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Original Research Article

DEVELOPMENT AND VALIDATION OF A SIMPLE UV SPECTROPHOTOMETRIC AND STRESS STUDIES METHOD FOR THE DETERMINATION OF VALACYCLOVIR HYDROCHLORIDE BOTH IN BULK AND MARKETED DOSAGE FORMULATIONS

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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 251 nm in distilled water and the method was validated for linearity, precision, sensitivity, and specificity. The drug obeyed linearity at concentration range of 2-32 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 99.1 –99.7%. Therefore, the proposed method could be applied for the routine analysis of pharmaceutical dosage forms containing Valacyclovir hydrochloride. Forced degradation studies were also performed.

Key words: Valacyclovir hydrochloride, UV spectrophotometric method, validation, method development.

Introduction: Forced degradation studies are also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used. But these guidelines are very general in conduct of forced degradation and do not provide details about the practical approach towards stress testing [16-19]. Knowledge of the stability of molecule helps in selecting proper formulation and package as well as providing proper storage conditions and shelf life, which is essential for regulatory documentation.

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Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule Although forced degradation studies are a regulatory requirement and scientific necessity during drug development, it is not considered as a requirement for formal stability program. A forced degradation study is an essential step in the design of a regulatory compliant stability program for both drug substances and products, and formalized as a regulatory requirement in ICH Guideline Q1A in 1993[20-25].

Valacyclovir HCl is the HCl salt of the L-valyl ester of the antiviral drug acyclovir Valacyclovir has a prodrug, an esterifies version of acyclovir that has greater oral bioavailability (about 55%) than acyclovir (10–20%). It has converted by esterase's to the active drug acyclovir, with the amino acid valine, via hepatic first-pass metabolism [1-10].Valacyclovir is L valine 2[(2amino 1, 6 di hydro 6oxo – 9H purine 9yl) methoxy] ethyl ester and exhibits antiviral activity against Herpes simplex virus and Varicella zoster virus. Valacyclovir exhibits similar potency but has more favorable pharmacokinetic characteristics, requiring less frequent dosing and achieving high blood plasma levels than acyclovir. The metabolism of valacyclovir to acyclovir probably occurs within the gut lumen prior to absorption, in the small intestine after uptake but before entry in to the portal blood system and in the liver before entry in to the systemic circulation [10-15].

IUPAC name: (S)-2-[(2-amino-6-oxo-6,9-dihydro-3H-purin9yl) methoxy] ethyl 2-amino-3-methylbutanoate

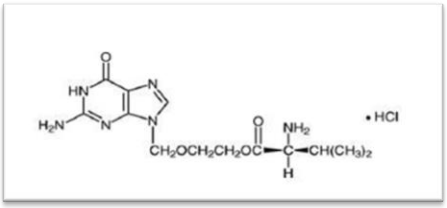


Fig1: structure of Valacyclovir hydrochloride

Introduction to UV spectroscopy

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Any molecule has either n, π or σ or combination of these electrons. These bonding (σ and π) and non-bonding electrons absorb the characteristic radiation and undergoes transition from ground state to excited state. By the characteristic absorption peaks and the nature of the electron present the molecular structure can be elucidated

UV spectroscopy obeys the Beer-Lambert law, Beer law:

This law can be stated as follows: "When a beam of monochromatic radiation is passed through a solution of absorbing substances, the intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substances exponentially".

$$I=I0*e-k1*c$$

Where, I0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

C = molar concentration of solute

K1=constant

Lambert's law: This law can be stated as follows "When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light".

$$I=I0*e-k2*l$$

Where, I0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

L = length of sample cell (cm.)

K2=constant

After combining equation 1 and 2 and deriving we get the following equation 3 of Beer-Lambert law as:

$$A = \log (I0/I) = ƐCl$$

Where, A = absorbance

I0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

C = molar concentration of solute
L = length of sample cell (cm.)
Ɛ = molar absorptivity

Materials and Methods:

Chemicals and reagents: Valacyclovir hydrochloride was gifted by pharma company, Hyderabad, Telangana, India. HPLC grade methanol procured from Rankem chemicals limited, New Delhi, India, distilled water, 0.1N H_2SO_4,

Instrumentation: Double beam UV spectrophotometer; Model: SL 210; Make: ELICO. The data was obtained using Spectra Treats the analysis was performed using UV SL120 using UV detector used for method development and validation. The output signal was checked and the acquisition and integration of data was performed using spectral threats. Software on a computer. The diluents are filtered through 0. 25µm.detection was monitored at 251nm.

Procedure:

Selection of wavelength: 10mg of Valacyclovir hydrochloride drug was accurately weighed and transferred into 10 ml of volumetric flask and the volume was made up to the mark with distilled water as Diluent .Then from this 0.1 ml was pipetted out and transferred into another 10 ml volumetric flask and the volume was made up to the mark with distilled water to give 10ppm solution and this was scanned between 200 to 400nm and its absorbance was measured at 251nm.(Figure-2).

