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DEVELOPMENT AND VALIDATION OF NEW SPECTROSCOPIC METHOD FOR THE ESTIMATION OF VALGANCYCLOVIR HCL IN BULK AND PHARMACEUTIC DOSAGE FORM

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ABSTRACT

The present study describes a simple, accurate, precise and cost effective UV-Spectrophotometric method for the estimation of Valgancyclovir Hcl, an anti-HIV drug, in bulk and pharmaceutical dosage form. The drug was first dissolved in 0.1N Hcl and final volume was made up with 0.1N Hcl. The λ_{max} or the absorption maxima of the drug was found to be 250nm. A linear response was observed in the range of 4-20µg/ml with a regression coefficient of 0.999. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Valgancyclovir Hcl in quality control of formulation without interference of the excipients.

Key Words: Valgancyclovir Hcl; UV-Spectrophotometric method; Validation; VALGAN tablets

INTRODUTION

Valganciclovir

Valganciclovir[1] is an L-valyl ester (prodrug) of ganciclovir that exists as a mixture of two diastereomers. After oral administration, both diastereomers are rapidly converted to ganciclovir by intestinal and hepatic esterases. Ganciclovir is a synthetic analogue of 2'-deoxyguanosine, which inhibits replication of human CMV in cell culture and in vivo. In CMV-infected cells ganciclovir is initially phosphorylated to ganciclovir monophosphate by

the viral protein kinase, pUL97. Further phosphorylation occurs by cellular kinases to produce ganciclovir triphosphate, which is then slowly metabolized intracellularly (half-life 18 hours). As the phosphorylation is largely dependent on the viral kinase, phosphorylation of ganciclovir occurs preferentially in virus-infected cells. The virustatic activity of ganciclovir is due to inhibition of the viral DNA polymerase, pUL54, synthesis by ganciclovir triphosphat

The chemical name of valgancyclovir[2] is 2-[(2-Amino 6,9,dihydro 3H purin 9-y1)methoxy] (2S) 2-Amino 3-metyl Butonate. The molecular formula of valgancyclovir $C_{14}H_{22}N_6O_5$.Hcl and it has the molecular weight of 390.83g/mol. It is freely soluble in methanol and water,insoluble in ether available as white crystals

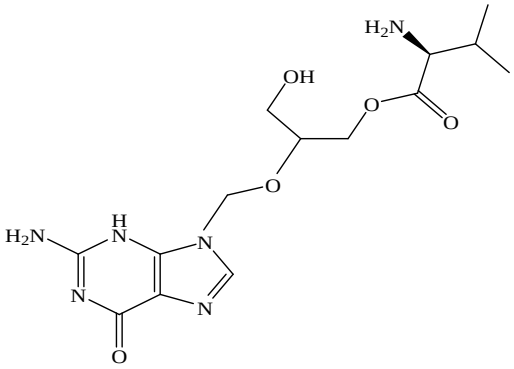

Figure 1: Chemical structure of Valgancyclovir

From the literature survey, we found that Valgancyclovir was estimated by different analytical methods like RP-HPLC[3-8], spectrophotometry[9]. The availability of an UV-Spectrophotometric method for estimation of Valgancyclovir will be very much useful for the determination in bulk and pharmaceutical formulations. This study aimed to develop a simple, precise, accurate and validated UV-Spectrophotometric method for the estimation of Valgancyclovir Hcl in bulk and pharmaceutical dosage form as per ICH guidelines. The statistical analysis proved that method is reproducible and selective for the simultaneous analysis of Valgancyclovir Hcl in bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS

Pharmaceutical grade Valgancyclovir Hcl was supplied by Hetro Drugs Ltd., Hyderabad, India and Hcl analytical reagent was purchased from Fisher scientific and commercially available VALGAN tablets, one (equivalent to 450mg mg of Ritonavir) of Cipla Ltd. was purchased from market for analysis.

The UV spectrophotometric estimation was done by using Roy instruments 1R-513C UV Visible spectrophotometer with 1 cm path length was used for spectral measurements with 1 cm matched quartz cells. Shimadzu balance (BL-220H) was used for all weighing.

METHOD DEVELOPMENT

Solubility Test

Solubility test for the drug Valganciclovir was performed by using various solvents, which includes distilled water, ethanol, chloroform, phosphate buffer (pH-4&8), N-N-dimethylformamide, 0.1M NaOH, acetone, 20% glacial acetic acid, 0.1M HCl, ethanol: water (1:9), and isopropyl alcohol. However, 0.1N Hcl was chosen as solvent for developing the method.

Preparation of Stock Solution

Weigh accurately 50mg of Valganciclovir and transferred to 50mL volumetric flask. Then add small amount of 0.1N Hcl and dissolve the drug. Then the final volume was made up with 0.1N Hcl.

