CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1427507

Available online at: http://www.iajps.com Research Article

# STABILITY INDICATING METHOD DEVELOPMNT AND VALIDATION OF UV METHOD FOR THE DETERMINATION OF ACYCLOVIR IN TABLET DOSAGE FORM

**Mrs. Prabhat Dessai* and Nirupa Fatrekar**

Postgraduate Department of Chemistry, Dnyanprassarak Mandal's College and Research Centre, Assagao Bardez - Goa, India.

**Abstract:**

*Acyclovir is an Antiviral medication. It is primarily used for the treatment of herpes, Simplex virus infections, Chickenpox, and shingles. Simple, precise, accurate and economical UV spectrophotometric methods have been developed and validated for the routine estimation of Acyclovir in bulk and pharmaceutical Dosage form. The Stability indicating method was developed and validated in the present work. The parameters Linearity, Precision, Accuracy, Robustness, LOD, LOQ, system suitability was studied as per the ICH guidelines. Acyclovir shows maximum wavelength at 252 nm. The method was found to be specific without any interference and precise with %RSD less than 2. The LOD & LOQ were found to be 0.030 µg/ml and 0.062 µg/ml. The robustness results were satisfactory by changing the λ max by ± 2nm. The accuracy of the method was determined by recovery studies obtained as 99.72%. The drugs showed degradation for acid, base and peroxide and no degradation for photolight while performing forced degradation studies. Thus the proposed method was found to be accurate, simple & can be used for routine analysis.*

**Keywords:** *UV Spectrophotometry, Acyclovir, Pharmaceutical Dosage form.*

**Corresponding author:**

**Mrs. Prabhat Dessai*,**

Postgraduate Department of Chemistry,

Dnyanprassarak Mandal's College and Research Centre,

Assagao Bardez - Goa, India.

**Email:** desaiprabhat@yahoo.com

QR code

*Please cite this article in press Prabhat Dessai and Nirupa Fatrekar.,* ***Stability Indicating Method Developmnt and Validation of UV Method for the Determination of Acyclovir in Tablet Dosage Form.,*** *Indo Am. J. P. Sci, 2018; 05(09).*

## INTRODUCTION:

Acyclovir is an Antiviral medication. It is primarily used for the treatment of herpes, Simplex virus infections, Chickenpox, and shingles. 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. Acyclovir is converted by viral thymidine kinase to acyclovir monophosphate, which is then converted by host cell kinases to Acyclovir triphosphate (ACV-TP). ACV-TP, in turn, competitively inhibits and inactivates HSV- specified DNA polymerases preventing further viral DNA synthesis without affecting the normal cellular processes.

Literature survey shows different HPLC methods for the estimation of Acyclovir. Several UV methods have been reported for estimation of acyclovir with water in bulk and in pharmaceutical dosage form. Present work includes estimation of Acyclovir in bulk and in pharmaceutical dosage form, in alkaline medium and analysing it on UV spectrophotometer and also stability studies were carried out.

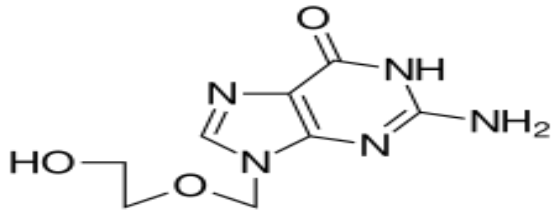



**Figure 1: structure of Acyclovir**

**Instrumentation:-**

UV visible Spectrophotometer (Make- LABINDIA UV 3000$^{+}$) was used with 1cm quartz cuvettes to record the UV spectra of Acyclovir (ACY). A digital weighing balance (make- Shimadzu Corporation) was used for all preparations. Sonicator was used for the sonicating the solution. DBK- MINI MAG STIRRER was also used.

