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Method Development And Validation Of Valacyclovir In Bulk & Tablet Dosage Form By Rphplc Method

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Abstract : *A reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the estimation of valacyclovir in bulk drug and pharmaceutical dosage form. The method is carried out using C8 agilent zorbax column 150cm x 4.6mm x 5micron and mobile phase consisting of phosphate buffer at pH 3.0: water : methanol (50:50 % v/v), at flow rate of 1 mL/min. The column temperature is 25°C. Eluents were monitored by UV detector set at 253 nm. The method was statistically validated in terms of linearity, accuracy, precision,systemsuitability and robustness in accordance with ICH guidelines.Linear regression analysis data for the calibration plot showed that there was a linear relationship between response and concentration in the range of 20-150 mcg/ml and the correlation coefficient is 0.9999.The retention time found to be 2.02min. Literature survey reveals analytical methods for the estimation of Valacyclovir from pharmaceutical dosage forms and also in biological fluids. The proposed method was found to be simple, precise, accurate, rapid and reproducible for the estimation of valacyclovir in bulk drug and tablet.*

Key words*:Valacyclovir, RP-HPLC Method,Validation,Linearity.*

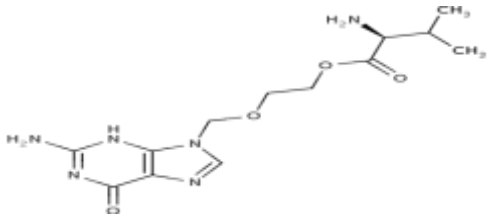
I. INTRODUCTION

Valacyclovir hydrochloride is *L*-valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy]ethyl ester, monohydrochloride.It is an antiviral agent which is used in the treatment of herpes zoster and herpes simplex virus. Literature survey reveals few analytical methods for the estimation of valacyclovir from pharmaceutical dosage forms and also in biological fluids. Due to 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 (5 min) with retention time 2.027 minutes 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.

2.1. DRUG PROFILE:

Name : VALACYCLOVIR

Structure :



ChemicalName : (*S*)-2-[(2-amino-6-oxo-6,9-dihydro-3*H*-purin-9yl)methoxy]ethyl- 2-amino-3- -methylbutanoate.

Molecular Formula : $C_{13}H_{20}N_6O_4$
Molecular Weight : 360.80
Nature : White –Offwhite capsule shaped film coated tablets
Solubility : soluble in water
Pka : 1.90, 7.47, and 9.43.
Therapeutic Category : Anti-viral Agent.

λ_{max} : 253nm.

Half life : <30min valacyclovir.2.5-3.6hrs acyclovir
Bioavailability : ~55% (oral)
Excretion : renal(40-50%)acyclovir, and fecal (47%)
Metabolism : Hepatic (50 – 70%)to acyclovir
Storage : stored under cool and dry place.

2.2. Chemicals/ Reagents and Solvents:

Table.3. Chemicals and Reagents

S. No.	Chemicals/standards and reagents	Grade	Make
1	Potassium dihydrogen phosphate	AR	Thomas Baker
2	Ortho phosphoric acid	AR	Merck
3	HPLC Grade Methanol	HPLC	Merck
4	HPLC Grade Acetonitrile	HPLC	Merck
5	Double Distilled Water	HPLC	Loba Chemi
6	Valacyclovir	N/A	MSN Laboratories

2.3. Instruments and Equipments:

Table.4.Instruments and Equipments

S.NO.	Instruments And Equipments	Software	Model	Company
1	HPLC	Empower 2	Alliance 2695, Detector 2487	WATERS
2	UV-Spectrophotometer	UV Win	UV-Visible SpectrophotometerT60 (model)	Analytical technologies Limited
3	Weighing Balance	N/A	ER200A	ASCOSET
4	Sonicator	N/A	SE60US	ENERTECH
5	pH Meter	N/A	AD102U	ADWA

Marketed Formulation: Valcivir– 500mg

II. METHODOLOGY

In the present investigation, a simple and sensitive HPLC method was developed for quantitative estimation of valacyclovirin bulk drug and pharmaceutical formulations. A Rapid, Precise, Accurate, Specific and Simple HPLC method was developed for determination of valacyclovir in pharmaceutical formulation using PDA detector. The presented method was simple, since diluted samples are directly used without an preliminary chemical derivatisation or purification steps.

3.1. Method Development and Optimization Of Chromatographic Parameters:

3.1.1. Selection of wavelength:

The sensitivity of the HPLC method which uses UV detection depends upon the proper selection of wavelength. An ideal wavelength is one that gives good response for all the drugs to be detected. A UV spectrum of Valacyclovir was recorded between 200-400nm (Fig.8).

3.1.2. Selection of chromatographic methods:

The proper selection of methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and stability. The drugs selected are polar, ionic and Reversed phase chromatography can be used because of its simplicity and suitability.

3.1.3. Development of RP-HPLC method for Valacyclovir:

The objective of this experiment was to optimize the assay method for estimation of Valacyclovir based on the literature survey made. So here the trials mentioned describes how the optimization was done. The Chromatograms were showed in Fig:7,8 and .

TRIAL: 1

- Buffer preparation: Transferred 7 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 70:30 and sonicated to degas.
- Chromatographic conditions:

Flow rate : 1.0 ml/min
Column : Thermo C_8 column, (4.6*150mm, 3.5µm)
Detector wavelength : 253nm
Column temperature : Ambient
Injection volume : 10µl
Run time : 10 min

- Observation: Peak shape is not good and Asymmetry is more than limit.

