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ORIGINAL ARTICLE **OPEN ACCESS**

Development and Validation of UV Spectrophotometric Method for Estimation of Acyclovir in Bulk and Pharmaceutical Dosage form by Area Under Curve Using Organic Solvent

Suresh Jadhav, Muskan Inamdar, Vrushali Naykodi, Shankar Dhoble, Dushyant Gaikwad
Vishal Institute of Pharmaceutical Education & Research, Ale, (Alephata), Dist. Pune
Correspondence Email: inamdarmuskan1998@gmail.com

ABSTRACT

The aim of present investigation is to establish simple, precise, and rapid Spectrophotometric method for the quantification of Acyclovir in Active Pharmaceutical Ingredient. In this, work is carried out to for estimation of Acyclovir bulk by utilizing an Area under Curve (AUC) method using UV – Visible Spectrophotometer. The study is designed to validate the developed methods as per ICH guidelines. For this purpose, the wavelength range between 200-400 nm was selected. Distilled water (50 ml methanol used for stock solution and serial dilution in 25 ml distilled water) was used as a solvent throughout the work. The method was validated for linearity, range, accuracy, precision, robustness, LOD and LOQ. Linearity was obtained in concentration range 10 to 60 µg/ml (r2 =0.998) for the method Accuracy was performed by using a recovery study. The amount of drug recovered was found to be in the range of 100.1-100.5 %. The % RSD value was found to be less than 2. The developed method was found to be simple, linear, accurate, precise and highly sensitive and which can be used for routine quality control analysis for Spectrophotometric estimation of Active Pharmaceutical Ingredient.

Keywords: *Acyclovir, AUC, methanol, Water, UV Spectrophotometer*

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INTRODUCTION

Acyclovir [2-amino-9(2-hydroxyethoxy) methyl)-1, 9 dihydro-6Hpurine-6-one] is an antiviral agent. Acyclovir is a white crystalline powder. Acyclovir is sparingly soluble in water freely soluble in dimethyl sulfoxide (DMSO) and very slightly soluble in alcohol.[1] It is used in the of treatment of virus infection, chickenpox and shingles. It is a synthetic moiety of deoxyguanosine in which the carbohydrate moiety is acyclic.1Acyclovir is used to control the symptoms of infection involving herpes simplex virus (HSV) type-1 and type-2 which causes herpes simplex, varicella zoster virus (VZV) causes shingles and chickenpox.[2-4] The aim of this present work is to develop simple, precise and accurate Spectrophotometric method for the routine determination of Acyclovir in bulk.[5-6]

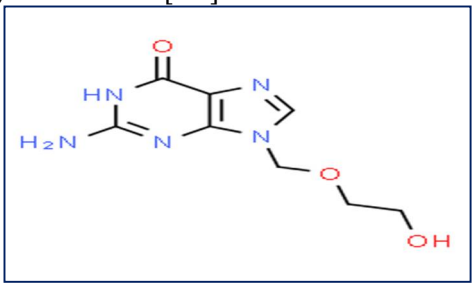

Figure no.1 Structure of Acyclovir

MATERIAL AND METHODS

Chemicals: Acyclovir was obtained as Gift sample from Zen vision pharma LLP, Navi Mumbai. Methanol and Distilled water were used as solvent throughout the experimentation.

Instrumentation: A Shimadzu (Kyoto, Japan) model UV- 1800 double beam UV- Visible spectrophotometer attached with computer operated by software with UV probe 2.33. Spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells was used to measure

absorbance of the resulting solutions. Digital Analytical balance, Mettler Toledo (Model JL 1503-C) was used for weighing purpose.[10]

Method: -

Experimental Work:

A) To check the solubility of Acyclovir: Qualitative solubility analyses of drugs were done by dissolving 5mg of Acyclovir in 5ml solvent such as methanol and distilled water.

