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Research Article

Method Development and Validation of Acyclovir by UV Spectrophotometric Method

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ABSTRACT

Background: Acyclovir, an antiviral agent active against herpes simplex virus types I and II and varicella zoster, is widely used in pharmaceutical formulations. Existing pharmacopoeial methods employ non-aqueous titration or spectrophotometry at 254 nm. However, simple, rapid, and validated UV spectrophotometric methods for routine estimation are limited. **Objectives:** To develop and validate a simple, sensitive, and cost-effective UV spectrophotometric method for the quantitative estimation of Acyclovir in bulk and pharmaceutical dosage forms as per ICH Q2 (R^1) guidelines. **Materials and Methods:** Acyclovir standard stock solution (1000 µg/mL) was prepared in distilled water. Working solutions (0.5-30 µg/mL) were scanned, and the wavelength of maximum absorbance (λ_{max}) was determined at 254 nm. Calibration curves were plotted in the concentration range of 0.5-30 µg/mL. Method validation was performed for assay, linearity, precision, accuracy, robustness, ruggedness, Limit of Detection (LOD), and Limit of Quantification (LOQ). **Results:** The method showed excellent linearity (R^2=0.996) in the concentration range of 0.5-30 µg/mL. Accuracy studies demonstrated percentage recoveries between 98.94% and 100.86%, within pharmacopeial limits (99-101%). Precision studies indicated low %RSD (<2%) for repeatability, intra-day, and inter-day analysis. Robustness and ruggedness tests confirmed stability under small deliberate variations in wavelength, analyst, and conditions. The method exhibited low LOD and LOQ values, indicating high sensitivity. **Conclusion:** The developed UV spectrophotometric method is simple, rapid, precise, accurate, robust, and cost-effective. It can be successfully applied for routine quality control and estimation of Acyclovir in bulk and tablet dosage forms.

Keywords: Acyclovir, Diazo Coupling, p-Dimethylaminobenzaldehyde, Spectrophotometric determination, Acyclovir, Temperature and Coupling Reaction Time.

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INTRODUCTION

Acyclovir [2-amino-1,9-dihydro-9-[(2-hydroxyethoxy) methyl]-6H-purina-6-one;acycloguanosine;9-[2-hydroxyethoxy) methyl] guanine, is an antiviral agent which is highly active *in vitro* against Herpes Simplex (HSV) type-I and II and varicella viruses, but its toxicity to mammalian cells is low. Acyclovir is phosphorylated to the active compound acyclovir triphosphate after entry into a herpes infected cell. Intracellular conversion of acyclovir by viral thymidine kinase to the triphosphate which acts as an inhibitor for the herpes specified DNA polymerase preventing further viral DNA-synthesis without affecting normal cellular processes.[1]

Acyclovir is the subject of monographs in both the British Pharmacopoeia (BP)[2] and the European Pharmacopoeia.[3] Both methods recommended non-aqueous titration of the raw material using perchloric acid as a titrant. For the tablets, the BP[2] described a spectrophotometric method based on measuring the absorbance of the extracts at 254 nm.

STRUCTURE OF ACYCLOVIR DRUG

Several methods have been reported for the analysis of acyclovir either in pure form or in pharmaceutical forms as well as in the biological fluids and tissues, viz. spectrophotometry,[4-6] HPLC,[7-9] fluorimetry,[10] radioimmunoassay[11-12] and enzymatic immunoassay.[13]

3-Methylbenzothiazolin-zone Hydrazine (MBTH), has been frequently used in pharmaceutical analysis, thus, it has been utilized as a color producing reagent for determination of acetaminophen and phenobarbital simultaneously,[14]

