Research Article

DEVELOPMENT AND VALIDATION OF A SIMPLE UV SPECTROPHOTOMETRIC AND FLUOROMETRIC METHOD FOR THE DETERMINATION OF VALACYCLOVIR HYDROCHLORIDE BOTH IN BULK AND MARKETED DOSAGE FORM

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ABSTRACT

Introduction: Several analytical methods such as high performance liquid chromatography (HPLC), Uv-spectrophotometry and colorimetry have been reported for quantitative estimation of Valacyclovir hydrochloride in bulk and pharmaceutical formulations. The aim of this study was to develop simple, easily accessible and economic UV spectrophotometric and newer fluorometric methods. **Methods:** A simple, rapid, specific and cost effective spectrophotometric method using different solvents like methanol (Method A), ethanol (Method B), water (Method C) and phosphate buffer of pH 7.4 (Method D) and fluorometric method using solvents such as methanol (Method A), water (Method B) and 0.1N HCl (Method C) has been developed to determine the Valacyclovir hydrochloride content in bulk and pharmaceutical dosage formulations. **Results:** The calibration graph are linear and obeys beer's law in the concentration range of 2-20 µg/mL for all four spectrophotometric methods with a correlation coefficient (r^2) of 0.998, 0.996, 0.999 and 0.997, respectively while the calibration graph are linear in the concentration range of 1-10 µg/mL for all three fluorometric methods with a correlation coefficient (r^2) of 0.998, 0.999 and 0.999, respectively. The accuracy and precision of the methods were evaluated based on the intra-day and inter-day variations. The accuracy of the methods was further confirmed by standard addition procedure. The other characteristics such as limit of detection (LOD) and limit of quantification (LOQ) values are also reported. **Conclusion:** The obtained results proved that the developed methods can be employed for the routine analysis of Valacyclovir hydrochloride in bulk as well as in the commercial pharmaceutical formulations.

Keywords: Valacyclovir hydrochloride; UV spectrophotometry; Fluorometry, Validation.

INTRODUCTION

Valacyclovir hydrochloride, 2-[(2-amino-1, 6-dihydro-6-oxo-9H-purin-9-l) methoxylethyl ester, is an antiviral drug. It is a prodrug of acyclovir and 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*[1, 2]. The inhibitory activity of acyclovir is very 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 enzyme[3, 4]. *In vitro*, acyclovir triphosphate stops replication of herpes viral DNA. This is accomplished in 3 ways: Competitive inhibition of viral DNA polymerase, incorporation and termination of the growing viral DNA chain and inactivation of the viral DNA polymerase. The grater antiviral activity of acyclovir against HSV compared with VZV is due to its more efficient phosphorylation by the viral thymidine kinase[4].

The literature survey revealed that several analytical methods such as high performance liquid chromatography (HPLC), Uv-spectrophotometry and colorimetry have been reported for quantitative estimation of Valacyclovir hydrochloride in bulk and pharmaceutical formulations[5-11]. In this study, efforts were made to develop a simple, easy and economic UV spectrophotometric and fluorometric methods using different solvents like methanol, ethanol, water, 0.1N hydrochloric acid and phosphate buffer of pH

7.2 for the determination of Valacyclovir hydrochloride in the bulk and in the marketed dosage formulation. The developed method was optimized and validated as per the guidelines of International Conference on Harmonization (ICH)[12] and demonstrated excellent specificity, linearity, precision and accuracy for Valacyclovir hydrochloride. The chemical structure of Valacyclovir hydrochloride is shown in Figure 1.

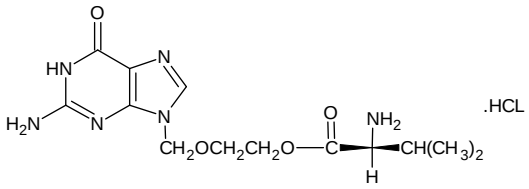

Fig. 1: Chemical structure of Valacyclovir

EXPERIMENTAL

Instruments

The spectrophotometric measurements were carried out using Agilent Technology Carry 60 UV-visible spectrophotometer and fluorometric measurements were carried out using Systronics photofluorometer.

Materials

Valacyclovir was supplied as a gift sample by Mylan pvt. Ltd. Hyderabad, India. Analytical grade methanol and ethanol were purchased from Molychem, Mumbai.