B) To identify the ʎ max of Acyclovir: Weigh 50 mg of the pure drug (Acyclovir) and dissolve it in small portion of methanol and make up the volume up to 50 ml using methanol to give a standard stock solution of 1000µm/ml. this solution is sonicate for 5 min to obtained clear solution. Form above solution 10ml of standard solution was withdraw in volumetric flask and diluted with 25 ml to prepare 100 ppm Solution. Suitable dilutions were made with to get standard solutions of concentration 10, 20, 30, 40, 50,60ug/ml (ppm).[7-9]

C)Preparation of Standard Solution: -The standard stock solution of Acyclovir was prepared by accurately weighing and transferring, 50 mg of API to 50 ml of volumetric flask. Using methanol Then take from that 10 ml and add to 100 ml volumetric flask and make up with water to get final standard stock solution (100 µg/ml) was further diluted with water to obtain 10-60 µg/ml Acyclovir solutions.

D)Assay of acyclovir tablet: -20 tablets weighed and powdered. The powder equivalent to 50 mg of acyclovir was weighed, transferred into 50 ml volumetric flask. It was dissolved sufficient amount of methanol. This solution was sonicated for 15 min and the final volume was made up to the mark with methanol. 10 ml of solution was transferred into 100 ml volumetric flask and diluted up to 100 ml with water. The absorbance of this solution was measured at 254 nm (table.no.1)

E) Area under Curve Method:

The AUC (Area under Curve) method is applicable for there is no sharp peak or broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths ʎ1 and ʎ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis.

Area calculation: (α+β) = $\int_{\lambda 2}^{\lambda 1} A d\lambda$

Where, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ1 and λ2 are wavelength range start and end point of curve region. The horizontal axis is selected by the entering the wavelength which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above-mentioned spectrums were used to calculate AUC. Thus, the calibration curve can be constructed by plotting concentration (10-60µg/ml) Vs AUC.[9]

Analytical Method Development and Validation:[13]

The above method was validated for various parameters such as Accuracy, Linearity, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH guideline.

1. **Linearity: -**The linearity was determined by using working standard solutions between 10-60 µg/ml. The areas under curve (AUC) of these solutions were recorded. Calibration curve of area under curve to concentration plotted on excel sheet and linear regression was performed. The correlation coefficient, regration Equation was calculated. The absorbance maxima and area under curve for the solutions was measured at 254 nm and range of to 200-400 nm for two methods respectively.

2. **Accuracy** The accuracy for the analytical method was evaluated at 50%, 100% and 150% was done to confirm the accuracy of developed method. Area under curve (AUC) was measured in wavelength range 254 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

3. **Precision** The precision of an analytical procedure expresses the closeness of an agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions intraday precision was studied by integrating area of standard solution of 20 µg/ml concentration at six independent series in the same day. Interday precision studies were performed by integrating area of standard solution of 20 µg/ml concentration on three consequent days. The % RSD was calculated.

4. **Limit of Detection and Limit of Quantification: -** The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula:

$$LOD = 3.3\ \sigma / S$$

The limit of quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified.

LOQ was calculated using the following formula:

$$LOQ = 10\ \sigma/S$$

Where, σ is standard deviation of the response and S is the slope of the calibration curve. LOD and LOQ of Acyclovir was found to be 1.320 µg/ml and 3.465 µg/ml respectively.

RESULTS AND DISCUSSION

The AUC (Area under Curve) spectra for Acyclovir were recorded at the wavelength of 242-262 nm.

A) Calibration Curve for Drug:

Absorbance maxima method:

The absorbance maxima of Acyclovir were found to 254 nm in Methanol + distilled water. Under the Experimental conditions described, the graph obtained for the absorbance maxima for pure drug showed linear relationship. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curve were y = 0.049x + intercept 0.005 R^2 = 0.998 at 254 nm for absorption maxima the range was found to be 10 to 60µm/ml by the UV Spectrophotometric analysis.