TRIAL: 2

- Buffer preparation: Transferred 2 ml of orthophosphoric acid into 1000 ml of water and the PH was adjusted to 2.5 with triethylamine, filtered through 0.45µm nylon membrane filter and degassed.
- Mobile phase: Buffer and Methanol were mixed in the ratio of 60:40 and sonicated to degas.
- Chromatographic conditions:

Flow rate : 1.2 ml/min
Column : Thermo C_8 column, (4.6*150mm, 3.5µm)
Detector wave length : 253nm
Column temperature : 30^0 c
Injection volume : 10µl
Run time : 10 mins

- Observation: Theoretical plates are less .

TRIAL: 3

- 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 50:50 and sonicated to degas.
- Chromatographic conditions:

Flow rate : 1.2 ml/min
Column : Agilent zorbax RP C-8column with 150 x 4.6 mm ,5 µm
Detector wavelength : 253nm
Column temperature : Ambient
Injection volume : 20µl
Run time : 10 min

- Observation: The retention time is more, peak tailing is observed.

After several trials with the different combination and ratio of solvents, the mobile phase composition of 0.1M Phosphate Buffer: Methanol (50 : 50) of pH 3.0 ± 0.05 was used and the chromatogram was recorded with flow rate of 1.0 ml / min with UV detection at 253 nm and was showed in Fig: 14. The retention time of valacyclovir was 2.02min and the run time was less than 6min. Linearity was observed over concentration range of 20 - 150 µg / ml.

3.1.4. Optimized Method: Fig.9:

- 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 50:50 and sonicated to degas.
- Chromatographic conditions:

Column	:	Agilent zorbax RP C-8column with 150 x 4.6 mm i.d.& 5 µm
Column temperature	:	25°C
Flow rate	:	1ml/min.
Injection volume	:	5 µl.
Wavelength	:	253 nm.
Run time	:	5min.
Diluent	:	methanol: water (50:50)
Mobile Phase	:	Buffer: diluents

The proposed method was validated for various parameters like Linearity, , Accuracy, Precision, Robustness, and System Suitability according to ICH guidelines. Commercial tablet formulations and laboratory prepared dilutions were successfully analyzed using the developed methods.

3.2. Experimental Details:

3.2.1. Apparatus and Soft ware:

The liquid chromatographic system consisted of following components: Waters 2695 HPLC model (automated) containing variable wavelength programmable PDA (Photo diode array) detector . Chromatographic analysis was performed using Empower2 software on a Agilent zorbax RP C-8column with 150 x 4.6 mm i.d. and 5 µm particle size.

3.2.2. Collection of solvents:

HPLC grade Methanol, Phosphate Buffer, Ortho Phosphoric acid, was used as solvents through out the experiment.

3.2.3. Reagents and Pharmaceutical Preparations:

Valacyclovir was kindly supplied by MSNlaboratories (Hyderabad, A.P and India). All the solvents used in HPLC method are of HPLC grade. Commercial pharmaceutical preparations of Valacyclovir(Valcivir) from local market . Which were claimed to contain 500 mg Valacyclovir was used in analysis.

3.2.4. Buffer:

A solution of 13.80 gm of potassium bi phosphate was dissolved in 900 ml of Hplc grade water, mix well by using sonicator, and make up the volume to 1000 ml with water. The pH of the resulting solution was adjusted to 3.0 ± 0.5 with 10 % ortho phosphoric acid. The above solution was filtered through a 0.45 µm pore size nylon filter and degassed by ultra sonicator.

3.2.5. 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 mcg / ml of drug concentration.From this 0.5ml make up to 10ml with mobile phase.(conc=50mcg/ml).

3.2.6. Pharmaceutical Preparations:.

Total of 10 tablets were accurately weighed and powdered in a mortar. An amount equivalent to 10 mg of valcyclovir 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 a100 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 mcg / ml of drug concentration.

Chromatographic conditions:

Mobile phase	:	Phosphate buffer: Methanol (50 : 50)
Flow rate	:	1.0 ml / min
Column	:	Agilent zorbax RP C-8, 150 × 4.6 mm, 5µm
Detector wave length	:	253nm
Column temperature	:	25ºC
Injection volume	:	5µl
Run time	:	5min

3.3. Assay Procedure:

Inject 20µL of the standard and sample solutions into the HPLC system and the chromatograms were recorded Fig.16, 17. Measured the areas for the Linezolid peaks and calculate the %Assay by using following formulae:

$$\% \text{ Assay} = \frac{At}{As} \times \frac{Ws}{Ds} \times \frac{Dt}{Wt} \times \frac{P}{100} \times \frac{avg.weight}{label\ claim} \times 100$$

Where,
At = average area counts of sample preparation.
As = average area counts of standard preparation.

Ws = Weight of working standard taken in mg.

Wt = Weight of sample taken in mg.

Dt = sample dilution

Ds = standard dilution

P = Percentage purity of working standard

$$\text{Amount found (mg)} = \frac{\%\ \text{of Drug}}{100} \text{ x label claim}$$

3.4. Validation of Analytical Method:

Validation of a method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical Parameters.

3.4.1. Accuracy:

Accuracy of the method was determined by recovery experiments. Recover y studies were carried out by adding known amount of standard drug solution to the sample solution. The % recovery was calculated and reported in Table:5.

3.4.2. Precision:

The Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The Precision expressed as standard deviation or relative standard deviation.The experiment was repeated three times in a day and the average % RSD values of the results were calculated. When the experiment was repeated on three different days the average % RSD values for determination of Valacyclovir were calculated.

