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Stability-Indicating RP-HPLC Method Development for Determination and Estimation of Valacyclovir in Raw and Tablet Formulation.

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ABSTRACT

A simple, gradient RP- HPLC method has been developed and validated for Valacyclovir bulk and tablet formulation. The method was validated to meet official requirements including selectivity, stability, linearity, precision and accuracy. Successful estimation was carried out for the drug product was developed on C(18) column reversed-phase using Phosphate buffer: methanol in the ratio (65:35 %. v/v) as mobile phase composition. The flow rate was adjusted to 1ml /minute and the absorption maxima were observed at 254 nm utilizing Shimadzu SPD-20A Prominence UV-Vis detector. Valacyclovir drug showed a good and precise linearity in the range 25-125 µg/mL. The HPLC, assay showed the purity ranging 99.49 to 100.17% for tablet formulation. The mean percentage purity was 99.95%. The chromatographic retention time of Valacyclovir was found to be 2.10 minutes. The statistical analysis showed the method accuracy. Various forced degradation studies were conducted on Valacyclovir International Conference on Harmonization (ICH) guidelines. The developed method can be successfully applied for estimation and determination of Valacyclovir in bulk and tablet formulations.

Keywords: Valacyclovir, methanol, buffer, HPLC and UV.

INTRODUCTION

Valacyclovir hydrochloride is L -valine, 2-[(2-amino-1,6-dihydro-6-oxo-9H -purin-9-yl) methoxy] ethyl ester, monohydrochloride[1]. It is an antiviral agent, which is used in the treatment of herpes zoster and herpes simplex virus[1]. Literature review shows minimal methods has been developed and reported for Valacyclovir estimation in biological fluids and there are two methods reported by UV spectroscopy[2-4], HPLC, UPLC and LC-MS methods were reported for estimation of Valacyclovir[5-8]. Method development of HPLC estimation for this Valacyclovir is new method will fulfil all requirements of validation according to ICH guidelines. The increasing importance of speed and reliability of analysis in pharmaceutical analytical laboratories, a new method for determination of valacyclovir in formulations with a short time of analysis is described in this work. It is fast and quick chromatographic

method in terms of retention time and run time when compared with other reported methods described in literature survey. The proposed aim of the study was to develop simple, accurate, specific and precise RP-HPLC method for the estimation of Valacyclovir in the bulk and pharmaceutical tablet formulation.

MATERIALS AND METHODS

Chemicals: The Valacyclovir reference standard (RS) was purchased from Sigma, Germany. The Valanext (Valacyclovir 1000mg) tablet marketed drug of Lifestar pharma pvt. Ltd, purchased from vellore, local Pharmacy, India. The HPLC grade acetonitrile, water and methnol was purchased from Sigma, German.

RP-HPLC instrumentation: Shimadzu LC-20 AT HPLC system, using SPD-10 detector (SPD- M20A, Japan). A Zodiac C(18) column (50mm x 4.6mm, 5µm) with Pore size 95Å. The column temperature was maintained at 27ºC and the flow rate was 1ml/min. The injection volume was 20µl, 254nm was set as a wavelength and the HPLC run time was set for 10 minutes.

Preparation of mobile phase: Buffer preparation: Transferred 5 ml of Triethylamine into 1000 ml of water and the PH was adjusted to 3 with Orthophosphoric acid, filtered through 0.45µm nylon membrane filter and degassed. Mobile phase: Buffer and Methanol were mixed in the ratio of 65:35 and sonicated to degas.

Approximately 350 ml of methanol was transferred into 1 liter volumaetric flask and 650 ml of Phosphate buffer was added and mixed thoroughly by shaking and the pH was adjusted to 6 by gradual adding of 0.5N phosphoric acid, the resulting solution was filtered with 0.45µ membrane filter. The final mobile phase was prepared by adding the ratio of 65:35 %. v/v Phosphate buffer: methanol.

Preparation of valacyclovir stock solution

Preparation of standard Valacyclovir solution: Accurately weighed 10 mg of pure drug was taken in clean, dry 100 ml volumetric flask and dissolved in small volume of mobile phase and made up the volume to 100 ml with mobile phase. This gave 100 µg/mL of drug concentration. The concentration of 25-125 µg/mL was achived by diluting the standard stock solution with mobile phase.

Pharmaceutical Preparations: Total of 10 tablets were accurately weighed and powdered in a mortar. An amount equivalent to 10 mg of valacyclovir was taken and dissolved in 50 ml of mobile phase and sonicate for 5min. About 50 ml of mobile phase was added and sonicate for further 5 minutes. The mixture was shaking well for 2 minutes and transferred to a 100 ml volumetric flask through a Whatman Filter paper No. 41.The residue was washed thrice with mobile phase and the combined filtrate was made up to the mark with mobile phase, this gave 100 µg/mL of drug concentration. The concentration of 25-125 µg/mL was achived by diluting the standard stock solution with mobile phase. Valacyclovir powder were very freely soluble in water.

