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Analytical Method Development and Validation of Valacylovir in Pharmaceuticals and Bulk Dosage Form by Using RP-HPLC

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ABSTRACT

A new simple, accurate, economic, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Valacyclovir in bulk form and its pharmaceutical dosage form. Chromatographic separation was carried out on Zorbax C18 (4.6mm x 250mm, 5µm, Make: X terra) column using a mixture of Acetonitrile: Methanol: Water (50:30:20% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 254 nm. The retention time of the Valacyclovir was found to be 5.462 ±0.02min. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 50-90 mcg/mL for Valacyclovir.The correlation coefficient was found to be 0.999. The LOD and LOQ for Valacyclovir were found to be 1.6µg/mL and 4.8µg/mL respectively. The proposed method was found to be good percentage recovery for Valacyclovir, which indicates that the proposed method is highly accurate. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and marketed pharmaceutical formulations.

Keywords: Valacyclovir, RP-HPLC, Method Development, Validation, ICH Guidelines.

INTRODUCTION

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use"[1]. Understanding of the physical and chemical characteristics of drug allows one to select the most appropriate high performance liquid chromatography method development from the available vast literature. Information concerning the sample, for example, molecular mass, structure and functionality, pKa values and UV spectra, solubility of compound should be compiled. The requirement of removal of insoluble impurities by filtration, centrifugation, dilution or concentration to control the concentration, extraction (liquid or solid phase), derivatization for detection etc. should be checked. For pure compound, the sample solubility should be identified whether it is organic solvent soluble or water soluble, as this helps to select the best mobile phase and column to be used in HPLC method development.

Method development in HPLC can be laborious and time consuming. Chromatographers may spend many hours trying to optimize a separation on a column to accomplish the goals. Even among reversed phase columns, there is astonishing diversity, owing to differences in both base silica and bonded phase characteristics. Many of these show unique selectivity. What is needed is a more informed decision making process for column selection that may be used before the chromatographer enters the laboratory. The method of column selection presented here involves a minimal investment in time initially, with the potential of

saving many hours in the laboratory.

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods that support safety and characterization studies or evaluations of drug performance are also to be evaluated. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate the potential degradation of the API in the presence of formulation excipients [2].

The three critical components for a HPLC method are: sample preparation (% organic, pH, shaking/sonication, sample size, sample age) analysis conditions (%organic, pH, flow rate, temperature, wavelength, and column age), and standardization (integration, wavelength, standard concentration, and response factor correction). During the preliminary method development stage, all individual components should be investigated before the final method optimization. This gives the scientist a chance to critically evaluate the method performance in each component and streamline the final method optimization[3]. The percentage of time spent on each stage is proposed to ensure the scientist will allocate sufficient time to different steps. In this approach, the three critical components for a HPLC method (sample preparation, HPLC analysis and standardization) will first be investigated individually [4].

The primary objective of proposed work is:

- ✓ To develop new simple, sensitive, accurate and economical analytical method for the estimation of Valaciclovir in bulk and marketed pharmaceutical dosage form.
- ✓ To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Valaciclovir in bulk and marketed pharmaceutical dosage form.

MATERIALS AND METHODS

INSTRUMENTS USED

Table 1: Instruments used

S.No	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Lab man

CHEMICALS USED

Table 2: Chemicals used

S. No.	Chemical	Brand names
1	Valacyclovir	Sura labs
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution: Accurately weigh and transfer 10 mg of Valacyclovir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.7ml of the above Valacyclovir stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Water and Methanol: Phosphate buffer with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer (pH-4.0) (35:65% v/v) respectively.

Optimization of Column: The method was performed with various columns like C18 column, X- bridge column, Xterra, and C18 column. Symmetry ODS C18 (4.6mm x 150mm, 5µm, Make: X terra) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used : Waters HPLC with auto sampler and PDA detector 996 model.

Mobile phase ratio : Acetonitrile: Methanol: Water (50:30:20% v/v)

Column : Zorbax C18 (4.6mm x 250mm, 5µm, Make: X terra)
Column temperature : 35^0C
Wavelength : 254 nm
Flow rate : 1ml/min
Injection volume : 20µl
Run time : 10min

PREPARATION OF MOBILE PHASE

Preparation of mobile phase: Accurately measured 500 ml (50%) of Acetonitrile, 300 ml (30%) of Methanol and 200 ml of HPLC Grade water (20%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µm filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