(a). System precision: System-suitability tests are an 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 100 µg / ml. The results were within acceptable limits and given in Table: 6

(b)Method precision: The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting six (6) repeated injections of 100% concentration from tablet solution within a day. Peak area and %RSD were calculated and reported in Table-7. Inter-day precision studies, was done by injecting six (6) repeated injections of 100% concentration from tablet solution for six consecutive days. Peak area and %RSD were calculated and reported in Table-7.

3.4.3. Linearity:

The linearity of the method was demonstrated over the concentration range of 20 – 150 µg / ml of the target concentration. Aliquots of 20, 50, 100, 120 and 150 µg / ml were prepared from stock solution. The chromatograms was recorded in Fig27-31..A Calibration curve was produced by analyzing different concentrations of the pure drug from the chromatogram in Fig.32.

The correlation coefficient for the peak area at each level versus concentration of analyte was calculated and reported in Table 9. and the calibration parameters of Valacyclovir were showed in Table 9.

Acceptance criteria:

1. Correlation Coefficient should be not less than 0.9990.

Observation:

1. The correlation coefficient was found to be 0.999.
2. From the above study it was established that the linearity of test method is from 20 % to 150% of the target concentration.

3.4.4. 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. Analyze the sample separately by deliberate changes in the analytical method as given below:

(a) Effect of variation of flow rate:

A study was conducted to determine the effect of variation in 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. Evaluate the system suitability parameters for 1.0 ml / min, 0.8 ml / min and 1.2ml / min flow. The chromatograms were recorded and were presented in Fig: 33 and 34.

Acceptance criteria:

1. The Tailing Factor of Valacyclovir standards should be ≤ 2.0 for Variation in flow.

(b) Effect of variation in Temperature:

A study was conducted to determine the effect of variation in temperature.Standard solution was prepared as per the testing procedure. By Changing the temperature i.e. 30ºc injected into the column and the chromatograms were recorded, presented as Fig: 35 and 36.

Acceptance criteria:

1. The Tailing Factor of Valacyclovir standard should be ≤ 2.0 for Variation in mobile phase concentration.

6. FIGURES AND TABLES:

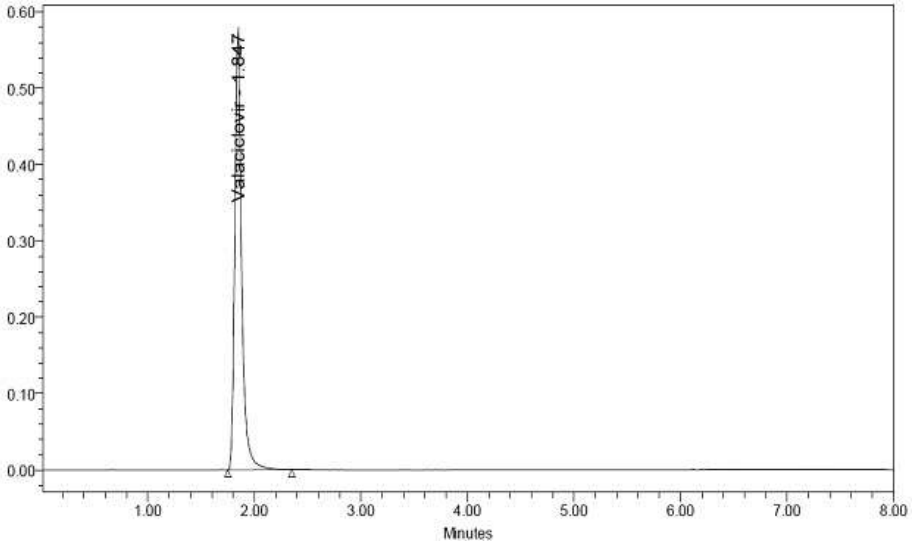
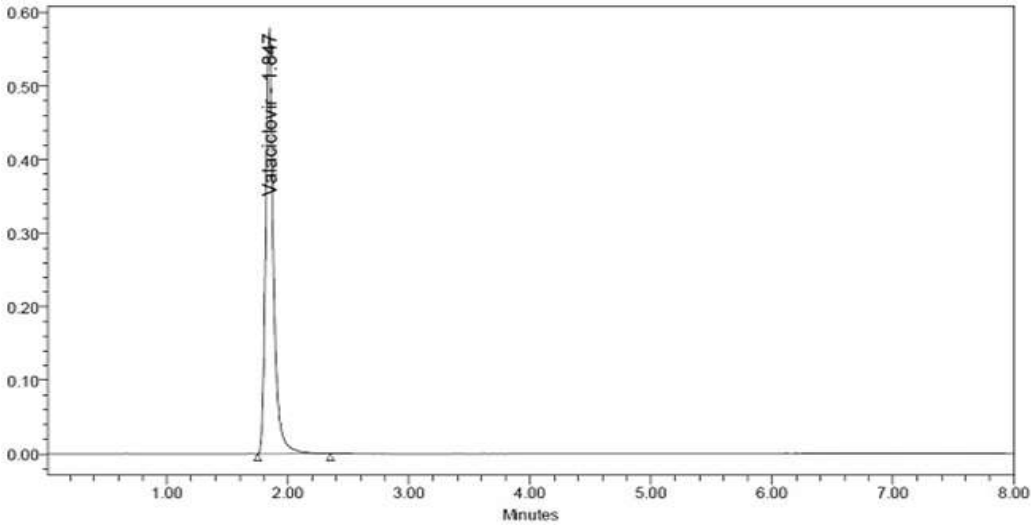


