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Method Development and Validation of Valacyclovir in Pharmaceutical dosage form by RP-HPLC Method

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A B S T R A C T

A Simple, accurate, precise and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for the analysis of Valacyclovir in pharmaceutical dosage form. The method development was carried out by using Hypersil-C_{18}, 250x 4.6mm, 5µ column as stationary phase with a mobile phase of Methanol:Orthophosphoric acid (60:40) v/v at a flow rate of 1 ml/min, injection volume was 20µl,column temperature was ambient, retention time was 3.007min and UV detector wavelength was 253nm.Linearity of this method was in the range of 20-70µl,the correlation coefficient was found to be 0.999.The developed method was validated for Linearity, Accuracy, Precision, Sensitivity and Robustness. All the validated parameters were within the limit. The proposed method was found to be simple, economic, fast, accurate, precise and hence can be used for the routine analysis of Valacyclovir in pharmaceutical dosage forms.

Keywords: Valacyclovir, RP-HPLC, Hypersil-C_{18} column, Method development and validation.

A R T I C L E I N F O

CONTENTS

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1. Introduction

Valacyclovir 2-[(2-amino-6-oxo-6,9-dihydro-3H-purin-9-yl) methoxy] ethyl (2S)-2-amino-3-methylbutanoate is an antiviral drug, is a prodrug, being converted in vivo to acyclovir and L-Valine. Thymidine kinase viral enzyme converts acyclovir into acyclovir-monophosphate, which is then converted into acyclovir diphosphate and triphosphate (active metabolite) by cellular enzymes. The mechanism of action of Valacyclovir inhibits herpes viral DNA replication by inhibition of viral DNA polymerase and also in the viral DNA chain termination. Valacyclovir is used to treat infections like shingles (herpes zoster), chickenpox (varicellazoster), genital herpes(herpes simplex genitalis) and cold sores(herpes labialis).The objective of the proposed method is to develop simple, accurate and precise method for the estimation of Valacyclovir in pharmaceutical dosage form by RP-HPLC [1,2,3].

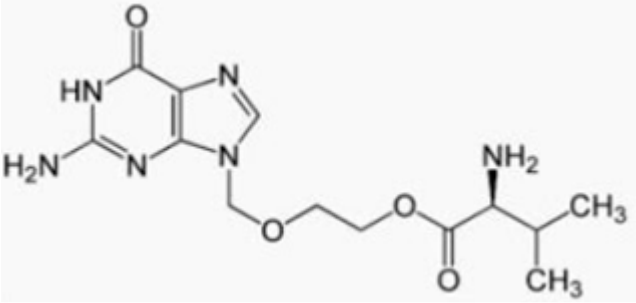

Figure 1: Structure of Valacyclovir

2. Materials and Methods

Preparation of Mobile phase:

HPLC Methanol and Orthophosphoric acid buffer (pH6) were in 60:40 ratios and the resulting solution was sonicated for 20 min then finally filtered through a 0.45 µm membrane filter and degassed.

Preparation of Buffer:

Take 250ml of HPLC grade water, mix with 0.28 ml of orthophosphoric acid having pH value at 6. Finally it was sonicated and filtered through 0.45 µm membrane filter and degassed.

Preparation of Standard Stock Solution:

Weigh accurately a quantity of 10 mg of Valacyclovir and transfer into a 10 ml dry, clean volumetric flask and the volume is made up to the mark with solvent (1000µg/ml). From this 0.4ml was pipette out into a 10 ml dry, clean volumetric flask and the volume is made up to mark with solvent (40 µg/ml).

Preparation of Sample:

Ten tablets were weighed and triturated into fine powder. An amount of powder equivalent to 20 mg of Valacyclovir was accurately weighed and transferred into 20 ml dry, clean volumetric flask and volume is made up to the mark with solvent (1000 µg/ml). From this 0.4 ml was pipette out into a 10 ml dry, clean volumetric flask and the volume is made up to mark with solvent (40 µg/ml).

Method Development:

Selection of Wavelength:

The known concentration of Valacyclovir was accurately weighed and dissolved in volumetric flask using solvent. The resulting solution was scanned in the range of 200 to 400 nm. The absorption curve showed characteristic absorption at 253 nm for Valacyclovir was shown in Figure no: 2.

Assay:

Inject 20 µL of the standard and sample solutions into the HPLC system and the chromatograms were recorded in Fig. 3,4 and Table no:10.Measure the areas for the peaks and calculate the % Assay by using following formulae:

% Assay = At/As x Ws/Ds x Dt/Wt x P/100 x Avg weight/Label claim x 100,Where

At= Sample area

As= Standard area

Ws= Standard weight

Wt= Sample weight

Dt= Sample dilution

Ds= Standard dilution

P = % purity of standard

Amount found= % of drug/100 x Label claim

Observation:% Purity of Valacyclovir by assay method was found to be 99.76%

Acceptance criteria:% Purity should lie within the range of 98-102%.

Method Validation:

System suitability:

System-suitability tests are integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 40 µg/ml. The results were within acceptable limits and are in shown in Table no: 11 and Fig no: 5-10.

Observation:

All system suitability parameters were within the acceptable limits.

Acceptance criteria:

1. Theoretical plates should be more than 2000.
2. Asymmetry factor NMT 2.
3. %RSD of peak area and Retention time NMT 2%.