VALIDATION

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

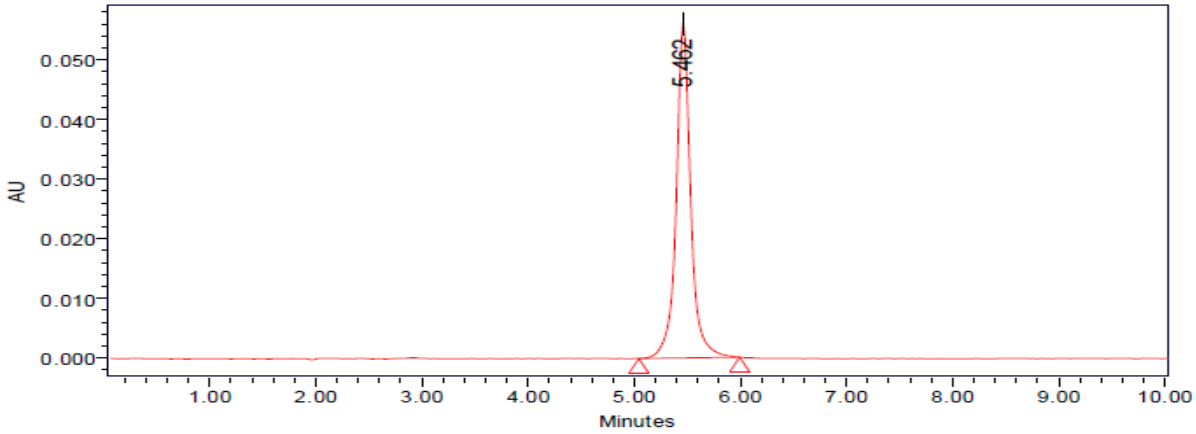

Fig 1: Optimized Chromatogram (Standard)

Optimized Chromatogram (Sample)

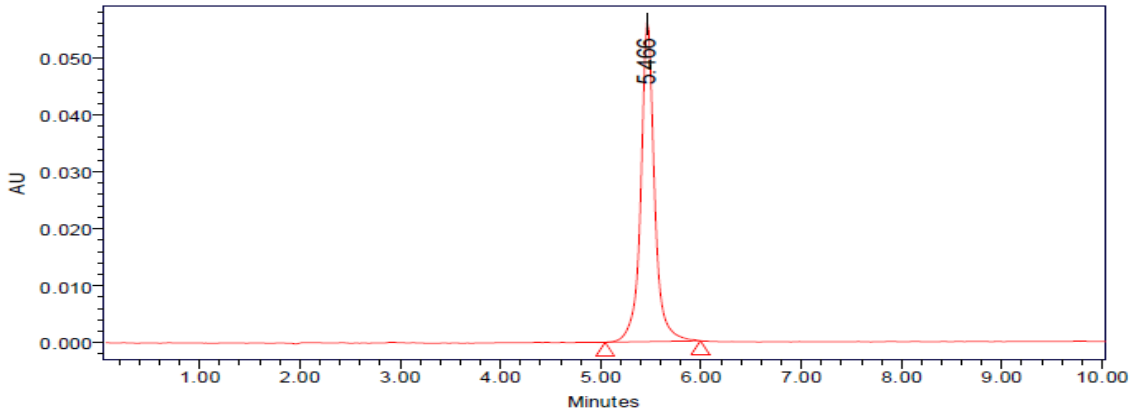

Fig 2: Optimized Chromatogram (Sample)

METHOD VALIDATION

System Suitability

Table 3: Results of system suitability for Valacyclovir

S. No.			Area	Height (µV)		
1	Valacyclovir	5.474	1052658	75842	9658	1.63
2	Valacyclovir	5.466	1058475	75481	9758	1.62
3	Valacyclovir	5.474	1059854	75162	9685	1.63
4	Valacyclovir	5.452	1054786	75241	9635	1.62
5	Valacyclovir	5.446	1052642	75468	9649	1.63
Mean			1055683			
Std. Dev.			3331.494			
% RSD			0.315577			

Specificity

Table 4: Peak results for assay standard

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate	Injection
1	Valacyclovir	5.427	1052689	75421	1.63	9674	1
2	Valacyclovir	5.430	1052854	75462	1.62	9657	2
3	Valacyclovir	5.443	1055365	75489	1.62	9625	3

Table 5: Peak results for Assay sample

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate	Injection
1	Valacyclovir	5.453	1065851	76854	1.63	9785	1
2	Valacyclovir	5.462	1065482	76352	1.64	9786	2
3	Valacyclovir	5.466	1063544	76586	1.63	9795	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Valacyclovir in pharmaceutical dosage form was found to be 99.58%.