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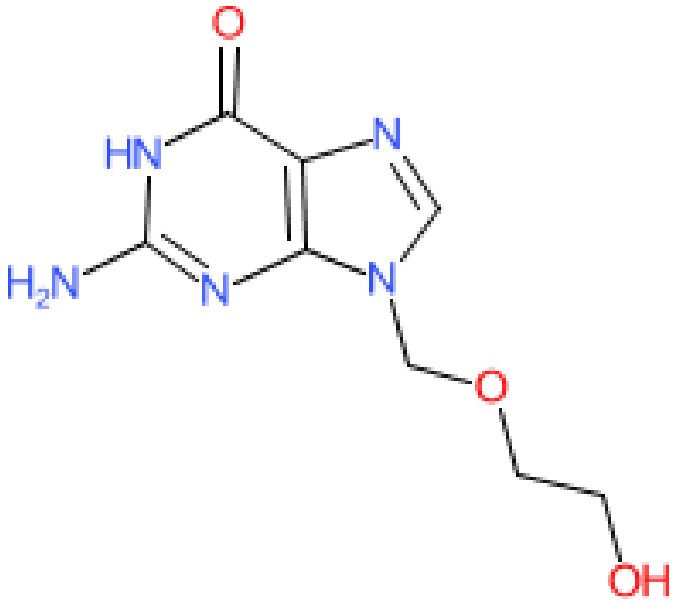

ritodrine hydrochloride and amoxicillin,[15] metoclopramide hydrochloride[16] and pefloxacin.[17]

Methods based on derivative and differential spectrophotometry have also been reported for the assay of acyclovir in dosage forms. To the best of our knowledge, there is no work in the literature reported about the visible spectrophotometric method for the analysis of acyclovir in either biological fluids or pharmaceutical formulations.[17] The purpose of this investigation was to develop a simple and sensitive visible spectrophotometric method for the quantitation of acyclovir in pure drug and in pharmaceutical formulations.[18]

MATERIALS AND METHODS

Materials

Drug Sample: Active pharmaceutical ingredient of Acyclovir is gifted as a sample from Aadhaar Life Sciences Pvt. Ltd., Solapur. Marketed formulation of Acyclovir was procured from the local pharmacy.[19]

Pharmaceutical Formulation: Marketed formulation of Acyclovir was procured from the local pharmacy (e.g., labelled as containing 400 mg or 800 mg of acyclovir).[20,21]

Chemicals and Reagents: Chemicals such as Distilled water and methanol were used throughout the analysis process.

Instruments: UV/Visible double beam spectrophotometer Systronic 2201. Standard cuvettes having 10 mm of path length are used for analysis.[22] Ultrasonicator (micro clean-103) was used to sonicate the formulation sample. The drug sample was weighed by using an electronic analytical balance (Shimadzu AY220).

Method Development

Preparation of Standard Stock Solution

A standard stock solution of Acyclovir was prepared by dissolving 100 mg of accurately weighed Acyclovir in 100 mL of distilled water (or methanol, depending on solubility) to obtain a concentration of 1000 µg/mL.[23] From this, a working standard solution of 100 µg/mL was prepared by appropriate dilution with distilled water.[24-26]

Determination of Maximum Absorbance (λ_{max})

To determine the wavelength for measurement, Acyclovir (50 µg/mL) solution was scanned in the range of 200-400 nm against distilled water as blank. Wavelength of maximum absorption was determined for the drug. Acyclovir showed maximum absorption at 254 nm.

Preparation of Calibration Curve

Aliquots (0.5,1,1.5,2,2.5 µg/mL) of prepared standard solution were transferred into series of 10 mL volumetric flasks and diluted by distilled water to give the concentration range of 0.5 -2.5 µg/mL.[27] The absorbance of each solution was measured at the determined λ_{max} against a reagent blank. A calibration curve was plotted with concentration on the X-axis and absorbance on the Y-axis.

Method Validation

The Method validation is an exercise to ensure the development method for appropriate purpose. In research, the UV spectrophotometric method for estimation of Acyclovir in pharmaceutical dosage forms was validated and verify as per ICH Q2 (R^1) guidelines. The parameters for validation like:

- Assay,
- Linearity,
- Precision,
- Robustness,
- Ruggedness,
- Accuracy,
- Limit of Detection,
- Limit of Quantification.

Assay

20 tablets weighed and powdered. The powder equivalent to 10 mg of acyclovir was weighed, transferred into 10 mL volumetric flask and dissolved in water. This solution was sonicated for 15 min and the final volume was made up to the mark with water. 1 mL of solution was transferred into 10 mL volumetric flask and diluted up to 10 mL with water. The absorbance of this solution was measured at 254 nm.

Linearity

Linearity is the initial and most significant test of method validation. It verifies that the response of the instrument (here, absorbance) is proportional to the sample drug concentration over a given range.