Linearity:

Linearity was determined in the range of 20-70 µg/ml with six different concentrations in the level of 20, 30, 40, 50, 60, 70 µg/ml concentrations. Plot a graph of peak area verses concentration (on X- axis concentration and on Y-axis peak area) and the correlation coefficient was

calculated and calibration curve was recorded in Table no: 12 and Fig no:11.

Accuracy:

Accuracy (Recovery) of the method was tested by spiking 50,100,150% of known amount of standard drug to the sample. The % Recovery was calculated and reported in the table no: 13and Fig no: 12-17.

Observation:% Recovery was within the limit.

Acceptance criteria:% Recovery should lie within the range of 98-102%.

Precision: Precision was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting six repeated injections of 40µg/ml concentration from tablet solution within a day. Peak area and %RSD were calculated and reported in Table no:15and Fig no:18-23. Inter-day precision studies were done by injecting six repeated injections of 40 µg/ml concentration from tablet solution for six consecutive days. Peak area and %RSD were calculated and reported in Table no: 16 and Fig no: 24-29.

Observation: %RSD was within the limit.

Acceptance criteria:% RSD of the six replicate injections NMT 2.0%.

LOD and LOQ:

LOD and LOQ were determined from standard deviation and slope method as per ICH guidelines.

Robustness

The Robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay.

Effect of variation of flow rate:

Standard solution was prepared as per the testing method and was injected into the HPLC system by keeping flow rates, 1.0 ml/min, 0.8 ml/min and 1.2 ml/min flow. The chromatograms were recorded and were presented in Fig no: 30, 31 and Table no: 17.

Observation: Tailing factor was within the limit.

Acceptance criteria: The Tailing factor of Valacyclovir standards should be ≤ 2.0 for variation in flow rate

Table 1: Instruments and Apparatus

S.No	Instruments and Apparatus	Software	Model	Make
1.	Weighing balance	_	BT 224S	Sartorius Micro Balance
2.	Sonicator	_	8510	Branson Ultrasonic Bath
3.	HPLC	Empower 2	2690	Waters
4.	UV Spectrophotometer	UV probe 2.10	UV- 1800	Shimadzu
5.	Glass ware	_	_	Borosilicate

Table 2: Chemicals and Reagents

S.No	Chemicals & Reagents	Grade	Make
1.	Methanol	HPLC	E.Merk, Mumbai, India.
2.	Orthophosphoric acid	AR	E.Merk, Mumbai, India.
3.	Puried water	HPLC	Milli-Q
4.	Valacyclovir	NA	Aurobindo Pharm Ltd, Hyderabad, A.P, India.
5.	Pharmaceutical dosage form (Tablet), Brand name: Valcivir 500mg		In local market manufactured by Cipla Pharma Ltd.

Table 3: Trials for method development for Valacyclovir

S.NO	Trials	Observation	Result
1.	Mobile phase: Methanol: Water(90:10%v/v) Column:HypersilC_{18},250x4.6mm,5µ Flow rate: 1 ml/min	Peak shape and base line was not good.	Not Satisfy
2.	Mobile phase: Methanol: Water(80:20%v/v) Column:HypersilC_{18},250x4.6mm,5µ Flow rate: 1 ml/min	Peak tailing was observed.	Not Satisfy
3.	Mobile phase: Methanol: Water(70:30%v/v) Column:HypersilC_{18},250x4.6mm,5µ Flow rate: 1 ml/min	Base line was not proper.	Not Satisfy
4.	Mobile phase: Methanol: Water(60:40%v/v) Column:HypersilC_{18},250x4.6mm,5µ Flow rate: 1 ml/min	Peak fronting and extra peak was observed.	Not Satisfy

5.	Mobile phase: Methanol: Orthophosphoricacid (90:10%v/v) Column:HypersilC_{18},250x4.6mm,5µ Flow rate: 1 ml/min	Peak tailing and base line was not proper.	Not Satisfy
6.	Mobile phase: Methanol: Orthophosphoricacid (80:20%v/v) Column:HypersilC_{18},250x4.6mm,5µ Flow rate: 1 ml/min	Peak broadening and peak tailing was observed.	Not Satisfy
7.	Mobile phase: Methanol: Orthophosphoricacid (70:30%v/v) Column:HypersilC_{18},250x4.6mm,5µ Flow rate: 1 ml/min	Peak tailing and base line was not proper.	Not Satisfy
8.	Mobile phase: Methanol: Orthophosphoricacid (60:40%v/v) Column:HypersilC_{18},250x4.6mm,5µ Flow rate: 1 ml/min	Peak shape, base line, retention time and tailing factor was good.	Satisfy

Table 4: Optimized chromatographic conditions of the proposed method

S.No.	Parameter	Value
1.	Mobile phase	Methanol : Orthophosphoric acid buffer (60:40)
2.	Column	Hypersil- C_{18}, 250x 4.6mm, 5µ
3.	Column temperature	Ambient
4.	Flow rate	1ml/min
5.	Injection volume	20µl
6.	Detection wavelength	253 nm
7.	Retention time	3.007

