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RP-HPLC Estimation of Valacyclovir HCl in Tablet Formulation

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A simple, rapid, sensitive and fully validated reverse phase HPLC method has been described for estimation of valacyclovir in tablet dosage form using mobile phase without any column degrading agent. Here in present method, chromatography was achieved on an ODS column, 0.015 M acetic acid and methanol (95:5 v/v) as the mobile phase with a flow rate of 1.1 mL/min and 254 nm as wavelength for monitoring eluent. The retention time of the drug was 3.0 min. The detector response was linear in the concentration of 6-90 µg/mL with correlation coefficient 0.999. The limit of detection (LOD) and limit of quantification (LOQ) were 3.4 and 4.6 µg/mL, respectively. The percentage assay of valacyclovir hydrochloride was 99.861-100.45 %. The method was validated by determining its sensitivity, accuracy and precision. This method is suitable not only on the parameters validated but also the mobile phase utilized which consists of only methanol and acetic acid both of them were not affects the column life and performance.

Key Words: Valacyclovir HCl, RP-HPLC, Estimation, Tablets.

INTRODUCTION

Valacyclovir (VALC), chemically l-valine-2-[(2-amino-1,6-dihydro -6-oxo-9-hipurin-9yl)methoxy]ethyl ester (Fig. 1), is l-valyl ester pro-drug of acyclovir and it is an antiherpes simplex virus type 1 (HSV-1), 2 (HSV-2) and vericella zoster virus[1]. Valacyclovir acts specifically by inhibit DNA polymeraze of virus[2,3]. Valacyclovir is converted rapidly into acyclovir after its oral administration by first-pass metabolism[4]. The oral bioavailability of valacyclovir is relatively higher than acyclovir[5-9].

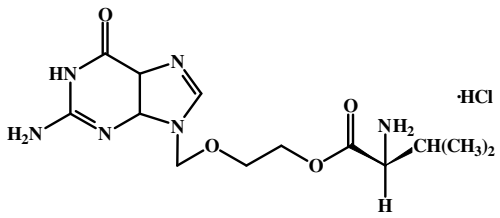

Fig. 1. Structure of valacyclovir HCl

The available literatures revealed that there are several methods were reported earlier for determination of valacyclovir and acyclovir in biological matrix by LC-MS/MS, estimation of the valacyclovir in formulation by HPLC with UV detection, HPLC by fluorescence detection, voltametric estimation in our earlier paper we reported a simple UV spectrophotometric method for its estimation[10-20]. RP-HPLC is the method of choice in pharmaceutical industries. Some of the methods described by HPLC were not suitable for estimation in formulation, the reported HPLC by UV utilizes a triple solvent system in the mobile phase with 20-70 % of acetonitrile as well as phosphate buffer about 30-53 % in total volume ratio, acetonitrile which is an organic modifier as well as polluting solvent and higher volume of buffer in the mobile phase will decrease the column life. Even with the organic modifier the method require longer run time for the analysis. Considering this our aimed is to develop simple, rapid, accurate and sensitive HPLC by UV without organic modifier and with reduction in run time of analysis.

EXPERIMENTAL

Waters Alliance 2695 separation module chromatographic system equipped with automatic injector with maximum injection volume 100 mL, Waters 2487 Dual alpha absorbance UV-visible detector and Empower software for data collection and integeration.

Valacyclovir HCl reference standard was received from Sigma-Aldrich (Germany), methanol HPLC grade from J.T.

Baker (Mallinckrodt Baker, Inc., USA), Milli-Q water obtained from Millipore water system (Billerica, USA). Acetic acid AR (Acros organics, NJ, USA), pharmaceutical tablet formulation containing 500 mg of valacyclovir was purchased from local pharmacy.

Chromatographic conditions: Chromatographic separation was achieved using an Inertsil ODS C_{18} (150 mm × 4.6 mm, 5 µ) analytical column, 0.015 M acetic acid and methanol (95:05 v/v) as mobile phase with 1.1 mL/min flow rate and detection wavelength was fixed as 254 nm. The column and HPLC system was kept in ambient temperature throughout the analysis.

