International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1512-1518 www.ijprajournal.com ISSN: 2456-4494

Method development and validation of acyclovir in bulk form by uv-visible spectroscopy

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Date of Submission: 15-08-2024 Date of Acceptance: 25-08-2024

ABSTRACT: An analytical method development and validation of Acyclovir was performed using UV- visible spectrophotometer according to the ICH guidelines .The Acyclovir is an antiviral agent. The Acyclovir is validated using 0.1N HCL as a solvent on the basis of solubility studies. The maximum absorption of Acyclovir was found at 299nm respectively .The linearity range was given at 10- 50µg/ml and represented on the graph. The method was validated for accuracy, precision, linearity, ruggedness .For accuracy it was 50%, 100%, and 150% and the %RSD values are obtained within limits. For precision i.e., intraday precision and Interday precision was performed and the %RSD values obtained are within the limits. For ruggedness it is performed as analyst 1, 2, 3 respectively and the % RSD values obtained are within the limits .The proposed method was statistically validated for relative standard deviation and the results were within the range. Hence the method was simple, cheap, economical, cost effective and robust.

KEYWORDS:UV-Visiblespectroscopy, Acyclovir

I. INTRODUCTION

UV-VISIBLE SPECTROSCOPY

Ultraviolet and visible spectroscopy, also known as electronic spectroscopy, is used to measure the number of double bonds and aromatic conjugation in a molecule. Spectroscopy is the measurement and interpretation of electromagnetic radiation that is absorbed or emitted when molecules or atoms or ions in a sample move from one energy state to another.

UV spectroscopy is a type of absorption spectroscopy in which a molecule absorbs light in the ultraviolet range (200-400 nm), as a result of which electrons are excited from the ground state to a higher energy state. The ultraviolet range corresponds to 400-200 nm and the visible range to 800-400 nm.

INTRODUCTON TO METHOD DEVELOPMENT AND VALIDATION

Method Development

It refers to the process of designing and optimizing analytical methods to accurately and reliably measure specific compounds or properties in a sample

Basic criteria for new method development of drug analysis

The drug or drug combination may not be official in any pharmacopoeias.

A proper analytical procedure for the drug may not be available in the literature due to patent regulations; Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.

Analytical methods for the quantization of the drug in biological fluids may not be available, Analytical methods for a drug in combination with other drugs may not be available the existing analytical procedures may require expensive reagents and solvents.

It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Steps involved in method development

1. Analyte standard characterization
2. Method requirements
3. Literature search and prior methodology
4. Choosing a method
5. Instrumental setup and initial studies
6. Optimization
7. Documentation of analytical figures of merit
8. Evaluation of method development with actual samples
9. Determination of percent recovery of actual sample and demonstration of quantitative sample analysis

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Method Validations

Analytical Method Validation can be defined as (ICH) "Establishing documented evidence, which provides 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 main aim of method validation is to produce proof that the method will what it is supposed to do, accurately, reliable and consistent.

The validation parameters as per ICH guidelines are described below:

(1) Linearity
(2) Accuracy
(3) Precision
(4) Detection Limit (LOD)
(5) Quantitation Limit (LOQ)
(6) Ruggedness

Table 1: Acceptance Criteria for the different characteristics of validation by ICH

Characteristics	Acceptance Criteria
Linearity	Linearity r2 ≥0.99, similar response ratios
Precision-System	Precision-System RSD<2%
Precision-Method	Precision-Method RSD<2%
Accuracy	Accuracy FDA 98-102%, EPA 50-150%
Specificity	Specificity No interference
Detection Limit	Detection Limit >2 times base line
Quantitative Limit	Quantitative Limit Signal-to-Noise=10:1

DRUG PROFILE

Name: Acyclovir

IUPACNAME:

2-amino-9-(2-hydroxyethoxymethyl)-1H-purin- 6-one

Molecular formula: C8H11N5O3

Molecular weight: 225.20g/mol.

Structure:

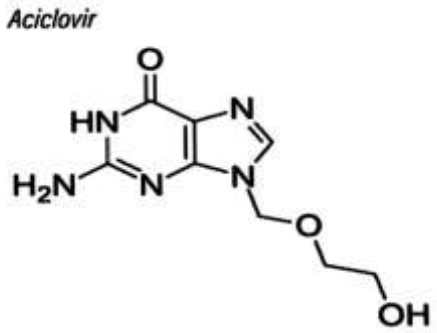

Figure 1: structure of acyclovir

Mechanism of action:

Acyclovir is a synthetic purine nucleoside analogue with in vitro and in vivo inhibitory activity against herpes simplex virus types 1 (HSV-1), 2 (HSV-2), and varicella-zoster virus (VZV). The inhibitory activity of acyclovir is highly selective due to is affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV.