3. Result and Discussion

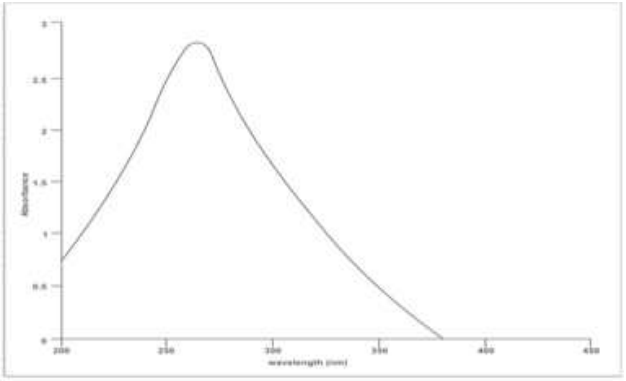

Figure 2: UV Spectra of Valacyclovir

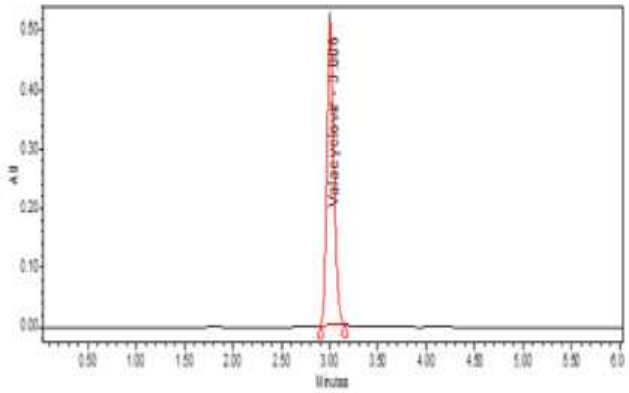

Figure 3: Chromatogram for Standard

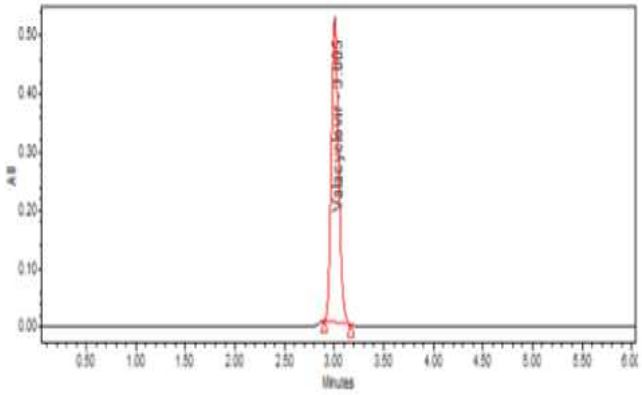

Figure 4: Chromatogram for Sample

Table 10: Observations for Assay of Valacyclovir

S.No	Particulars	Valacyclovir
1.	Standard area	3224612
2.	Sample area	3225531
3.	Sample weight	20.92mg
4.	Standard weight	10mg
5.	Purity of std	99.8%
6.	Amount found	498.8mg
7.	Label claim	500mg
8.	% Purity	99.76%

