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RP-HPLC Estimation of Valacyclovir in Tablets

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A simple, reliable and reproducible HPLC method has been developed for the analysis of valacyclovir in tablet dosage forms. Chromatography was carried out on a C_{18} column using phosphate buffer-acetonitrile (30 : 70) (v/v) as the mobile phase at a flow rate of 1 mL/min. Adenine was used as an internal standard. Detection was carried out at 230 nm. Linearity was observed in the concentration range 5–60 μg/mL of valacyclovir with a limit of detection of 5 ng/mL. Parameters of validation obtained prove the precision of the method and its applicability for the determination of valacyclovir in tablet formulations.

Key Words: Valacyclovir, Tablets, RP-HPLC method.

INTRODUCTION

Valacyclovir (L-valine 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl) methoxy] ethyl ester) is a prodrug of acyclovir, which exhibits antiviral activity[1]. The drug has more favourable pharmacokinetic characteristics resulting in less frequent dosing schedule and achieves higher blood plasma levels than acyclovir[2, 3]. The activity of valacyclovir appears to be entirely due to its conversion to acyclovir[4, 5]. After oral administration, valacyclovir is rapidly absorbed and extensively converted to acyclovir *via* first-pass metabolism[6].

In this paper, the authors present an RP-HPLC method for the determination of valacyclovir with adenine as internal standard. The method is simple, reliable and accurate for the determination of valacyclovir in tablet dosage forms.

EXPERIMENTAL

A Jasco (Japan) HPLC instrument was employed for this study. A PU-2080 pump was used to deliver the mobile phase to the Supelcosil (Japan) analytical column (4.6 × 250 mm; 5 μ). Sample injection was performed with a Rheodyne 7725 injection valve *via* a 20-μL loop. Detection was achieved with a Jasco UV-2075 detector. Jasco-Borwin software was used for quantitative determination of the eluent peaks. Degassing of solvents was achieved by helium purging before

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use. Dissolution of compounds was enhanced by sonication on a Bandelin Sonerex sonicator. A UV spectrum of valacyclovir for selecting the working wavelength of detection was taken using a Jasco V-550 model UV-Vis spectrophotometer.

A pure sample of valacyclovir and valcivir tablets (containing 500 and 1000 mg of valacyclovir) were obtained from Cipla Limited (Mumbai, India). Adenine (HMR Ltd, Mumbai) was used as an internal standard. Purified water was prepared using a Millipore Milli-Q water purification system and acetonitrile (HPLC grade) was a product of Merck Limited (India). AR grade orthophosphoric acid and potassium dihydrogen orthophosphate were used for preparing the buffer solution.

Preparation of drug and internal standard solutions: Stock solution of the drug was prepared by dissolving 100 mg of valacyclovir in a 100 mL volumetric flask containing 70 mL acetonitrile. The solution was sonicated for about 20 min and then made up to volume with acetonitrile. Working standard solution of valacyclovir was prepared by suitable dilution of the stock solution with the mobile phase. Similarly stock solution of the internal standard was prepared by dissolving 100 mg of adenine in 100 mL of acetonitrile.

Chromatographic conditions: The mobile phase used in this study was a mixture of phosphate buffer (0.02 M, pH 3.0) and acetonitrile (30 : 70 v/v) with a flow rate of 1 mL/min. The mobile phase was filtered through a 0.45 μ membrane filter and degassed before use. The retention times obtained for valacyclovir and adenine were 6.2 and 3.0 min respectively. All the experiments were carried out at 23 ± 1°C. The detection was carried out at 230 nm. The identification of the separated valacyclovir and adenine was confirmed by running the chromatograms of the individual compounds under identical conditions.

Recommended procedure: After a systematic and detailed study of the various parameters involved, the following procedure and conditions are recommended for the determination of valacyclovir in bulk samples and in pharmaceutical formulations.

