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Spectrophotometric Method Development and Validation of Valacyclovir Hydrochloride in Bulk and Pharmaceutical Dosage Form

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Short Communication

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ABSTRACT

A simple, accurate, precise UV spectrophotometric method was developed in pure and pharmaceutical formulations for Valacyclovir hydrochloride. It is a antiviral drug. Valacyclovir hydrochloride exhibiting maximum absorbance at 255 nm in 0.1 M sulphuric acid and the method was validated for linearity, precision, sensitivity, and specificity. The drug obeyed linearity at concentration range of 4-12 µg/ml. The proposed method was validated statistically with significant high value of correlation coefficient 0.999. The percentage recovery value for Valacyclovir hydrochloride was in the range of 99.4 – 100.9 %. Therefore, the proposed method could be applied for the routine analysis of pharmaceutical dosage forms containing Valacyclovir hydrochloride.

INTRODUCTION

Valacyclovir hydrochloride, 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-l)methoxy]ethyl ester, monohydrochloride (Fig 1), is a antiviral drug. It is a prodrug. It is rapidly converted to acyclovir which has antiviral activity against herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and Varicella-zoster virus (VZV) both in vitro and in vivo. The inhibitory activity of acyclovir is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV. This viral enzyme converts acyclovir into acyclovir monophosphate, a nucleotide analogue. The monophosphate is further converted into diphosphate by cellular granulate kinase and into triphosphate by a number of cellular enzymes. In vitro, acyclovir triphosphate stops replication of herpes viral DNA. This is accomplished in 3 ways: 1) competitive inhibition of viral DNA polymerase, 2) incorporation and termination of the growing viral DNA chain, and 3) inactivation of the viral DNA polymerase. The greater antiviral activity of acyclovir against HSV compared with VZV is due to its more efficient phosphorylation by the viral thymidine kinase.

A few analytical methods have been reported for its quantitative estimation in pharmaceutical formulations by HPLC, UV and colorimetric method[1,2,3,4,5,6,7,8,9]. The objective of the work is to develop UV method for its estimation and validation in bulk and tablet dosage form with good accuracy, simplicity, precision and economy. The present method was validated according to the International Conference on Harmonization (ICH) for the determination of Valacyclovir hydrochloride in bulk and tablet dosage forms.

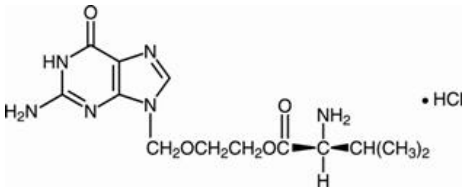

Figure 1: Chemical Structure of Valacyclovir hydrochloride

MATERIALS AND METHODS

Chemicals

Valacyclovir hydrochloride (standard) is obtained from Aurobindo Pharma Ltd., Hyderabad, A.P, India. Sulphuric acid (AR grade), Water (AR grade) were purchased from E. Merck (India) Ltd., Worli, Mumbai, India. Distilled water was used throughout the study. Pharmaceutical dosage form (tablet) was obtained from local market, manufactured by Cipla Pharma Ltd., as a brand name of Valcivir 500mg which was used as sample.

Apparatus

Analysis was performed by using UV-Spectrophotometry of Perkin Elmer equipped detector (UV-2500). Data acquisition was made with Lambda 25 software. Analytical Balance (BSA224S-CW, Sartorius), was used for the study.

Selection of Wavelength

The known concentration of Valacyclovir hydrochloride was weighed separately and dissolved in volumetric flasks using 0.1M sulphuric acid. The resulting solution was scanned in the range of 200 nm to 400 nm. The absorption curve showed characteristic absorption at 255 nm for valacyclovir hydrochloride.

Preparation of Standard Stock Solutions

The quantity containing 100 mg of valacyclovir hydrochloride was accurately weighed and transferred into a 100 ml dry, clean volumetric flask and the volume made up to the mark with solvent (1000 µg/ml). From this 1 ml was pipetted out in to a 10 ml dry, clean volumetric flask and the volume is made up to mark with solvent (100 µg/ml).

Working Standard Solution

From the standard stock solution 1ml was pipetted out in to a 10 ml dry, clean volumetric flask and the volume is made up to mark with solvent (100 µg/ml).

Sample Preparation

Ten tablets were weighed and triturated into fine powder. An amount of powder equivalent to 500 mg of valacyclovir hydrochloride was accurately weighed into 100 ml clean, dry volumetric flask and volume made up to mark with solvent (1000 µg/ml). From this 1ml was pipetted outinto a 10 ml dry, clean volumetric flask and the volume is made up to mark with solvent (100µg/ml).

Method Validation

The method was validated by evaluating linearity, accuracy, method and system precision, limit of detection (LOD), limit of quantification (LOQ) and ruggedness were performed accordance with ICH guideline Q2 (R1).

Linearity

From the standard stock solution of concentration 1000 µg/ml, 0.4, 0.6, 0.8, 1, 1.2 ml was transferred to five 10ml flasks and made up the volume with solvent. The concentration of Valacyclovir hydrochloride was found to be 4-12 µg/ml. The calibration curve was shown in Fig 3. The results of linearity were shown in table 1and figure 2.

Accuracy

Accuracy of the method was carried out by standard addition method at three levels of concentrations (80%, 100%, and 120%). The results of recovery (%) and %RSD were shown in table 2.

Method and System Precision

Precision of the method was verified by repeatability (system precision) and intermediate (method precision) studies. Repeatability studies were performed by three replicate absorbances of Valacyclovir hydrochloride on the same day. The studies were replicated on different days to determine intermediate precision. The results of the system and method precision were shown in Table 3 and 4.