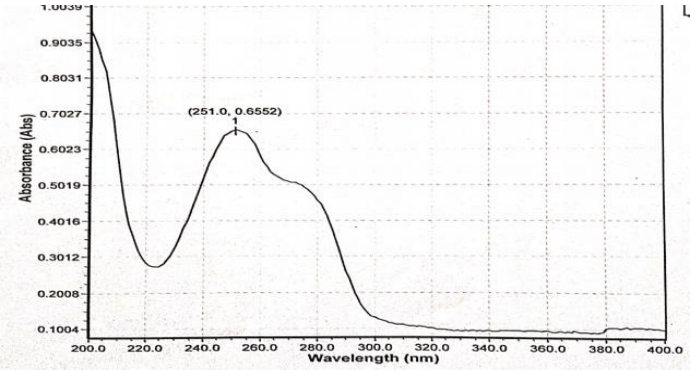


Fig 2: UV spectrum of Valacyclovir hydrochloride

Assay:

Preparation of standard solution: 10mg of Valacyclovir hydrochloride drug was accurately weighed and transferred into 10ml of volumetric flask and the volume was made up to the mark with distilled water to get concentration of 1000ppm.From this 0.1 ml was pipetted out and transferred into 10ml of volumetric flask and the volume was made up to the mark to get 10ppm solution and its absorbance was measured at 251nm.

Preparation of test solution: 20 tablets were weighed and powdered. Powdered tablet equivalent to 10 mg of valacyclovir hydrochloride was weighed accurately and it was taken into 10ml volumetric flask then volume was made up to the mark with distilled water. From the above solution 0.1 ml of solution was pipetted out and taken in 10ml volumetric flask. The volume was made up to 10ml to get 10ppm solution and its absorbance was measured at 251nm.

The % Assay is calculated by using the following formula:

% Assay= ((absorbance of the sample/absorbance of the standard) *(concentration of the standard/concentration of the sample)) *100

Method validation parameters

Method validation: ICH guidance for industry was followed for validation of the method. Linearity, Accuracy, Robustness, LOD, LOQ were assessed during method validation.

Linearity: Calibration standard solutions were prepared in plasma from the working solutions. Five calibration curves ranging from the 2 to 32 ppm were run to establish the linearity by using linear regression analysis. From the stock solution0.2ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2ml, 1.5ml, 1.8ml, 2.0ml, 2.2ml, 2.4ml, 2.6ml, 2.8ml, 3.0ml, 3.2ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml with distilled water give 2 ppm, 4 ppm, 6 ppm, 8ppm, 10ppm, 12ppm, 15ppm, 18ppm, 20ppm, 22ppm, 24ppm, 26ppm, 28ppm, 30ppm, 32ppm. concentration.

Respectively and absorbance was measured at 251nm using distilled water as blank and the calibration curve is plotted.

Precision: 10ppm standard solution of Valacyclovir HCl pure drug is selected for Precision study. From the standard stock solution 0.1ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml using distilled water to give 10ppm solution. This procedure is repeated 6 time and observances of all were measured at 251nm using distilled water as blank and its %RSD was calculated by using the formula

%RSD = (standard deviation of the measurement / mean value of measurement) *100

Accuracy: Quality control of samples was prepared at four different levels. The concentration of Valacyclovir HCl was calculated from a standard calibration curve that was concurrently obtained. Accuracy was analyzed at each level by comparing the observed concentration as a mean relative percentage recovery. Standard quantity equal into 50%, 100% and 150 % is to be added in sample.2ml of 5ppm of standard solution was spiked with 2ml of5ppm of sample solution, 2ml of 2.5ppmof standard solution was spiked with 2ml of 5ppm of sample solution, 2ml of 7.5ppm of standard solution was spiked with 2ml of 5ppm of sample solution. Absorbance was measured for three times at 251nm.Repeated three times and their absorbances are measured at 251nm and the %recovery is calculated by using the formula:

% Recovery = (amount found / amount added) *100

Limit of detection: The detection limit (DL) may be expressed as:

$$DL = 3.3*\sigma/ S$$

Where σ = the standard deviation of the response S = the slope of the calibration curves the slope S may be estimated from the calibration curve of the analyte.

Limit of quantification

The Quantitation limit (QL) may be expressed as:

$$QL = 10*\sigma/ S$$

Where σ = the standard deviation of the response S = the slope of the calibration curves the slope S may be estimated from the calibration curve of the analyte.

Robustness: Robustness:6 aliquots of 6ppm of standard solution was prepared and it was scanned at wavelength at (±)1nm of λmax. The absorbance was noted down

Ruggedness:10ppm standard solution was prepared and scanned for 6 times by different analyst and different instruments.

