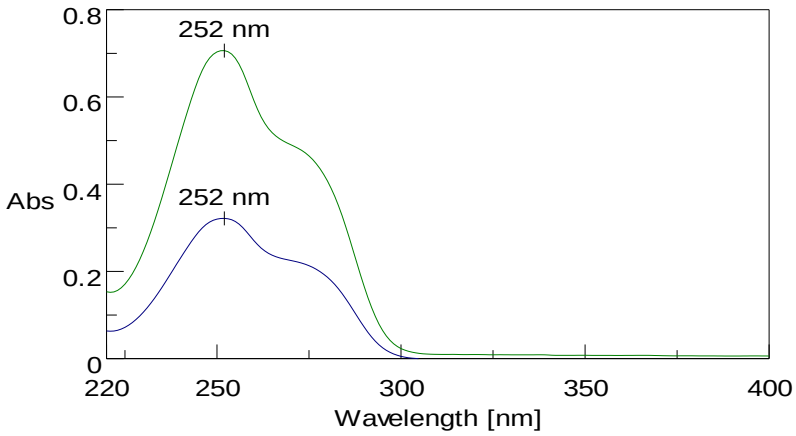


Figure No.: 1:Overlain spectra of Acyclovir 5 & 10 µg/mL (D_0)

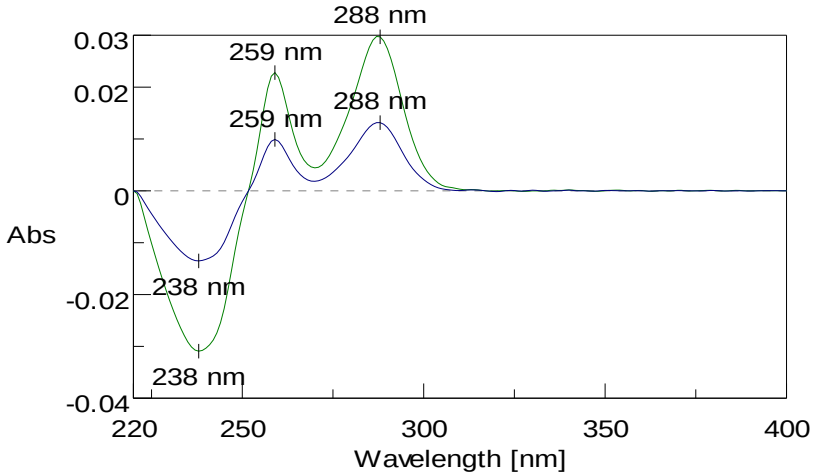


Figure No.: 2 : Overlain spectra of Acyclovir 5 & 10 µg/mL (D_1)

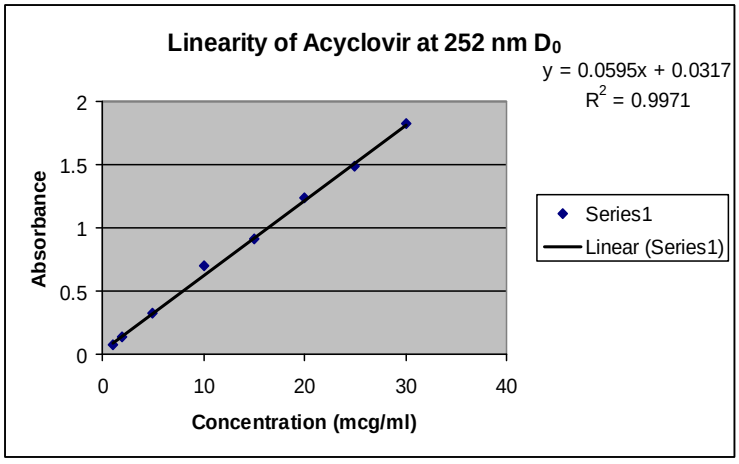


Figure No.: 3 : Linearity of Acyclovir at 252 nm (D_0)