Acyclovir + ATP becomes acyclovir monophosphate due to the action of viral HSV-thymidine kinase. Acyclovir monophosphate is converted to the diphosphate form by guanylate kinase. Acyclovir diphosphate is converted to acyclovir triphosphate by cellular kinase, nucleoside diphosphate kinase, pyruvate kinase, creatine kinase, phosphoglycerate kinase, succinyl-CoA synthetase, and phosphoenolpyruvate carboxykinase and adenylosuccinate synthetase. Acyclovir triphosphate has higher affinity for viral DNA polymerase than cellular DNA polymerase and incorporates into the DNA where the missing 2' and 3' carbons causes DNA chain termination. In other cases acyclovir triphosphate competes so strongly for viral DNA polymerase that other bases cannot associate with the enzyme, inactivating it. Acyclovir triphosphate in the presence of HSV DNA polymerase binds with and gets incorporated in viral DNA and stops lengthening of DNA strand. The terminated DNA inhibits the DNA polymerase irreversibly. And also Acyclovir triphosphate inhibit

herpes virus DNA polymerase competitively. It inhibit viral DNA synthesis and viral replication process.

Acyclovir inhibits viral DNA synthesis . Its selectivity of action depends on interaction with two distinct viral proteins. Cellular uptake and initial phosphorylation are facilitated by HSV thymidine kinase. The affinity of acyclovir for HSV thymidine kinase is about 200-fold greater than for the mammalian enzyme. Cellular enzymes

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convert the monophosphate to acyclovir triphosphate, which is present in 40- to 100- fold higher concentrations in HSV-infected than in uninfected cells, and competes for endogenous deoxyguanosine triphosphate (dGTP).

Uses:

Acyclovir is used to treat the symptoms of chickenpox, shingles, herpes virus infections of the genitals (sex organs), the skin, the brain, and mucous membranes (lips and mouth), and widespread herpes virus infections in newborns. 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. It is also the drug of choice for treatment of herpes simplex encephalitis.

EXPERIMENTAL METHODOLOGY

Materials and methods

Instruments

The present work was carried out with UV-Visible spectrophotometer having double beam detector configuration. The absorption spectra of reference and test solution were carried in a 1cm quartz cuvette over the range of 200-800nm.

Chemicals

Acyclovir (API) was procured from pharma industry in Hyderabad.

Solubility study

Few gm. of Acyclovir (API) dissolved in few ml of 0.1N HCL in a beaker stirred for few minutes at room temperature and examined visually.

Method development

Preparation of standard stock solution of ACYCLOVIR:

Accurately weighed 100mg of Acyclovir drug and transferred to 100ml of volumetric flask and dissolved in few ml of solvent0.1N HCL and volume was made up to the mark with 0.1N HCL.

Determination of wavelength of maximum absorbance (λmax):

Pipette out 10ml of standard stock solution (having conc.1000µg/ml) and transfer it into 100ml volumetric flask and volume was made up to 100ml with 0.1N HCL Then from solution having conc.100µg/ml, 1ml of the solution is taken in 10ml volumetric flask and then volume was made up to the mark with 0.1N HCL. The absorbance of this solution was scanned in the U.V range of 200-400nm against 0.1N HCL as a blank. The λ max obtained was 299nm.

Method validation:

Linearity

Form solution having conc.100µg/ml, various conc ranges 10 µg/ml - 50 µg/ml solution was prepared and linear relationship was observed between absorbance and concentration.

Precision:

Precision of the methods was studied as intra-day, inter-day.

Intra-day Precision- Intra-day study was performed by examining a 10µg/ml, concentration of drug for three times in the same day.

Inter-day Precision– Inter-day precision was performed by examining a 10µg/ml, concentration of drug for three times in next day.

Accuracy

The Accuracy of the method was evaluated by recovery studies at three different levels i.e. 50%, 100%, and 150%. The recovery studies were carried out by the addition of a known amount of standard solution of acyclovir to pre-analyzed solutions. The resulting solutions then re-analyzed by the proposed method.