**Chemicals and reagents:-**

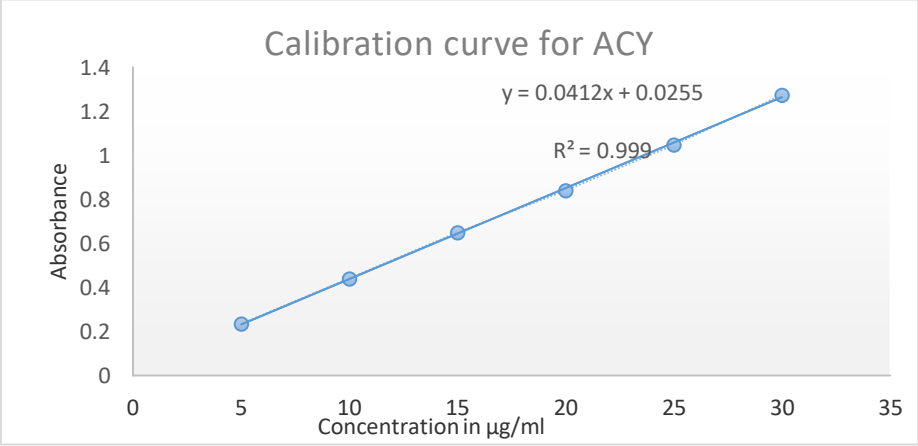
The reference samples of Acyclovir were obtained from CIPLA. LTD kumrek, Rangpo, Sikkim India. Hydrogen Peroxide solution was purchased from Finar chemicals PVT Ltd. Gujrat India. Sodium Hydroxide pellets extra pure AR was purchased from Sisco Research Laboratories PVT. Ltd. HCL was purchased from Loba Chemie PVT. Ltd. Acivir-200 DT (200mg) was purchased from local market.

**Preparation of stock solution:-**

100 mg of ACY was accurately weighed and transferred into 100 ml standard volumetric flask. To that 80 ml 0.1N NaOH was added & sonicated for 10 min. After 10 min volume was made up to the mark with the solvent. Filter the above solution through Whatmann no.42 discard first few drops. Pipette out 10 ml into 100 ml std. flask and make further dilution to get 10 µg/mL. That solution was used as a stock solution.

**Selection of the wavelength:-**

Working standard solutions of 10 µg/mL were scanned in the entire UV range of 400-200 nm to obtain the absorbance spectra. The drug shows maximum absorption at 252 nm. Six working standard solutions for drug having concentration 5, 10, 15, 20, 25 and 30 µg/mL were prepared in 0.1N NaOH 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.




**Graph 1:- Calibration curve for Acyclovir**

**Sample preparation:-**
Twenty tablets were weighed accurately and reduced to fine powder; drug equivalent to 100 mg of acyclovir was weighed and dissolved in 80 ml of 0.1 N NaOH in a 100ml volumetric flask, sonicated for about 10min and final volume was made with 0.1N NaOH. The above solution was filtered by using Whattmann filter paper No.42. From the above filtrate 10 mL of solution was diluted to 100 mL with 0.1N NaOH to get 10 µg/mL of Acyclovir. Absorbance of above solution was found at 252 nm. This procedure was followed for the various validation parameters such as specificity, accuracy, linearity, precision, robustness, LOQ, LOD and stability.

**Method validation:**
As per the International Conference on Harmonization (ICH) guidelines, the method validation parameters like linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and specificity were experimentally determined and the method was validated.

**System suitability:**
System suitability parameter was studied as per ICH, system suitability of the method was determined from the six replicate absorbance of the standard drug mixture using UV visible spectroscopy.

**Selectivity/specificity:**
Specificity was demonstrated by resolution of compound. Specificity is the degree to which the procedure is applies to a single analyte and is checked in each analysis. The specificity of the method was investigated by the analysis of blank preparations spiked with standard and sample of Acyclovir.

**Precision**
Precision of the method was studied as Repeatability & Intermediate Precision. Repeatability was performed by analysing, the six measurements of 100% concentration of drug on the same day. Whereas Intermediate Precision was studied on the different day by using different six 100% concentration of drug & using different glassware.

**Linearity**
Series of mixed standard solutions of Acyclovir was prepared. Linearity of the method was studied by finding the absorbance of five concentrations of the standard solution in the range of 1600-2400 µg /ml for Acyclovir respectively.

**Accuracy**
The accuracy of the method was determined by recovery studies. The recovery studies were performed by standard addition method; at 80%, 100%, 120% level i.e. three different levels. Accuracy of the method was studied by calculating recovery of the spiked samples.