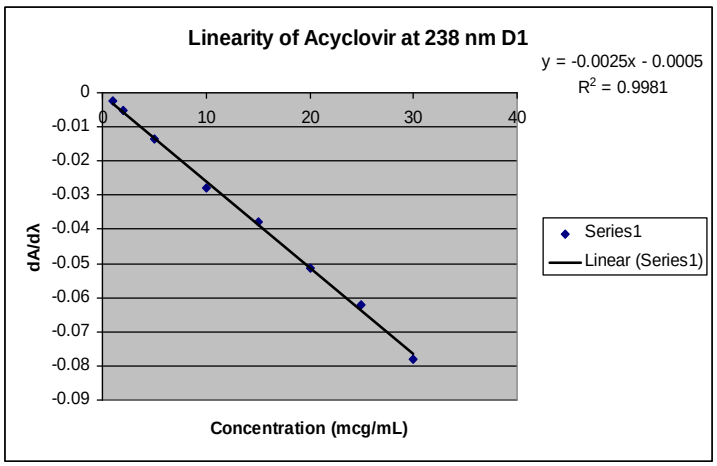


Figure No.: 4 : Linearity of Acyclovir at 238 nm (D_1)

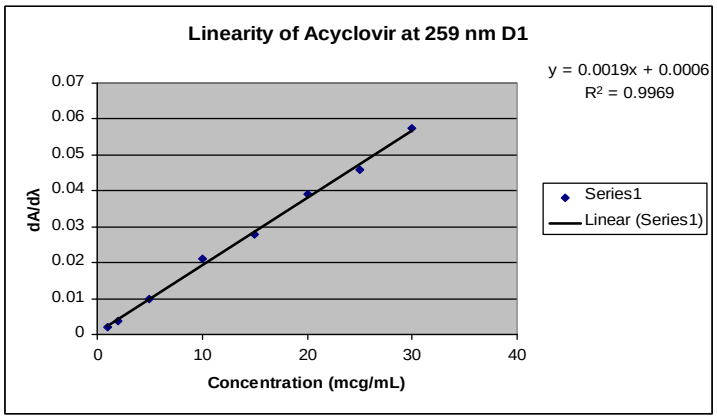


Figure No.:5: Linearity of Acyclovir at 259 nm (D_1)

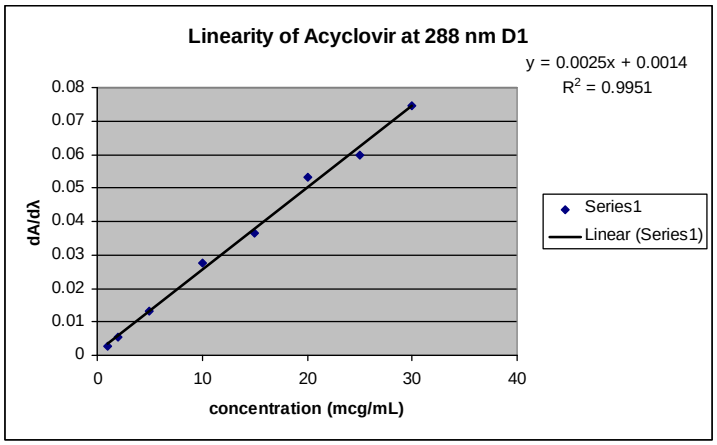


Figure No.:6: Linearity of Acyclovir at 288 nm (D_1)

Analysis of tablet

Twenty tablets were weighed accurately and reduced to fine powder, drug equivalent to 25mg of acyclovir was weighed and dissolved in 10 ml of distilled water in a 100ml volumetric flask, final volume was made with distilled water and sonicated for about 10min. The above solution was filtered by using Whattmann filter paper No.:41. From the above filtrate 0.4 mL of solution was diluted to 10 mL with distilled water to get 10 µg/mL of acyclovir. Analysis procedure was repeated six times with tablet formulation. Aliquot was scanned in the UV range (200-400nm). The same spectrum was derivatised into first order, amplitude of the trough at 238nm,crest at 259nm and 288 for D_1. The amount of drug present in the tablet was calculated from the standard graphs (Table No.:2).

Method Validation:

Linearity

Appropriate concentration of stock solution was assayed as per developed methods.Beer-Lambert's concentration range was found to be 1- 30µg/ml. The linearity data for both methods are presented in Table No.1.

Accuracy

The accuracy of the methods was determined by performing recovery studies on tablet formulation and for prepared solutions containing known amount of drug by standard addition method in which preanalyzed samples were taken and standard drug was added at three different levels 80%.100% and 120% as per ICH guidelines. The recovery study performed three times at each level. The results are shown in Table No.:2

Precision

To check the degree of repeatability of methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablets formulation. The standard deviation, coefficient of variance and standard error was calculated. The results of statistical evaluation are shown in Table No.:2.

