UV Spectrophotometric Method for the Estimation of Valacyclovir HCl in Tablet Dosage Form

M. GANESH^{§*}, C.V.NARASIMHARAO[§], A.SARAVANA KUMAR[§],
K.KAMALAKANNAN[§], M.VINOBA[#], H. S. MAHAJAN[§] and T. SIVAKUMAR[§]

[§]Department of Pharmaceutical Analysis,
Nandha College of Pharmacy, Erode-638 052, India.

[#]Department of Chemical Engineering,
Alagappa College of Technology, Anna University, Guindy,
Chennai-600 025, Tamilnadu, India.

chemgans@gmail.com

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Abstract: A simple, sensitive, highly accurate UV spectrophotometric method has been developed for the determination of valacyclovir in bulk and tablet dosage form. Solution of valacyclovir in 0.1N HCl shows maximum absorbance at 255 nm. Beer's law was obeyed in the concentration range of 5-25 mcg mL⁻¹ with 1.0910×10^4 mol⁻¹ cm⁻¹, the slope, intercept, correlation coefficient, detection and quantitation limits were also calculated. The proposed method has been applied successfully for the analysis of the drug in pure and in its tablets dosage forms. Result of percentage recovery and placebo interference shows that the method was not affected by the presence of common excipients. The percentages assay of valacyclovir HCl in tablet was 99.82%. The method was validated by determining its sensitivity, accuracy and precision which proves suitability of the developed method for the routine estimation of valacyclovir in bulk and solid dosage form.

Keywords: Valacyclovir HCl, UV spectroscopy, Estimation, Tablets.

Introduction

Valacyclovir, *L*-valine-2-[(2-amino-1,6-dihydro-6-oxo-9-hipurin-9-yl) methoxy]ethyl ester (Figure 1), is the *L*-valyl ester prodrug of the antiviral drug acyclovir that exhibits activity against herpes simplex virus types, 1 (HSV-1) and 2 (HSV-2) and varicellazoster virus¹. The mechanism of action of acyclovir involves the highly selective inhibition of herpes virus DNA replication, via enhanced uptake in herpes virus-infected cells and phosphorylation by viral

thymidine kinase. The substrate specificity of acyclovir triphosphate for viral, rather than cellular, DNA polymerase contributes to the specificity of the drug^{2,3}. After oral administration, valacyclovir is converted rapidly and extensively to acyclovir as a result of first-pass intestinal and hepatic metabolism through enzymatic hydrolysis⁴. The oral bioavailability of acyclovir is higher after administration of valacyclovir relative to acyclovir itself⁵⁻⁹.

Figure 1. Structure of valacyclovir HCl.

In previous studies, only one assay has been reported for the simultaneous determination of valacyclovir and acyclovir in human serum and urine by UV detection¹⁰. Recently, the chemical and enzymatic stability of valacyclovir has been investigated by HPLC with UV detection¹¹. Valacyclovir has also been quantified in pharmaceutical preparations, human serum and biological fluids by HPLC with UV detection and enantioselective HPLC with UV detection¹²⁻¹⁴.

Although the ultraviolet spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories because of their simplicity, selectivity, and sensitivity. As of our knowledge no report has been mentioned in the literature for the determination of valacyclovir by UV method. The aim of the present work was to develop simple, rapid, accurate, and sensitive UV spectrophotometric method for the determination of valacyclovir in pure and pharmaceutical formulation.

Experimental

Pharmaceutical grade of valacyclovir hydrochloride was procured from Dr.Reedy's Laboratories, India. All the chemicals were of analytical reagent grade of Merck (Germany) unless otherwise specified. Doubly distilled water was used to prepare all solutions. Freshly prepared solutions were always employed. Different brands of tablets of valacyclovir were supplied from local stores.

Instrumentation

PerkinElmer Lambda-35 UV-Visible double beam spectrophotometer with 1 cm matched quartz cell and Elico UV/VIS SI-164 spectrophotometer with 1 cm matched cells.

Valacyclovir stock solution

Standard stock solution was prepared by dissolving 50 mg of valacyclovir in 100 mL of 0.1 N HCl to get concentration of 500 µg/mL solution.

Method development

Aliquots of stock solution were further diluted with 0.1 N HCl to get working solution of 5, 10, 15, 20 and 25 µg mL⁻¹ and the working standards were scanned between 200-400 nm which shows the maximum absorbance at 255 nm (Figure 2). The same λ_{max} was used for the further measurement of the drug.

Procedure for calibration curve

Aliquots of stock solution were further diluted with 0.1 N HCl to get working solution of 5, 10, 15, 20 and 25 µg mL⁻¹. Finally, the prepared standards were measured after standing for

5.0 min at λ_{max} as recorded in (Table 1), in each case against a solvent blank similarly prepared. A calibration graph of the absorbance *versus* the concentration of the drug was plotted (Figure 3).

