World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

DEVELOPMENT AND VALIDATION OF SIMPLE AND SENSITIVE UV-VISIBLE SPECTROSCOPIC METHOD FOR ESTIMATION OF ACYCLOVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Article Received on 24/09/2020 **Article Revised on 14/10/2020** **Article Accepted on 04/10/2020**

ABSTRACT

A simple, sensitive, accurate, novel, rapid, precise, reproducible and cost effective spectrophotometric method for the quantitative estimation of Acyclovir in bulk drug and pharmaceutical formulation and validated according to the ICHQ2 (R1) guideline. The acyclovir drug solution was scanned over UV-visible range for determining the wavelength at which the maximum absorbance is occurs. At wavelength of maximum absorbance of acyclovir various calibration standards of acyclovir were prepared and recorded the absorbance. The calibration curve of concentration vs. absorbance was plotted and linearity and range was calculated. Various analytical method validation parameters viz. accuracy, precision, limit of detection, limit of quantification, and ruggedness were calculated using QC standards. The maximum absorbance (λ max) of acyclovir was found to be 253.2 nm. The drug obeyed beer lamberts' law in the concentration range of 1-10 µg/ml and the correction coefficient was 0.991 at 253.2 nm. The overall % recovery was taken at three level 80%, 100% and 120% which is found to be 99.69 to 100.89 %, 99.02 to 100.92% and 99.24 to 100.7% respectively, which reflects that the method was free from the interference of the impurities and other excipients used in the formulation. The low value of % RSD was indicative of accuracy and reproducibility of the method. The % RSD for inter-day and intra-day precision was found to be of 0.42 to 1.75 & 0.48 to 1.23 respectively which is < 2% which proved that method is precise. The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. The developed method can be adopted in routine analysis of acyclovir in bulk dosage as well as form tablet dosage form.

KEYWORDS: UV- visible spectrometry, Validation, Acyclovir.

INTRODUCTION

Acyclovir, 2-amino-9-(2-hydroxyethoxymethyl)-1H-purin-6-one, (fig. 1) is an antiviral drug and Acyclovir is used intravenously in the treatment of severe initial and recurrent mucocutaneous infections caused by HSV-1, HSV-2 and varicella-zoster virus (chickenpox virus) in adults and children.[1-2] Acyclovir is a purine derivative antiviral drug. Acyclovir is converted by viral thymidine kinase to acyclovir monophosphate, which further converted to acyclovir triphosphate, thus they competitively inhibits and inactivates HSV-specified DNA polymerases preventing further viral DNA synthesis without affecting the normal cellular processes.[3-4] It is a white crystalline powder, it is soluble in dilute hydrochloric acid; slightly soluble in water; insoluble in alcohol. Acyclovir is official in IP, USP, BP etc.[5-6] The investigation of literature shows the UV spectro-photometric, HPLC analytical methods were developed on different wavelengths for analysis of Acyclovir in Human serum, Plasma, plasma fluids and pharmaceutical bulk drugs or sample tablet dosage form.[7-11] The rationale of this work is to develop a sensitive, simple, accurate, rapid, reproducible, precise and very cost effective spectro-photometric method for the quantitative determination of Acyclovir. By using this method, we determine Acyclovir in bulk drug and tablet dosage form and validation as per International Conference on Harmonization (ICH) Guideline.

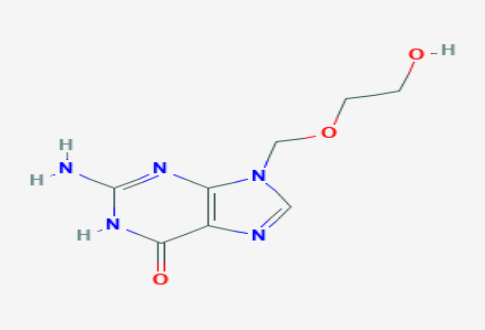

Fig. 1: Chemical structure of Acyclovir.