For checking the linearity of Acyclovir, a set of standard solutions were prepared between 5 µg/mL and 30 µg/mL concentrations. They were each scanned at the maximum drug absorbance wavelength (λ_{max}), which was approximately 254 nm. A calibration curve was then created by plotting concentration on the X-axis and absorbance on the Y-axis.[28]

The curve should be a straight line. To ensure this, the correlation coefficient (R^2) was determined. A figure of 0.999 or more shows that there is excellent linearity and the method can detect and quantify Acyclovir with accuracy at different concentrations

Precision

Accuracy verifies correctness, while precision verifies consistency. In another sense, precision informs us if we obtain corresponding results every time we conduct the test, even if the values differ slightly from the actual one.

Precision was measured in two forms

- **Repeatability (Intra-day Precision):** The same concentration was tested by the same analyst several times during the same day with the same instrument. Repeatable readings show that the method is reproducible under the same conditions.[29]
- **Intermediate Precision (Inter-day or Analyst-to-Analyst Precision):** The same sample was analysed by various analysts or on different days. This is used to determine if external factors influence the reliability of the method.

Robustness

Robustness tests whether the method doesn't vary with small, deliberate changes in experimental conditions. In actual lab environments, these changes are unavoidable-perhaps the analyst adjusts the wavelength slightly, or the solvent mix slightly different.[30]

Minor variations were made in testing robustness to:

- Wavelength (e.g., ±2 nm from 252 nm),
- Solvent mix,
- Time between preparation and analysis.

If the results are consistent under these changes, the procedure is robust. The readings of absorbance and % recovery were shown to be negligible variation in this instance, which confirmed robustness.

Ruggedness

Ruggedness is a step beyond precision. It checks the reproducibility of the method under varying operating conditions: various analysts, various instruments, or even various laboratories.

To determine ruggedness, the procedure was conducted by two analysts on alternate days employing identical equipment.[31,32] Results were reproducible, with low % RSD values, showing that the procedure is reproducible despite minor external variability.

Accuracy

Accuracy informs us if the method is capable of measuring the actual amount of the drug in the sample. It provides the answer to the question: Are we getting the correct number?

To prove this, recovery experiments were performed. This consisted of spiking the sample (i.e., a previously analysed tablet solution) with amounts of pure Acyclovir at three levels: 50%, 100%, and 150% of the nominal concentration.[29,30] The solutions were then analysed and the percentage of recovered Acyclovir computed.

If the method is precise, the recovery should be in the ranges, and the replicate variation (expressed as %RSD) should be low most often less than 2%.

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

These two variables address the method's sensitivity

LOD (Limit of Detection)

The lowest amount of acyclovir that can be found but may not be precisely measured Understand it as the point when the device can "see" that the drug is present.

LOQ (Limit of Quantitation)

The smallest amount that can be precisely and accurately quantified is referred to as the limit of quantitation, or LOQ.[33]

They are calculated using this formula:

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

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

Where:

σ is the standard deviation of the response (usually the y-intercept),

S is the slope of the calibration curve.

Lower LOD and LOQ values indicate that the method is highly sensitive and suitable for detecting even small amounts of Acyclovir.

RESULTS AND DISCUSSION

Linearity

Linearity was observed within 0.5-3 µg/mL and 10 mg/mL concentration the acceptable limit is, it should be linear in the specified range and the correlation coefficient, 0.99. The correlation coefficient, R=0.996. Hence the relationship between the concentrations and the absorbance of acyclovir showed linearity. (Figure 1 is wavelength maxima of acyclovir and Figure 2 is Linearity curve).

Determination of active ingredients in Tablets

Each tablet contains 400 mg of Acyclovir. Magnesium stearate, microcrystalline cellulose, sodium starch glycollate, pregelatinised starch and colloidal anhydrous silica. Hence the average percentage recovery of 98% was found to be within the acceptance limit. %RSD values did not exceed the acceptance limit of 2%.

Accuracy

According to IP acyclovir contains not less than 99.0% and not more than 101.0% of acyclovir. The results showed that drug content was within specified limits and the %RSD values did not exceed the accepted limit of 2%. Hence the method can be said to be accurate.