ANALYTICAL METHOD DEVELOPMENT

Preparation of Stock Solution

10 mg Valacyclovir was dissolved in 10 mL methanol, ethanol, distilled water, 0.1N hydrochloric acid and phosphate buffer of pH 7.2 separately to get solutions of concentration 1000 µg/mL. Above stock solution were further diluted with same solvent to get the final concentrations of 100 µg/mL and these solutions were used as standard stock solutions.

Determination of λ max

The standard stock solution of Valacyclovir having the concentration 100 µg/mL in different solvents was scanned in the range of 200-400 nm using UV spectrophotometer.

Preparation of Working Solutions

From standard stock solutions of concentration 100 µg/mL, 0.2, 0.4, 0.6, 0.8, 1.0 mL etc solutions were withdrawal and final volume was adjusted upto 10 mL with methanol **(Method A)**, ethanol **(Method B)**, water **(Method C)** and phosphate buffer of pH 7.4 **(Method D)** separately to get solutions of concentration 2, 4, 6, 8 and 10 µg/mL etc. respectively and analyzed by Uv-Spectrophotometer. Similarly 0.1, 0.2, 0.3, 0.4 mL etc were withdrawal from the same stock solutions and final volume was adjusted upto 10 mL with methanol **(Method A)**, water **(Method B),** 0.1N HCl **(Method C)** separately to get solutions of concentration 1, 2, 3, 4 and 5 µg/mL etc. respectively and analyzed by fluorometry.

Construction of Calibration Curve

The absorbance of working solutions prepared was measured at λ_{max} found above against solvents as a blank and recorded. The calibration curve was plotted using absorbance v/s concentration. The percent relative transmittance also measured and the calibration curve was plotted using percent relative transmittance v/s concentration. Then the liner equation and regression coefficient were calculated.

Method Validation

Validation is a process of 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. Method was validated by evaluating linearity, accuracy, precision, limit of detection and limit of quantification[13, 14].

Linearity and Range

The linearity of an analytical method is its ability to produce test results that are directly proportional to the concentration of analyte in sample within a given range. Linearity and range of methods were determined by taking absorbance by spectrophotometry and percent relative transmittance by fluorometry of working solutions prepared using different solvents. Finally the linear equation and regression coefficient were calculated and range was decided.

Precision

The precision is measure of the degree of reproducibility or repeatability of an analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as standard deviation, relative standard deviation or coefficient of variance of a series of measurements. The two types of precision study intra- day and inter- day were performed by analyzing the diluted working solutions for three times within a day (intra-day) and analyzing the same

solutions for three different days (inter day) precision study.

Accuracy and Recovery Study

This study was performed as per ICH guidelines. 20 tablets were weighed and powdered. The powder sample equivalent to 300 mg of active ingredients was weighed and dissolved in 300 mL of different solvents (1000 µg/mL) and allowed to sonicate for 10 mins. The study was performed at three levels by preparing sample solution concentration of 2.0 µg/mL, 4.0 µg/mL and 6.0 µg/mL using solution of concentration 10 µg/mL. The readings (absorbance and percent relative transmittance) of these concentrations were recorded. Then the % RSD of the concentrations was calculated. The accuracy of the proposed methods was assessed by recovery studies at three different levels. Recovery studies were carried out by standard addition method. It was performed by adding known amount of Valacyclovir solution of the pure drug to pre-analyzed tablet solutions. The resulting solutions were then reanalyzed by proposed methods.

LOD and LOQ

The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy. The LOD and LOQ were determined by using following formulae[15].

$$LOD = \frac{3.3\ \sigma}{S} \quad and \quad LOQ = \frac{10\ \sigma}{S}$$

σ = Standard deviation, S= Slope of the calibration curve

RESULT AND DISCUSSION

λmax

The λ max of drug by different method was found to be 255 nm.

Construction of Calibration Curve

The calibrations curves were constructed by measuring the absorbance of the working solutions of concentration 2-20 µg/mL in different solvents at λmax of 255 nm by Uv spectrophotometer and the percent relative transmittance of the solutions of concentration 1-10 µg/mL in different solvents by the fluorometer. The calibration curve of Valacyclovir by different method is shown in figure 2 and 3.

