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Research Article

UV-SPECTROPHOTOMETRIC ESTIMATION OF ACYCLOVIR IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Narayana Raju Padala[1], Dey Baishakhi[2], Fathi H Assaleh[3], Prakash Katakam[3,4]* and Babu Rao Chandu[3]
[1]KLR College of Pharmacy, Khammam, India
[2]School of Medical Science and Technology, IIT Kharaghpur, India
[3]Faculty of Pharmacy, University of Zawia, Zawia, Libya
[4]Nirmala College of Pharmacy, Guntur, India
*Corresponding Author Email: pkatakam9@gmail.com
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ABSTRACT
Analytical method development being a vital part of pre formulation-formulation research and development obviates the need to develop reliable, effective, eco friendly and cost effective methodologies for routine analysis of active pharmaceutical ingredients. UV spectroscopy is one of the earliest, yet of wide applications in drug analysis in different stages of formulations and quality control; despite the availabilities of sophisticated chromatographic techniques and other hyphenated techniques. Current research attempts to develop simple, sensitive, accurate, precise and economical UV spectrophotometric methods for the routine analysis of acyclovir in bulk and pharmaceutical dosage forms using two separate alkaline media, 0.1N NaOH (method A) and 0.1N KOH (method B) and validate them as per ICH guidelines. In both the methods maximum absorbance was observed at 264 nm. Beer's law was obeyed in the concentration of 2.5-40 µg / mL in method A and 2.5-30 µg / mL in method B with correlation coefficient of 0.999. The % recovery carried out by adding known amount of standard drug to pre-analyzed tablet solutions was 98.75 ± 0.52 % to 99.78 ± 0.69 % (method A) and 98.55 ± 0.31 % to 99.78 ± 0.22 % (method B). Intra and interday precision expressed in % RSD were 0.38 ± 0.01 and 0.27 ± 0.02 - 0.44 ± 0.01 respectively and the percent purity was 99.85 ± 0.05 %. The methods were validated statistically as per ICH guidelines and the results obtained were within the acceptance criteria for the parameters relating to linearity, accuracy, precision.
Keywords: Acyclovir, dosage forms, UV spectrophotometric, alkaline medium, validation.

INTRODUCTION

Development of a properly validated, stability indicating, specific analytical method for the routine analysis of drugs is of imperative necessity since the design of the drug delivery system is related to it. Analytical method development is an integral part of pre formulation and formulation development research[1]. Sophisticated chromatographic methods with HPLC, HPTLC which are being employed for analysis are relatively expensive; many methods necessitate analyte extraction from respective sample matrices and hence complicated sample preparation steps, use of internal standards for analysis thus increasing the time required and error in recovery[2]. Hence there is always a need to develop a simple, sensitive, cost effective and less time consuming method for the selected drug to aid during various steps of formulation design. UV-vis spectrophotometric method is one of the earliest, yet easy, sensitive, relatively cost effective method applied for drug estimations in both small and large scale RandDs[1-3]. Acyclovir (ACV), chemically known as 9-[(2-hydroxyethoxy) methyl] guanine is a purine nucleoside analog active against *Herpes Simplex* Virus (HSV) both type 1 and type 2 and marketed under trade names like Zovirax, Zovir (GSK), Acivir, Axovir, Herpex etc. ACV is official in USP and BP, very selective in action and low in cytotoxicity (Figure 1)[1-3]. ACV interacts with HSV thymidine kinase and DNA polymerase and inhibits viral DNA synthesis. Cellular enzymes convert ACV to ACV-triphosphate which competitively inhibits viral DNA polymerase and acts as a chain terminator when incorporated into viral DNA. ACV causes suicide inactivation when the terminated DNA template containing ACV binds the viral DNA polymerase leading to its irreversible inactivation[3-5]. Literature surveys have reported some HPLC and immunoassay techniques, specctrofluorometry and UV-spectrophotometric techniques for the analytical estimation of ACV[1,6-9]. Some of the spectrophotometric methods are based on oxidative-coupling reactions using mostly ceric ammonium sulphate or potassium persulfate as oxidizing reagents followed by coupling with 3-methylbenzothiazoline 2-one hydrazone (MBTH reagent) to form colored chromogens detectable at 630 nm. Another method reported oxidative-coupling reaction with MBTH in presence of $FeCl_3$ and the deep green colored species detectable at 616 nm[3,10-12]. One method also mentioned about the reaction of ACV with metals like Cu (II) and Co (II) using borax or NaOH buffers in nonaqueous media and the absorbance's of the formed complexes measured at 290 nm[3]. The objective of the current study was to develop a simple, accurate UV-method in two separate alkaline media for the routine estimation of ACV with minimum processing steps.

MATERIALS AND METHODS

Chemicals

Acyclovir was a kind gift sample from Torrent Pharma Ltd., Ahmedabad. NaOH and KOH (AR grades) and acyclovir tablets were purchased from local market. All reagents were prepared a fresh with double distilled water.

Instrumentation

All the spectral and absorbance measurements were made on an ELICO SL-59, UV-VIS spectrophotometer by using 1 cm match quartz cells.

