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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF VALACYCLOVIR HYDROCHLORIDE IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Valacyclovir HCl in tablet dosage form. **Methods:** The drug is soluble in Analytical Grade Water. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in Analytical Grade Water. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Valacyclovir in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in Water, soluble in DMSO and slightly soluble in Ethanol. So, the analytical grade water is used as a diluent in method. The melting point of Valacyclovir was found to be 161-162˚C (uncorrected). It showed absorption maxima 230 nm in analytical grade Water. On the basis of absorption spectrum the working concentration was set on 15 µg/ml (PPM). The linearity was observed between 5-25 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.8, 99 and 98.6% for three levels respectively. The % RSD for precision and ruggedness was found to be 077% and 0.24% respectively. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Valacyclovir in tablet dosage form. The method could be considered for the determination of Valacyclovir in quality control laboratories.

KEYWORDS: Valacyclovir, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Valacyclovir hydrochloride is a hydrochloride salt of L-valyl ester of acyclovir. It is chemically 2-[(2-amino-6-oxo-3, 9-dihydropurin-9yl) methoxy] ethyl-2-amino-3-methylbutanoate.[1] It is an antiviral drug used in the treatment of herpes simplex and herpes zoster. It inhibits viral DNA synthesis. It is prodrug intended to increase the bioavailability of acyclovir by increasing lipophilicity, valacyclovir converted by esterase to active drug acyclovir via hepatic first pass metabolism.The substrate specificity of acyclovir triphosphate for viral, rather than cellular, DNA polymerase contributes to the specificity of the drug.[2] After oral administration, valacyclovir is converted rapidly and extensively to acyclovir as a result of first-pass intestinal and hepatic metabolism through enzymatic hydrolysis . The oral bioavailability of acyclovir is higher after administration of valacyclovir relative to acyclovir itself.[3] The mechanism of action of acyclovir involves the highly selective inhibition of herpes virus DNA replication, via enhanced uptake in herpes virus-infected cells and phosphorylation by viral thymidine kinase. The substrate specificity of acyclovir triphosphate for viral, rather than cellular, DNA polymerase contributes to the specificity of the drug.[4,5]

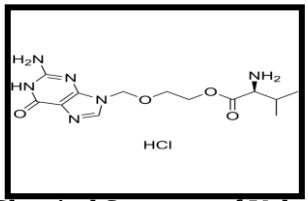

Fig. 1: Chemical Structure of Valacyclovir.

From literature review it's found that one method was reported on spectrophotometry for simultaneous estimation, zero order of Valacyclovir.[6,7,8] Also the method was reported on LCMS[9] on Valacyclovir. Lot of work was done on HPLC method development for Valacyclovir drugs, impurity profiling.[10,12] But very few methods were reported on estimation of Valacyclovir in tablet dosage form for UV spectroscopic method. This indicates that so far no UV method exists for the estimation and determination of Valacyclovir in tablet dosage forms.

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair. Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance. Melting point apparatus of Equiptronics were used.

- **Materials**

Valacyclovir was obtained as a gift sample. Valacyclovir tablets were procured from local pharmacy. Analytical Grade Water was used throughout the experiment as a diluent. Freshly prepared solutions were employed.

Method development

A. Determination of ƛ max (15 PPM)[14,16]

50 mg weighed amount of Valacyclovir was dissolved into 100 ml of volumetric flask with Water. Pipette out 1.5 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with Water. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

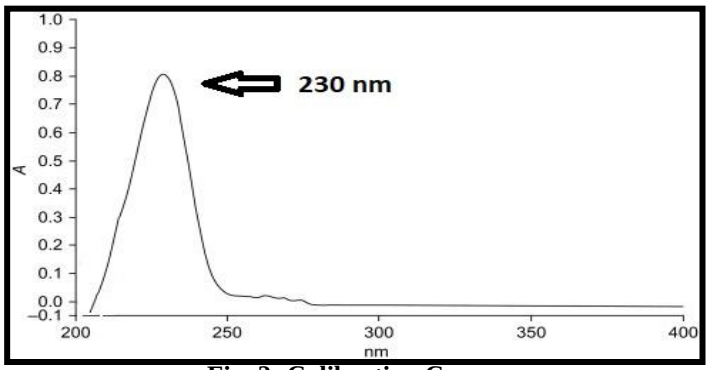

Fig. 2: Calibration Curve.

B. Preparation of Working concentration[14]

Preparation of Standard stock solution

Standard stock was prepared by dissolving 50 mg of Valacyclovir in 100 ml of Water to get concentration of 500 µg/ml (PPM).