Preparation of standard stock solution: About 50 mg of valacyclovir working standard was weighed and transferred into 50 mL volumetric flask, add little amount of 0.015 M acetic acid (diluent), sonicate to dissolve it completely, finally the volume made with diluent to get 1 mg/mL stock solution.

Preparation of sample solution: Powder equivalent to 750 mg of valacyclovir was accurately weighed from the well crushed powder of 20 tablets and transferred into 500 mL volumetric flask. Add about 375 mL of diluent and sonicate for 0.5 h with frequent shaking, make the remaining volume with diluents, a quantity of this was centrifuged at 4000 rpm for 10 min. Pipette 2 mL of the above solution into 50 mL volumetric flask and dilute to volume with diluent, mixed well and filtered using 0.45 µm nylon membrane filter.

RESULTS AND DISCUSSION

The method was validated for the following parameters such as system suitability, precision, accuracy, bench top stability, limit of detection (LOD), limit of quantification (LOQ), ruggedness and robustness with reference to ICH and USP guidelines for analytical method validation[21,22].

Method optimization: Method was optimized after different trials with various mobile phase compositions to achieve the symmetric peak with less retention time and less tailing. A better base line resolved peak with good symmetry and good theoretical plate was observed with 0.015 M acetic acid and methanol (95:05 v/v) with flow rate of 1.1 mL (Fig. 2(b), Table-1). All the remaining parameters were validated with this optimized chromatographic condition.

TABLE-1
RESULTS OF CALIBRATION CURVE AND SYSTEM SUITABILITY DATA

Drug	Valacyclovir
Detection wavelength (nm)	254
Concentration range (µg/mL)	6-90
Slope (m)	1.6770×10^4
Intercept (b)	3.086×10^3
Correlation coefficient (r^2)	0.999
RSD (%)	0.2
System suitability parameters	
Theoretical plates (N)	2091
Tailing factor	0.9

System suitability: The system suitability tests were carried out on freshly prepared standard stock solution of valacyclovir. This was evaluated by studying the theoretical plates, retention time and tailing factor, the results were summarized in table

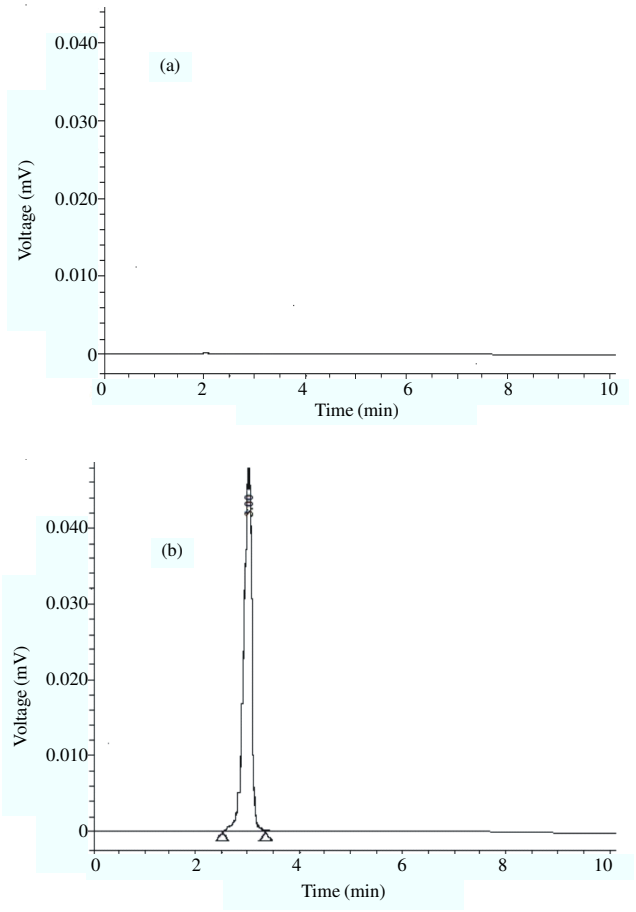

Fig. 2. Chromatogram of (a) blank (b) standard

which shows that all the parameters studied were good in agreement with guide lines (Table-1).