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**
The LOD and LOQ of ACY by the proposed methods were determined on the basis of absorbance and slope of the regression equation. LOD and LOQ values were calculated using the formula 3.3×s/S and 10×s/S, respectively, where S is the slope of calibration curve and s is the standard deviation of y-intercept of regression equation.

**Robustness**
The robustness of the developed method was determined according to ICH guidelines. Experimental conditions were deliberately altered. Robustness of the method was studied by changing the wavelength ± 2nm.

**Stability:-**
The absorbance of a standard solution was taken & the same solution was stored at 25°c for one day and after one day absorbance was found out.

**Forced Degradation Studies:**

**Degradation by Hydrochloric Acid (Acid Treated Sample):**
Randomly 20 tablets were selected from a batch and powder was made, and powder was weighed accurately (equivalent to 200 mg of Acyclovir) and it was transferred to 50ml volumetric flask. Then 10ml of acid (0.1N HCL) was added, and the flask was shake on the rotator shaker for 30 min and sonicated for 1hr with intermediate shaking then 10ml base (0.1NaOH) was added and made up to the volume with 0.1N NaOH.
5ml of above clear solution was pipetted out into 50ml volumetric flask and was made up to the volume with 0.1N NaOH and further dilutions were made. Absorbance of above solution was found out at 252 nm.

**Degradation by Sodium Hydroxide (Base Treated Sample):**
Randomly 20 tablets were selected from a batch and powder was made, and powder was weighed accurately (equivalent to 200 mg of Acyclovir) and it was transferred to 50ml volumetric flask. Then 10ml of base (0.1N NaOH) was added, and the flask was shake on the rotator shaker for 30 min and sonicated for 1hr with intermediate shaking then 10ml acid (0.1N HCL) was added and made up to the volume with 0.1N NaOH.
5ml of above clear solution was pipetted out into 50ml volumetric flask and was made up to the volume with 0.1N NaOH and further dilutions were made. Absorbance of above solution was found out at 252 nm.

**Degradation by Hydrogen Peroxide (Peroxide Treated Sample):**
Randomly 20 tablets were selected from a batch and powder was made, and powder was weighed accurately (equivalent to 200 mg of Acyclovir) and it was transferred to 50 ml volumetric flask. Then 10 ml 1% peroxide was added and flask was shake on the rotator shaker at 60˚C and sonicated for 1hr with intermediate shaking and volume was made with 0.1N NaOH.
5ml of above clear solution was pipetted out into 50ml volumetric flask and was made up to the volume with 0.1N NaOH and further dilutions were made. Absorbance of above solution was found out at 252 nm.

**Degradation by Photo Light:**
Randomly 20 tablets were selected from a batch and powder was made, and powder was weighed accurately (equivalent to 200 mg of Acyclovir) and it was transfer to 50ml volumetric flask and flask was kept in sunlight for 55hrs. Then 10 ml of 0.1 N NaOH was added & flask was shake on rotator shaker for 30min and sonicated for 1hr with intermediate shaking and made up to the volume with 0.1N NaOH.
5ml of above clear solution was pipetted out into 50ml volumetric flask and was made up to the volume with 0.1N NaOH and further dilutions were made. Absorbance of above solution was found out at 252 nm.

**Degradation by 0.1N NaOH:**
Randomly 20 tablets were selected from a batch and powder was made, and powder was weighed accurately (equivalent to 200 mg of Acyclovir) and it was transferred to 50ml volumetric flask. Then 10ml of 0.1N NaOH was added, and the flask was shaken on the rotator shaker for 30 min and sonicated for 1hr with intermediate shaking and made up to the volume with 0.1N NaOH.
5ml of above clear solution was pipetted out into 50ml volumetric flask and was made up to the volume with 0.1N NaOH and further dilutions were made. Absorbance of above solution was found out at 252 nm.