Fig6: Chromatogram of Blank Standard 0.1mg/ml



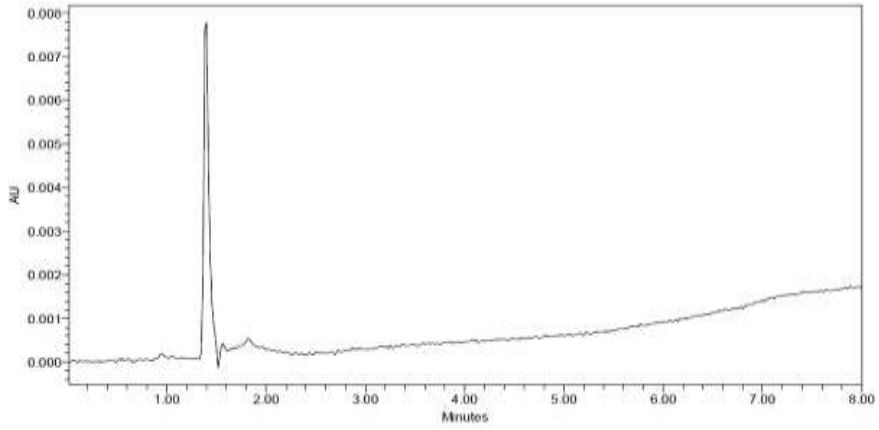


Fig7: Chromatogram Of Standard Mobile Phase 60:40: Water: Methanol

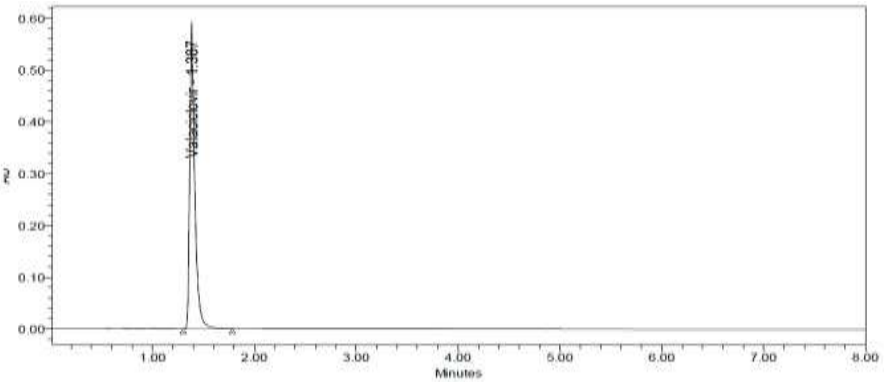


Fig: 8. Chromatogram for Standard Mobile phase pH 3.2 Trial-2

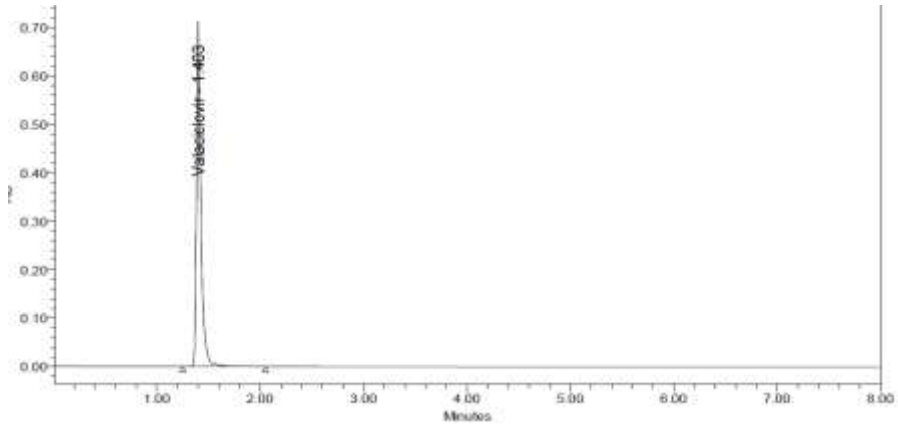


Fig:9. Chromatogram for standard 30 degree Trial-3

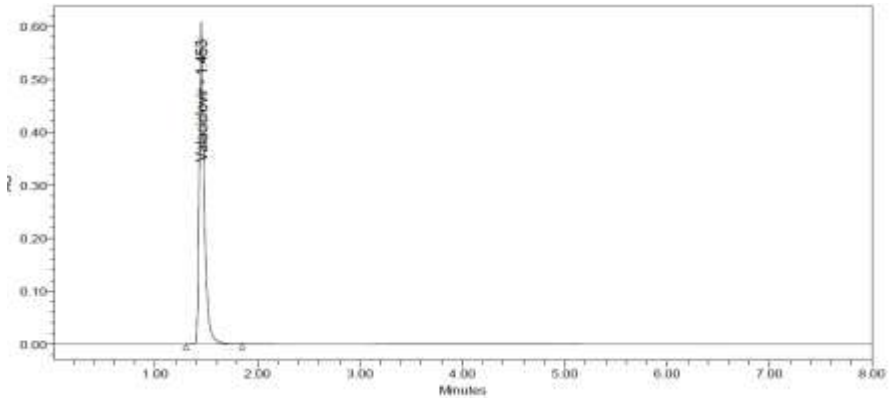


Fig:10. Chromatogram for Standard Mobilephase 60:40 using AcetoNitrile

ASSAY RESULTS:

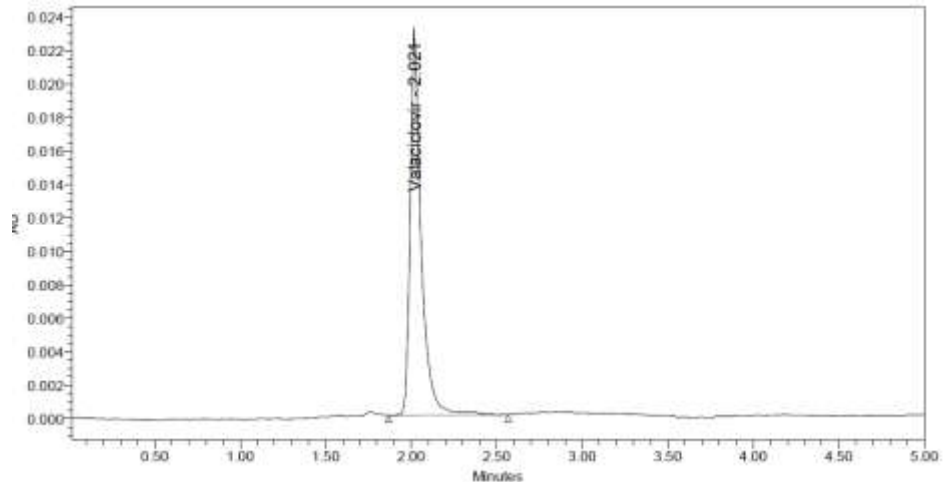


Fig: 11. Chromatogram for Sample 1 inj 1

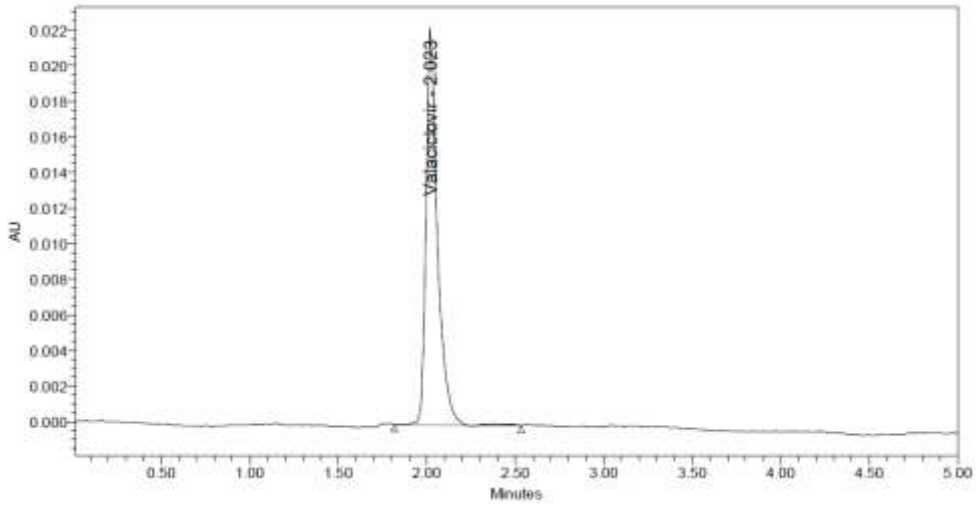


Fig12: Chromatogram for Sample 1 inj 2

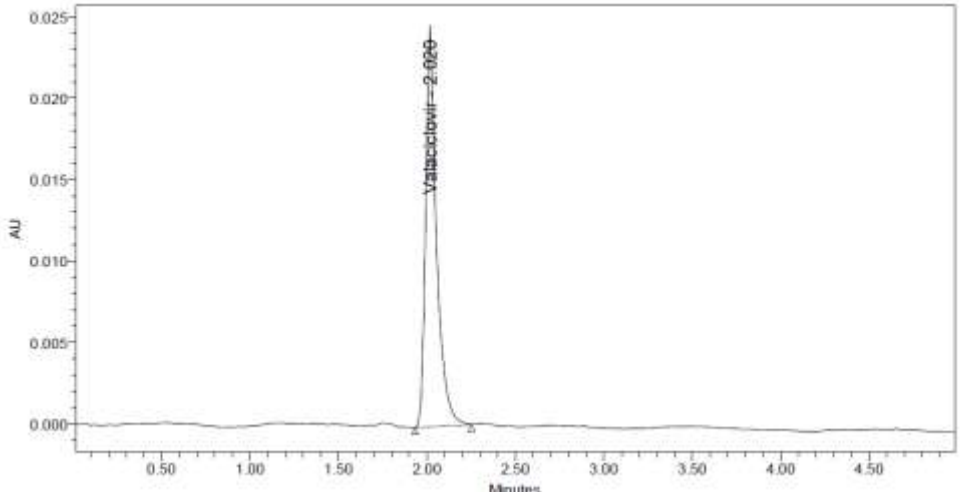


Fig13: Chromatogram For Sample 2 inj 1

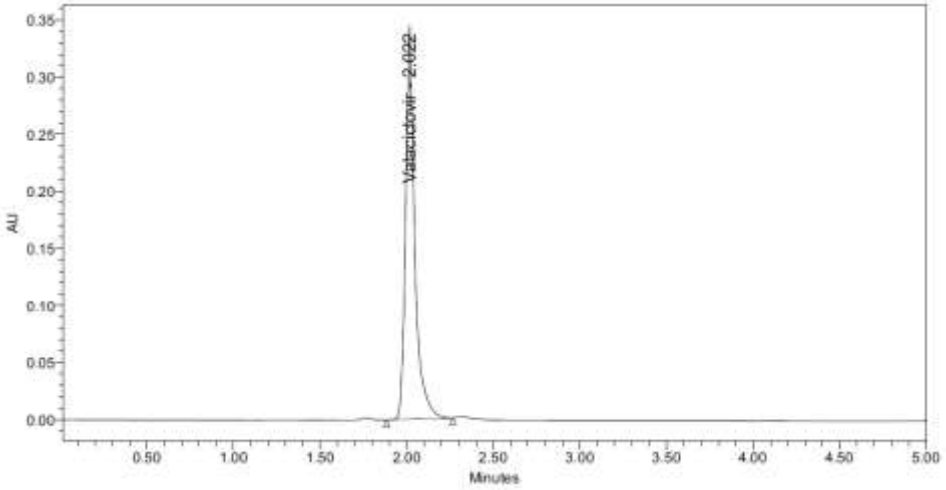


Fig14: Chromatogram for Sample 2 inj 2

Table 3: Assay Results for valacyclovir

S.NO	Formulation	Label claim	Amount found	%Assay
1	Valcivir Tablets	500	499.5	99.92

Table: 4. Parameters for HPLC

Instrument	WATERS 2695 HPLC Model
Column	Agilent zorbax C8 (150x4.6mm,5µm)
Wavelength	253nm
Flow rate	1ml/min
Injection volume	5µl
Mobile phase	Phosphate buffer : methanol (50:50v/v)
Temperature	25ºC
Run time	5min

VALIDATION OF ANALYTICAL METHOD:

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

1. Accuracy:

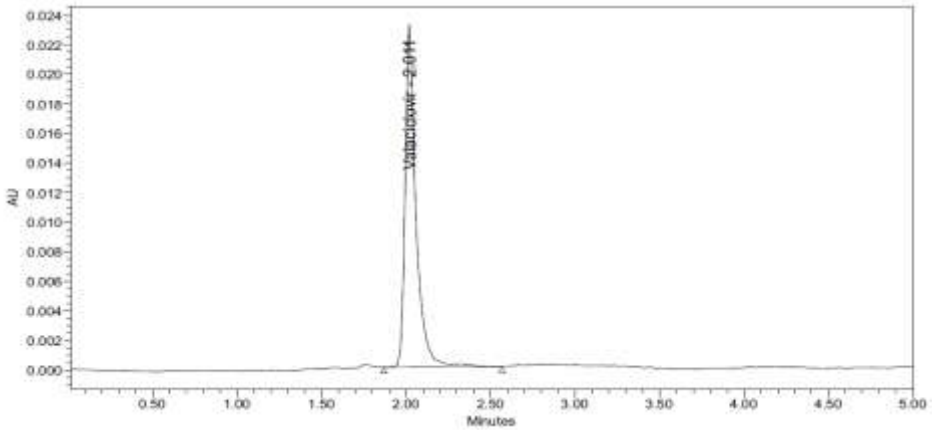


Fig 14. Chromatogram for Accuracy 50% 1st injection

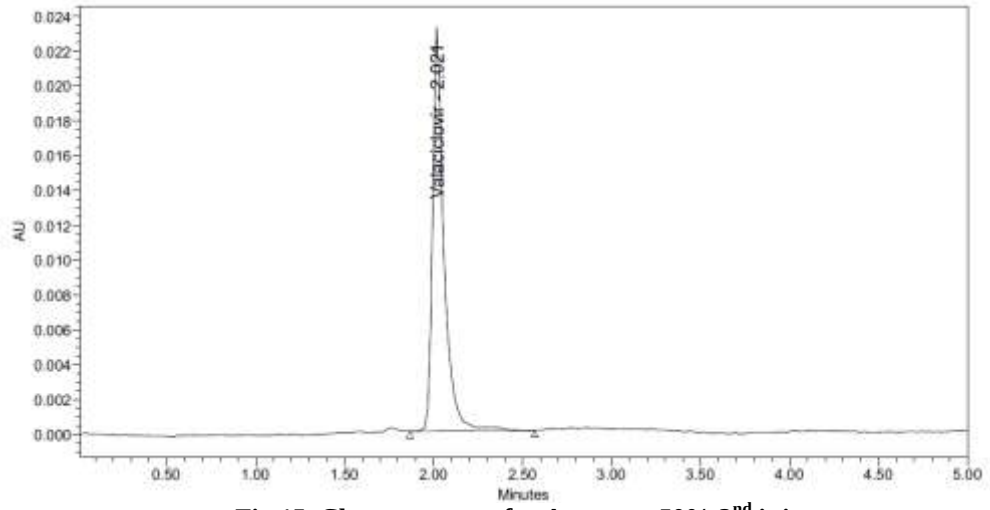


Fig 15. Chromatogram for Accuracy 50% 2nd inj