MATERIALS AND METHOD

Materials

Acyclovir was gifted from Healing Pharma Drugs and Pharmaceutical, Mumbai, India. The commercially available tablets Zoviclovir 800mg (Batch No. M13014), Triclovir 400 mg (Batch No.15621ME). And distilled water was obtained from the Water purification unit.

Instruments Used

Shimadzu UV1800 double beam spectrophotometer with UV Probe software version 2 was used to develop the analytical method. The instruments had automatic wavelength accuracy 0.1 nm and matched quartz cells with 1 cm cell path length, Ultrasonicator (OSCAR 220V, 2.5 L) and Weighing balance (KERN ABS-N) were used for this work.

Method Development

Preparation of standard stock solution

A Standard stock solution of Acyclovir was prepared by accurately weighing 50 mg Acyclovir in 50 ml of volumetric flask and dissolved in sufficient sulfuric acid (0.1 N) and volume make up with water to obtain a concentration 1 mg/ml or 1000 µg/ml (standard Stock I). Further diluting 2.5 ml of stock I solution to 25 ml mixture of distilled water to get desired concentration of 100 µg/ml (standard stock II).

Selection of wavelength for analysis of Acyclovir

The 10 µg/ml of Acyclovir solution is prepared by accurately measuring 1 ml of standard stock II solution, was transferred into a 10 ml volumetric flask and diluted to 10 ml with distilled water. This 10 µg/ml of Acyclovir solution was used for initial spectral scan in the UV range of 400-200 nm to detect wavelength at which maximum absorbance and this λmax is used for development of method, and further dilutions for linearity were prepared from the stock II solution by alligation method.

Preparation of serial dilutions

The series of different dilutions were prepared by using the standard stock II solution to get a respective concentration of 5. 6, 7, 8, 9 & 10 µg/ml.

Method Validation

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, ruggedness, sensitivity and specificity according to ICH Q2 (R1) guideline and USP guidelines.[12-13]

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration of an analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of an analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The linearity of the analytical method was demonstrated over the concentration range investigated by triplicate analysis (n = 3) at a concentration range of 5-10 µg/ml. The absorbance obtained at respective concentration was recorded, and the graph is plotted as concentration (µg/ml) versus absorbance. The linear regression equation and the coefficient correlation were obtained from the UV probe software

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. The accuracy of proposed method was determined on the basis of recovery study. Recovery study was carried out by spiking standard working solution to sample solution (formulation) at three different levels 80%, 100% and 120%. The final concentration of Acyclovir was determined at each levels of the amount; three determinations were performed. The percentage recovery was calculated as mean±standard deviation.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day precision study, three different solutions of same concentration were prepared and analyzed in the same day (morning, noon and evening), whereas in the inter-day precision study, the solutions of same concentration were prepared and analyzed, for three consecutive days, and the absorbance's were recorded. All study was performed in triplicates. The result was indicated by calculating % RSD.

Ruggedness

The ruggedness is a degree of reproducibility of test result under verification of condition like a different analyst, different instruments and different days. To establish ruggedness of the proposed method, the solutions of 10 µg/ml of standard Acyclovir solution was prepared and analyzed with the change in the different analyst.

Limit of Detection (LOD)

The LOD of the developed UV method was calculated by using following formula

$$LOD=3.3\times SD/S$$

Where, SD= Standard deviation of Y-intercepts

S= Slope

Limit of Quantitation (LOQ)
The LOQ of the developed UV method was calculated by using following formula
LOQ= 10×SD/S
Where, SD= Standard deviation of Y-intercepts
S= Slope

Assay of marketed tablet formulation
The Acyclovir content in its marketed formulation (Zoviclovir 800mg & Triclovir 400 mg) was estimated using pre-validated UV spectrophotometric method. Twenty tablets were accurately weighed, and average weight was calculated, they were crushed to fine powder. The powder equivalent to 50 mg Acyclovir was dissolved in sufficient amount of sulfuric acid (0.1 N) with the help of sonication and volume was made up using distilled water up to the mark of 25 ml volumetric flask. The solution was filtered using Whitman filter paper. This solution was further diluted to obtain 10 µg/ml concentration of the solution by using distilled water as a solvent (n=5) and observed by UV analysis.