Preparation of Standard solution

Pipette out 1.5 ml from standard stock solution and diluted up to 50 ml with Water to get concentration of 15 µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with Water. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[14]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Valacyclovir was accurately weighed and transferred into the 100 ml of volumetric flask, added 60 ml Water, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with Water. Filtered the solution through whatmann filter paper. Pipette out 1.5 ml of the above solution and diluted up to 50 ml with Water. The absorbance was measured at 230 nm. The absorbance was recorded.

Table 1: Absorbance of Dosage Form.

Cipla Pharmaceutical Limited Valcivir® 500 (500 mg)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0000
2	Standard	0.7448
3	Sample	0.7314

Table 2: Dosage Form Specifications.

Type	Company	M.D.	E.D.	Batch No.	Average weight (g)	Assay (%)
1	Cipla Pharma LTD Valcivir® 25	08/2023	07/2026	ZSA 1654	0.624	98.20

E. Method of validation[13,15,18]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 5 - 25 PPM at 230nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample Concentration	Absorbance
1	5 PPM	0.2585
2	10 PPM	0.4881
3	15 PPM	0.7241
4	20 PPM	0.9391
5	25 PPM	1.1461
Correlation coefficient		**0.9986 ~ 0.999**

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 50ml of volumetric flask with Analytical Grade Water and measuring the absorbance at 230nm.

Table 4: Accuracy Studies.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.79	98.75 ~ 98.8
100	1	0.99	99.0
120	1.2	1.18	98.55 ~ 98.6

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the % RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.7451
2	Sample Solution 2	0.7314
3	Sample Solution 3	0.7325
4	Sample Solution 4	0.7374
5	Sample Solution 5	0.7327
MEAN		**0.7358**
SD		**0.0057**
% RSD		**0.7715 ~ 0.77**

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.7501	0.7443	99.93	0.2379 ~ 0.24
		0.7384			
2	Analyst 2	0.7351	0.7418	99.59	
		0.7484			

RESULTS

1. Solubility of Valacyclovir

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	Water	Freely Soluble
2	DMSO	Soluble
3	Ethanol	Slightly soluble

2. Melting point of Valacyclovir

The melting point of Valacyclovir was found to be 161-162˚C (uncorrected).

3. Results for linearity for assay method of Valacyclovir [Conc Vs Absorbance]

The linearity of method was determined at concentration level ranging from 5 to 25 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9986 ~ 0.999.**

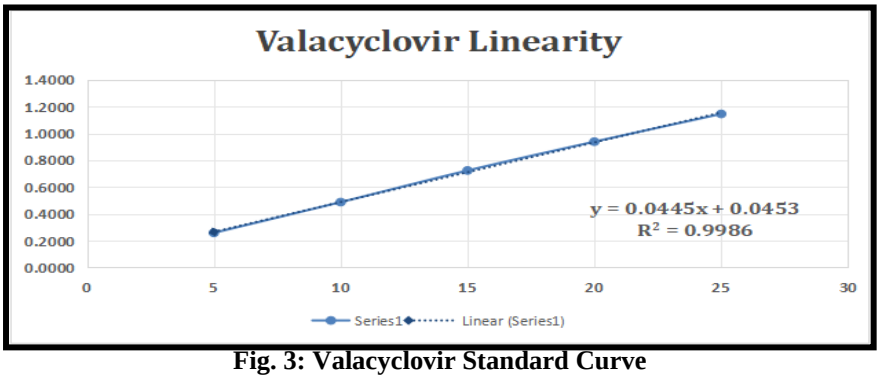

Fig. 3: Valacyclovir Standard Curve

4. Results for accuracy for assay method of Valacyclovir

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Valacyclovir

The % RSD for different sample of precision was found to be 0.77 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Valacyclovir

The % RSD for different sample of ruggedness was found to be 0.24 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Valacyclovir in tablet form has been developed. From the spectrum of Valacyclovir, it was found that the maximum absorbance was 230 nm in Analytical Grade Water. A good linear relationship was observed in the concentration range of 5-25 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Valacyclovir in solid dosage forms. Hence, the method could be considered for the determination of Valacyclovir in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. DNA - Deoxyribonucleic Acid
4. LCMS - Liquid Chromatography and Mass Spectroscopy
5. HPLC - High Performance Liquid Chromatography
6. UV - Ultra violet
7. FDA - Food and Drug Administration
8. NaOH - Sodium Hydroxide
9. ICH - International Council for Harmonization
10. RSD - Relative Standard Deviation
11. SD - Standard Deviation
12. Qty - Quantity
13. C - Celsius
14. M.D. - Manufacturing Date
15. E.D. - Expiry Date.

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