Validation of Analytical Method

The above methods are validated for linearity and range, precision, accuracy and recovery, LOD and LOQ according to ICH guidelines.

Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 2-20 µg/mL was linear with a correlation coefficient (R^2) greater than 0.97 for spectrophotometric method and percent relative transmittance of the samples in the range of 1-10 µg/mL was linear with a correlation coefficient (R^2) greater than 0.99 for fluorometric method. The linearity and range profile of all the methods represented in the Table 1 and 2.

Precision Study

The intra-day and inter-day precision study (Table 3 and 4) of the developed method confirmed adequate sample stability and method reliability where all the RSDs were <2%

Accuracy and recovery

To further ascertain the accuracy and reliability of the proposed methods, recovery experiments were performed via standard addition procedure. Results within the range of 100.12–101.88 % for spectrophotometry and 100.71-102.42 % for fluorometry ensure an accurate method (Table 5 and 6) as well as indicate non-interference with the excipients of formulation.

LOD and LOQ

The LOD and LOQ were calculated for all methods and mentioned in the Table 7 and 8.

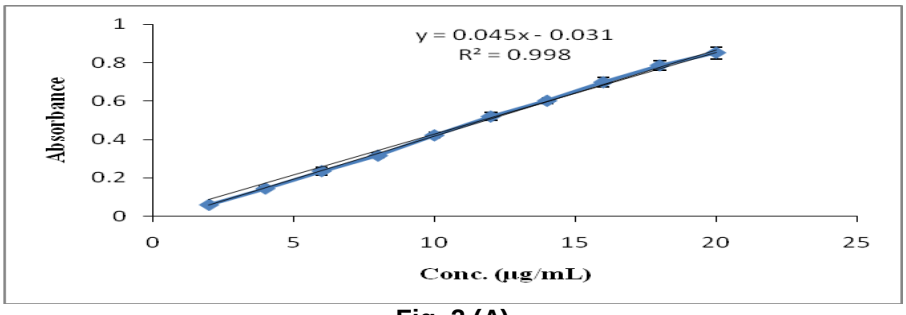

Fig. 2 (A)

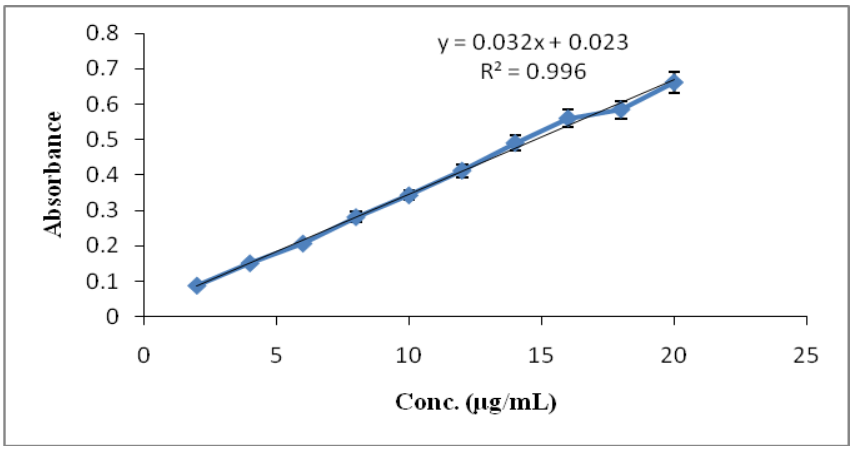

Fig. 2 (B)

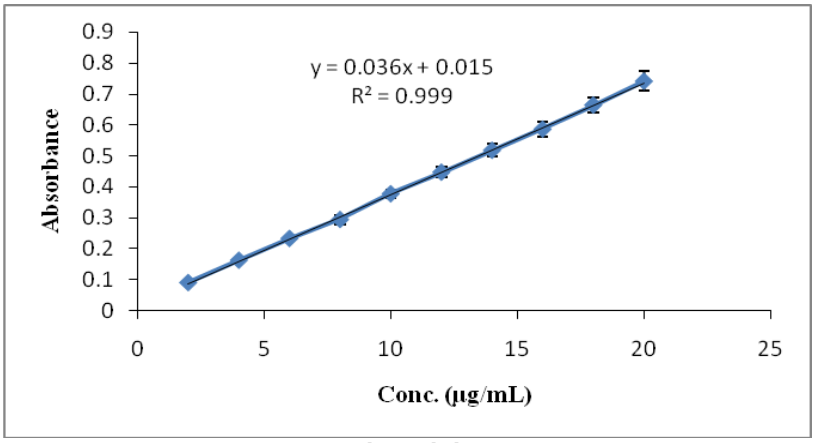

Fig. 2 (C)

