STABILITY INDICATING DETERMINATION OF VALACYCLOVIR BY RP-HPLC METHOD

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ABSTRACT

A simple Stability indicating reverse phase HPLC method was developed for the determination of Valacyclovir present in pharmaceutical dosage forms. Valacyclovir represents a clear advance in the prevention and treatment of viral infection.The mobile phase was simple to prepare and economical. A Hypersil ODS C-18 (250 x 4.6 mm, packed with 5 micron) in an isocratic mode with mobile phase Acetonitrile: Phosphate buffer (pH- 3.6) (50:50%v/v) was used. The flow rate was 0.8ml/ min and effluent was monitored at 252 nm. The retention time was 2.842 min Valacyclovir. The linearity range was found to be 0.5 - 200 µg/ml. It was found that the percentage recovery values of pure drug from the pre-analyzed solutions of formulations were in between 98.1337- 101.4633. All the degradation products formed during forced decomposition studies were well separated from the analyte peak demonstrates that the developed method was specific and stability-indicating.

KEYWORDS: *Valacyclovir, Acyclovir, Prodrug, Valcivir.*

INTRODUCTION

Valacyclovir was a prodrug, an esterified version of acyclovir that has greater oral bioavailability (about 55%) than acyclovir (10-20%). It was converted by esterase to the active drug acyclovir via hepatic first-pass metabolism. It comes under the category of Antiviral Drugs. It was initially approved by the FDA in 1995 and marketed by GlaxoSmithKline. Valacyclovir was the L-valine ester of acyclovir. It was a member of the purine (guanine) nucleoside analog drug class. This class of drugs forms an important part of hepatitis, HIV, and cytomegalovirus drug regimens. The chemical name was 2-[(2-amino-6-oxo-3, 9-dihydropurin-9-yl) methoxy] ethyl-2-amino-3-methyl-butanoate with molecular formula of $C_{13}H_{20}N_6O_4$. It was soluble in soluble in methanol and maximum soluble in water. It was a white crystalline powder. Acyclovir was selectively converted into a monophosphate form by viral thymidine kinase, which was far more effective (3000 times) in phosphorylation than cellular thymidine kinase. The objective of this study was to develop and validate a simple and cost effective stability indicating method for rapid estimation of Valacyclovir in presence of its stress degradation related impurities. Hence the proposed method can be useful as a rapid analytical technique for the degradation kinetics and to establish the degradation pathways as shown in **Fig. 1.**

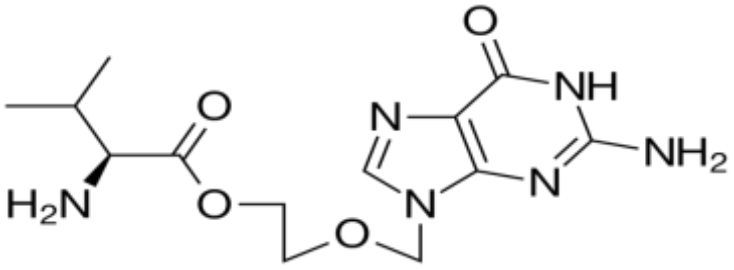

Fig. 1: Structure of Valacyclovir.

MATERIAL AND INSTRUMENT

SHIMADZU HPLC with UV-Visible Detector (SPD-10A), PUMP (LC-10AT) and (LC-10AT VP) was used. UV-Visible (ELICO SL-159), Electronic Balance (AFCOSET), Ultra Sonicator (ENERTECH), and pH Analyzer (ELICO) was used. Valacyclovir was gifted sample by Cipla pharmaceuticals limited (India). Acetonitrile (RANKEM), Disodium hydrogen phosphate anhydrous (Merck) and Citric acid (Merck) were used.