Linearity of the method: The linearity of the peak area response of the standards with respect to their concentration of standards was examined using optimized HPLC conditions. The valacyclovir shows linear response in the concentration range over 6-90 µg/mL with regression equation of $y = 1.6770 \times 10^4 x + 3.086 \times 10^3$ by the method of least squares, where 'x' is the concentration and correlation coefficient (R^2) 0.999 (Fig. 3).

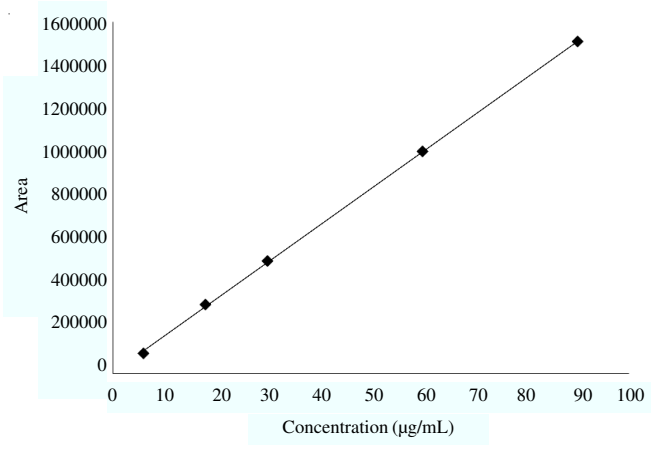

Fig. 3. Calibration graph

Specificity: The specificity of the method is the evidence for suitability of method to the specific analyte and its freedom from interfering substance either in formulation or in mobile

TABLE-2
RESULTS OF ACCURACY AND PRECISION

Concentration of preanalyzed sample (µg/mL)	Accuracy			Precision				
					Intra day		Inter day	
	Spike level (%)	Recovery* (%)	RSD (%)*	Concentration (µg/mL)	Mean amount found	RSD (%)*	Mean amount found	RSD (%)*
30	80	100.012	0.741	30	29.465	0.781	30.103	0.976
	100	99.861	0.654					
	120	100.453	0.585					

*Mean of six determinations.

phase. Specificity was proved by placebo studies and blank run. For the placebo, solution of common excipients used in tablet formulation was prepared and injected into the chromatogram. There were no additional peaks, interfering peak near by the retention time of valacyclovir observed in the chromatogram obtained using blank run and placebo (Fig. 2a and 4b).

Limits of detection and limits of quantification: The limit of detection is the estimation of sensitivity based on how much low concentration can be detected by the method which was found to be 3.4 µg/mL (S/N > 3). In addition the limit of quantification was 4.6 µg/mL (S/N > 10) for valacyclovir.

Precision: The precision of a measurement system also called as reproducibility or repeatability is the degree to which repeated measurements under unchanged conditions show the same results. For this solution containing same concentration of the drug was injected repeatedly at different time in the same day (intraday), for three days (inter-day). The results of the analysis shows that RSD of intra-day and inter-day precision were 0.781, 0.976, respectively (Table-2).

Accuracy: Accuracy is a measure of degree of closeness of measurements of a quantity to its actual (true) value. Recovery study was used for this purpose, in which a known quantity of the drug containing solution at three levels (80, 100 and 120 % with reference to the concentration of preanalyzed sample solution) were spiked into the preanalyzed sample and the final solution was injected into the chromatographic system. The results of accuracy is good in agreement with limit given in guidelines for accuracy (Table-2).

Stability: To determine the stability of the standard in the diluents, the solution containing definite concentration of standard was kept at 4 ºC for 3 days then the temperature was get down to room temperature and injected. There were no extra peaks found in the chromatogram, which proved that the analyte of interest was stable up to 3 days without degradation.

Ruggedness: The study of variation upon the method was evaluated using different HPLC system and by different analyst. This was established by determining valacyclovir in dosage formulation using the two different chromatographic system and by two analysts. The percentage RSD % for analyst variation and inter system variation were 0.610-0.891 (limit < 2.0 %) and 0.763, 0.668 (limit < 2.0 %). This indicates that the method was rugged (Table-3).