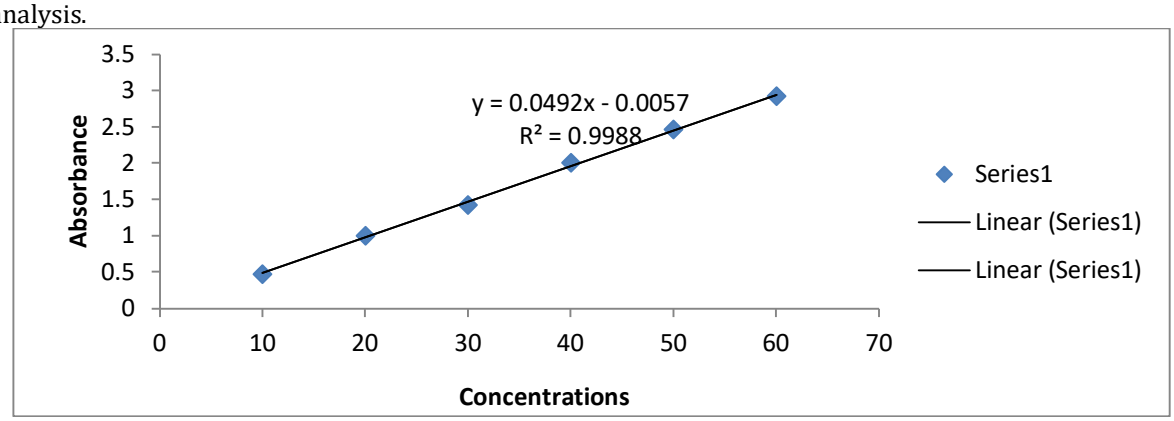

Figure 2: Calibration curve of Acyclovir

B] Area under Curve Method:

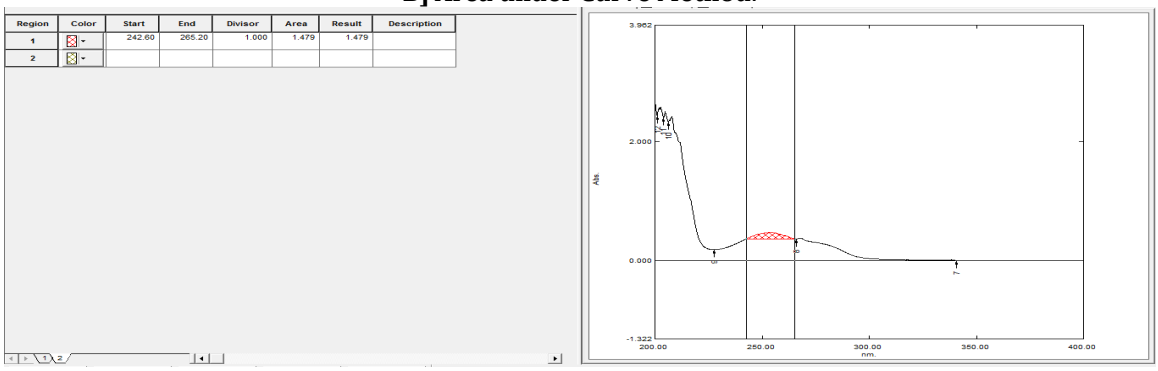

Figure 3: λmax of Acyclovir Spectrum peak pick

In the Experimental conditions described, the graph obtained of the Area under Curve (AUC) spectra shows linear relationship. Regression analysis was made of the slope, intercept and R2 values. The equation is Y = 0.049x + intercept 0.005 R^2 = 0.998 at 254 nm in between range 200 – 400 nm for Area under Curve Spectrophotometric analysis. The range was found to be 10 to 60µm/ml for the Area under Curve of UV Spectrophotometric analysis.

METHOD VALIDATION:

1) **Linearity:** The linear relation between absorbance and concentration of drug was evaluated using 3 replicates over concentration range in 10-60µg/ml by making the replicates (table 1 and fig 3). The wavelength for linearity was scanned at 254nm.By taking six different concentrations for linearity the regression coefficient was found to be 0.998 i.e., in the limit of standard. Hence the linearity parameter was found to be validated. (Table 2 and fig 3)

2)Accuracy: - Accuracy of the method was confirmed by recovery studies from marketed formulation at three different levels of standard i.e., 50%, 100%, 150% was done to confirm the accuracy of the developed method. The amount of acyclovir is calculated at each level and percentage recoveries were calculated (table 3).

3) Precision : Precision of the developed method expressed in terms of the relative standard deviation of the absorbance. The solution was analyzed in 6 replicates for intra-day precision and in two successive

days for inter-day precision. The % RSD value was found to be less than . Results confirmed that the precision of the method was found to be accepted. Precision results were given in (table 4 and table 5) for intra and inter-day precision respectively

4) Limit of detection (LOD): - Limit of detection of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value. LOD was found to be 1.320

5) **Limit of quantitation (LOQ)**: -Limit of quantitation of an individual analytical procedure is the lowest amount of an analyte in the sample which can be quantified as an exact value. LOQ was found to be 3.465.