SELECTION OF WAVE LENGTH

The sensitivity of the HPLC method with UV detection depends upon the proper selection of wavelength. To ascertain the maximum wavelength, λ_{max} of the proposed method, the drug solution (10 µg/ml) was scanned between the wavelength ranges of 200 – 380 nm in UV-Visible Spectrophotometer found to be 252 nm as in **Fig. 2** and **Fig. 3.**

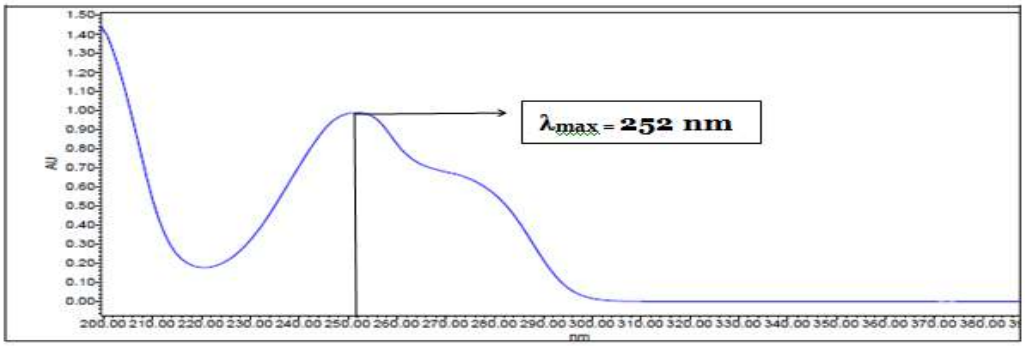

Fig. 2: Maximum Wavelength λ_{max} of Valacyclovir.

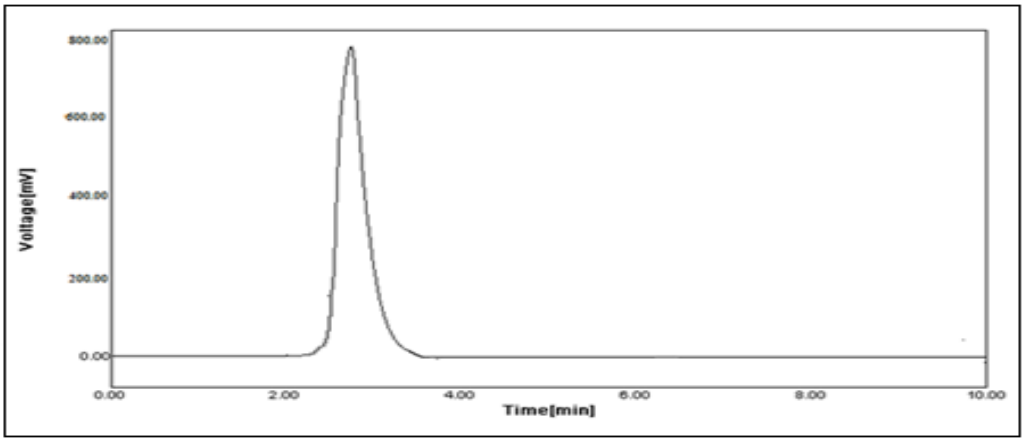

Fig. 3: Optimized Chromatogram of Valacyclovir.

PREPARATION OF MOBILE PHASE AND BUFFER

Acetonitrile and Phosphate buffer (p^H- 3.6) were properly mixed in the ratio of 50:50.
0.9g of anhydrous disodium hydrogen phosphate and 1.298g of citric acid monohydrate were weighed, mixed and volume made with double distilled water (1000ml).

PREPARATION OF STANDARD SOLUTIONS

Stock solution of Valacyclovir (1 mg/ml) was prepared by dissolving 25 mg of Valacyclovir in 25 ml of volumetric flask containing 10 ml of Acetonitrile and 10ml of Phosphate buffer (pH- 3.6). The solution was sonicated for about 10 min and then made up to volume with mobile phase. Daily working standard solutions of Valacyclovir was prepared by suitable dilution of the stock solution with appropriate mobile phase. Working standard solutions of Valacyclovir were prepared by taking suitable aliquots of drug solution from the standard stock solution 1000µg/ml and the volume was made up to 10 ml with mobile phase.

PREPARATION OF SAMPLE SOLUTION

Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of Valacyclovir was extracted with Acetonitrile and Phosphate buffer (pH- 3.6) in a 25ml volumetric flask using ultra sonicator. This solution was filtered through 0.45µm filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined.

ASSAY PROCEDURE

The amount of drug present in pharmaceutical formulation was calculated through area under curve of drug by standard calibration curve (concentration in µg/ml was taken on X-axis and area under curve on Y-axis) method. A typical chromatogram of Valacyclovir in formulation was shown in **Table 1** and **Fig. 4.**

Table 1: Amount of Valacyclovir in Tablets.