Preparation of Working Standard Solution

From stock solution 10mL was further diluted to 100mL with 0.1N Hcl to get the solution having concentration 100µg/mL.

Determination of λmax

From the above working standard solution, 5mL was pipette out into a 10mL volumetric flask and the volume was made up to the mark with 0.1N Hcl to prepare a concentration of 50µg/mL. Then the sample was scanned in UV-VIS Spectrophotometer in the range 400-200nm using 0.1N Hcl as a blank and the wavelength corresponding to maximum absorbance (λmax) was found to be 250nm (fig:2).

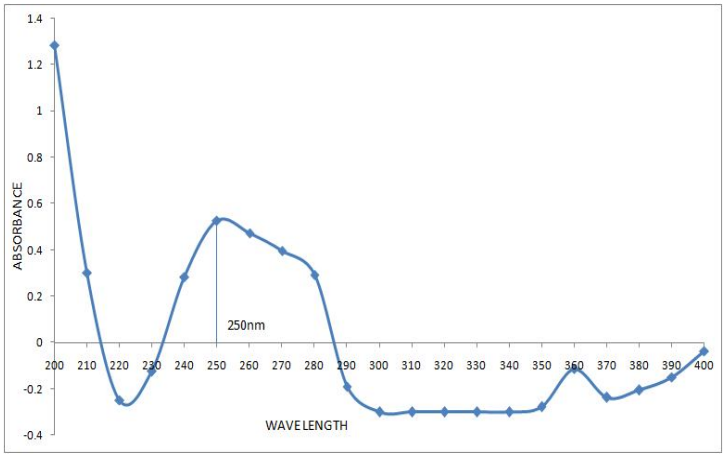

Fig: 2. UV Spectrum of valganciclovir (50µg/mL)

Preparation of Calibration Curve:

From the working standard solution, pipette out 0.4mL, 0.8mL, 1.2mL, 1.6mLand 2.0mL then diluted to 10mL using 0.1N Hcl to produce 4µg/mL, 8µg/mL, 12µg/mL, 16µg/mL and 20µg/mL solutions respectively. Then measure the absorbance of these solutions at the λmax of 250nm using 0.1N Hcl as the blank. Then, the calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis (fig: 3.4.2). The curve showed linearity in the concentration range of 4-20µg/mL. The correlation coefficient (r^2) was found to be 0.999.

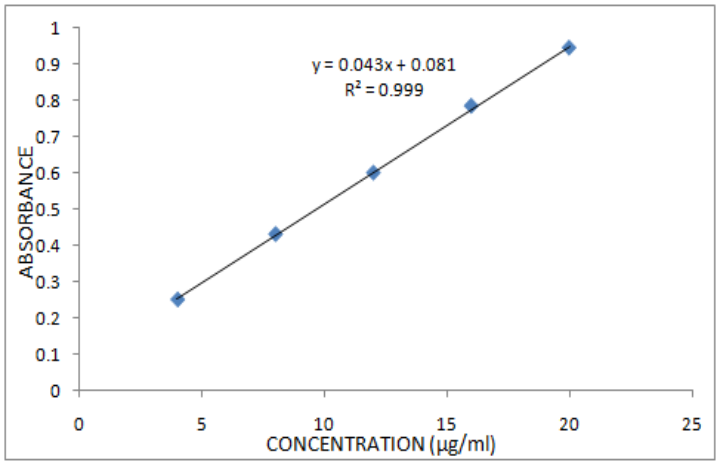

Fig: 3. Calibration Curve of Valganciclovir

Assay of Valganciclovir (VALGAN- 450mg)

A quantity of powder equivalent to 100mg of Valganciclovir was taken in a 100mL volumetric flask and it was first dissolved in small amount of 0.1N Hcl by shaking the flask for 3 to 5 minutes and diluted up to the mark with 0.1N Hcl. Then the solution was filtered using Whatmann filter paper No.40. From this filtrate, appropriate dilutions were made with

0.1N Hcl to obtain the desired concentration (12µg/mL). These solutions were analyzed in UV and the result was indicated by % recovery given in Table: 1.

Table: 1. Analysis of Formulation

DRUG	LABEL CLAIM (mg/ Tablet)	AMOUNT FOUND* (mg/ Tablet)	% AMOUNT FOUND	%RSD
VALGANCI-CLOVIR	450	448.20	99.60	0.48

*Mean of three readings

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.

The method was validated as per ICH guidelines[10-11] for different parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Linearity

Various aliquots were prepared from the working standard solution (100µg/mL) ranging from 4-20µg/mL. The samples were scanned in UV-VIS Spectrophotometer using 0.1N Hcl as blank. It was found that the selected drug shows linearity between the 4-20µg/mL (Table: 3).

Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation (VALGAN-450mg) was kept constant (8µg/mL) and the amount of pure drug was varied that is 6µg/mL, 8µg/mL and 10µg/mL for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (table: 2 & 5).