Figure 2. UV spectra of valacyclovir HCl.

Figure 3. Standard plot of valacyclovir HCl.

Procedure for dosage forms

For analysis of commercial formulations, twenty tablets were taken and powdered. Tablet powder equivalent to 68 mg of valacyclovir HCl was transferred to 100 mL volumetric flask and dissolved in 0.1N HCl. Then the solution was sonicated for 30 min and filtered and it was further diluted to get the required concentration. The absorbance of the prepared sample solution was measured against 0.1N HCl blank at 255 nm. A standard additions technique was also used to confirm the accuracy and precisions.

Results and Discussion

The absorption spectrum of valacyclovir was measured in the range 200–400 nm against the blank solution 0.1N HCl similarly prepared (Figure 3). The standard solution show maximum absorbance at λ_{max} for each three systems as recorded in Table 1. And the method was validated by studying the following parameters as ICH guide lines (ICH guide lines 1995) for method validation¹⁵.

Table 1. Parameters for determination of valacyclovir HCl against 0.1N HCl.

Parameters	Values
λ_{max} , nm	255
Beer's law limit, $\mu\text{g mL}^{-1}$	5– 20
Molar absorptivity, $\text{L mol}^{-1} \text{cm}^{-1}$	1.0910×10^4
Range of Errors, %	-0.29-0.34
Regression equation ^a Slope (b)	0.0299
Intercept (a)	0.0075
Correlation coefficient (r)	0.9999
<i>t</i> -value(2.55) ^b	1.32
<i>F</i> -value(5.06) ^b	2.65

^a $Y = a + bc$ *c* is the concentration in $\mu\text{g mL}^{-1}$ ^b Values in parentheses are the theoretical values for *t*- and *F*-values at 95% confidence and five degree of freedom.

The precision of the method was investigated with respect to repeatability. For intra-day precision, standard solution of fixed concentration was analyzed at various time interval and %RSD was noted (limit %RSD<2.0%). And the day-to-day precision was studied by taking the absorbance of the same concentration of standard solution at various days and the %RSD was calculated (%RSD<2.0%) as shown in Table 2.

Table 2. Results of Assay and Precision Studies.

Sample	Label claim, mg/tab	Amount		C.V*	Precision**		
		found, mg /tab	(%)*		Repeatability	Interday	Intraday
Valacylovir	1000	998.2	99.8±	0.2473	0.317	0.579	0.786
		±0.232	0.4121				

* Mean of six determinations. **%RSD of six determination.

Accuracy of the method was performed by recovery studies. The recovery of valacyclovir hydrochloride was performed by spiking pure drug to the preanalyzed sample at five concentration levels 5,10,15,20 and 25 $\mu\text{g mL}^{-1}$ (Table 3). And the results were compared with already existed HPLC method¹³.

The specificity of the method was conducted to prove that the free from determined interference of solvent and commonly used tablet excipients. This is evidenced by the lack of absorbance at the specified λ - max for the excipients in the placebo and blank solutions.

The applicability of the proposed method for the assay of valacyclovir in tablet formulation was examined by analyzing formulations and the results were tabulated in Table 2. The results obtained were good agreement with the label claims. The results were reproducible with low %RSD values. The results of analysis of the commercial tablets and the recovery study of drug suggested that there is no interference from any excipients (such as starch, lactose, titanium dioxide, and magnesium stearate) which are commonly present in tablets.

The results obtained for the proposed methods were compared with those obtained using the HPLC method¹³. The calculated student's *t*-values and *F*-values did not exceed the theoretical ones at 95% confidence level. Therefore, there is no significant difference between the proposed method and HPLC methods.

Table 3. Accuracy of the method.

Sample $\mu\text{g mL}^{-1}$	Amount added, $\mu\text{g mL}^{-1}$	Amount found Proposed, $\mu\text{g mL}^{-1}$	Amount found by HPLC	% Recovery	%RSD*	RE, %
20	5	5.02	5.04	100.40	0.915	0.04
	10	9.89	10.03	98.90	1.010	-0.10
	15	15.09	15.09	100.67	0.804	0.67
	20	20.04	20.02	100.20	0.674	0.20
	25	24.94	25.00	99.76	0.903	-0.24

*Mean of 6 determinations.

Conclusions

A method for the determination of valacyclovir in the bulk drug and tablet formulation has been developed. From the spectrum of valacyclovir hydrochloride as shown in Figure 2, it was found that the maximum absorbance is at about 255 nm in 0.1 N HCl. A good linear relationship (0.9998) was observed between the concentration ranges of 5-25 $\mu\text{g/mL}$. The assay of valacyclovir tablet was found to be 99.82%. The high percentage recovery indicates the high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, accurate and reproducible. Thus the developed method can be easily used for the routine quality control of valacyclovir in bulk and tablet dosage form.

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