Prior to injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The prepared dilutions containing concentrations of valacyclovir in the range 5–60 μg/mL and fixed concentration (50 μg/mL) of internal standard (adenine) were injected into the chromatograph. The amount of drug present in each pharmaceutical formulation was calculated through peak area ratio of the drug to that of the internal standard by making use of the standard calibration curve.

Method validation

Accuracy and precision: Five separate sample solutions of valacyclovir of 50 μg/mL strength were prepared from the stock solution and analyzed as per the procedure.

Linearity: Six dilutions of the standard drug solution in the range 5–60 μg/mL were prepared. Each dilution was injected five times and analyzed.

Specificity: Five sample solutions of valacyclovir 50 μg/mL were prepared from the stock solution and analyzed.

Limit of detection (LOD) and limit of quantitation (LOQ): LOD and LOQ were calculated on the basis of signal to noise ratio. Experiments were performed to analyze the actual concentration that can be accurately quantified or detected by the method.

Ruggedness: It was determined for the method by varying the analyst, instrument and columns from different manufacturers.

Robustness: The percentage of acetonitrile, buffer strength, pH and sonication time was varied and the effects on retention time and peak parameters were studied.

Estimation of valacyclovir from the commercial formulations by the proposed method: Twenty tablets were weighed and pulverized. The sample of the powdered tablet, claimed to contain 100 mg of active ingredient was extracted with acetonitrile and suitably diluted to get a stock solution of 1 mg/mL. This solution was filtered through a 0.45 μ membrane filter. This solution was further diluted stepwise with the mobile phase to get the different concentrations required. From the area under peak, the drug content per tablet (on average weight basis) was calculated.

RESULTS AND DISCUSSION

To know the percentage recovery of valacyclovir from tablets, adenine was used as the internal standard because of their similar properties. A typical chromatogram of valacyclovir and adenine in tablet extract is shown in Fig. 1, which indicates a good base line separation. The chromatograms of valacyclovir and adenine were also recorded individually under identical chromatographic conditions. The order of the elution was adenine followed by valacyclovir. The calibration curve plotted for valacyclovir was later used to determine concentrations of the drug in tablets. The calibration curves were plotted by using 5–60 μg/mL concentrations of valacyclovir.

To optimize the chromatographic conditions, various combinations of acetonitrile with phosphate buffer and acetonitrile with water were tested. Besides, buffers of different concentrations (0.01–0.06 M) and pH (2.5–6.5) alone or with acetonitrile were also tested. The use of a 30 : 70 v/v mixture of phosphate buffer (0.02 M, pH 3.0) and acetonitrile resulted in peaks with good shape and resolution.

It is interesting to note that the values of the chromatographic parameters are similar for the pure sample and tablet extract, which indicates the robustness of the proposed HPLC method. It has been observed that the recovery of valacyclovir from the tablet was 99.8%. The validation of the developed method was ascertained by carrying out five replicates (n = 5) of the chromatographic runs under identical conditions. The results are tabulated in Table-1.