LINEARITY
CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

Table 6: Chromatographic Data for Linearity Study

Concentration	Average
50	786789
60	945685
70	1102689
80	1265241
90	1405476

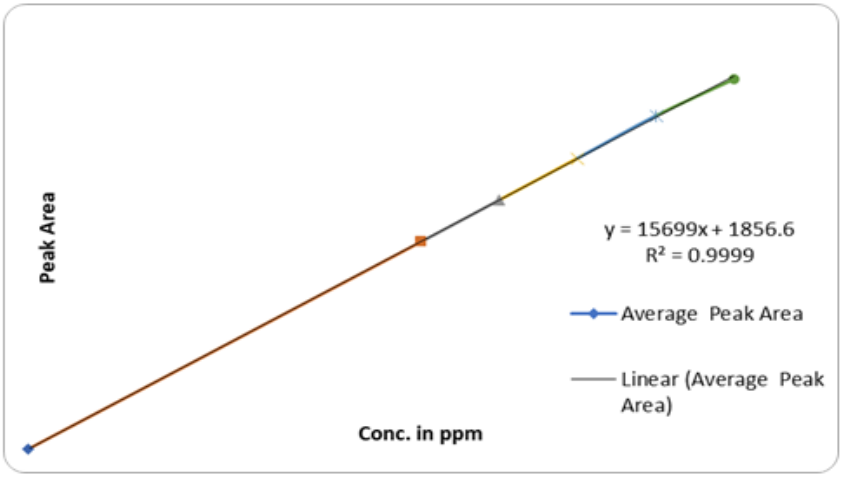

Fig 3: Calibration Curve of Valacyclovir

Correlation Coefficient (r) is 0.99, and the intercept is 1856. These values meet the validation criteria.

PRECISION
REPEATABILITY

Table 7: Results of Repeatability for Valacyclovir:

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Valacyclovir	5.419	1052658	76231	9658	1.63
2	Valacyclovir	5.405	1056854	75898	9667	1.62
3	Valacyclovir	5.478	1052468	75452	9652	1.63
4	Valacyclovir	5.466	1052774	75468	9635	1.62

5	Valacyclovir	5.466	1055245	76214	9658	1.63
Mean			1054000			
Std.dev			1958.724			
%RSD			0.185837			

Intermediate precision

Table 8: Results of Intermediate precision for Valacyclovir

S.No.			**Area (µV*sec)**	**Height (µV)**		
1	Valacyclovir	5.484	1075846	76985	9785	1.65
2	Valacyclovir	5.493	1078254	76854	9748	1.64
3	Valacyclovir	5.406	1078598	76254	9786	1.65
4	Valacyclovir	5.419	1075461	76859	9726	1.65
5	Valacyclovir	5.446	1075236	75898	9742	1.64
6	Valacyclovir	5.452	1075842	76985	9785	1.65
Mean			1076540			
Std. Dev.			1483.688			
% RSD			0.13782			

Table 9: Results of Intermediate precision Analyst 2 for Valacyclovir

S.No.			**Area**	**Height**		
1	Valacyclovir	5.493	1068545	78574	9865	1.65
2	Valacyclovir	5.493	1068547	78546	9854	1.64
3	Valacyclovir	5.478	1063588	78452	9826	1.65
4	Valacyclovir	5.466	1063542	78542	9824	1.65
5	Valacyclovir	5.478	1065243	78563	9863	1.66
6	Valacyclovir	5.419	1065874	78632	9875	1.66
Mean			1065890			
Std. Dev.			2251.215			
% RSD			0.211205			

ACCURACY

Table 10: The Accuracy Results for Valacyclovir

%Concentration	**Area**	**Amount**	**Amount**	**% Recovery**	**Mean**
50%	553829	35	35.159	100.454%	100.29%
100%	1105114	70	70.275	100.392%	
150%	1650868	105	105.039	100.037%	

LIMIT OF DETECTION FOR VALACYCLOVIR

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where
σ = Standard deviation of the response
S = Slope of the calibration curve
Result: =1.6µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where
σ = Standard deviation of the response
S = Slope of the calibration curve
Result: =4.8µg/ml

ROBUSTNESS

Table 12: Results for Robustness

Parameter used for sample analysis	**Peak Area**	**Retention Time**	**Theoretical**	**Tailing factor**
Actual Flow rate of 1.0 mL/min	1052689	5.453	9625	1.62

Less Flow rate of 0.9 mL/min	1015241	5.599	9155	1.54
More Flow rate of 1.1 mL/min	1023654	4.576	9254	1.56
More Organic phase	1015853	3.827	9147	1.54
Less organic phase	1002514	7.415	9256	1.53

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

The HPLC method developed is accurate, precise, repeatable and specific. The method is linear over a wide range and utilizes a mobile phase which can be easily prepared. The column used is a widely available reversed phase Zorbax C18 (4.6mm x 250mm, 5µm, Make: X terra). This method is suitable for the routine quantification of Valacyclovir in bulk drug and in the tablet dosage forms. The validation of this method was proved to be simple, fast and reliable. The method was validated for its performance parameters Linearity, Repeatability, Accuracy, Precision, Ruggedness, and Robustness etc. The developed method offers several advantages in terms of simplicity in mobile phase, isocratic mode of elution and sample preparation steps and comparative short run time makes the method specific, repeatable and reliable for its intended use in determination of Valacyclovir in bulk form and pharmaceutical dosage form.

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