Precision

The %RSD values for repeatability, intraday and inter day precision data were well below the specified limit of 1% and 2%, respectively. Hence, the method was found to be precise in the specified range. (Precision results are displayed in Tables 1 and 2).

Robustness

The assay was done under different temperature and wave length conditions. The results showed %RSD values to be with in the acceptance criteria of 2%. Hence, the method was found to be robust in the given conditions. (Robustness results are displayed in Table 3).

Ruggedness

Ruggedness of the method was assessed by analysing Acyclovir (10 µg/mL) using two different analysts. The mean absorbance values obtained were 0.0743 and 0.0738 with %RSD of 1.09% and 1.58%, respectively. As the %RSD values were within the acceptable limit (<2%), the method was found to be rugged and reproducible. (Ruggedness results are displayed in Table 4).

Accuracy

Accuracy of the proposed method was evaluated through recovery studies at three concentration levels (50%, 100%, and 150%). The percentage recovery for Acyclovir was found to be 100.71% at 50% level, 100.80% at 100% level, and 98.94% at 150%

Table 1: Intermediate Precision (Inter-day or Analyst-to-Analyst Precision).

Sl. No.	Concentration (µg/mL)	Absorbance (254 nm)
1	5	0.089
2	10	0.128
3	15	0.197
4	20	0.259
5	25	0.315
6	30	0.381
Sl. No.	**Concentration (µg/mL)**	**Absorbance (254 nm)**
1	5	0.089
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3	15	0.197
4	20	0.259
5	25	0.315
6	30	0.381

Table 2: Inter-day precision (Reproducibility) data of proposed method.

Sl. No.	Concentration (µg/mL)	Absorbance at (254 nm)	
		Morning	Evening
1	10	0.075	0.076
2	10	0.076	0.074
3	10	0.073	0.075
4	20	0.074	0.074
5	20	0.075	0.075
6	20	0.074	0.077
Average		0.0745	0.075167
Standard Deviation		0.001049	0.001169
%RSD		1.4%	1.55%

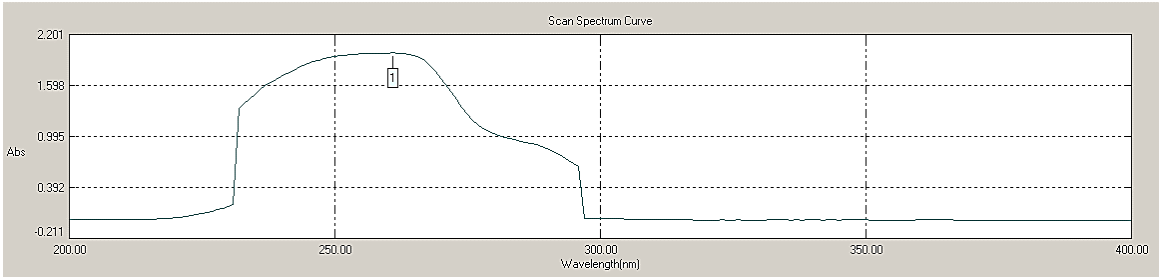

Figure 1: Wavelength maxima of acyclovir.

Table 3: Robustness studies data of proposed method.

Sl. No.	Concentration (µg/mL)	Absorbance (at 254 nm)	
		Morning	Evening
1	10	0.074	0.072
2	10	0.073	0.075
3	10	0.074	0.074
4	20	0.075	0.072
5	20	0.072	0.073
6	20	0.075	0.073
Average		0.073833	0.073167
S.D		0.001169	0.001169
%R.S.D		1.58%	1.59%

Table 4: Ruggedness studies data of proposed method

Sl. No.	Concentration (µg/mL)	Absorbance at 254 nm	
		Analyst 1	Analyst 2
1	10	0.075	0.074
2	10	0.073	0.075
3	10	0.074	0.072
4	10	0.075	0.074
5	10	0.074	0.073
6	10	0.075	0.075
Average		0.074333333	0.073833333
Standard Deviation		0.000816	0.001169
%RSD		1.09%	1.58%

Table 5: Percentage of recovered Acyclovir computed

Sl. No.	Spike Level	µg/mL added	µg/mL found	% of recovery	Mean % recovery
1	50%	10	10.07	100.71	100.71%
2	50%	10	10.07	100.71	
3	50%	10	10.07	100.71	
1	100%	20	20.17	100.86	100.80%
2	100%	20	20.13	100.69	
3	100%	20	20.17	100.86	
1	150%	30	29.69	98.97	98.94%
2	150%	30	29.69	98.97	
3	150%	30	29.65	98.86	