Labeled Amount	Amount Recovered	% Drug recovered	% RSD
500 mg	501.3 mg	100.275	0.2045

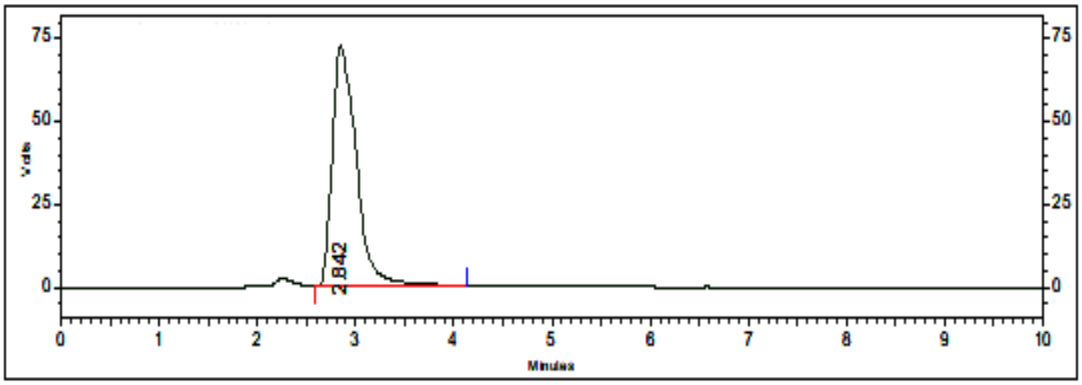

Fig. 4: Chromatogram of Valacyclovir in formulation.

METHOD VALIDATION

LINEARITY

The retention time, areas under curve of drug were recorded. Taking conc. plotted a graph on X-axis and areas under curve of drug on Y-axis. The linearity range was found to be in between 0.5-200 µg/ml for Valacyclovir as shown in **Table 2** and **Fig. 5.**

Table 2: Linearity Result of Valacyclovir.

Concentration (mg/ml)	Absorbance
0.5	33620
1	70467
10	582534
50	2663426
100	5178819
200	10284367
SD	0.205
% RSD	0.204

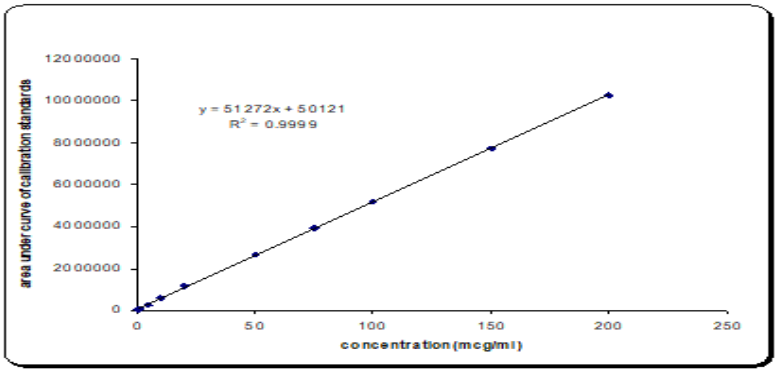

Fig. 5: Calibration Curve of Valacyclovir.

PRECISION

The precision of each method was ascertained separately from the areas under the curve obtained by actual determination of six replicates of a fixed amount of drug. The precision of the assay was also determined in terms of intra-and inter-day variation in the peak areas for a set of drug solutions on three different days as shown in **Table 3.**

Table 3: Precision Results of Valacyclovir.

Concentrations (µg/ml)	Absorbance	Statistical analysis
10	582534	Mean = 590218.5 SD = 10408.85 %RSD = 1.764
10	601255	
10	596824	
10	572155	
10	582456	
10	600345	

ACCURACY

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples of Valacyclovir within the linearity ranges were taken and added to the pre-analyzed formulation of concentration 10 µg/ml. From that percentage recovery values were calculated as shown in **Table 4.**

Table 4: Accuracy Results of Valacyclovir.

Sample	Concentration (µg/ml)		% Recovery	Statistical Analysis
	Pure drug	Formulation		
80 %	8	10	98.38	Mean = 98.133
			97.56	SD = 0.498
			98.46	% RSD = 0.507
100 %	10	10	101.56	Mean = 101.463
			101.89	SD = 0.482
			100.94	% RSD = 0.475
120 %	12	10	99.61	Mean = 99.253
			99.28	SD = 0.370
			98.87	% RSD = 0.373

SYSTEM SUITABILITY

System suitability parameters were defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The system suitability parameters were calculated and the proposed RP-HPLC method for the estimation as shown in **Table 5.**

Table 5: System Suitability Results of Valacyclovir.