Forced degradation studies:

Acid degradation: From the 10ppm of drug solution,1 ml of the 10ppm solution was taken into 10ml volumetric flask to that added 1 ml of 0.1 N HCl kept for 24 hours at room temperature. After 24 hours neutralize the solution with 1 ml of 0.1N NaOH and measured its absorbance at 251nm

Alkali degradation: From the 10ppm of drug solution,1 ml of 10ppm solution was taken into 10 ml volumetric flask to that added 1ml of 0.1N NaOH Was kept for 24 hours at room temperature. After 24 hours neutralize with 1 ml of 0.1 N HCl and Measured its absorbance at 251nm

Photolytic degradation: 10mg of drug powder was exposed to UV light in UV chamber for 3hrs by placing the drug in Petri dish. After 3hrs Sample was diluted to get concentration of 10 µg/ml and absorbance was measured at 251nm

Thermal degradation: Drug was exposed to dry heat 40ºC in oven at for 3hrs by placing the drugs in Petri dish. Weighed 10mg of drug and diluted to get a final concentration of 10 µg/ml. Measure the absorbance at 251nm and calculate the percentage of Degradation.

Peroxide degradation: From the 10ppm of drug solution, 1 ml of the drug solution was taken into 10 ml volumetric flask to that added 1 ml of 3% hydrogen peroxide solution kept for 24 hours at room temperature After 24 hours

dilute with water to get concentration of 10 µg/ml and measured its absorbance at 251nm

Results and discussion

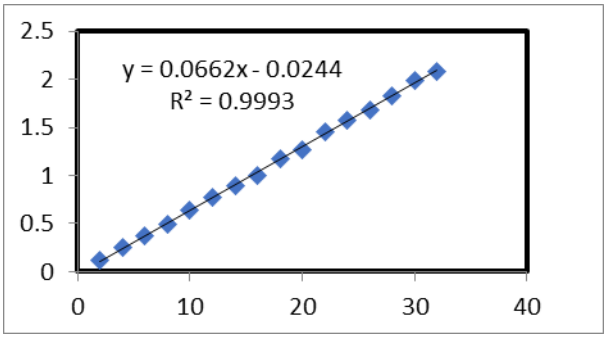


Figure 2: linearity curve of valacyclovir HCL

Table1: Conc.Vs Abs. table for Linearity Study.

Concentration(ppm)	Absorbance(nm)
1ppm	0.0982
2ppm	0.1987
4ppm	0.2998
6ppm	0.3993
8ppm	0.4989
10ppm	0.6552
12ppm	0.7702
15ppm	0.8793
18ppm	1.0021
20ppm	1.1201

Table 2: Evaluation data of precision study

S.No	**Concentration**	**Absorbance**
1	10ppm	0.6435
2	10ppm	0.6434
3	10ppm	0.6431
4	10ppm	0.6429
5	10ppm	0.6427
6	10ppm	0.6428
7	Mean	0.6430
8	SD	0.0003265
9	%RSD	0.050777

Table 3: Accuracy data

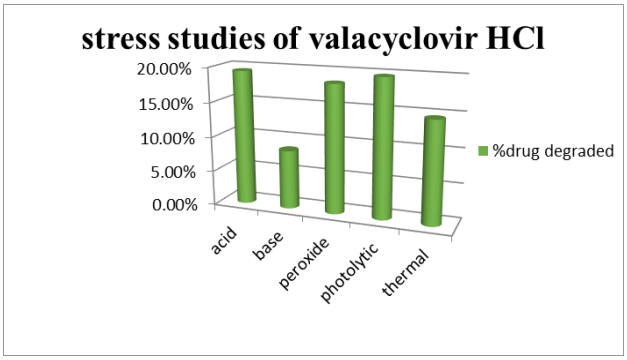
%Level	Absorbance(nm)	%Recovery	Mean% Recovery
50% (5ppm+2.5ppm)	0.5082	99.2%	99.1%
	0.5079	99.1%	
	0.5072	99.0%	
100% (5ppm+5ppm)	0.6509	99.8%	99.7%
	0.6501	99.7%	
	0.6502	99.7%	
150% (5ppm+7.5ppm)	0.7543	99.9%	99.8%
	0.7540	99.8%	
	0.7538	99.8%	

Table: 4 Robustness data

S. No	Concentration(ppm)	Absorbance(nm)	
1		250nm	252nm
	10ppm	0.6442	0.6420
2	10ppm	0.6441	0.6419
3	10ppm	0.6442	0.6417
4	Mean	0.6441	0.6418
5	SD	0.000057735	0.0001 5275
6	%RSD	0.0089636	0.0238 006

Table 5: Ruggedness

S.NO	Concentration(ppm)	Absorbance(nm)	
		Analyst-1	Analyst-2
1	10ppm	0.6436	0.6449
2	10ppm	0.6433	0.6446
3	10ppm	0.6432	0.6447
4	10ppm	0.6432	0.6445
5	10ppm	0.6430	0.6446
6	10ppm	0.6431	0.6445
7	Mean	0.6432	0.6446
8	SD	0.00020655	0.000150554
9	%RSD	0.032112	0.0233562



Assay:

%Assay= Absorbance of Sample x Concentration of Standard x 100
Absorbance of Standard Concentration of Sample

$$= \frac{0.6325}{0.6552} \times \frac{10}{9.65} \times 100$$

= 99.4%

Conclusion: The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. Therefore, this method can be used for the determination of Valacyclovir hcl either in bulk or in the dosage formulations without interference with commonly used excipients and related substances.

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