Table 1: Results for Assay of Tablet Dosage Form

Sr.No.	Concentration(µg/ml)	Amount Found (%)	Mean (%) Found	%RSD
1	10	98.59	99.28	0.581
2	10	100.07		
3	10	99.19		

Table 2: Results for Linearity

Concentration (ppm)	Absorbance(nm)
Solvent (Blank)	0.000
10ppm	0.471
20ppm	1.003
30ppm	1.430
40ppm	2.008
50ppm	2.465
60ppm	2.923

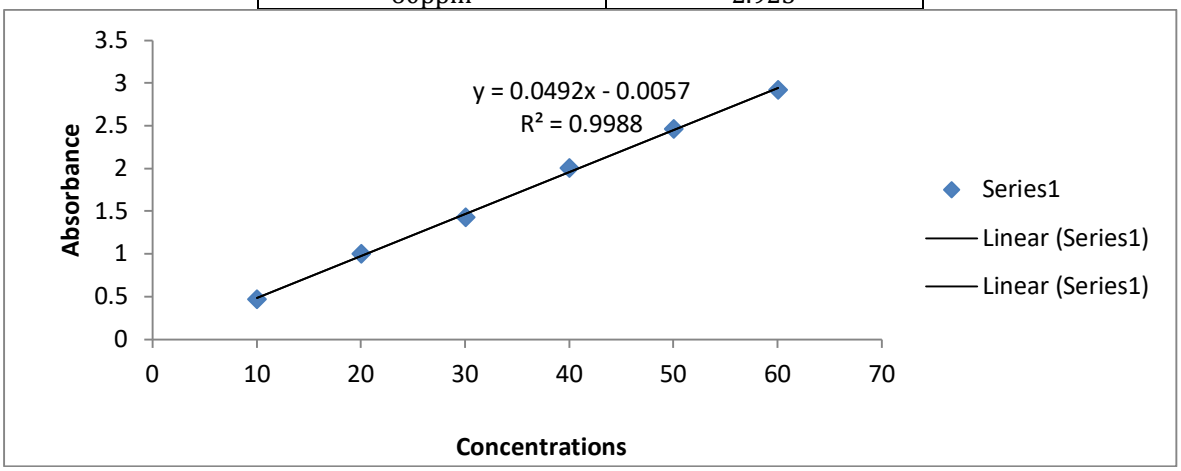

Figure 4: Calibration curve of Acyclovir

Table 3: Results for Accuracy Test

Name of Drug	Recovery levels	Concentration(µg/ml)	Amount Recovered	% Recovery with SD
	50%	10	10.001	100.01±0.70
Acyclovir	100%	20	20.001	100.03±0.13
	150%	30	30.004	100.05±0.25

Table 4: Results for intra-day precision

Sr.No.	Conc	Absorbance (Day 1)	Absorbance (Day 2)
1	20	1.151	1.153
2	20	1.152	1.152
3	20	1.15	1.153
4	20	1.152	1.151
5	20	1.153	1.153
6	20	1.152	1.152
SD		0.001032796	0.000816497
%RSD		0.08%	0.07%

The method was validated for linearity, range, accuracy, precision, LOD and LOQ. Linearity was found in the range of 10- 60µg/ml. Accuracy was performed by using a recovery study. The amount of drug recovered was found to be in the range of 100.1-100.5 %. The % RSD value was found to be less than 2.

CONCLUSION:

Spectrophotometry methods development for their determination of drugs has increased considerable in recent year because of their importance in pharmaceutical analysis. A new method has been developed for the spectrophotometric estimation of acyclovir. [11]

Area under curve method was developed for the determination of Acyclovir based on analytical technique. The method was validated and found to be simple, sensitive, accurate, and precise. Hence, this method can be used successfully for routine analysis of pharmaceutical dosage forms of Acyclovir in bulk and pharmaceutical dosage form. Therefore simple, fast and precise method for area under curve was developed by UV spectrophotometrically for the routine analysis of Acyclovir. [12] The developed method can be concluded as simple, accurate, sensitive and precise and can be easily applicable in the pharmaceutical formulation.

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