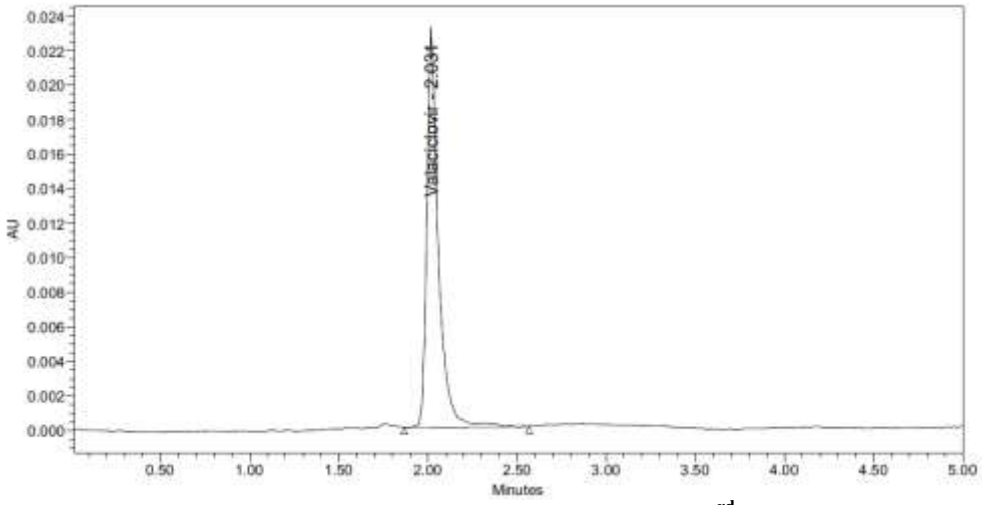


Fig 16. Chromatogram for Accuracy 50% 3rd inj

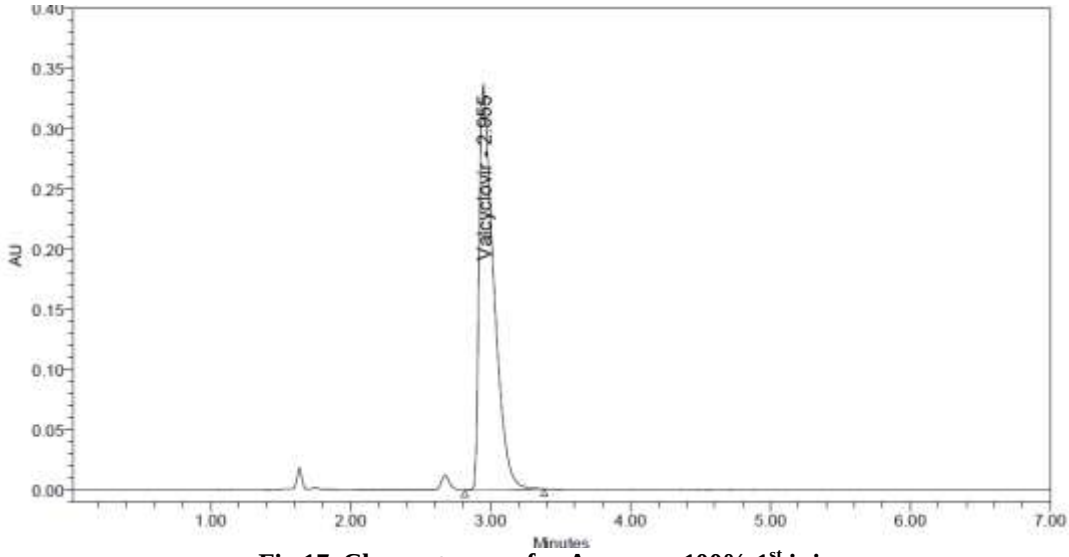


Fig 17. Chromatogram for Accuracy 100% 1st inj

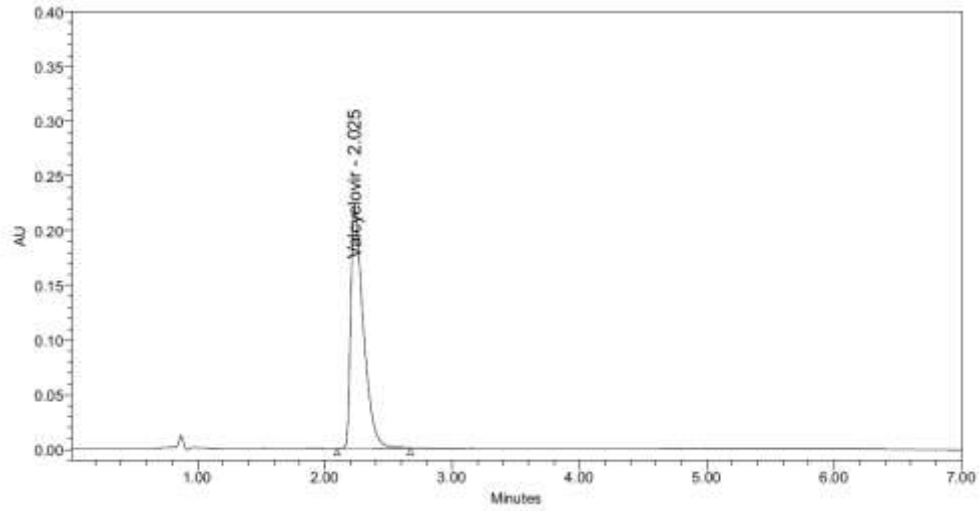


Fig 18. Chromatogram for Accuracy 100% 2nd inj