Method Development

An accurately weighed amount of acyclovir (pure and tablet powder) equivalent to 100 mg was dissolved 100 mL of 0.1N

NaOH (method A) and 100 mL of 0.1 N KOH (method B) and further dilutions were made with 0.1 N NaOH and 0.1 N KOH in method A and method B respectively. A series of standard solutions containing 2.5-40 µg / mL of acyclovir were prepared in 0.1 N NaOH and a series of standard solutions containing 2.5-30 µg / mL of acyclovir were prepared in 0.1 N KOH and absorbances were measured at 264 nm against reagent blank. Recovery studies were carried out by adding a known quantity of pure drug to the pre-analyzed formulation and following the same methodology. Percentage recovery was calculated from the amount of drug found.

Method Validation

Method validation is the process of establishing documented evidence to provide a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The analytical method development of ACV in bulk and pharmaceutical dosage forms was validated in terms of accuracy, intra and inter day precision, linearity and percent recovery[13,14].

Linearity

Appropriate concentrations of stock solutions were assayed as per the developed methods. Beer-Lambert's concentration ranges were found to be 2.5-40 µg / mL in (method A) and 2.5-30 in (method B).

Accuracy

Accuracy was determined by performing recovery studies on marketed formulations (tablets) and for prepared solutions containing known amount of drug by standard addition method in which standard drug were added at three different concentration levels (80 %, 100 %, 120 %) to pre analyzed samples as per ICH guidelines. The recovery studies were carried out in triplicates at each level.

Precision (intraday and inter day)

Precision was demonstrated by intraday and inter day variation studies. Intraday precision was determined by taking different solutions of same concentration (5 µg / mL) and analyzed thrice a day, results indicated by % RSD. In the inter day study solutions of same concentration (5 µg / mL) were prepared, analyzed and results presented as % RSD.

Drug Content Estimation in Formulations

ACV content in the marketed formulations was estimated by this method. Twenty tablets were weighed; the mean weight was determined and finely powdered. An accurately weighed tablet powder equivalent to its labeled amount (200, 400 or 800 mg) of ACV was transferred into 100 mL volumetric flask containing either 0.1 N KOH or NaOH and sonicated for 10 minutes. After achieving complete solubility of the drugs, the volume was made up to the mark using the alkali solution. The resulting solution was filtered through 0.45 µm membrane filter. From the filtrate, a 5 mL of solution was transferred into 10 mL volumetric flask and volume was made up to mark with alkali solution to obtain the concentration of 25 µg mL^{-1} ACV which were then subjected to proposed methods and the amount of ACV was determined using calibration curves using the two developed methods.

Recovery Studies

To further validate the accuracy of the method developed, analytical recovery studies were performed by adding known amount of pure drug to pre-analyzed samples of the tablet formulations with 2, 10 and 20 µg / mL concentration range. Percent analytical recovery values were obtained by comparison between concentrations obtained from spiked samples against actual added concentrations (2, 10 and 20 µg / mL) covering the specified range.

RESULTS

Calculation of percent purity

$$\%\ \text{purity} = \frac{A}{A}\%1cm \times \frac{1}{100} \times \frac{250}{wt\ .taken} \times \frac{50}{5} \times \frac{50}{5}$$

$$\%\ \text{purity} = \frac{\text{amount present}}{\text{dose of tablet}} \times 100$$

The percent purity of the drug was found to be 99.85 ± 0.05 %. Data for optical characteristics and linearity of ACV are presented in Table 1 and the calibration curve of ACV for method A and method B in Figure 2.

Table 1: Optical Characteristics and Linearity of Acyclovir

Parameters	Method A	Method B
Maximum absorbance (λ_{max}, nm)	264	264
Beer's law limits (µg / mL)	2.5-40 µg / mL	2.5-30 µg / mL
Sandell's sensitivity (µg / cm^2 / 0.001 absorbance unit)	0.01930	0.01780
Molar extinction coefficient (L $mole^{-1}$ cm^{-1})	1.156 x 10^4	1.257 x 10^4
% Range of error		
0.05 confidence limits	0.24750	0.26422
0.01 confidence limits	0.37594	0.39091
Correlation coefficient (r)	0.9998	0.9996
Regression equation (Y^*)		
Slope (a)	0.0518	0.5657
Intercept (b)	-0.00114	-0.00242

$Y^* = b + aC$, where C is concentration in µg / mL and Y is absorbance unit.