Solution stability: The prepared drug solution stability was analysed during the time of analysis and also repeated the same analysis method on same day with different time intervels. The same analysis was repeated after 24hrs by keeping the drug solution under laboratory temperature (37 ± 1°C) and in refrigeration (5 ± 1°C).

Method validation: The present method was preceded to obtain new, sensitive and easy method for simultaneous estimation by HPLC from capsule formulation. According to the ICH guidelines recommendations the experimental was validated and USP-30 for parameters such as, system suitability, accuracy, precision, linearity and specificity.

System suitability: System suitability parameters like resolution, retention time, tailing factor and column theoretical plates was performed by injecting six replicates of standards and two replicates of sample preparation at a 100% level to cross verify the accuracy and precision of the chromatographic system.

RESULTS AND DISCUSSION

The RSD values are below 2% indicating the method precision and the accuracy of the method shown by the low standard error values. This shows

Table 1: HPLC conditions for estimation of Valacyclovir.

Parameters	Description
Column	Zodiac C(18) column (50mm x 4.6mm, 5µm)
Column temperature	27 ± 1°C
Mobile phase	Phosphate buffer: methanol (65:35 %. v/v)
Detection	Photodiode array detection at 230 nm
Injection volume	20 µl
Flow rate	1 ml min-1

Table 2: HPLC linearity data for Valacyclovir

Concentration (µg/ml)	Peak area
25	254567.67
50	502344.22
75	754352.35
100	1006097.45
125	1247649.79

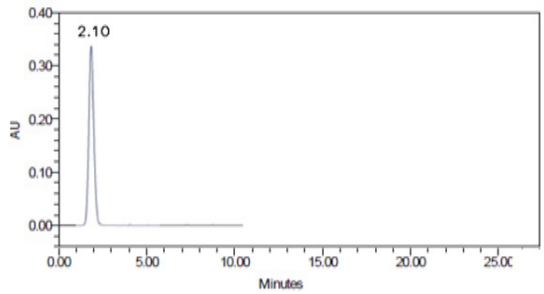

Fig. 1: A Typical Chromatogram of Valacyclovir Standard

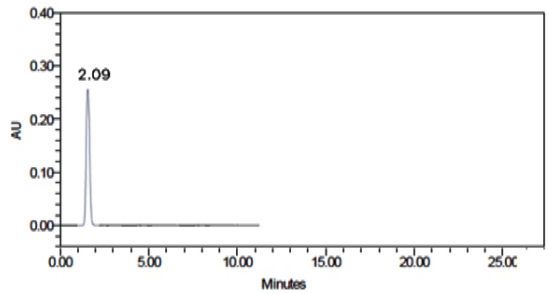

Fig. 2: A Typical Chromatogram of Valacyclovir tablet formulation

a good index of accuracy and reproducibility of the developed method. All the parameters including flow rate, detection wavelength sensitivity was maintained constant. The typical RP-HPLC conditions are presented in Table 1.

The HPLC chromatogram of Valacyclovir standard and Valacyclovir is presented in figure 1 and 2.

Linearity: The proposed method linearity was examined for five different concentrations. The concentration ranges from 25-125 µg/ml. The valacyclovir standard linearity was determined by the plotting graph concentration vs peak area. By peak area as a functional of analyte concentration linearity was evaluated for valacyclovir. The linearity graphs presented in figure 3, and data presented in Table 2. The system suitability is demonstrated by the linearity analysis.

Accuracy

The recovery experiment showed the accuracy of the method. The good recovery showed the method was accurate. The analysis for recovery was performed by known amount of valacyclovir working standard added to pre-analyzed solution of the formulation in the test concentration range of (40%, 60% and 100 %). For each recovery level three samples was prepared and repeated for 3 consecutive days. The statistical results for recovery study are well within the range (S.D. < 2.0). The valacyclovir tablet formulation recoveries results are presented in Table 3.