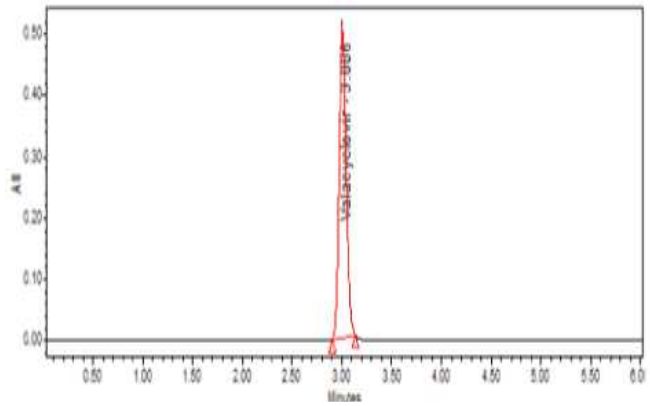

Figure 5: Chromatogram for System suitability Injection 1

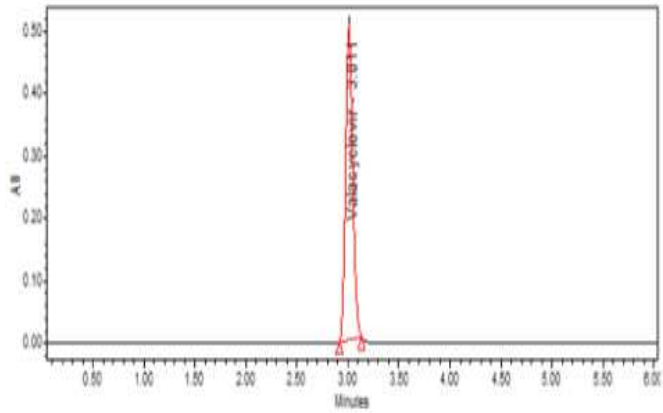

Figure 6: Chromatogram for System suitability Injection 2

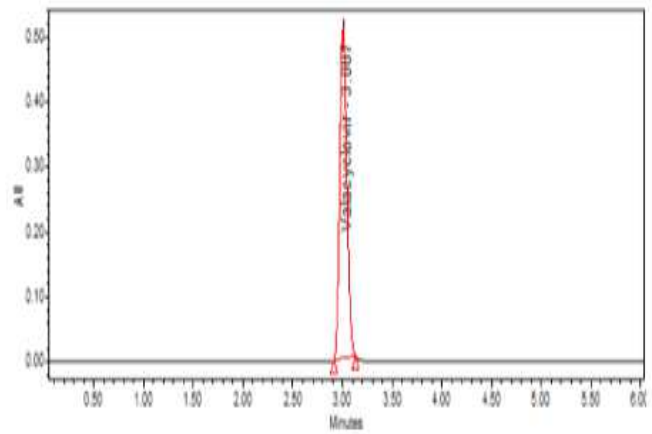

Figure 9: Chromatogram for suitability for Injection 5

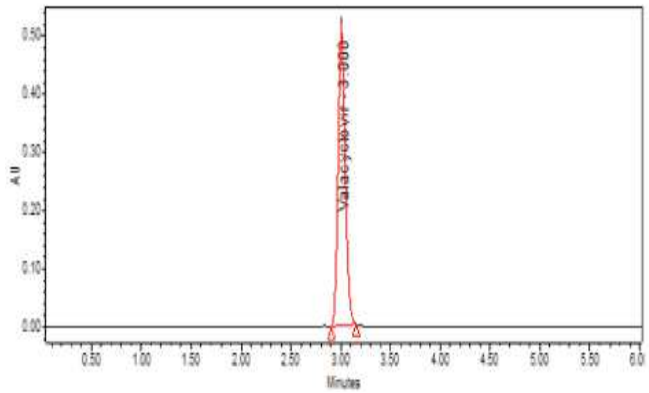

Figure 7: Chromatogram for System suitability Injection 3

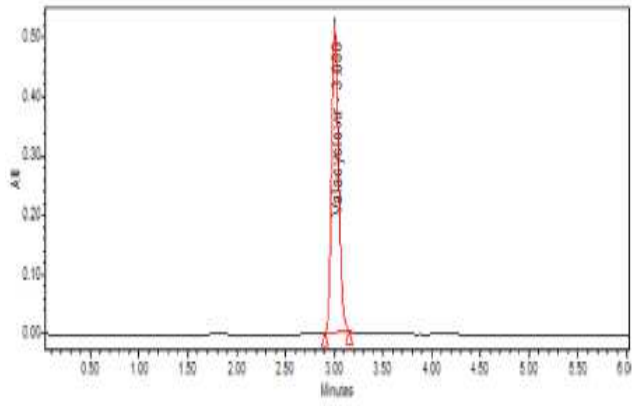

Figure 8: Chromatogram for System suitability Injection 4

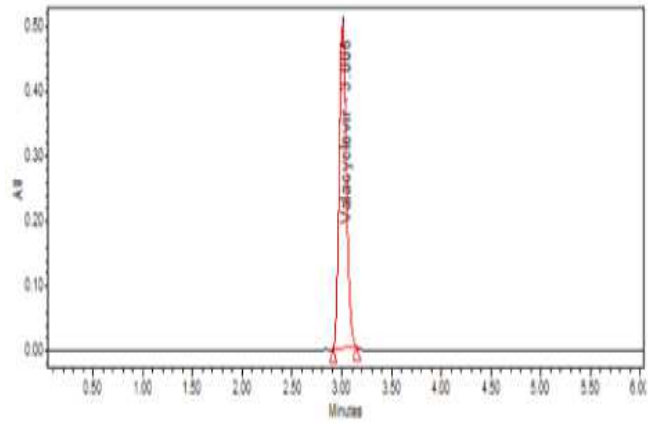

Figure 10: Chromatogram for System suitability Injection 6

Table 11: System suitability parameter for Valacyclovir

Standard 40µg/ml	**Retention time(min)**	**Peak area**	**Asymmetry factor**	**Theoretical plate count**
Injection 1	3.006	3228239	1.346475	3270.253952
Injection 2	3.011	3228403	1.347635	3223.752718
Injection 3	3.008	3224598	1.360519	3220.809123
Injection 4	3.008	3225546	1.311324	3223.379589
Injection 5	3.063	3225373	1.347499	3260.613384
Injection 6	3.008	3229234	1.343196	3252.940057
Mean	3.0173	3226898.833	_	_
SD	0.0304	345.3	_	_
%RSD	1.0075	0.0107	_	_

Table 12: Linearity for Valacyclovir

S.No	Concentration(µg/ml)	Peak area(vs.)	Statistical data
1.	20	1615163	Regression equation(y) = 79371x +45241 Slope = 79371 Intercept = 45241 Correlation coefficient(r)=0.999
2.	30	2423239	
3.	40	3225382	
4.	50	4038096	
5.	60	4842826	
6.	70	5556812	