**Results and Discussion:-**
The goal of this present study was aimed at developing a sensitive, precise and accurate Stability indicating UV method for the analysis of Acyclovir in its bulk and pharmaceutical dosage form. The results of intraday and interday precision values are represented in (Table3). The % RSD for assay of drugs during intra-day and inter-day were 0.211 and 0.313. The calibration curve showed linearity in the concentration range of 1600-2400 µg/mL respectively (Graph2). The regression coefficients of concentration over their peak areas were found to be 0.9994 (Table 4). Assay of drugs using the developed method showed acceptable relative error values that are less than 2 indicating that the method is highly precise. The percentage mean recovery of individual analyte was high, satisfactory and indicates that the proposed method is accurate (Table 5). In robustness study, Wavelength was deliberately altered, which illustrates good robustness of the developed method. (Table7). The solution was found to be stable for one day and found degraded in acid, base and peroxide where as dry powder was stable in photo light (Table 9).

**Table1:- Result of System suitability**

| Sr. No. | Parameters | Acceptance criteria | Acyclovir |
|---|---|---|---|
| 1 | SD | Less than 2 | 0.2102 |
| 2 | RSD | Less than 2 | 0.0021 |

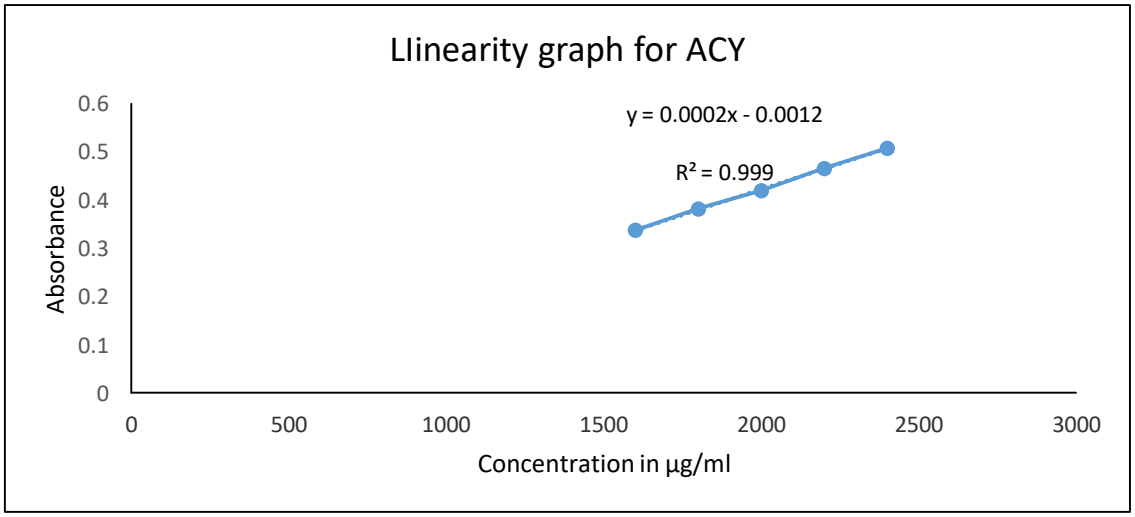
SD- standard deviation, RSD – relative standard deviation

**Table 2:- Result of Specificity**

| Conc. (%) | % Purity |
|---|---|
| 100% | 99.75% |

**Table 3:- Result of precision**

| Drugs (Label claim) | Percentage obtained | | % RSD | |
|---|---|---|---|---|
| | Intraday | Interday | Intraday | Interday |
| Acyclovir 200 mg Mean | 99.763 | 99.96 | 0.211 | 0.313 |




**Graph 2:- Linearity graph of Acyclovir**

**Table 4:- Result of linearity study from the graph**

| Drug | Conc.(µg/ml) | $R^2$ |
|---|---|---|
| ACY | 1600-2400 | 0.9994 |

**Table 5:- Result of Accuracy study**

| | Concentration of solution in percentage | Amount Spiked (µg/ml) | Amount Recovered (µg/ml) | % Recovery |
|---|---|---|---|---|
| **ACY** | 80% | 1600 | 160.37 | 100.23 |
| | 100% | 2000 | 199.76 | 99.88 |
| | 120% | 2400 | 240.39 | 100.16 |
| Mean % Recovery | | | | 100.09 |

**Table 6:- Result of LOD & LOQ**

| Parameter | Acyclovir |
|---|---|
| Limit of detection | 0.030 µg/ml |
| Limit of Quantification | 0.062 µg/ml |

