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Spectrophotometric Determination of Valacyclovir Hydrochloride in Bulk and Pharmaceutical Formulations

D.V. PRASAD REDDY, B.M. GURUPADAYYA*, Y.N. MANOHARA and V. VIJAYA BHASKAR
Department of Pharmaceutical Analysis, National College of Pharmacy Shimoga-577 201, India
E-mail: bm_guru2004@yahoo.co.in

Two simple, sensitive, rapid and accurate colorimetric methods (Method A and B) have been developed for the estimation of valacyclovir hydrochloride in bulk and pharmaceutical dosage forms. Methods A and B are based on the formation of the coloured schiff's base in acidic conditions. The presence of heterocyclic amino group in valacyclovir hydrochloride enabled the use of its condensation reaction with aromatic aldehydes like *p*-dimethyl aminobenzaldehyde (PDAB) and vanillin. Method A is based on yellow coloured complex formation between valacyclovir hydrochloride and vanillin, which shows maximum absorbance at 428 nm. Method B is based on light yellow coloured complex formation between valacyclovir hydrochloride and PDAB, which shows maximum absorbance at 388 nm. The linearity was found to be 20-100 and 100-500 µg/mL for Method A and B, respectively. Proposed methods were validated statistically and the recovery was carried out by standard addition method.

Key Words: Vanillin, Valacyclovir hydrochloride, *p*-Dimethyl aminobenzaldehyde, Spectrophotometric determination.

INTRODUCTION

Valacyclovir hydrochloride is chemically L-valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy]ethyl ester and monohydrochloride. Valacyclovirhydrochloride[1-7] is rapidly converted to acyclovir, which has demonstrated antiviral activity against herpes simplex virus types, 1 (HSV-1) and 2 (HSV-2) and varicella zoster virus (VZV) both *in vivo* and *in vitro*. The inhibitory activity of acyclovir is highly selective due to its affinity for the enzyme thymidine kinase 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 guanylate kinase and into triphosphate by a number of cellular enzymes. *In vitro* acyclovir triphosphate stops replication of herpes viral DNA.

The therapeutic importance of this compound justifies research to establish analytical methods for its determination in bulk drug and pharmaceutical formulation. In literature, no analytical methods were reported

for the determination of valacyclovir hydrochloride. In this paper, two methods are proposed for the determination of valacyclovir hydrochloride both in bulk and in pharmaceutical formulation.

EXPERIMENTAL

A shimadzu model 1700 double beam UV-Visible spectrophotometer with a pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Gift sample of valacyclovir hydrochloride was obtained from Cipla (Protec), Goa. Double distilled water, nitric acid and ethanol of A.R. grade were used in the study. Reagents such as vanillin and ethanolic solution of *p*-dimethyl aminobenzaldehyde (PDAB) were used in the colorimetric estimation. 4 N Nitric acid is being prepared and is used in the estimation.

Vanillin (0.5 %): Prepared by dissolving 500 mg in 100 mL of distilled water.

Ethanolic PDAB: Prepared by dissolving 1 g of PDAB in a mixture of 30 mL of 95 % ethanol, 180 mL of 1-butanol and 30 mL of HCl.

Nitric acid (4 N): Prepared by diluting 25.2 mL of nitric acid to 100 mL with distilled water.

Preparation of standard solutions: Valacyclovir hydrochloride standard stock solution (1 mg/mL) was prepared by dissolving 100 mg of drug in distilled water and made up to volume with distilled water in a 100 mL volumetric flask. The standard stock solution (1 mg/mL) is used as such for Method A and B. Vanillin and ethanolic PDAB solutions are prepared as per the procedures mentioned above.

Method-A

Visible spectrophotometric method using vanillin: Aliquots of standard drug solution 0.2-1.0 mL (1 mL = 1000 µg) were pipetted out into a series of 10 mL volumetric flasks. Each flask is being added with 2 mL of vanillin and 1 mL of nitric acid (4N). The solutions were warmed on a water bath for 10 min at 60-70ºC. The resulting solutions are cooled to the room temperature and the volume is made up to the mark with distilled water. The absorbances of the yellow coloured species were measured at 428 nm against the reagent blank. The amount of valacyclovir hydrochloride present in the sample was computed from calibration curve.

