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RP-HPLC method for the determination of Valacyclovir in bulk and Pharmaceutical formulation

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ABSTRACT

A reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the estimation of valacyclovir in bulk drug and pharmaceutical dosage form. The quantification was carried out using C_{18} column and mobile phase consisting of 0.067 M phosphate buffer at pH 6.5: acetonitrile : methanol (70:20:10 % v/v), at flow rate of 0.5 mL/min. The separation was performed at ambient temperature. Eluents were monitored by UV detector set at 244 nm. The method was statistically validated for the linearity, precision, accuracy, LOD and LOQ. The linearity was found to be in the range of 5-30 µg/mL. The proposed method was found to be simple, precise, accurate, rapid, economic and reproducible for the estimation of valacyclovir in bulk drug and tablet.

Key Words : Valacyclovir , RP-HPLC-method, LOD, LOQ, validation.

INTRODUCTION

Valacyclovir hydrochloride is a hydrochloride salt of L-valyl ester of acyclovir [1]. It is chemically 2-[(2-amino-6-oxo-3, 9-dihydropurin-9-yl) methexy] ethyl-2-amino-3-methylbutanoate [2]. It is an antiviral drug used in the treatment of herpes simplex and herpes zoster. It inhibits viral DNA synthesis [3-6]. It is a prodrug intended to increase the bioavailability of acyclovir by increasing lipophilicity. Valacyclovir converted by esterase to active drug acyclovir via hepatic first pass metabolism. Extensive literature survey reveals that only HPLC and LC-MS method for the determination of valacyclovir in plasma samples has been reported [7-9]. The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of valacyclovir in bulk drug and in its tablet formulation using the most commonly employed RP-C_{18} column with UV-detection.

MATERIALS AND METHODS

The pure drug sample of valacyclovir was obtained as gift sample from Dr.Reddy's Lab, Hyderabad. The formulation (valcivir-500mg tablets) used was purchased from local pharmacy. Qualigens fine chemicals, Mumbai, supplied HPLC grade acetonitrile , methanol and water, sodium dihydrogen orthophosphate AR grade and sodium hydroxide AR grade. An isocratic high pressure liquid chromatograph (Shimadzu HPLC class VP series) with LC- 10 ATVP pump, variable wavelength programmable UV /Vis detector SPD-10 AVP system and operating software winchrome was used. The chromatography column used was a reverse phase phenomenax C_{18} column (250mm ×4.6 mm i.d, particle size 5 µ). A mixture consisting of 0.067 M phosphate buffer at pH 6.5 (adjusted using sodium hydroxide solution) : acetonitrile : methanol (70:20:10 %v/v) was used as mobile phase and was filtered before use through 0.45µ membrane filter. The flow rate of mobile phase was maintained at 0.5 ml/ min. Detection was carried out at 244 nm at ambient temperature.

Preparation of standard stock solution

About 25 mg of valacyclovir was weighed accurately and dissolved in a minimum quantity of mobile phase and the total volume was brought to 25 ml with more mobile phase to get the concentration of 1000 µg/mL.

Assay procedure

From the standard solution, 1 mL was transferred into a 10ml standard flask and made up to the mark to produce 100 µg/mL. To the series of five 10ml standard flask, added (0.5-3ml) of above solution and made up to the mark to obtain the concentration range from 5 to 30 µg/ml and the calibration curve was plotted between concentration and peak area.

Quantification of valacyclovir in formulation

Twenty tablets containing 500 mg of valacyclovir were accurately weighed and finely powdered. An accurately weighed sample of powdered tablets equivalent to 250 mg of valacyclovir was placed in a 250 mL volumetric flask, added 50ml mobile phase and shaken vigorously for few minutes and repeated the extraction consequently by four times (4×50) to produce 250 mL with mobile phase and filtered through a 0.45 µ membrane filter paper. Further dilutions were made to get a concentration of 15 µg/mL of drug solution. An aliquot of 20 µL of test solution was injected and the amount of drug was calculated from the calibration curve.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drug by RP-HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products.

The mobile phase containing 0.067 M phosphate buffer at pH 6.5, acetonitrile and methanol in the proportion 70:20:10 %v/v was selected because it was found to give a peak for valacyclovir with minimal tailing. With the above mentioned composition of mobile phase, sharp peak was achieved with reasonable short run time of 10 min. The criteria employed for assessing the suitability of above said solvent system were cost. Time required for analysis, solvent noise,

preparatory steps involved in the use of same solvent system for the extraction of the drug from formulation excipient matrix for the estimation of drug content. UV detection was carried out at 244 nm as valacyclovir showed good absorbance at this wavelength. The retention time for valacyclovir was found to be 3.74 min. A typical chromatogram of test solution is shown in Figure 1.