RESULTS AND DISCUSSION

Selection of wavelength
The spectra of Acyclovir in methanol showed absorption at 253.2 nm shown in fig. 2, which is complying with reported λmax. Hence, it was selected as λmax of Acyclovir in sulfuric acid distilled water for further use.

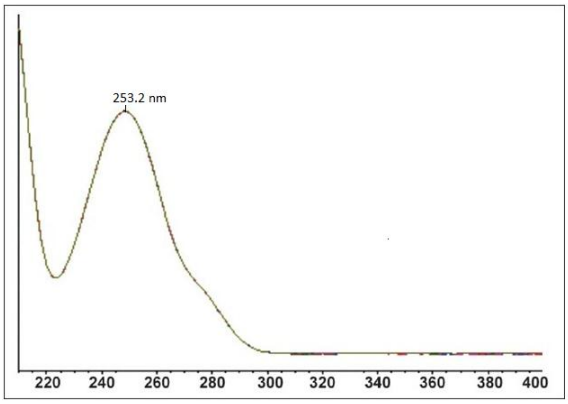

Fig. 2: UV spectrum of Acyclovir.

Preparation of calibration curve
Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and an equation stating correlation between concentration and the response. As compare to graphical method, above stated method is widely accepted and reproducible in nature. Considering the utility of quantitative analysis of Acyclovir, calibration curve for Acyclovir was developed using seven different calibration standards. The absorbance of different calibration standards at 253.2 nm was recorded using fixed wavelength mode of UV-Visible spectrophotometer. Calibration curve was repeated five times and the mean values ± deviation was reported as shown in Table 1.

Table 1: Calibration standard data for Acyclovir.

Concentration (µg/ml)	Absorbance
5	0.241±0.0028
6	0.401±0.0031
7	0.576±0.0042
8	0.755±0.0057
9	0.878±0.0061
10	1.013±0.0065

Method validation
Linearity and Range
Linearity and range are the key parameters of analytical method that demonstrates the limit within which the intended method is to be used for its optimum performance. Considering the prime importance of linearity and the range, seven point calibration curve of Acyclovir covering a range of 5-10 µg/ml was plotted. Details of concentrations and the respective mean absorbance values are depicted in Table 1. Calibration curve when subjected to least square regression analysis yielded an equation; y = 0.15628x+0.5281 with correlation coefficient 0.9978 shown in Figure 3. From the linearity study, it was revealed that, developed UV method was linear in the pre-defined concentration range of calibration standards.

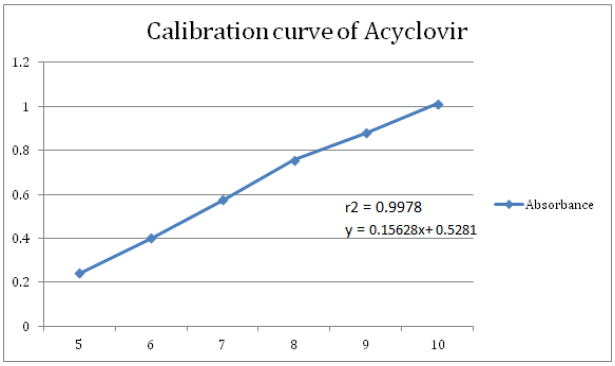

Fig. 3: Calibration curve for Acyclovir.

Accuracy
Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable. In case of UV method for Acyclovir, accuracy was established using recovery studies. At 80 % standard addition, mean recovery of Acyclovir was found to be 100.18% whereas at 100 and 120 % standard addition, it was found to be 100.26 and 99.30% respectively. % RSD was found to be less than 2 for the Acyclovir recovery studies as shown in Table 2. From the results of accuracy studies, it was observed that developed UV method is highly accurate as the percent recovery was in between 98 to 102% and the % RSD was well below 2%.

Table 2: Accuracy data of UV method for Acyclovir.