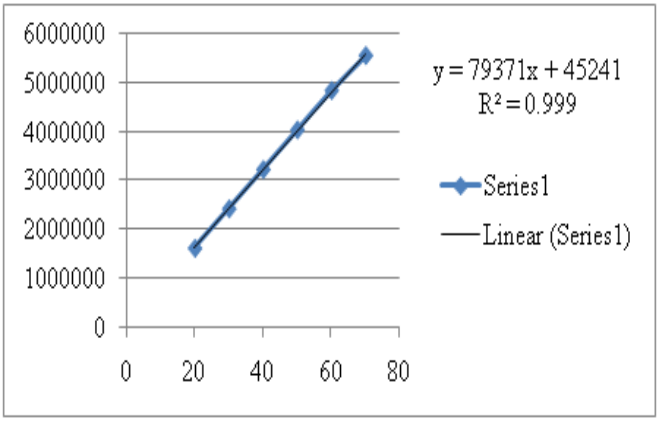

Figure 11: Calibration curve for Valacyclovir

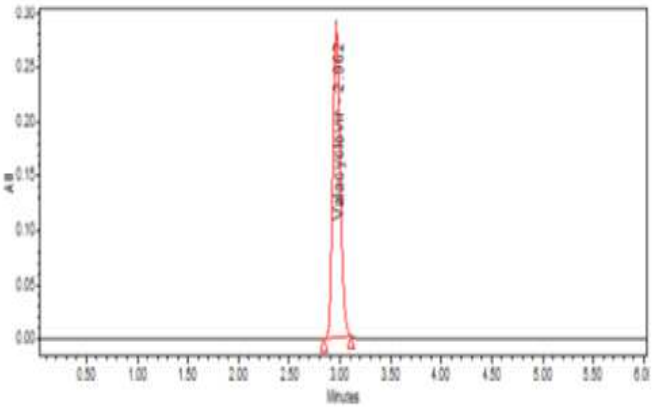

Figure 12: Chromatogram for Accuracy 50% Injection 1

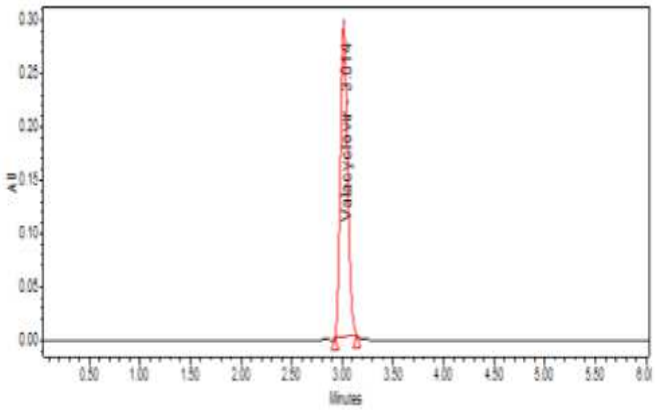

Figure 13: Chromatogram for Accuracy 50% Injection 2

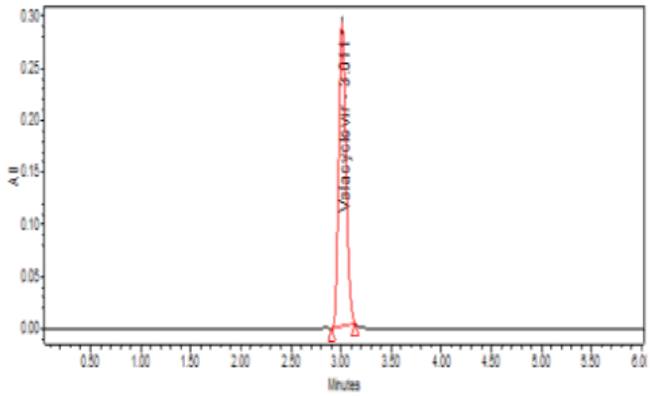

Figure 14: Chromatogram for Accuracy 50% Injection 3

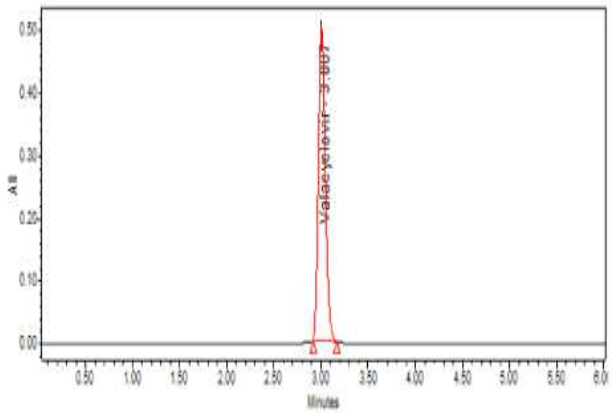

Figure 15: Chromatogram for Accuracy 100% Injection 1

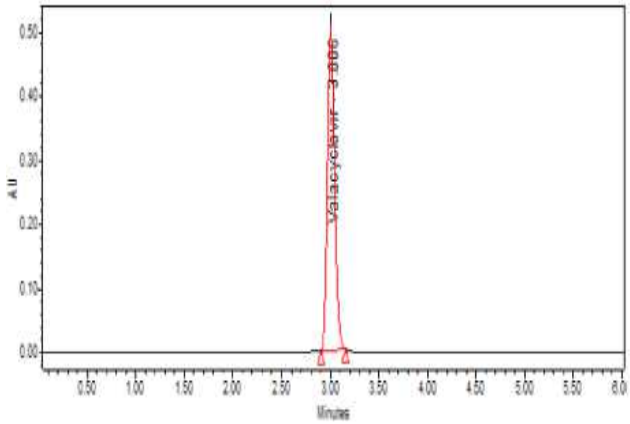

Figure 16: Chromatogram for Accuracy 100% Injection 2

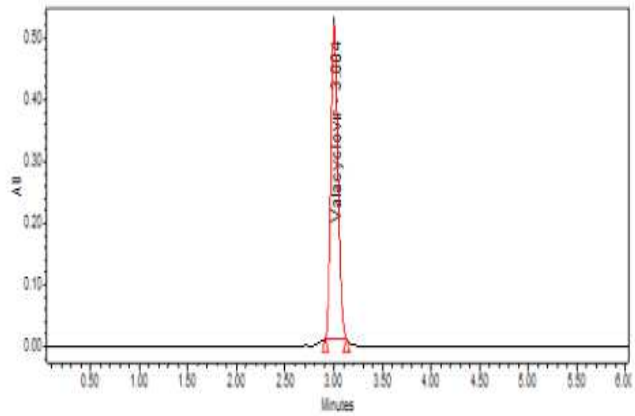

Figure 17: Chromatogram for Accuracy 100% Injection 3

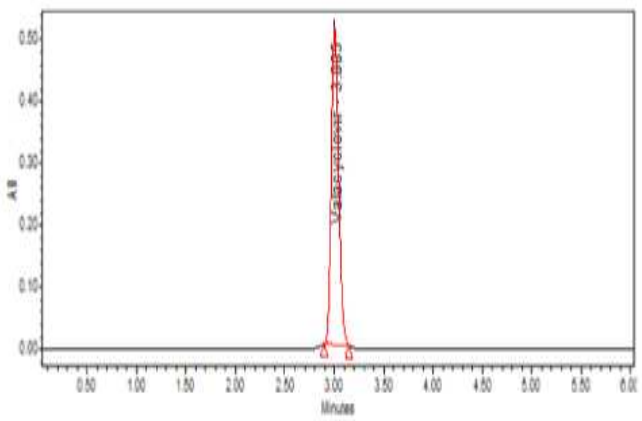

Figure18: Chromatogram for Intra-day precision Injection1

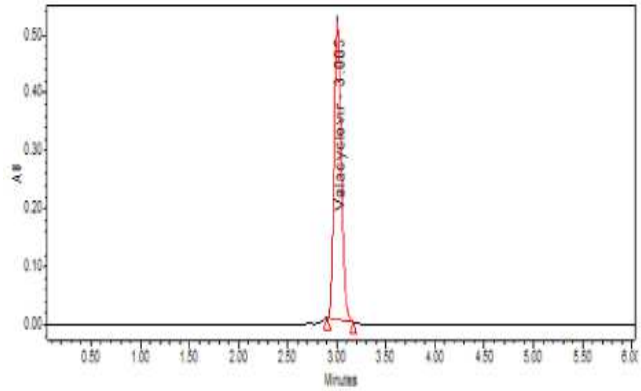

Figure19: Chromatogram for Intra-day precision Injection5

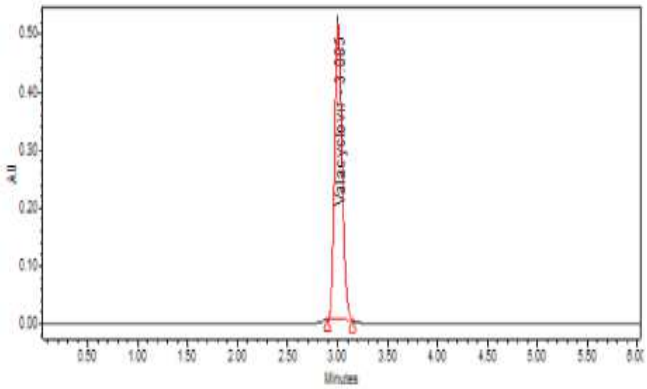

Figure20: Chromatogram for Intra-day precision Injection6

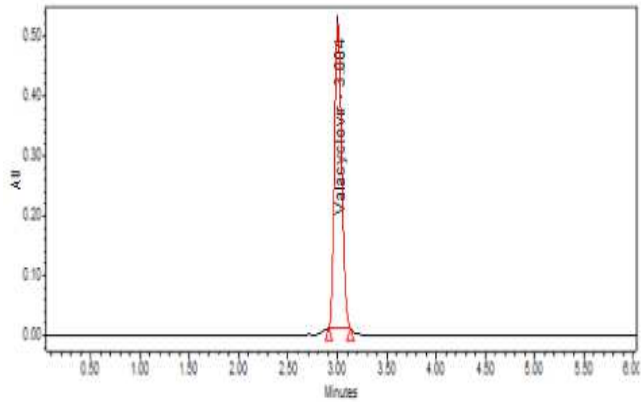

Figure21: Chromatogram for Intra-day precision Injection 2

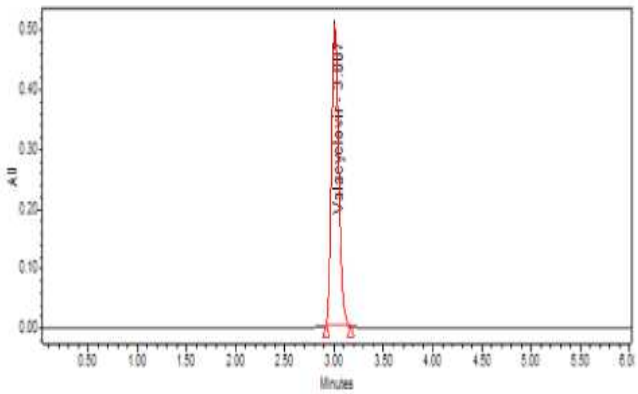

Figure22: Chromatogram for Intra-day precision Injection 3