Method-B

Visible spectrophotometric method using ethanolic PDAB: Aliquots of standard drug solution 1.0-5.0 mL (1 mL = 1000 µg) were pipetted out into a series of 10 mL volumetric flasks. Each flask is being added with 2 mL of ethanolic PDAB and 1 mL of nitric acid (4 N). The solutions were warmed on a water bath for 10 min at 60-70ºC. The resulting solutions are

cooled to the room temperature and the volume is made up to the mark with ethanol. The absorbances of the light yellow coloured species were measured at 388 nm against the reagent blank. The amount of valacyclovir hydrochloride present in the sample was computed from calibration curve.

Analysis of formulation: The contents of three formulations were emptied into a beaker and mixed. Weight equivalent to 100 mg of valacyclovir hydrochloride was quantitatively transfered into a 100 mL calibrated flask and diluted to the mark with distilled water. The solution was then analyzed after dilution by Visible spectrophotometric method. Appropriate aliquots of drug solution were taken and the individual assay procedure was followed for the estimation of drug content in the tablets. The concentrations of drug in the tablets were calculated using calibration curve. The recovery experiments were carried out by standard addition method. The results of analysis are given in Table-2.

RESULTS AND DISCUSSION

Methods A and B are based on the formation of the coloured Schiff base in acidic conditions. The presence of heterocyclic amino group in valacyclovir hydrochloride enabled the use of its condensation reaction with aromatic aldehydes like *p*-dimethyl aminobenzaldehyde and vanillin. The determination conditions for both these methods were established by varying one parameter at a time and keeping the others fixed by observing the effect produced on the absorbance of the coloured species. The various parameters involved for maximum colour development for both the methods were optimized. For method A, parameters like volume of nitric acid and vanillin solutions and time for optimization of reaction were studied and it was found to be 1 mL, 2 mL and 10 min, respectively. For method B, parameters like volume of nitric acid, ethanolic PDAB solutions and time for optimization of reaction were studied and it was found to be 1 mL, 2 mL and 10 min, respectively. Measuring the absorbance values at time intervals of 20 min for 3 h in both methods, to find out the stability of the formed chromogen and it was found that the chromogen was stable for more than 3 h in methods A and B respectively. The proposed methods were validated statistically and by recovery studies.

The molar absorptivity and sandell's sensitivity values show the sensitivity of both methods, while the precision is confirmed by % RSD, which are mentioned in Table-1. Assay results and results of recovery studies are given in Table-2. The results are in good agreement with labeled value. The per cent recovery obtained indicates non-interference from the common excipients used in the formulations. The reproducibility, repeatability and accuracy of these methods were found to be good, which is evidenced by low standard deviation. The proposed methods were simple, sensitive,

accurate, precise and reproducible and can be successfully applied for the routine estimation of valacyclovir hydrochloride in bulk and pharmaceutical dosage forms.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF PROPOSED METHODS

Parameters		Methods	
		A	B
Beer's law limits (μg/mL)		20-100	100-500
Molar absorptivity (L $mol^{-1}cm^{-1}$)		3.463×10^3	2.842×10^3
Sandell's sensitivity (μg/cm^2/0.001 abs. unit)		0.00134	0.00323
Correlation coefficient (γ)		0.9997	0.9998
Regression equation (Y)*	Slope (a)	0.0478	0.009
	Intercept (b)	0.0102	0.006
% RSD†		0.30337	0.4242
Range of errors			
Confidence limit with 0.05 level		0.00195	0.0017
Confidence limit with 0.01 level		0.00288	0.0025

*Y = a + bx, where Y is the absorbance and x is the concentration of valacyclovir hydrochloride in μg/mL; †For eight replicates.

TABLE-2
RESULTS OF ANALYSIS OF VALACYCLOVIR HYDROCHLORIDE IN TABLETS

Formulation	Label claim (mg)	Method	% of Label claim ±SD*	% Recovery ±SD**
Tablets	100	A	99.89 ± 0.189	99.92 ± 0.122
		B	99.78 ± 0.256	99.85 ± 0.265

*Average ± standard deviation of eight determinations.
**Average ± standard deviation of eight determinations (known quantity of the standard drug was added in the solution and then final solution was analyzed).

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