Limit of detection (LOD)

LOD for Acyclovir by the proposed method was determined on the response and slope of the regression coefficient.

$LOD = 3.3 \times \sigma/S$

Where, σ = standard deviation, S = linearity curve slope

Limit of quantization (LOQ)

Limit of quantization for acyclovir by the proposed method was determined on the response and slope of the regression coefficient.

$LOQ = 10 \times \sigma/S$

Where, σ = standard deviation, S = linearity curve slope.

Ruggedness

The ruggedness is a degree of reproducibility of test result under verification of condition such as a different analyst, different instruments, and different days. To determine ruggedness of the proposed method, the sample

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solutions of 10µg/ml of Acyclovir was prepared by different analysts and analyzed.

II. RESULT AND DISCUSSION

Solubility study:

0.1N HCL was selected for the study of Acyclovir as the drug was freely soluble in it.

Determination of wavelength of maximum absorbance (λ max):

The absorbance of 100µg/ml solution was scanned in the U.V range of 200-400nm against 0.1N HCL as a blank. The λ max obtained was 299nm.

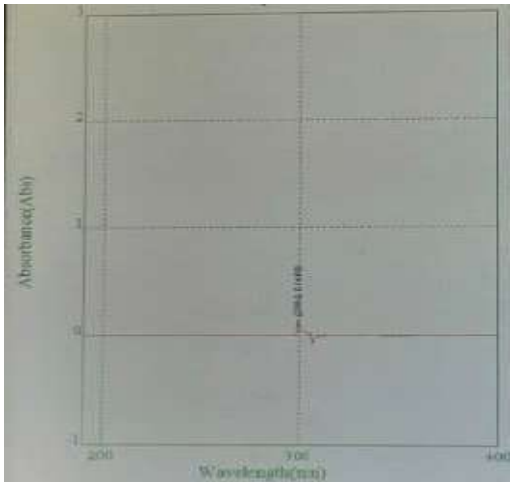

Figure 2: UV Spectrum of Acyclovir

Method validation:

Linearity

The linearity of this technique was found at concentrations between 10 and 50 µg/ml. The calibration curve was obtained by plotting the absorbance v/s concentration data. The calibration equation for Acyclovir obtained was y = 0.009x + 0.01 with the calibration coefficient R^2 = 0.99.

Table 2: Data for linearity of Acyclovir

concentration	Absorbance
10 µg /ml	0.12
20 µg /ml	0.19
30 µg /ml	0.26
40 µg /ml	0.38
50 µg /ml	0.46

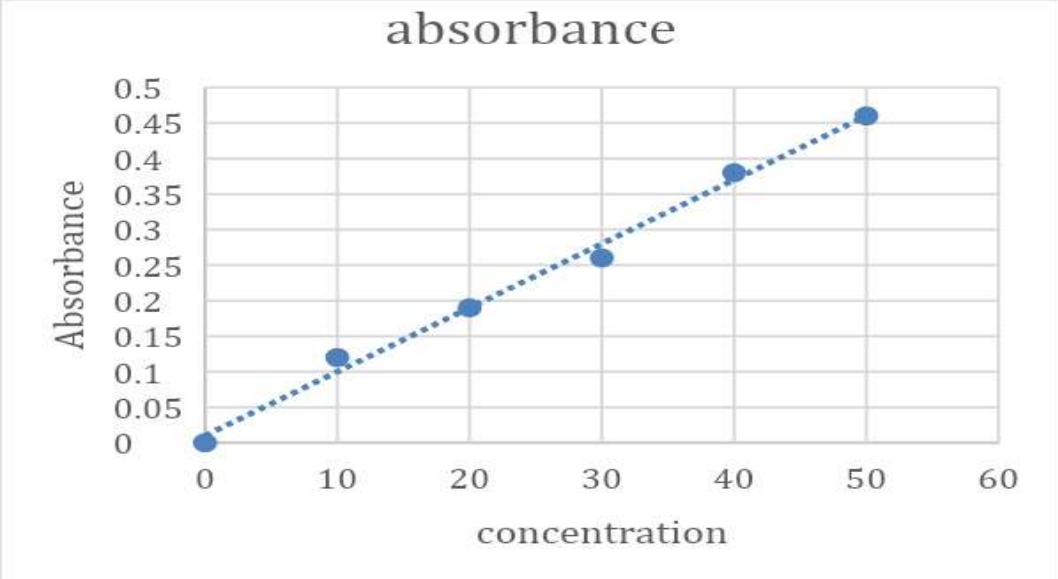

Figure 3: Calibration Curve of Acyclovir

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Table 3: Optimization Parameters of Acyclovir

Parameters	Method values
Maximum wavelength	**299nm**
Beer's Law	**10-50 µg /ml**
Correlation coefficient (r^2)	**0.99**
Regression Equation	**y = 0.009x+0.01**
Slope(m)	**0.009**
Intercept(c)	**0.01**

Precision:

The precision (measurement of intra-day, inter-day,) results showed good reproducibility, %RSD was below 2%.