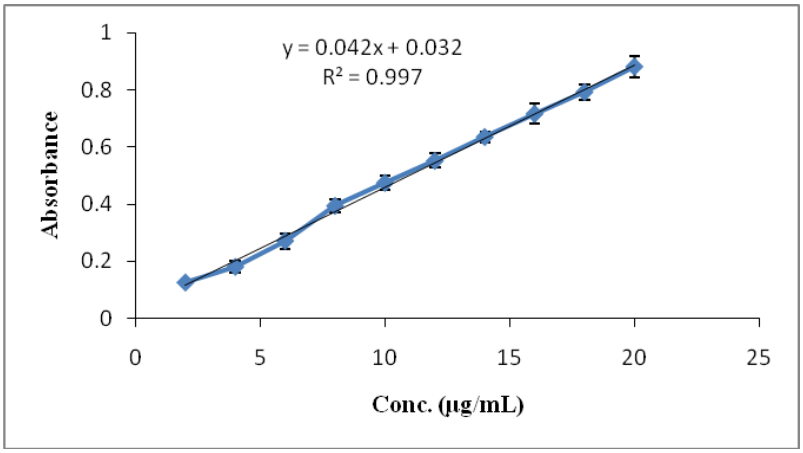

Fig. 2 (D)

Fig. 2: Calibration curve of Valacyclovir in (A) Methanol, (B) Ethanol, (C) Distilled water and (D) 7.2 pH PBS by Spectrophotometry

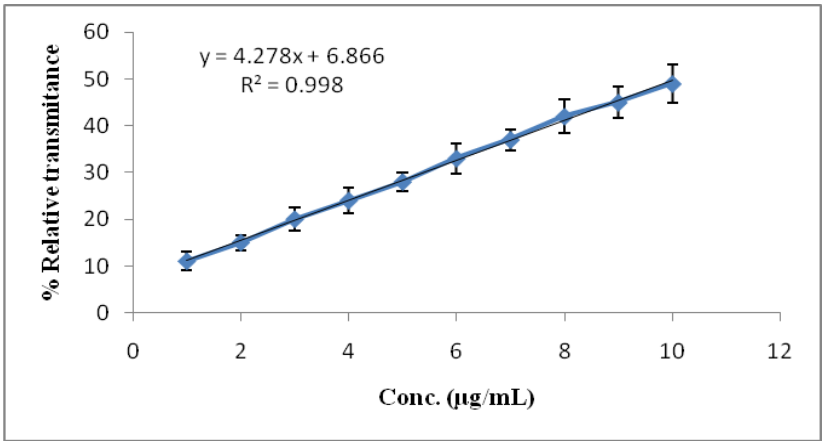

Fig. 3 (A)

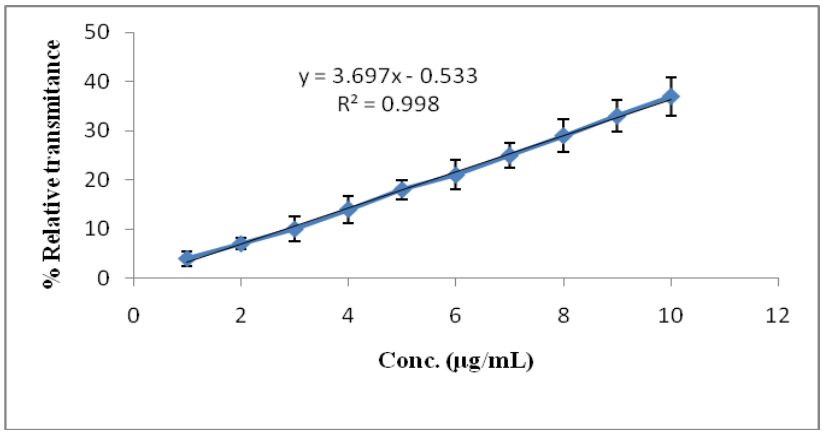

Fig. 3 (B)