Precision: The proposed method precision (repeatability) experiment results of are shown in Table 4. In the proposed method intraday and

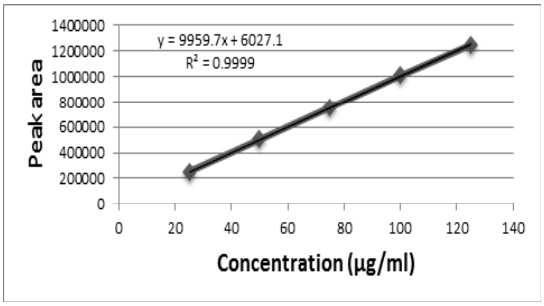

Fig. 3: Calibration graph of Valacyclovir 25-125 µg/ml precision

Table 3: Recovery studies of Valacyclovir tablet formulation

Drug	% Level	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean recovery
Valacy	50	50	100	99.77	
clovir	50	100	149	99.45	99.59
	50	150	199	99.55	

Table 4: Method precision data of Valacyclovir by RP-HPLC method

Valacyclovir 50µg/ml (n=4)	Retention time	Area
1	2.101	501523.22
2	2.091	501193.20
3	2.107	499634.79
4	2.103	500553.52
Mean	3.10	507751.18
S.D a	0.0201	101.71
% CVb	0.68	2.07

n=4 observations

interday precision was examined by analyzing the responeses of the sample on the same day for 4 repeatations and 3 alternate days for 2-10 µg/ml concentration range of valacyclovir. The obtained results are represented in % RSD. The % CV of the proposed method was precise as the values < 1.0 % for the repeatability study. The precision data are presented in Table 5.

Limit of detection and quantitation

The limit of detection and quantification for valacyclovir is presented in table 6. Limit of detection (LOD) and limit of quantification (LOQ): LOD and LOQ were estimated by injecting the diluted known concentration solution, which gave minimum

Table 5: Intermediate precision data of Valacyclovir by RP-HPLC method

Valacyclovir µg/ml	Inter-day measured mean area ± S.D.[a]	%CV[b] (n^c=4)	Intra-day measured mean area ± S.D.[a]	%CV[b] (n^c=4)
75	4057.25± 2.19	0.0872	4497.44± 4.65	0.0125
100	7919.75±2.22	0.0617	7172.75±2.15	0.0157
125	99613.12±2.11	0.0722	9576.12±4.26	0.1056

nc = 4 observations

detectable peak area. This was multiplied thrice to get LOD and by 10 times to get LOQ with suitable precession as per the International Conference on Harmonization guidelines. LOD and LOQ were calculated which was found at concentrations of 0. 55µg/mL and 1.05 µg/mL respectively.

Table 6: Results of Limit of detection & limit of quantification

Parameters	Valacyclovir
LOD (µg/ml)	0.55
LOQ (µg/ml)	1.05

Table 7: Results of system Suitability parameters

SNo	Parameters	Valacyclovir
1.	Theoretical plates	4790
2.	Tailing factor	0.968
3.	Resolution factor	2.11
4.	Retention time	2.1± 0.1
5.	Calibration range or Linear dynamic range	25-125µg/ml

Table 8: Quantitative estimation (Assay) data of Valacyclovir

Drug	Label claim (mg)	Amount found (mg)	Mean amount found (mg/ ml)	Percentage purity (% w/w)	Mean purity (% w/w)	% Deviation
Valacyclovir	50	50.01		100.17		+ 0.2
		49.77		99.49		+0.1
		50.21	50.01	100.21	99.95	+0.2
		49.93		99.77		+1.0
		50.14		100.14		+0.4

Table 9: Reslts of statistical parameters
Statistical parameters

SNo	Parameters	Valacyclovir
1.	Standard deviation (SD)	1.17
2.	Relative standard deviation (RSD)	0.0576
3.	% RSD	0.576
4.	Standard error (SE)	0.02077
5.	Correlation Coefficient (r)	0.9999
6.	Slope (a)	9959.7
7.	Intercept (b)	6027.1

Specificity: Specificity of chromatographic method was established by the separation of drug peak from the adjacent resolving peaks. Specificity was verified by running a blank, placebo and drug working solution to check any interference at the retention time of the standard peak. The valacyclovir standard reference and the drug formulation show the specificity of the method. The RP-HPLC chromatogram of valacyclovir both bulk and the tablet formulation are presented in figure 1, 2. The valacyclovir standard reference and tablet formulation retention time was found to be 2.10 min. For the tablet formulation there was no excipient interference was detected, which shows the specificity of the method. The proposed method showed the ability to determine the analyte in presence of excipients.

The system suitability

For the system suitability parameters five repeats of standards and two repeats of sample preparation are injected, the data is presented in table 7. The Assay data of valacyclovir presented in table 8.

Statistical Parameters: The results of assay obtained are subjected to the following statistical analysis, standard deviation, relative standard deviation, coefficient of variation and standard error. are presented in table 9.

CONCLUSION

The proposed and developed RP-HPLC method is precise, accurate, and sensitive. The method is rapid, reproducible, and economical and does not have any interference due to the excipients in the pharmaceutical preparations.

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