Intra-day precision

Table 4: Intra-day precision results of Acyclovir

Intra-day precision results			
Conc.	Absorbance		
	1hr	2hr	3hr
10 µg	0.125	0.202	0.298
10 µg	0.127	0.202	0.302
10 µg	0.128	0.205	0.305
10 µg	0.128	0.206	0.305
10 µg	0.129	0.206	0.305
10 µg	0.129	0.211	0.307
Absorbance Mean	0.127666667	0.20533	0.30367
Absorbance SD	0.001505545	0.00333	0.0032
% RSD	1.179278307	1.62013	1.05516

Inter-day precision:

Table 5: Inter-day precision results of Acyclovir

Inter-day precision results			
Conc.	Absorbance		
	Day 1	Day 2	Day 3
10 µg	0.125	0.197	0.175
10 µg	0.127	0.201	0.175
10 µg	0.127	0.203	0.176
10 µg	0.128	0.205	0.176
10 µg	0.129	0.207	0.18
10 µg	0.129	0.207	0.183
Absorbance Mean	0.1275	0.20333	0.1775
Absorbance SD	0.001516575	0.00388	0.00327
% RSD	1.189470658	1.90897	1.84287

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Accuracy:
The accuracy study was performed at three levels 50%, 100% and 150%. The results are shown in table.

Table 6: Accuracy results of Acyclovir

Accuracy					
Conc taken 50%(5 µg /ml)		**Conc taken 100%(10 µg /ml)**		**Conc taken 150%(15 µg /ml)**	
Absorbance	**Conc.found**	**Absorbance**	**Conc.found**	**Absorbance**	**Conc.found**
0.249	4.78	0.283	9.71	0.318	14.78
0.251	5.07	0.283	9.71	0.318	14.78
0.251	5.0	0.286	10.14	0.325	15.79
Mean conc	4.97	**Mean conc**	9.85	**Mean conc**	15.12
SD of conc	0.167	**SD of conc**	0.251	**SD of conc**	0.585
%Recovery	99.51	**%Recovery**	98.55	**%Recovery**	100.80

Limits of detection and Quantitation (LOD and LOQ):
The LOD and LOQ were 0.358µg/ml and 0.720µg/ml respectively.

Ruggedness:
Ruggedness of the proposed method was determined by analysis of sample solution (10µg/ml) prepared by different analysts.

Table 7: Ruggedness results of Acyclovir

Ruggedness			
Conc.	Absorbance		
	analyst 1	analyst 2	analyst 3
10	0.127	0.128	0.133
10	0.127	0.129	0.133
10	0.128	0.129	0.133
10	0.128	0.131	0.137
10	0.131	0.133	0.137
10	0.131	0.133	0.139
mean	0.128666667	0.1305	0.135333333
SD	0.001861899	0.00216795	0.00265832
% RSD	1.447071507	1.66126309	1.964276063

III. CONCLUSION

The method that was developed for measuring acyclovir in API was simple, accurate, sensitive, repeatable, and inexpensive. In compliance with ICH criteria, the method's Linearity, Range, Precision, Accuracy, Limits of detection and Quantitation (LOD and LOQ) and ruggedness were all validated. Linearity and Range were assessed by evaluating the method's response at various concentration levels, ensuring consistent performance across a specified range. Precision was demonstrated through repeated measurements, which yielded consistent results, highlighting the method's repeatability. Accuracy was confirmed by comparing the measured values to known standards, showcasing the method's reliability. The Limits of Detection and Quantitation were determined to ensure that even the smallest amounts of acyclovir could be accurately detected and quantified. Ruggedness was evaluated by testing the method under different conditions and by different analysts. The proposed UV spectroscopic approach can be used for routine examination of acyclovir, based upon the results obtained.

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