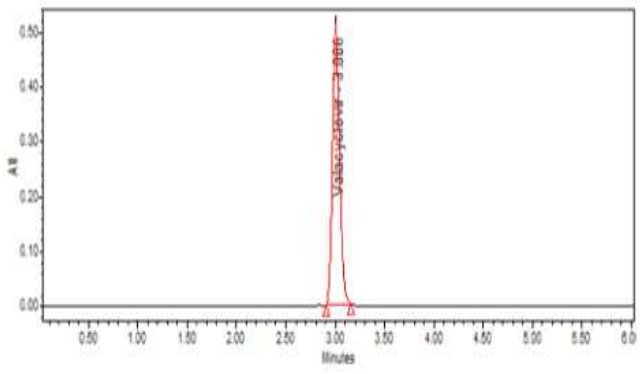

Figure23: Chromatogram for Intra-day precision Injection 4

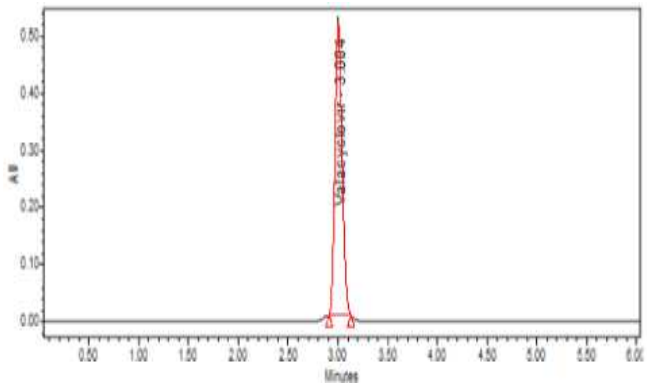

Figure24: Chromatogram for Inter-day precision Injection1

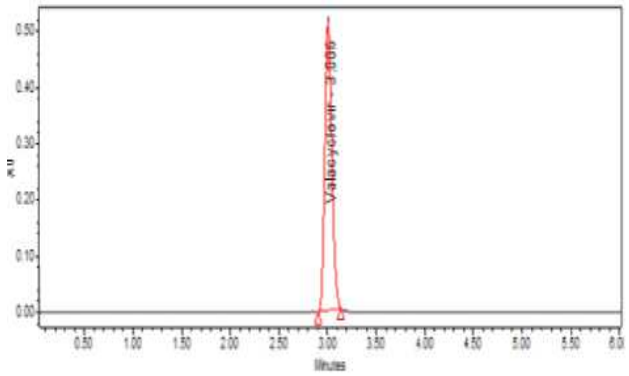

Figure25: Chromatogram for Inter-day precision Injection 2

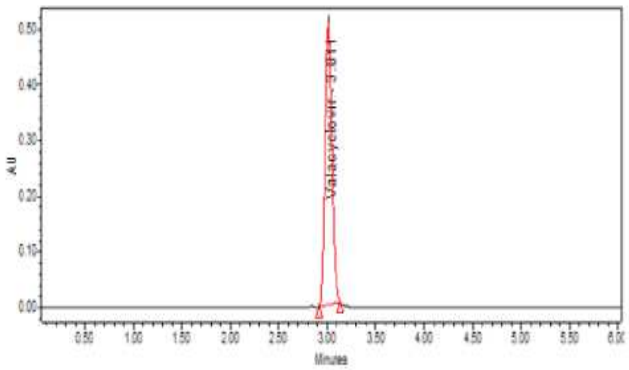

Figure26: Chromatogram for Inter-day precision Injection 3

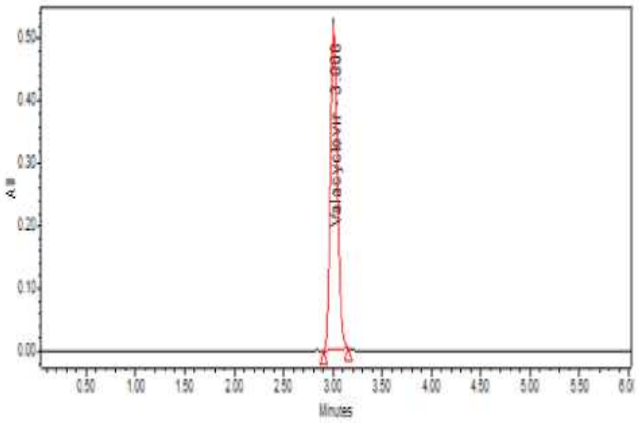

Figure27: Chromatogram for Inter-day precision Injection 4

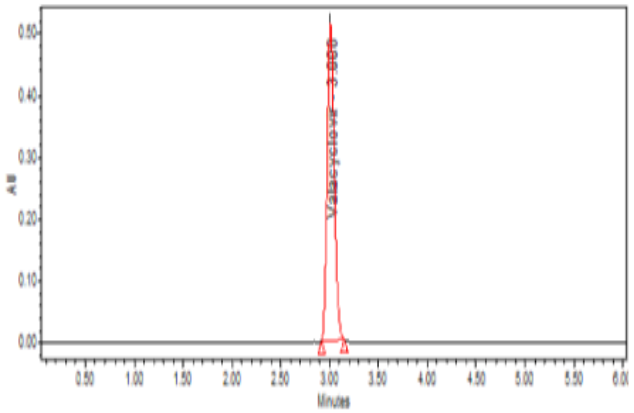

Figure28: Chromatogram for Inter-day precision Injection 5

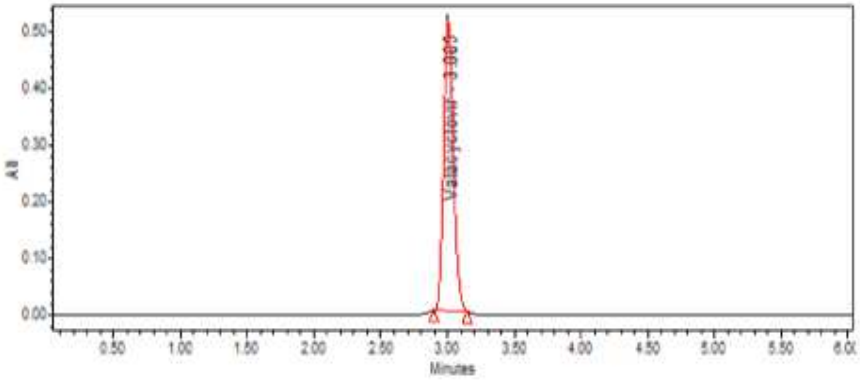

Figure 29: Chromatogram for Inter-day precision Injection 6

Table 14: Accuracy studies for Valacyclovir

Concentration of spiked level	Amount added	Amount found	%Recovery	% Recovery mean	RSD
50% Injection1	20	20.27	101.39	101.2766	0.04134
50% Injection 2	20	20.25	101.29		
50% Injection 3	20	20.23	101.15		
100%Injection1	40	40.56	101.40	101.4933	0.03935
100%Injection2	40	40.61	101.54		
100%Injection3	40	40.61	101.54		
150%Injection1	60	60.95	101.59	101.6533	0.01929
150%Injection2	60	61.01	101.69		