S.No	Parameters	Obtained Values
1.	Theoretical plates (N)	4369
2.	Resolution (R) of the drug	2.57
3.	Tailing factor (T)	1.024

RUGGEDNESS

Ruggedness was the degree of reproducibility of the results obtained under a variety of conditions. It was checked that the results are reproducible under differences in reagents, analysts and experimental periods and hence the proposed method as shown in **Table 6.**

Table 6: Ruggedness Result of Valacyclovir.

Variables	Retention Time	% RSD
Analyst-I	2.75	1.81
Analyst-II	2.85	1.75

ROBUSTNESS

The percent recovery of Valacyclovir was good under most conditions and didn't show any significant change when the critical parameters were modified. The tailing factor for Valacyclovir was always less than 2.0 and the components were well separated under all the changes carried out. The test solutions were injected with deliberate variations in method parameters like flow rate, temperature, pH and mobile phase composition as in **Table 7.**

Table 7: Robustness Results of Valacyclovir.

Parameters (n=6)	Variables	Statistical analysis	
		RT Mean ±SD	% RSD
Flow rate (ml/min)	0.7	2.92±0.05	1.70
	0.8	2.85±0.05	1.75
	0.9	2.63±0.09	3.42
Mobile Phase (Buffer : ACN)	55:45	2.93±0.10	3.41
	50:50	2.85±0.05	1.75
	45:55	2.75±0.09	3.27
Temperature ºC	26	2.88±0.09	3.12
	28	2.85±0.05	1.75
	30	2.76±0.04	1.44
pH	3.4	2.75±0.05	1.81
	3.6	2.85±0.05	1.75
	3.8	2.79±0.06	2.15

FORCED DEGRADATION STUDIES

About 10 mg of Valacyclovir pure drug was accurately weighed and transferred to 10 ml volumetric flask which was further treated with different stress conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient as shown in **Table 8.**

ACIDIC DEGRADATION

About 10 mg of Valacyclovir pure drug was accurately weighed and transferred to 10 ml volumetric flask and 1 mL of 0.1 N HCl was added and kept aside for one hour and made up to volume with mobile phase. Then from this 10 µg/ml solution was prepared and injected in HPLC system to obtain chromatograms as shown in **Fig 6.**

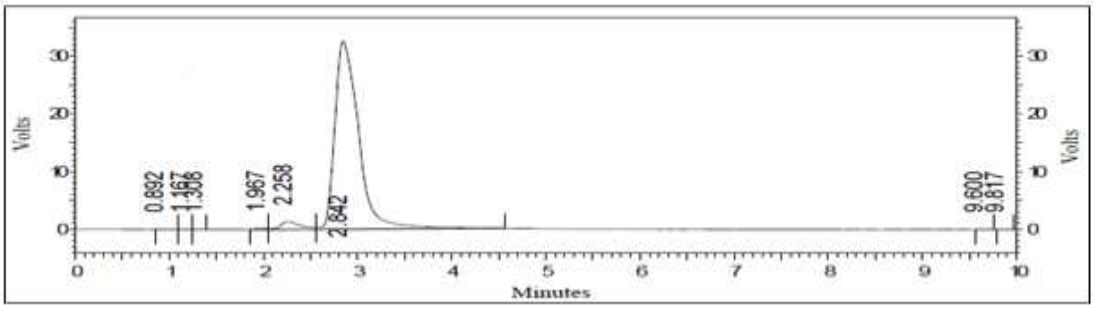

Fig. 6: Chromatogram for Acidic Degradation.