S No.	Concentration (%)	Origin level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % Recovery	% RSD
1	80	6	4.8	99.96	100. 18	0.6295
2	80	6	4.8	100.89		
3	80	6	4.8	99.69		
4	100	6	6	100.84	100.26	1.0746
5	100	6	6	99.02		
6	100	6	6	100.92		
7	120	6	7.2	100.7	99.87	0.7502
8	120	6	7.2	99.67		
9	120	6	7.2	99.24		

Precision

Precision is a measure of degree of scatter. It expresses the reproducibility of the measurements. It is expected that an analytical method should generate outcomes that are reproducible. Precise analytical method leads to accurate results. Considering the importance of reproducible yet accurate results, intra- and inter-day precision of developed UV method was established at 5, 7 and 10 µg/ml levels of Acyclovir. The results in terms of mean absorbance values, percent assay and % RSD for the intra- and inter-day precision study are demonstrated in Table 3 and Table 4 respectively. % RSD values of intra-day precision study were found to be in between 0.42 and 1.75 whereas those of inter-day precision study were in between 0.48 and 1.23. Overall, % RSD values of less than 2 showed the precision of developed UV method.

Table 3: Intra-day precision data of UV method for Acyclovir.

	Morning			Afternoon			Evening		
Concentration Range (µg/ml)	Amt. found	% Assay	% RSD	Amt. found	% Assay	% RSD	Amt. found	% Assay	% RSD
5	4.93	98.60	0.60	5.01	100.2	0.75	4.98	99.60	0.60
7	7.01	100.14	0.42	6.95	99.28	1.25	6.99	99.85	0.89
10	9.97	99.70	0.67	9.89	98.90	1.05	10.01	100.1	1.75

Table 4: Inter-day precision data of UV method for Acyclovir.

	Day 1			Day 2			Day 3		
Concentration Range (µg/ml)	Amt. found	% Assay	% RSD	Amt. found	% Assay	% RSD	Amt. found	% Assay	% RSD
5	5.04	100.8	0.89	4.99	98.80	0.60	5.05	101	1.05
7	7.03	100.42	0.73	6.98	99.71	0.49	6.94	99.14	1.23
10	9.98	99.80	0.48	9.95	99.50	0.89	9.97	99.70	1.05

Ruggedness

Ruggedness of analytical method is the degree of reproducibility of test results obtained by analysis of the same samples under a variety of conditions, such as different laboratories, different analyst, different instruments, different lots of reagent, different temperatures etc. In order to determine the ruggedness of proposed spectro-fluorimetric method, Acyclovir solutions were prepared and analyzed by different analysts. Sample analysis and data processing resulted into % RSD values between 0.30 and 0.34. Results of ruggedness studies revealed that proposed spectrofluorimetric method was rugged as it showed % RSD values less than 2 (Table 5).

Table 5: Ruggedness data of Spectrofluorimetric method for Acyclovir.

S. No.	Concentration (µg/ml)	Analyst	Amt. found	% Amt. found	% RSD
1	7	I	6.98	99.60	0.3409
2	7	II	6.99	99.80	0.3008

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

LOQ represents the lower most concentration that can be analyzed with acceptable accuracy and precision. Generally, LOQ is the first calibration standard. LOD and LOQ of proposed UV method was found to be 0.456 and 0.956 µg/ml respectively as shown in Table 6. Lower LOQ value indicated that proposed method would be suitable for analyzing the samples containing even small quantities of Acyclovir.

Table 6: LOD, LOQ data of Acyclovir.

1	LOD	0.456 µg/ml
2	LOQ	0.956 µg/ml

Estimation of Acyclovir content in marketed formulation

The developed UV method was successfully applied for estimation of Acyclovir content in Zoviclovir 800mg and Triclovir 400 mg. By proposed UV method, Acyclovir content in the tablet was found to be 98.38± %. & 99.74± % respectively.

CONCLUSION

The sensitive, simple, rapid, precise, and economical spectrophotometric method has been developed for the quantitative estimation of Acyclovir in Bulk and pharmaceutical formulation. The method is validated as per the ICH and USP guidelines, and it is found that the developed method is robust and sensitive. Hence, this method can be successfully and suitably acquired for routine quality control analysis of Acyclovir in bulk and pharmaceutical dosage form.

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