150%Injection3	60	61.00	101.68		

Table 15: Intra-day precision for Valacyclovir

S.No	Retention time	Peak area	Asymmetry factor	Theoretical plate count
1.	3.005	3220319	1.347459	3274.547030
2.	3.004	3228356	1.346475	3270.253542
3.	3.007	3223974	1.359211	3229.456426
4.	3.006	3224603	1.363488	3268.417147
5.	3.005	3225531	1.356668	3277.913348
6.	3.005	3220415	1.347459	3274.547030
Mean	3.0053	3223866.333	_	_
SD	0.0423	466.5778	_	_
RSD	1.4075	0.01447	_	_

Table 16: Inter-day precision for Valacyclovir

S.No	Retention time	Peak area	Asymmetry factor	Theoretical plate count
1.	3.004	3228354	1.346475	3270.253542
2.	3.006	3228237	1.346475	3270.253952
3.	3.011	3238402	1.347635	3223.752718
4.	3.008	3224596	1.360519	3220.809123
5.	3.008	3225548	1.311324	3223.379589
6.	3.005	3225520	1.356668	3277.913348
Mean	3.007	3226776.167	_	_
SD	0.04	310.9666	_	_
RSD	1.3302	0.0963	_	_

Limit of Detection (LOD):

Calculation of LOD from Linearity curve as per formula given below:

$$LOD = \frac{3.3\sigma}{S}$$

Where, σ = the standard deviation of the response.

S =the slope of the calibration curve (of the analyte).

Limit of Quantification (LOQ):

Calculation of LOQ from Linearity curve as per formula given below:

$$LOQ = \frac{10\sigma}{S}$$

Where, σ = the standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

Table 17: Limit of Detection and Limit of Quantification by using Slope and SD of system suitability

Parameters	Slope	SD of System suitability	Result
LOD	79371	345.3	0.0143
LOQ	79371	345.3	0.0435

Robustness: Effect of Flow rate:

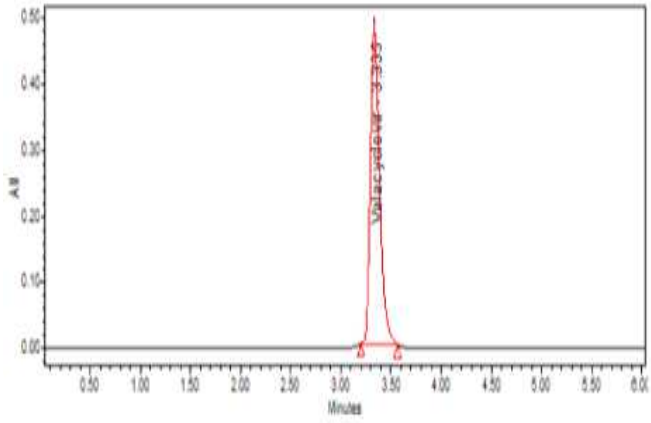

Figure 30: Chromatogram for low flow rate (0.8ml)

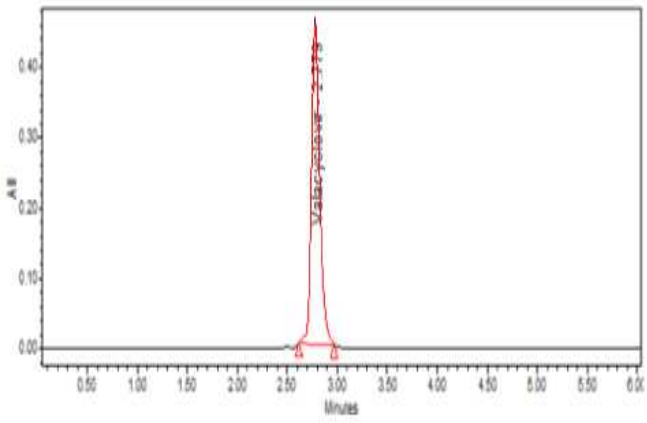

Figure 31: Chromatogram for High flow rate (1.2ml)

Table 18: Robustness for Valacyclovir

Flow rate(ml/min)	Retention time	Peak area	Tailing factor	USP Plate count
0.8	3.314	4278453	1.372843	3658.097540
1.2	2.779	3011885	1.309990	3322.505826

4. Conclusion

The proposed RP-HPLC method is suitable technique for determination of Valacyclovir. All the parameters for this drug met the criteria of ICH guidelines for method validation. The developed method is recommended for routine and QC analysis of the investigated drugs to provide simple and accurate quantitative analysis for the determination of Valacyclovir.

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