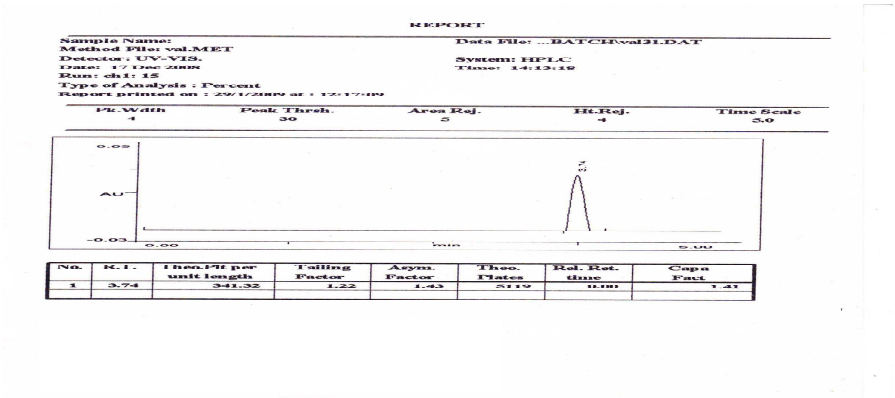

Figure 1 Typical chromatogram of the sample solution at optimized RP- HPLC condition

The peak shape was symmetrical and asymmetry factor was less than 2. When the concentrations of valacyclovir and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r=0.9998) was observed between the concentration of valacyclovir and the respective peak areas in the range of 5-30 µg/ mL. The regression of valacyclovir was found to be Y= 578169.2X-7876.82, where Y is the peak area and X is the concentration of valacyclovir. The regression equation was used to estimate the amount of valacyclovir either in tablet formulations or in validation study. The RP-HPLC method developed in the present study has been used to quantify valacyclovir in tablet dosage forms. Valacyclovir tablets were analyzed as per procedure described above and the average drug content was found to be 99.07% of the labeled amount (Table 1). The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y- intercepts of regression lines and slope of the calibration curves were used to calculate LOD and LOQ. The LOD and LOQ values are 0.2515 and 0.7623 µg/mL respectively.

Table 1 Quantification of formulation (valcivir) by RP-HPLC method

Labeled claim (mg/tab)	Amount found(mg)	Percentage purity obtained	Average amount± S.D.	% RSD	S.E
500	496.34	99.26	495.38± 0.4764	0.4808	0.213
500	492.5	99.51			
500	496.55	99.31			
500	493.29	98.65			
500	498.24	99.64			

The proposed method was validated as per the standard analytical procedures [10]. Each sample was injected six times and the retention times were same. Accuracy of the method was calculated by recovery studies (n=6) at three levels. Standard drug solution containing drugs in the range, 50%, 75% and 100% of nominal concentration was added to previously analyzed test solution. Amount of drug recovered at each level was calculated. The sample recovery in the formulation (Table 2) was in good agreement with the label claim.

Table 2 Recovery studies of formulation – valacivir by RP-HPLC method

percentage	Amount present (µg/ml)	Amount Added (µg/ml)	Total Estimated* (µg/ml)	Amount recovered* (µg/ml)	% recovery	Mean±S.D	%RSD	S.E
100	14.9	15	29.74	14.84	98.93	98.57±0.3061	0.3105	0.17673
75	14.9	10	24.74	9.84	98.40			
50	14.9	5	19.82	4.92	98.40			

** Mean of three observations*

High percentage recovery and low % RSD value showed that the method was free from interference of the excipients used in the formulation. System suitability parameters of the proposed method for valacyclovir are given in Table 3.

Table 3 System suitability parameters at optimized RP- HPLC condition

Parameter	Value
Retention time	3.74
Tailing factor	1.22
Capacity factor	1.41
Asymmetric factor	1.43
No of theoretical plates	5119

No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

CONCLUSION

The results of the study indicate that the proposed HPLC method is simple, precise, accurate , sensitive, economic and less time consuming. Therefore, this method can be applied for the routine quality control analysis of valacyclovir in its tablet dosage form.

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