Table 2: Estimation of Acyclovir from its Pharmaceutical Formulations

Sample	Label Amount (mg)	Amount obtained (mg)		% Recovery by proposed method	
		Method A	Method B	Method A	Method B
Tablet1	200	197.5 ± 0.9	197.1 ± 0.5	98.75 ± 0.52	98.55 ± 0.31
Tablet 2	400	393.0 ± 1.2	396.2 ± 1.7	98.25 ± 0.35	99.05 ± 0.19
Tablet 3	800	798.3 ± 1.6	796.9 ± 1.8	99.78 ± 0.69	99.78 ± 0.22

Table 3: Table for Accuracy Data of Acyclovir

Sample No. (%)	Conc (µg / mL)	Pure drug	% Recovery	Statistical mean	Standard deviation (± SD)	% Recovery SD
S1:80	10	6	0.0681	0.0681	1.0×10^{-4}	0.15
S2:80	10	6	0.0682			
S3:80	10	6	0.0680			
S4:100	10	8	0.0821	0.0820	1.5×10^{-4}	0.19
S5:100	10	8	0.0822			
S6:100	10	8	0.0819			
S7:120	10	10	0.0979	0.0977	1.5×10^{-4}	0.16
S8:120	10	10	0.0978			
S9:120	10	10	0.0976			

Table 4: Table for Interday Precision of Acyclovir

Sample No. (5 µg / ml)	Abs 1	Abs 2	Abs 3	Average % RSD
S1	0.0424	0.0425	0.0424	0.13
S2	0.0426	0.0425	0.0425	0.13
S3	0.0423	0.0423	0.0424	0.14
S4	0.0427	0.0426	0.0427	0.13
S5	0.0422	0.0423	0.0424	0.23
S6	0.0424	0.0424	0.0425	0.14
% RSD	0.44	0.28	0.27	

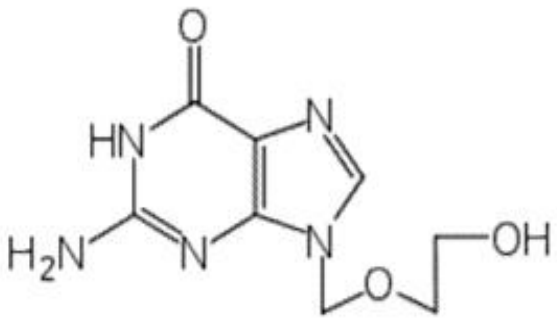

Figure 1: **Structure of Acyclovir**

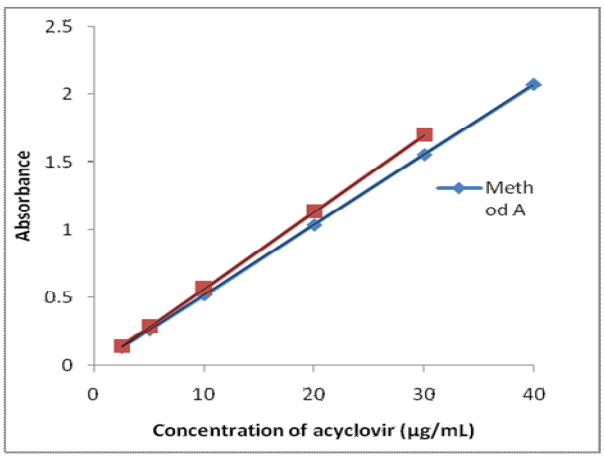

Figure 2: Calibration Curves of Acyclovir for Developed Methods A and B

The values obtained for the determination of acyclovir in tablet formulations by the proposed method were provided in Table 2. To evaluate the validity and reproducibility of the methods known amounts of pure drug was added to previously analyzed pharmaceutical preparations and the mixtures analyzed by proposed method and the percent recoveries are given in Tables 2. Results of validation studies relating to accuracy, precision (intra- and inter day) are presented in Tables 3 and 4. For intraday precision studies average of 8 samples were taken with mean value (0.0424 ± 0.02), SD values (1.6×10^{-4}) and % RSD (0.38 ± 0.01). The relationship amongst different RSD values i.e. RSD amongst samples and RSD amongst different absorbances are presented in Table 4.

DISCUSSION

The purpose of the current research was to develop an economical, easy, time saving, reliable UV spectroscopic method for the routine estimation of ACV in bulk and pharmaceutical formulations. As per the developed methods, Beer's law was obeyed in the concentration range of 2.5-40 µg / mL in (method A) and 2.5-30.0 µg / mL in (method B) with a correlation coefficient (r) of 0.9998 and 0.9996 for methods A and B respectively, indicating a good linearity of the developed methods. Sandell's sensitivity of 0.01930 µg / cm^2 / 0.001 (method A) and 0.01780 µg / cm^2 / 0.001 (method B) absorbance units showed that the methods was quite sensitive. Method validations followed by statistical analysis were done as per ICH guidelines[13,14]. The percentage recovery studies carried out by adding known amount of standard drug to pre-analyzed tablet solutions were 98.75 ± 0.52 % to 99.78 ± 0.69 % (method A) and 98.55 ± 0.31 % to 99.78 ± 0.22 % (method B). Accuracy studies carried out with different concentrations levels (80, 100 and 120 %) showed the percentage recovery SD values of 0.15-0.19 %. Results of the intra- and interday precision studies expressed in % RSD were 0.38 ± 0.01 and 0.27 ± 0.02 - 0.44 ± 0.01; respectively[10-14]. Thus the developed methods were found sensitive, accurate, precise and reproducible and could be used for the routine estimations of acyclovir in bulk and pharmaceutical formulations. Moreover the methods are very simple, use of analytical grade lab reagents makes it more cost effective, doesn't require any sample pre treatment or any complicated methods of sample preparation and expected to be beneficial in small and large analytical and formulation RandDs.

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