TABLE-3
RESULTS OF ROBUSTNESS AND RUGGEDNESS

Robustness (RSD %)*				Ruggedness (RSD %)*			
Flow rate (mL)		Wave length (nm)		Analyst		System	
1.0	1.2	253	255	1	2	1	2
0.670	0.532	0.591	0.799	0.610	0.891	0.763	0.688

*n = 5.

Robustness: Robustness is the measures of stability of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here, the detection wavelength was varied ± 1 nm, whereas the flow rate was varied ± 0.1 mL min^{-1}. The percentage RSD value of assay determined under developed method and under the varied conditions are 0.670, 0.532 and 0.591, 0.799 for flow rate change and wave length change, respectively which indicating that the developed method is robust over the deliberate change (Table-3).

Application of the method to formulation: The method was used for determination of valacyclovir in tablet formulation as described above. The results obtained (Table-4) showed with high assay values and low percentage RSD which confirms the method is suitable for routine determination of this component in its pharmaceutical preparation. Typical chromatogram obtained from the analysis of tablet formulation was given in Fig. 4a.

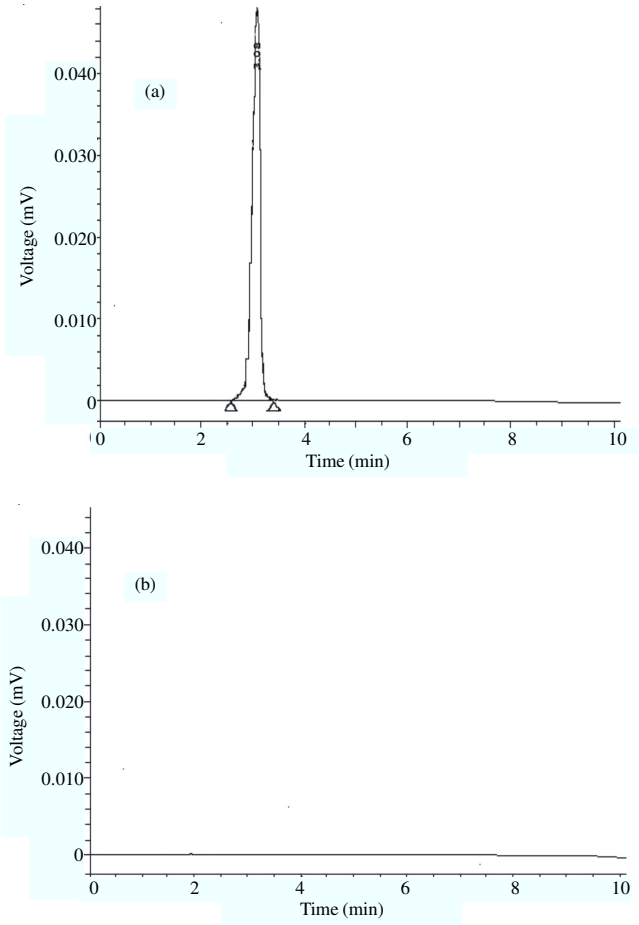

Fig. 4. Chromatogram of (a) tablet formulation (b) placebo

TABLE-4
RESULTS OF TABLET ASSAY

Label claim (mg/tablet)	Amount found* (mg/tablet)	Assay (%)	SD*	RSD* (%)
500	498.12	99.62	0.294	0.653

*Result of five determinations.

Conclusion

A simple and reliable HPLC method for estimating valacyclovir in pharmaceutical formulation has been developed and validated. However many methods have reported already, were less sensitive, longer run time, more tailing in the analyte peak and partially validated this method and some methods utilised phosphate buffers, acetonitrile both were consider to reduce the life time of column which leads increase the cost of the method too. Here in we used a simple organic acid (acetic acid of low concentration) and methanol which were not affect the column as well as low cost solvent compared to acetonitrile. Moreover present methods is more advantageous in terms of its shorter runtime, less tailing of component peak, simple sample preparation and clean up and fully validated. Hence, it is concluded that present method is suitable for the routine quality control analysis of valacyclovir in formulation.

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