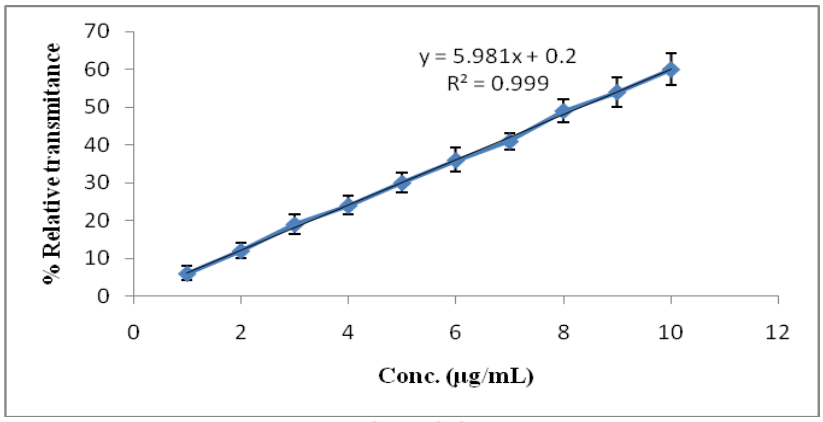

Fig. 3 (C)

Fig. 3: Calibration curve of Valacyclovir in (A) Methanol, (B) Distilled water and (C) 0.1 N HCl by Fluorometry

Table 1: Linearity and Range by Spectrophotometry

Statistical Parameters	Results			
	Method A	Method B	Method C	Method D
Wavelength (nm)	255	255	255	255
Range (µg/mL)	2-20	2-20	2-20	2-20
Correlation coefficient	0.998	0.996	0.999	0.997
Slope	0.045	0.032	0.036	0.042
Intercept	0.031	0.023	0.015	0.032

Table 2: Linearity and Range by Fluorometry

Statistical Parameters	Results		
	Method A	Method B	Method C
Range (µg/mL)	1-10	1-10	1-10
Correlation coefficient	0.998	0.998	0.999
Slope	4.278	3.697	5.981
Intercept	6.866	0.533	0.200

Table 3: Precision by Spectrophotometry

Method	Intraday precision (n=3)		Interday precision (n=3)	
	SD	% RSD	SD	% RSD
A	0.0011	0.47	0.0024	0.76
B	0.0044	1.0	0.0061	1.3
C	0.0014	0.44	0.0039	1.3
D	0.0019	0.36	0.0048	1.14

Table 4: Precision by Fluorimetry

Method	Intraday precision (n=3)		Interday precision (n=3)	
	SD	% RSD	SD	% RSD
A	0.2424	0.9440	0.3331	1.071
B	0.3723	1.6499	0.4107	1.92
C	0.6732	2.0	0.6636	1.99

Table 5: Accuracy by Spectrophotometry

Statistical Parameter	Method			
	A	B	C	D
% Recovery (Mean)	101.04	100.12	100.77	101.88
SD	2.7	2.4	1.64	3.67
% RSD	2.6	2.3	1.62	3.58

Table 6: Accuracy by Fluorimetry

Statistical Parameter	Method		
	A	B	C
% Recovery (Mean)	100.71	101.94	102.42
SD	1.82	2.33	2.74
% RSD	1.81	2.28	2.66

Table 7: LOD and LOQ by Spectrophotometry

Statistical Parameter	Method			
	A	B	C	D
LOD (µg/mL)	3.44	7.5	2.6	4.08
LOQ (µg/mL)	10.44	22.81	8.16	12.38

Table 8: LOD and LOQ by Fluorometry

Statistical Parameter	Method		
	A	B	C
LOD (µg/mL)	0.64	0.87	0.54
LOQ (µg/mL)	3.94	5.27	3.29

CONCLUSION

The simple, sensitive, easily accessible and economical UV spectrophotometric and fluorometric methods are developed for estimation of Valacyclovir. The results obtained with use of solvents like water, 0.1N HCl and ethanol. Results of developed methods were calculated as per analytical parameters and statically expressed. It was observed that all parameters were within standard limit. Thus the developed methods are simple, precise, rapid, specific and accurate that can be used to estimate Valacylcovir in bulk and in formulation.

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