**Table 7:- Result of Robustness**

| | ACYCLOVIR | | |
|---|---|---|---|
| | **252 nm** | **250 nm** | **254nm** |
| **Content in mg/tablet** | 200 | 199.20 | 200.75 |

**Table 8:- Result of assay from tablet dosage form**

| Drug | Content (mg) | % purity |
|---|---|---|
| Acyclovir | 200 | 99.76 |

**Table 9:- Forced degradation study results**

| Test | % Degradation | % Assay |
|---|---|---|
| Acid | 17.062 | 82.938 |
| Base | 5.214 | 94.786 |
| Peroxide | 18.247 | 81.753 |
| Light | No degradation of dry powder | 100 |
| 0.1 N NaOH | No degradation | 99.53 |

## CONCLUSION:

From the experimental studies it can be concluded that simple Stability indicating UV method has been developed for the estimation of Acyclovir in dosage forms. UV method is more reproducible, also it is more sensitive, precise, specific, and accurate. The developed method is simple, economic, accurate, precise, and reproducible and can be adapted to routine quality control analysis.

## ACKNOWLEDGEMENTS:

The authors wish to thank CIPLA LTD kumrek, Rangpo, Sikkim India, for providing the gift samples of Acyclovir for this work. The author is also thankful to the Assistant Prof. Prabhat Dessai, Professor at DM's College & Research Centre Assagao Bardez-Goa, for her guidance, kind help and constant encouragement at every step during the progress of my work. I am also grateful to my friends for their kind help from time to time at each and every step of my project work.

## REFERENCES:

1. ICH HARMONISED TRIPARTITE guideline; VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY Q2 (R1). Current step 4 version Parent Guideline dated 27 October 1994.
2. Yadav Purushotam Kumar, Chandanam Sreedhar, Rao Srinivas T, Akkamma HG, Bharadwaja Reddy Gangavarapu, Deepa Moye Shrestha, New Analytical Method Development And Validation Of Acyclovir By RP-HPLC Method, UJPBS, 2016, 04 (02): 20-26 .
3. G.S. Holkar, M.D. Rokade, R.R. Yadav and V.N. Daphal, Determination of Assay and Peak Purity Evaluation of Acyclovir and Valacyclovir by RP-HPLC Method, International Journal of Theoretical & Applied Sciences, 2012, 4(2): 56-65.
4. Umesh S. Dongare, Satyam Z. Chemate., Shweta A. Jadhav and Vaibhav R. Pawar, Spectrophotometric Determination And Validation Of Acyclovir In Tablet Dosage Form, International Journal of PharmTech Research, 2012, 4(4): 1840-1845.
5. Shilpa Chaudhari, Amatul Mannan and Shubhanki Daswadkar, Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Acyclovir and Silymarin in Niosome Formulation, Scholars Research Library, 2016, 8 (5):128-133.
6. Shital S.Patil, P. A. Salunke, R. S. Wagh, Dr. S. D. Barhate, Development And Validation Of RP-HPLC Method For Quantification Of Acyclovir In Tablets, IAJPS, 2016, 3 (1), 31-36.
7. Somsubhra Ghosh, Anirban Sahu, D. Sathis Kumar, Satyabrata Jena1, A. Harani, T.Praveen Kumar Reddy, D. Tanuja, Method development and validation for acyclovir in tablet dosage form by RP-HPLC, Journal of Pharmacy Research, 2012,5(3),1785-1786.
8. P. Susantakumar1, Ajay Gaur2, Piush Sharma et al, Development And Validation Of LC-MS/MS Method For The Estimation Of Acyclovir In Pharmaceutical Dosage Form An International Research Journal, 2011, Vol. 2 (4), 173-187.
9. V.R. Sinha , Monika , A. Trehan , Manoj Kumar , S. Singh , and J.R. Bhinge , Stress Studies on Acyclovir, Journal of Chromatographic Science, 2007, 4(5), 319-324
10. Selvadurai Muralidharan, Jayarajakumar Kalaimani, Subramani Parasuraman, and Sokkalingam Arumugam Dhanaraj, Development and Validation of Acyclovir HPLC External Standard Method in Human Plasma: Application to Pharmacokinetic Studies, Hindawi Publishing Corporation,2014, 3(2), 1-5.