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Estimation of Valacyclovir in Tablets and Human Serum by RP-HPLC Method

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A simple, rapid, sensitive and precise reverse phase-high performance liquid chromatographic (HPLC) method has been developed for the estimation of valacyclovir in tablets and human serum. In this method, RP-C_{18} column (150 × 4.6 mm I.D., 5 μm particle size) with mobile phase consisting of acetonitrile and 0.03 M phosphate buffer pH 2.99 in the ratio of 50 : 50 (v/v) in isocratic mode was used. The detection wavelength is 230 nm and the flow rate is 0.8 mL/min. In the range of 0.05–8 μg/mL, the linearity of valacyclovir shows a correlation coefficient of 0.9999. The mean recovery of the drug from the spiked serum solution was found to be 97.82%. The proposed method was validated by determining sensitivity, accuracy, precision and system suitability parameters. The high percentage of recovery indicates that there is no interference from the other serum contents.

Key Words: Valacyclovir, RP-HPLC, Human serum.

INTRODUCTION

Valacyclovir[1] is an orally administered anti-viral drug which is a nucleic acid synthesis inhibitor[2, 3]. Chemically it is L-valine, 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl) methoxy] ethyl ester, which is a prodrug of acyclovir[4, 5], works by disrupting the process by which herpes virus duplicates itself and spreads to other cells. Literature survey reveals that no specific method was reported for the estimation of valacyclovir in tablets as well as in human serum.

EXPERIMENTAL

A binary HPLC (Waters) pump, was used to deliver the mobile phase to the analytical column, symmetry C_{18} column (150 × 4.6 mm I.D., particle size 5 μm), was used. Sample injection was performed *via* a Rheodyne 033381 injection valve with a 20 μL loop. Detection was achieved by Waters 2457 UV Dual λ absorbance detector and the software was Waters Breeze GPC.

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Valacyclovir standard was provided by M/s. Cipla Ltd., Mumbai. Acetonitrile of HPLC grade was obtained from Qualigens (Mumbai). All the chemicals were of analytical grade; potassium dihydrogen phosphate, disodium hydrogen phosphate, triethylamine and orthophosphoric acid were from S.D. Fine Chemicals Ltd., Mumbai.

HPLC conditions: The mobile phase components acetonitrile and 0.03 M phosphate buffer (pH 2.99) adjusted with orthophosphoric acid were filtered through 0.45 μm membrane filter before use and were pumped from the solvent reservoir at a ratio of 50 : 50 (v/v) to the column at a flow rate of 0.8 mL/min. The volume of each injection was 20 μL. The run time was 4 min and the detection wavelength of valacyclovir was set at 230 nm.

Procedure: Stock solution of standard valacyclovir was prepared by dissolving 28 mg of valacyclovir HCl in 25 mL standard volumetric flask containing methanol. A 0.03 M solution of phosphate buffer (pH 2.99) was prepared by dissolving 4.082 g of potassium dihydrogen phosphate and 5.338 g of disodium hydrogen phosphate in 800 mL water and diluting to 1000 mL with water followed by addition of 1 mL of triethylamine. The pH was adjusted to 2.99 with orthophosphoric acid. From the stock solution 100 μg/mL of valacyclovir working standard solution was prepared.

Calibration of standards: From the working standard solution, solutions ranging from 0.05–8 μg/mL were prepared. A 20 μL aliquot was injected into the analytical column.

Assay of valacyclovir in tablets: Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 5 mg of valacyclovir was placed in a 50 mL volumetric flask and a few mL of methanol was added, shaken well and allowed to stand for half an hour with intermittent shaking to ensure complete solubility of the drug. The mixture was then made up to volume with methanol, thoroughly mixed and filtered through a 0.45 μm membrane filter. The filtrate was further diluted with mobile phase to 4 μg/mL solution.

Serum treatment: Serum samples were taken in seven centrifuge tubes each (1 blank + 6 drug solutions) and spiked with appropriate amounts of valacyclovir working standard solution. To these each 1 mL of acetonitrile was added and mixed thoroughly by mixing on a Cyclo mixer CM101 followed by centrifugation for 15 min at 3000 rpm (REMI Centrifuge BGL C-637, Remi Motors, Mumbai). The supernatant was collected and again it was extracted with 2 mL of methanol followed by centrifugation. The same procedure was repeated two times for complete extraction of the drug. The supernatants were pooled up and filtered through 0.2 μm membrane filters (Swinney 13 mm diameter millipore filter). The final valacyclovir concentration was in the range of 0.05–8 μg/mL.

Precision and accuracy: The precision was determined interms of intra- and inter-day variation in the peak area for a set of drug solutions on five different days (n = 5). The accuracy of the method was assessed by adding known amount of the drug to a drug solution of known concentration and subjecting the samples to the proposed HPLC method. The accuracy was expressed in terms of the recovery (Table-2).

RESULTS AND DISCUSSION

The run time of the method was set at 4 min and valacyclovir appeared on the chromatogram at 2.45 min. When the same drug solution was injected 5 times, the retention time of the drug was same. Table-1 gives the information about the calibration of the proposed HPLC method in both standard and in serum solutions. The regression of valacyclovir concentration over its peak area was found to be $Y = 5267.3 + 209524.7X$ ($r = 0.9997$) for standard solutions and $Y = 2696.07 + 206302.3X$ ($r = 0.9994$) for serum solutions where Y is the peak area and X is the concentration of valacyclovir. The coefficient of variation in the peak area of the drug for five replicate injections in standard and serum solutions was found to be less than 2 and 2.5%, respectively. Thus, the results showed that the proposed HPLC method is highly reproducible. The high percentage of recovery in both standard and serum solutions indicates that the proposed method is highly accurate (Table-2). No interference peaks were found in the chromatogram, indicating that the excipients used in the tablet formulation as well as serum contents did not interfere with the estimation of the drug by the proposed HPLC method (Table-3).

TABLE-1
CALIBRATION OF THE PROPOSED HPLC METHOD

Concentration of valacyclovir (μg/mL)	Average peak area*		C.V. (%)
	Std.	Serum	
0.05	11055	10564	1.814
0.1	22006	21108	1.131
0.2	43108	42254	0.695
0.4	85980	83818	0.930
0.8	169790	165563	0.087
1.0	220302	211271	1.226
2.0	438520	422160	0.085
4.0	859980	838170	0.990
8.0	1669567	1646357	1.765

*Mean of five determinations.

TABLE-2
RECOVERY AND ASSAY OF VALACYCLOVIR IN TABLETS

Brand of the tablet	Labelled amount (mg)	Observed amount* (mg)	C.V. (%)	Recovery (%)
Valacycloivir	500	497.5	1.267	99.5
(Cipla)	1000	998.0	1.097	99.8

*Mean of five determinations.

TABLE-3
SYSTEM SUITABILITY PARAMETERS OF VALACYCLOVIR

S.No.	Parameter	Value
1.	Theoretical plates (N)	5000
2.	Tailing factor (T)	1.01
3.	Capacity factor (K)	1.81
4.	HETP	0.003
5.	Plates per meter	33333
6.	Symmetry factor	1.17

Mean of 5 determinations.

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