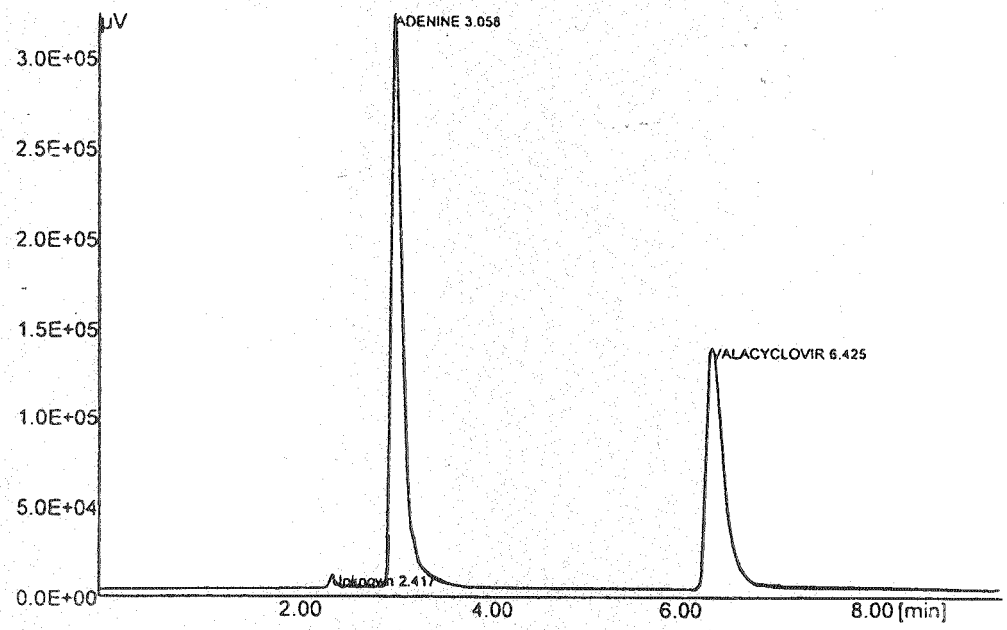

Fig. 1. Model Chromatogram for valacyclovir

TABLE-1
VALIDATION REPORT

Analytical parameters	Results
Accuracy (%)	99.89 ± 0.15
Precision (%)	99.92
RSD*	0.0766
Specificity (50 µg/mL of valacyclovir)	2213853 ± 0.09 (AUC)
Linearity	5–60 µg/mL
Limit of detection	5 ng/mL
Limit of quantitaion	8 ng/mL
Rugged ness (%)	99.88 ± 0.70

* Relative standard deviation

The peak area ratio of the drug to that of the internal standard *vs.* concentration was found to be linear. The linear regression for the proposed method was $Y = 0.0117 + 0.0169X$ ($r = 0.9991$), where Y is the peak area ratio and X is the concentration of valacyclovir.

Intra- and inter-day studies: Intra-day precision and accuracy were studied by five replicate measurements at three concentration levels. The inter-day precision and accuracy were conducted during routine operation of the system over a period of seven consecutive days. Statistical evaluation revealed that relative standard deviation of valacyclovir at different concentrations for five injections was less than 2.0 (Table-2).

TABLE-2
THE INTRA- AND INTER-DAY DATA FOR VALACYCLOVIR

S.No.	Concentration taken (μg/mL)	Intra-day			Inter-day		
		Measured concentration (μg/mL) ± S.D.	C.V. (%)	Relative error (%)	Measured concentration (μg/mL) ± S.D.	C.V. (%)	Relative error (%)
1.	5	4.95 ± 0.089	0.99	−1.0	4.95 ± 0.01	1.20	−1.00
2.	15	15.11 ± 0.025	0.17	+ 0.7	15.10 ± 0.02	0.18	+ 0.60
3.	30	30.20 ± 0.48	1.01	+ 0.6	30.45 ± 0.05	0.19	+ 1.50

Recovery studies: Recovery studies were conducted by analyzing tablet formulations in the first instance for the active ingredient by the proposed method. Known amounts of pure drug were then added to each of the previously analyzed formulations and the total amount of the drug was once again determined by the proposed method after bringing the active ingredient concentration within the limits. High percentage recoveries of valacyclovir ranging from 99.6–100.1 were observed with the tablet dosage forms.

Interference studies: The effect of wide range of excipients and other additives usually present in the tablet formulation of valacyclovir in the determination under optimum conditions was investigated. The common excipients like starch, talc, magnesium stearate, methyl and propyl parabens, cellulose derivatives and propylene glycol have been added to the sample and injected. They have not disturbed the elution pattern or quantification of drug or internal standard. In fact, many of them have no absorption at this detection wavelength.

Conclusion

The proposed HPLC method is simple, precise, accurate and rapid for the determination of valacyclovir in tablet dosage forms. Hence it can be easily and conveniently adopted for routine quality control analysis.

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