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Difference UV Spectrophotometric Method for the Estimation of Valacyclovir in Bulk and Pharmaceutical Formulation

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ABSTRACT

A simple, precise, economical and accurate difference spectroscopic method has been developed for the valacyclovir in bulk and in pharmaceutical dosage form. The proposed method is based on the principle that valacyclovir exhibits two different chemical forms that differs in the absorption spectra in acidic and basic solution. The absorptions were measured in acidic and basic solution separately against reagent blank. Valacyclovir has exhibited maximum absorbance at 252 and 262nm in acidic and basic solution respectively. Difference in absorbance between these two maxima was calculated to find out the amplitude. The amplitude plotted against concentration showed linear response in the concentration range of 0.5 – 2.5µg/mL with linear regression value 0.999. The proposed method was applied to pharmaceutical formulation and the common excipient present in the formulation does not interfere in the analysis of the drug. The method was validated as per ICH guidelines and statistical results of analysis were found to be satisfactory.

Keywords Valacyclovir, difference spectrophotometry, overlay spectra, tablet dosage form, validation.

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INTRODUCTION

Valacyclovir is chemically {L-valine-2-[(2-amino-1,6-dihydro-6-oxo-9-hipurin-9-yl) methoxy]ethyl ester}(Figure – 1). It is an antiviral agent. Valaciclovir is phosphorylated by viral thymidine kinase to acyclovir triphosphate (the active metabolite) which then inhibits herpes viral DNA replication by competitive inhibition of viral DNA polymerase and by incorporation into and termination of the growing viral DNA chain. Literature survey reveals that analytical methods like UV[1-2], VIS[3-4] and HPLC[5-8] have been reported for the estimation of VAL. As per our knowledge there was no difference spectroscopic method was found to be reported in literature. Hence the main objective of the study was to develop a simple and accurate method for the analysis of VAL in bulk and pharmaceutical dosage form is upon altering the spectral characteristics, no interference with the common excipients is observed.

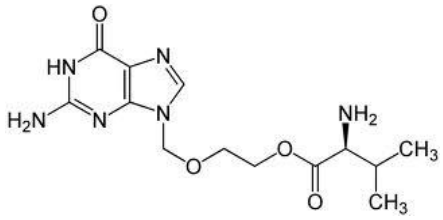

Figure1: Structure of Valacyclovir

MATERIALS AND METHOD

Chemicals and reagents

The working standard Valacyclovir (purity - 99.85%) was obtained as a gift sample from Hetero Laboratories Ltd, Hyderabad. The commercially available tablet, Valcivir 500mg (CIPLA) containing 500mg of VAL was procured from the local market and used for analysis. Freshly prepared 0.1M hydrochloric acid, 0.1M sodium hydroxide and distilled water were used in the present analysis. Whatmann filter paper no.41 and methanol (ar) was also used.

Instruments

A Shimadzu UV – 1800 double beam spectrophotometer with 1 cm path length supported by shimadzu UV – probe software, version 2.21 was used for spectral measurements with 1 cm matched quartz cells. Analytical balance Shimadzu (220h) was used for weighing purpose.

Difference spectroscopy

The selectivity and accuracy of spectrophotometric analysis of sample containing absorbing interference may be markedly improved by the technique of difference spectrophotometry. The essential feature of difference spectrophotometric assay is that the measured value is the difference in absorbance (ΔA) between two equimolar solutions of the analyte in different

chemical forms which exhibit different spectral characteristics. This is simplest and most economically employed for altering spectral properties of analyte by means of adjustment of pH by means of acid, alkali and buffer.

Preparation of standard

The working standard VAL (100mg) was weighed accurately and transferred to two (100mL) volumetric flasks. It was dissolved in 30mL of 0.1M NaOH and 0.1M HCl separately and finally made upto volumes with the 0.1M NaOH and 0.1M HCl. The solutions are further diluted with suitable diluents to get the final concentration 10µg/mL .

Construction of calibration curve

From the above solution, series of aliquots of 0.5-2.5 µg/mL concentrations were prepared using 0.1M NaOH and 0.1M HCl separately. These solutions were used to determine absorption maxima and linearity.

Determination of λmax

The above prepared solutions were scanned over the range of 400-200nm against reagent blank. From the spectrum obtained, the λmax was found to be 252 and 262nm in acidic and basic solutions respectively. The spectras are overlayed inorder to get the isobestic point (figure – 2). The intermediate wavelength 216.6nm was found from the overlay spectra. The difference in absorbance was calculated to find out the amplitude.

Analysis of tablet formulation

For the preparation and analysis of sample solution, each tablet containing 500mg of VAL, 5 tablets were accurately weighed and average weight per tablet was determined. The tablets were powdered and the powder equivalent to100mg drug was taken and treated in similar manner as that of standard using methanol. This solution was then filtered through Whatmann filter paper no. 41. Further dilutions of the stock solutions were made in both 0.1N HCl and 0.1N NaOH to get required concentration of 6µg/mL solutions. In this method, the concentrations were determined by measuring absorbance of sample solutions at 252nm and 262nm respectively. The nominal contents were determined either from the previously plotted calibration graphs or using the corresponding regression equations as shown in the table 2.

Method validation

The method was validated for different parameters like linearity, accuracy and precision according to ICH guidelines.