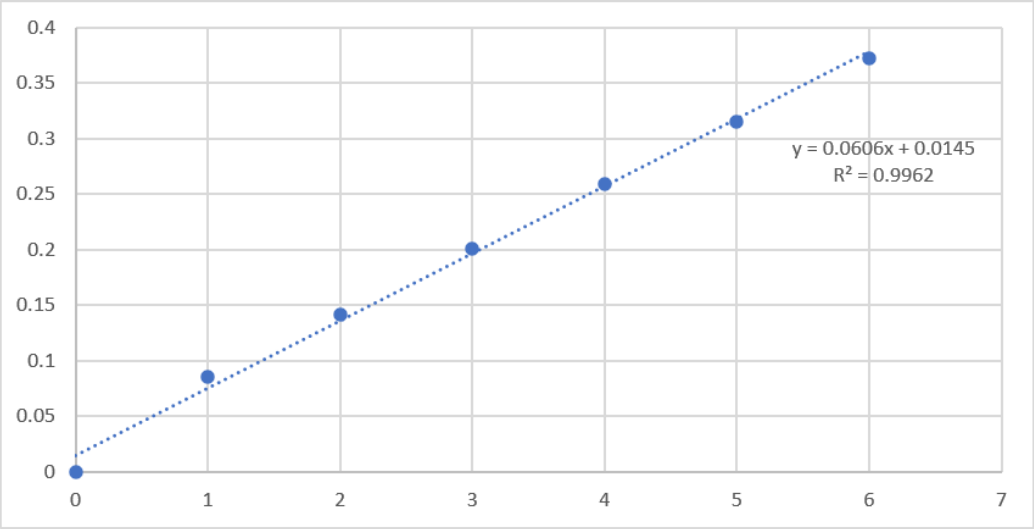

Figure 2: Linearity curve.

level. As all values were within the acceptable recovery range (98-102%), the method was confirmed to be accurate and reliable for the estimation of Acyclovir. (Accuracy results are displayed in Table 5).

CONCLUSION

The proposed method was simple, sensitive, and cost-effective. Method was validated in Terms of precision, linearity and accuracy. The results are reproducible, and can be used successfully for the estimation of acyclovir in active pharmaceutical formulations. The developed UV spectrophotometric method for the estimation of Acyclovir in bulk and pharmaceutical dosage forms is simple, accurate, precise, and cost-effective. Validation as per ICH Q2 (R^1) guidelines confirmed excellent linearity, accuracy, precision, robustness, and ruggedness, with acceptable recovery and %RSD values. The low LOD and LOQ values demonstrated the sensitivity of the method. Hence, this method can be reliably applied for routine quality control and quantitative analysis of Acyclovir in pharmaceutical formulations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

API: Active Pharmaceutical Ingredient; **UV:** Ultraviolet; **HPLC:** High Performance Liquid Chromatography; **λ_{max}:** Wavelength of Maximum Absorbance; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation; **RSD:** Relative Standard Deviation; **SD:** Standard Deviation; **ICH:** International Council for Harmonisation; **IP:** Indian Pharmacopoeia

SUMMARY

A simple, accurate, and cost-effective UV spectrophotometric method was developed and validated for the estimation of Acyclovir in bulk and pharmaceutical dosage forms. Acyclovir exhibited maximum absorbance at 254 nm, and the calibration curve showed excellent linearity in the range of 0.5-30 µg/mL with a correlation coefficient (R^2) of 0.996.

The method was validated according to ICH Q2 (R^1) guidelines. Accuracy studies showed recovery values within 98.94-100.86%, while precision results confirmed low %RSD values (<2%) for repeatability, intra-day, and inter-day analysis. Robustness and ruggedness testing demonstrated reliability under small variations in wavelength, analyst, and experimental conditions. The LOD and LOQ values confirmed the method's high sensitivity.

Overall, the method proved to be simple, sensitive, precise, robust, and economical, making it suitable for routine quality control of Acyclovir in both bulk drug and tablet formulations.

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