ALKALINE DEGRADATION

About 10 mg of Valacyclovir pure drug was accurately weighed and transferred to 10 ml volumetric flask and one ml of 0.1 N NaOH was added and kept aside for one hour and made up to volume with mobile phase. Then from this 10 µg/ml solution was prepared and injected in HPLC system to obtain chromatograms as shown in **Fig 7.**

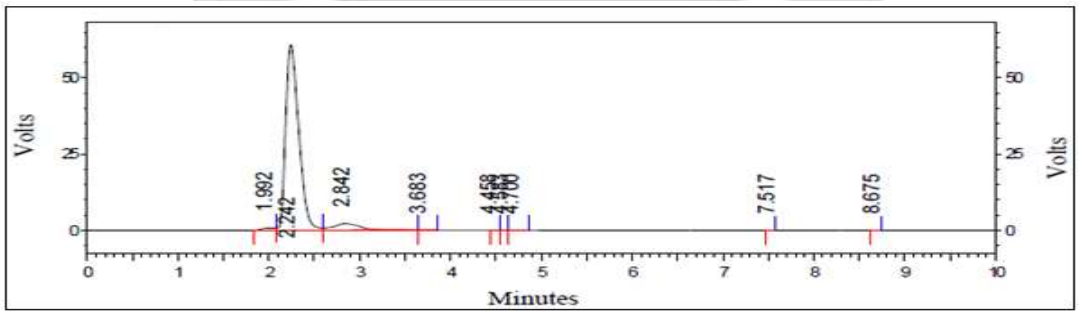

Fig. 7: Chromatogram for Alkaline Degradation.

OXIDATIVE DEGRADATION

About 10 mg of Valacyclovir pure drug was accurately weighed and transferred to 10 ml volumetric flask and one ml of 3%w/v of hydrogen peroxide (H_2O_2) was added and kept side for two hours and made up to volume with mobile phase. Then from this 10 µg/ml solution was prepared and injected in HPLC system to obtain chromatograms as in **Fig 8.**

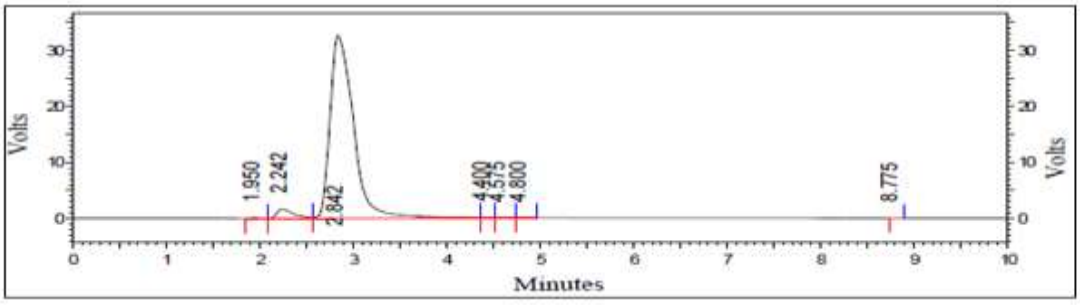

Fig. 8: Chromatogram for Oxidative Degradation.

PHOTOLYSIS

About 10 mg of Valacyclovir pure drug was accurately weighed and transferred to 10 ml volumetric flask and made up to volume with mobile phase and kept aside for 8 hours under direct sunlight. Then from this 10 µg/ml solution was prepared and injected in HPLC system to obtain chromatograms as shown in **Fig 9.**

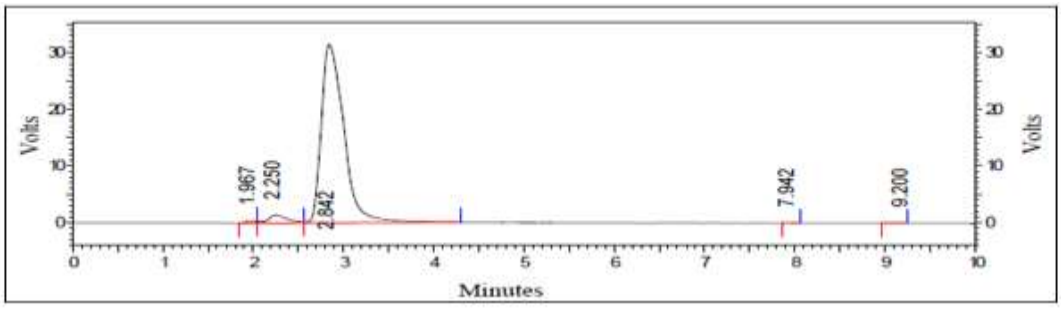

Fig. 9: Chromatogram for Photolytic Degradation.

Table 8: Results of Stability Indicating Assay.

S.No	Conditions	Peak area	% Recovered
1.	Standard	582534	100.0
2.	Acidic	573961	98.52833
3.	Alkaline	47643	81.78578
4.	Oxidative	572307	98.24439
5.	Photolysis	544850	93.53102

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