Precision

Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study, 6 different solutions of same concentration that is 8µg/mL were prepared and analysed three times in a day i.e. morning, afternoon and evening and the

absorbances were noted. The result was indicated by % RSD (table 2 & 6). In the inter-day variation study, 6 different solutions of same concentration (8µg/mL) were prepared and analysed three times for three consecutive days and the absorbances were noted. The result was indicated by % RSD (table 7).

Robustness

Robustness of the method was determined by carrying out the analysis at three different wavelengths (i.e. 250±1nm). The respective absorbances were noted and the result was indicated by % RSD (table: 2 & 8).

Ruggedness

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances were noted. The result was indicated by % RSD (table: 8).

Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (table: 9).

LOD= 3.3xSD/S

Where, SD = Standard deviation of Y-intercepts

S = slope

Limit of Quantification

The LOQ is the concentration that can be quantified reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (table: 9).

LOQ= 10xSD/S

Where, SD = Standard deviation of Y-intercepts

S = slope

RESULTS AND DISCUSSION

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (98.00% to 101.1%) of the drug were

obtained at each added concentration, which indicates that the method was accurate. The LOD and LOQ were found to be in sub-microgram level, which indicates the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%. The results of assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % Assay (99.51%). Summary of validation parameters of proposed spectrophotometric method is shown in table: 3.4.2.

Table: 2. Summary of Validation

Parameter	Result
Linearity indicated by correlation coefficient	0.999
Precision indicated by %RSD	1.1
Accuracy indicated by % recovery	98.00-101.4 %
Limit of detection (LOD), µg /mL	0.549
Limit of quantification (LOQ), µg /ml	1.666
Linear regression equation	y=0.134x+0.052
Robustness indicated by %RSD	
Ruggedness indicated by %RSD	
Assay indicated by % purity	99.60

Table: 3. Linearity table of Valganciclovir

S. No.	Concentration (µg/mL)	Absorbance
1.	4	0.182
2.	8	0.330
3.	12	0.455
4.	16	0.587
5	20	0.725

Table: 4. Optical Characteristic of Valgancyclovir

Optical characteristics	Result
Beer's law limit (µg/mL)	4-20
Molar extinction coefficient (L/Mol. cm)	16023.45
Correlation coefficient (r^2)	0.999
Regression equation	y=0.134x+0.052
Slope (a)	0.0339
Intercept (b)	0.052

Table: 5. Accuracy Studies of Valganciclovir

S.NO	Con.of (µg/mL)		% drug added	Amount recovered* (µg/mL)	% recovered	Mean	SD	%RSD
	Tab	pure drug						
1.	8	6	80	5.91	98.49	99.22	0.99	1.00
2.	8	8	100	7.90	98.83			
3.	8	10	10	10.03	100.36			

*Average of three values (n=3)

Table: 6. Intra-Day Precision

S.NO	Concentration (µg/mL)	Absorbances			Avg. % RSD
		Morning*	A. noon*	Evening*	
1.	8	0.330	0.329	0.330	0.175

* Average of six values (n=6)

Table: 7. Inter-Day Precision

S.NO	Concentration (µg/mL)	Absorbances			Avg. % RSD
		Day 1*	Day 2*	Day 3*	
1.	8	0.329	0.330	0.329	0.175

* Average of six values (n=6)

Table: 8. Robustness and Ruggedness of method for Valgancyclovir

Parameter		% RSD
Robustness	Change in λmax (± 1nm)	1.732
Ruggedness	By different Analysts	0.424

Table: 9. LOD & LOQ of Valgancyclovir

Standard	LOD (µg/mL)	LOQ (µg/mL)
Valgancyclovir	0.549	1.666

SUMMARY AND CONCLUSION

An attempt has been made to develop the validated and UV- Visible method for estimation of Valganciclovir and bulk and in dosage form. As the literature survey revealed that few methods are available for estimation of Valganciclovir bulk and in dosage form but there is a need for a simple, economical and proper method for estimation of Vlganciclovir bulk and in dosage form.

The UV spectrophotometric estimation was done by using Roy instruments 1R-513C UV Visible spectrophotometer. The estimation of Valgancyclovir done by using 0.1N Hcl as the solvent and the λmax was found to be 250nm. In this proposed UV method the selected drugs showed good linearity.

Results for the recoveries of selected drug were found within the limits (98-102%). These indicate that the proposed method was accurate for the analysis. The developed UV method for the determination Assay of selected drug is simple, accurate, precise, robust and economical. The solvent used in the proposed method is simple to prepare and economically reliable.

The sample recoveries were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. The method can be used in laboratories for the routine analysis of selected drug.

Since the system validation parameters UV method used for estimation of selected drugs in pure and have shown satisfactory, accurate and reproducible results (without any interference of excipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose.

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