Linearity

The linearity of the method is the ability to elicit the results that are directly proportional to the

concentration of the analyte in samples. From the working standard, series of dilutions were made to 10mL with 0.1M NaOH and 0.1M HCl separately to get concentration range of 0.5-2.5µg/mL. The absorbance was measured at 252 and 262nm in acidic and basic solutions respectively against reagent blank. Calibration curve was prepared by plotting concentration vs. difference in absorbance and found to be linear in the concentration range of 0.5-2.5µg/mL (figure-3)

Precision

Precision of the method was determined by performing inter day variation, intraday variation and repeatability studies and expressed in forms of %RSD. In inter day variation the absorbance of working standard solutions of VAL 0.5-2.5µg/mL was measured on three consecutive days. In intraday variation the absorbance were measured three times a day. In repeatability study, six determinations of the fixed concentration of both acidic and basic drug were analyzed separately the results of precision data are given in the table 3.

LOD and LOQ

In this study, LOD and LOQ were based on the standard deviation of the response (σ) and the slope of the corresponding curve (S) using the following equation-

$LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$

The results of validation parameters are shown in table 1.

Accuracy

Accuracy of the proposed method was determined by calculating the recoveries of VAL by standard addition method. It was prepared by preparing different solutions at different concentrations of 50%, 100 %, 150% in which the amount of marketed formulation was kept constant and the amount of pure drug was varied that is 2, 4, 6 for 50%, 100%, 150% respectively. The amount of VAL recovered estimated by applying obtained values to the regression line equation. The results were given in the table 4.

RESULTS AND DISCUSSION

The optical characteristics such as Beer's law limits within the concentration of 0.5 – 2.5µg/mL, regression equation of 0.999, % relative standard deviation and recovery data, SD were less than 2% and were found to be within the limits and satisfactory. All of the validation parameters for the proposed method were determined according to ICH guidelines. The method was found to provide high degree of precision and reproducibility. The recovery studies showed that the results were within the limit indicating no interference with the marketed formulation. The

proposed method is simple, accurate, precise and economic and can be successfully employed for the routine analysis of the valacyclovir in pharmaceutical formulations.

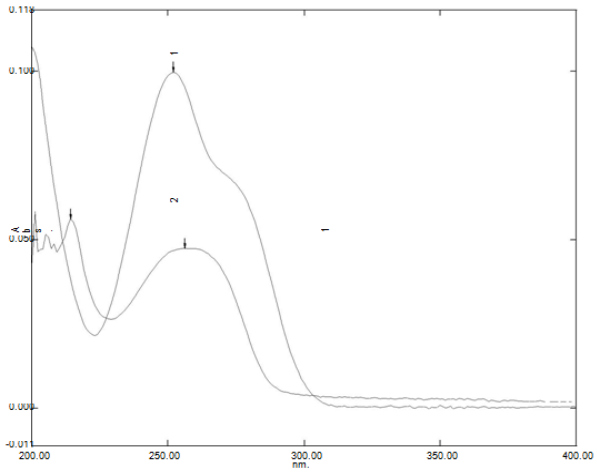

Figure 2: overlay spectra

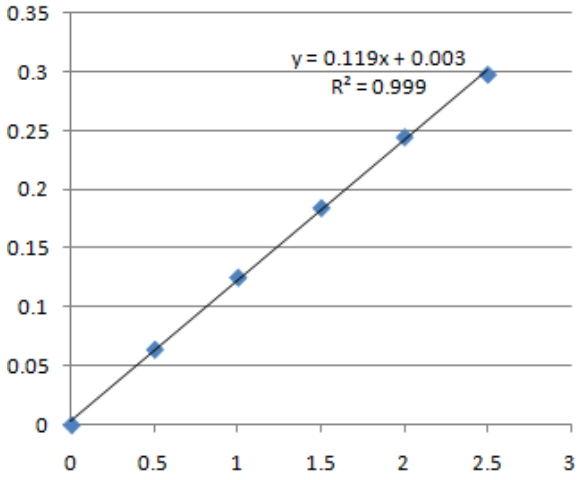

Figure 3: calibration curve of valacyclovir

Table1: validation parameters of valacyclovir

Parameters	Result
λmax	262nm(0.1N NaOH) 252nm(0.1N HCl)
Beer's limit (µg/mL)	0.5-2.5
Molar absorptivity (mol/lit)	125×10^{-3}
Precision indicated by %RSD	0.534
Accuracy indicated by average % recovery	100.71%
LOD (µg/mL)	0.43
LOQ (µg/mL)	0.12

Table 2: Analysis of tablet formulation

Label claim (mg)	Amount found (n=6)	% recovery	%RSD
500 mg	6	99.98%	0.02

Table 3: precision data

Intra day			Inter day		
Amount taken	**Amount taken ± SD**	**% RSD**	**Amount taken**	**Amount taken ± SD**	**% RSD**
1	0.99 ± 0.01		1	0.97 ± 0.07	
2	1.97 ± 0.05	0.53	2	1.95 ± 0.03	0.52
3.5	3.48 ± 0.04		3.5	3.72 ± 0.02	

Table 4: results of % recovery in tablet formulation

formulation	**Estimation of VAL in tablet formulations**			**% recovery of valacyclovir**				
Valacyclovir tablets	**Label claim(mg)**	**Amount found(mg)**	**% RSD**	**% of drug added**	**Concentration(g/mL)**		**% of drug recovered**	**% RSD**
					drug	**Formul.**		
	5	4.91		50%	2	10	97.5	0.87
	5	4.89	0.6	100%	4	10	100.4	0.62
	5	4.85		150%	6	10	104.25	0.43

CONCLUSION

The proposed method was found to be simple, accurate and economical with the use of solvents such as 0.1M HCl, 0.1M NaOH. The method is rapid as it do not require any sophisticated instruments for chromatographic method. This method is suitable for the routine analysis of VAL as upon altering the spectral characteristics, there is no interference with the common excipients. The preparations of solvents for this method was simple and economical.

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