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

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions. LOD & LOQ was calculated by using standard deviation and slope values obtained from calibration curve.

L.O.D. (3.3 × σ/m)	3.745807µg/ml
L.O.Q. (10 × σ/m)	11.35093 µg/ml

RESULTS AND DISCUSSION

The proposed method for Valacyclovir hydrochloride showed the maximum absorbance at wavelength of 255 nm. Linearity was observed in the concentration range of 4-12 µg/ml. Concentration of the drug present in the tablet sample solution was determined by solving the single component analysis at 255 nm. The drug content was found to be 100.24 % for Valacyclovir hydrochloride. The correlation coefficient (r^2) value of the drug was found to be 0.999. The RSD% for three replicates was found to be less than 2.0 % for both method and system precision. The LOD & LOQ value for Valacyclovir hydrochloride was found to be 3.74µg/ml and 11.3µg/ml respectively. The percentage recovery value for Valacyclovir hydrochloride was in the range of 99.4 – 100.9 %. It indicates that there is no interference from the excipients present in laboratory mixture. The results of assay validation of the proposed method show that they are accurate and precise.

CONCLUSION

An economic, simple and rapid UV spectrophotometric method has been developed for the determination of Valacyclovir hydrochloride in tablet dosage forms. The methods were validated for linearity, precision, accuracy, and LOD and LOQ. Therefore, the proposed method could be applied for the routine analysis of pharmaceutical dosage forms containing Valacyclovir hydrochloride.

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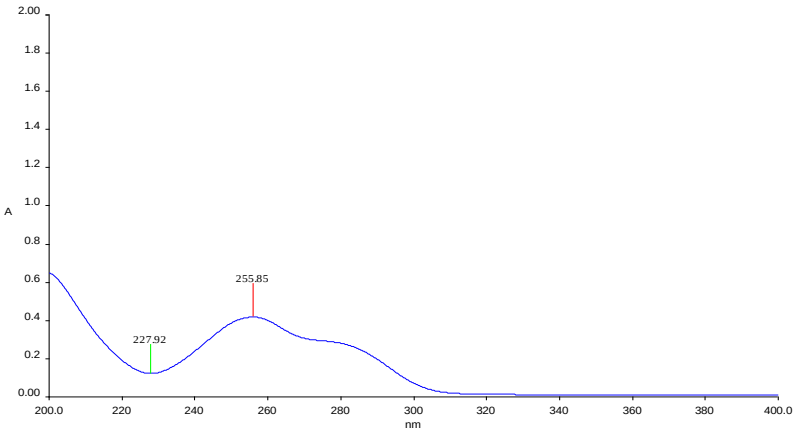

Figure 2: λ_{max} of Valacyclovir hydrochloride

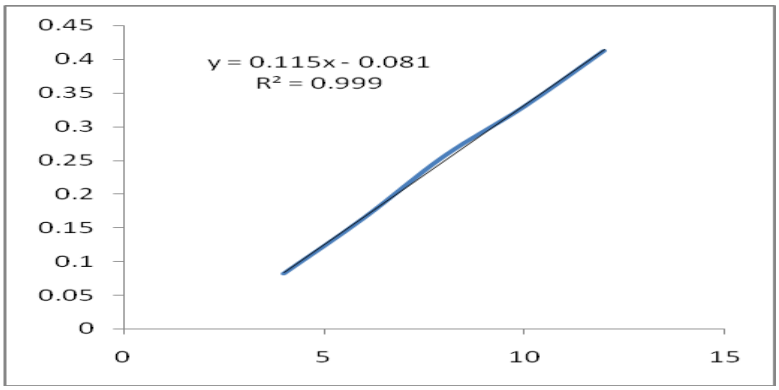

Figure 3 : Calibration Curve of Valacyclovir hydrochloride

Table 1: Assay Results for Valacyclovir hydrochloride

S.No	Particulars	Valacyclovir
1	Standard absorbance	1.6017
2	Sample absorbance	1.5802
3	Sample weight	150mg
4	Standard weight	100mg
5	Potency	99.8%
6	Amount found	501.22 mg
7	Label claim	500mg
8	% Purity	100.24%

Table 2: Regression Data for Valacyclovir hydrochloride

Concentration(µg/ml)	Absorbance	Statistical Analysis
4	0.0824	
6	0.1648	Slope = 0.115
8	0.2562	Intercept = 0.081
10	0.3296	Correlation coefficient = 0.999
12	0.4126	

Table 3: Accuracy Results for Valacyclovir hydrochloride

Level	Amount Added	Absorbance	Mean	Amount Recovery(mg)	% recovery
80%	5	1.5675 1.5662 1.5672	1.566967	13.01684	100.1295
100%	5	1.7962 1.7959 1.7971	1.7964	14.92398	99.49323
120%	5	2.0872 2.0852 2.0846	2.085667	17.32569	101.9158

Table 4: Method Precision Results for Valacyclovir hydrochloride

S.No	Absorbance
1	1.6017
2	1.6023
3	1.6029
Mean	1.6023
SD	0.0006
RSD %	0.037446

Table 5: System Precision Results for Valacyclovir hydrochloride

S.NO	Valacyclovir HCl	
	Day 1	Day 2
1	1.6023	1.6017
2	1.6025	1.6027
3	1.6017	1.6031
Avg.	1.602167	1.6025
S.D	0.000416	0.000721
%R.S.D	0.025986	0.044999

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