Intermediate Precision (Interday and Intraday precision)

The experiments were repeated three times in a day to determine intraday precision and on three different days to determine interday precision. The results of the same are presented in Table No.:3.

Selectivity

The selectivity of the methods was checked by monitoring a standard solution of Acyclovir in presence of excipients at the same concentration level as used in tablet using the method described in the procedure for calibration curve in pharmaceutical tablets.

Table No.: 1 Optical Characteristics for Acyclovir

Parameters	Values			
	(D_0)	(D_1)		
Working lmax	252nm	238nm	259nm	288nm
Beer's law limit (µg/ml)	1-30	1-30	1-30	1-30
Correlation coefficient*	0.9971	0.9981	0.9969	0.9951
Intercept*	0.0317	-0.0005	0.0006	0.0014
Slope*	0.0595	-0.0025	0.0019	0.0025

* Average of eight estimation

Table No.:2: Analysis of Tablet formulation, Statistical Validation and Recovery studies.

Method		Label Claim mg/tab	Amount found mg/tab	Label Claim (%)	S.D.*	% COV	S.E.*	Amount Added		% Recovery #
								%	mg/ml	
D_0	At 252 nm	200	198.808	99.404	0.1181	0.1188	0.0484	80	160	99.045
								100	200	98.954
								120	240	100.984
D_1	At 238 nm		200.689	100.345	0.1546	0.1540	0.0663	80	160	98.745
								100	200	99.458
								120	240	100.458
	At 259 nm		200.508	10.254	0.1722	0.1717	0.0706	80	160	98.884
								100	200	99.485
								120	240	100.024
	At 288 nm		196.227	99.386	0.1290	0.1311	0.0529	80	160	98.458
								100	200	100.080
								120	240	99.148

S.D.: Standard deviation., COV: Coefficient of variation., S.E.: Standard error *Average of six estimation of tablet formulation., # Average of three estimation at each level.

Table No.:3: Validation Parameters

Method		Precision(%COV)			
		Intraday n=3	Interday*		
			First day	Second day	Third day
D_0	At 252 nm	0.1171	0.7781	0.1258	0.9431
D_1	At 238 nm	0.0941	0.9879	0.9065	0.8278
	At 259 nm	0.1249	0.1547	0.1893	0.1299
	At 288 nm	0.9016	0.1027	0.9924	0.8945

COV: Coefficient of variation., *Average of six determination

RESULTS AND DISCUSSION

The proposed methods are simple, rapid and precise and do not suffer from any interference due to excipients of tablet. Various optical characteristics are shown in the Table No.1. The proposed spectrophotometric methods were found to be linear in the range of 1- 30µg/ml at 252nm in Do with correlation coefficients (R^2) 0.9971 while in D_1 1-30 µg/ml at 238nm, 259nm and 288nm. with correlation coefficients (R^2) for D_1were found to be 0.9981,0.9969 and 0.9951 respectively.

The methods were validated in terms of accuracy, precision, repetabiltiy and the results are recorded in Table No.:2 and 3.The accuracy of the method was determined by performing recovery studies by standard addition of method in which preanalyzed samples were taken and standard drug was added at three different levels. Values of recovery greater than 98.0% indicate that proposed method is accurate for the analysis of the drug.

The precision of the proposed method was estimated in terms of interday precision and intraday precision wherein the method was repeated on three different days and repeated for three different time periods in the same day respectively. The results shown in Table No.:3. SD less than 2% at each level clearly indicate that the proposed method is precise enough for the analysis of the drug.

The selectivity of the method was checked by monitoring a standard solution of acyclovir in presence of excipients at the same concentration level as used in tablet using the method described in the procedure for calibration curve in pharmaceutical tablets. The excipients did not show any effect on the estimation of acyclovir. Hence, the determination of acyclovir in the tablet is considered to be free from interference due to the excipients. Rigorous analysis of the results indicates that the presence of excipients in tablet formulation did not interference with the final determination of the active component. This reveals that the potential utility of this method for the routine analysis of acyclovir in pharmaceutical preparations.

CONCLUSION

Two new, simple precise, accurate and selective spectrophotometric methods were developed for the analysis of acyclovir in bulk and in pharmaceutical formulation. The D_0 method is useful for tablet formulations where there is no interference of excipients in the absorbance of acyclovir and method D_1 can be utilized for formulations containing any interfering excipients. The developed methods were also validated and from the statistical data, it was found that methods were accurate, precise, reproducible and can be successfully applied to the pharmaceutical formulations without interference of excipients.

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