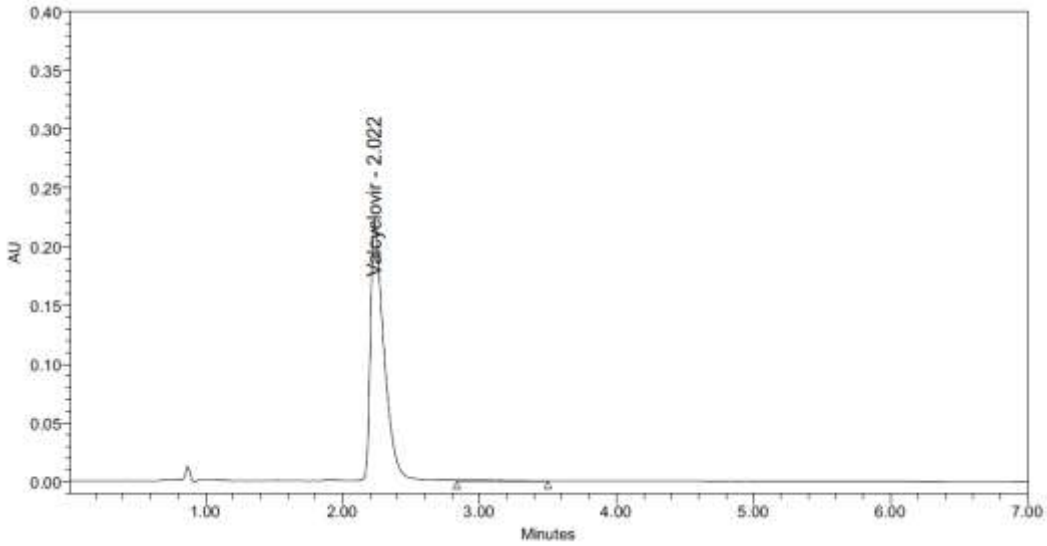


Fig 19. Chromatogram for Accuracy 100% 3rd inj

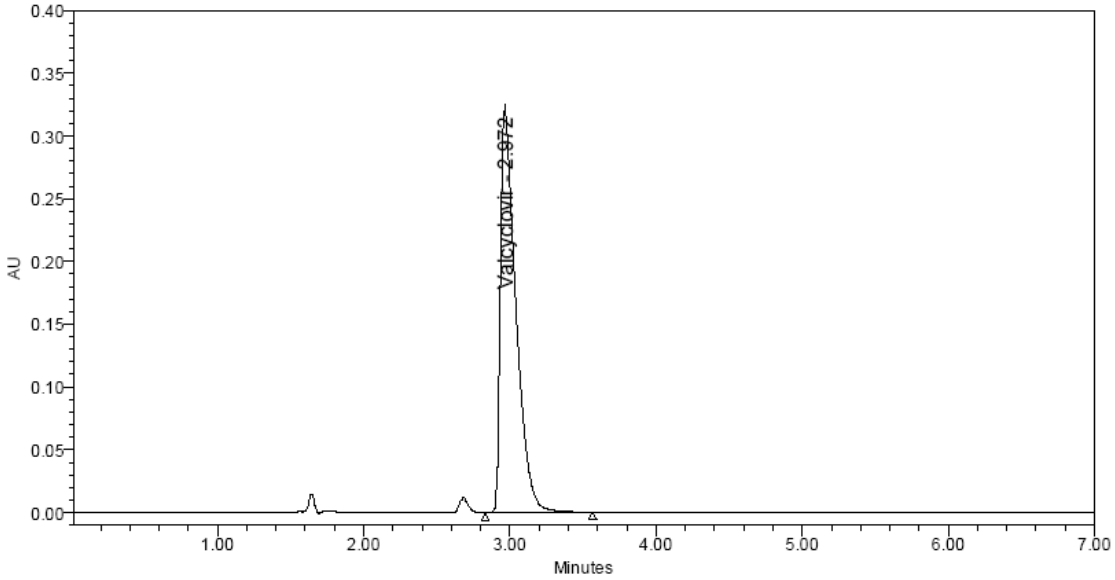


Fig 20. Chromatogram for Accuracy 150% 1st inj

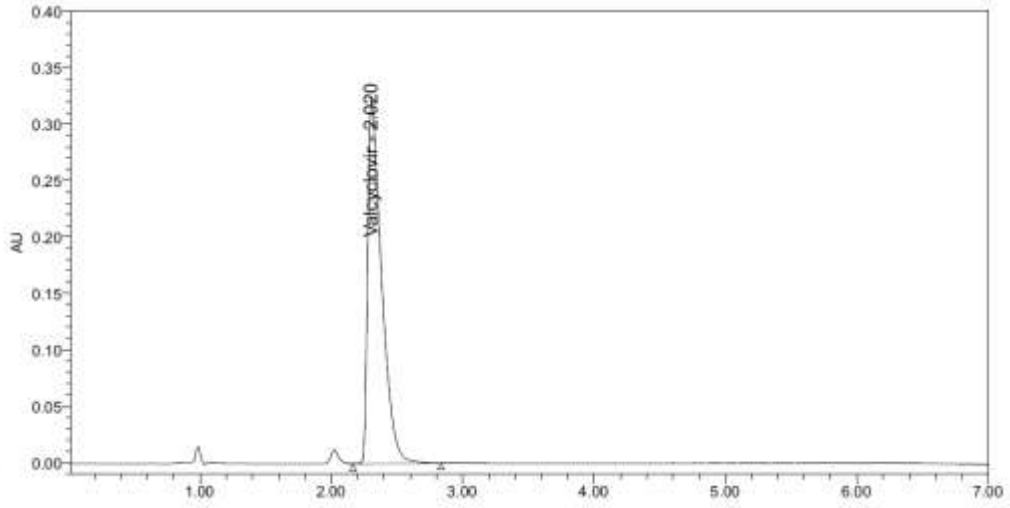


Fig 21. Chromatogram for Accuracy 150% 2nd inj

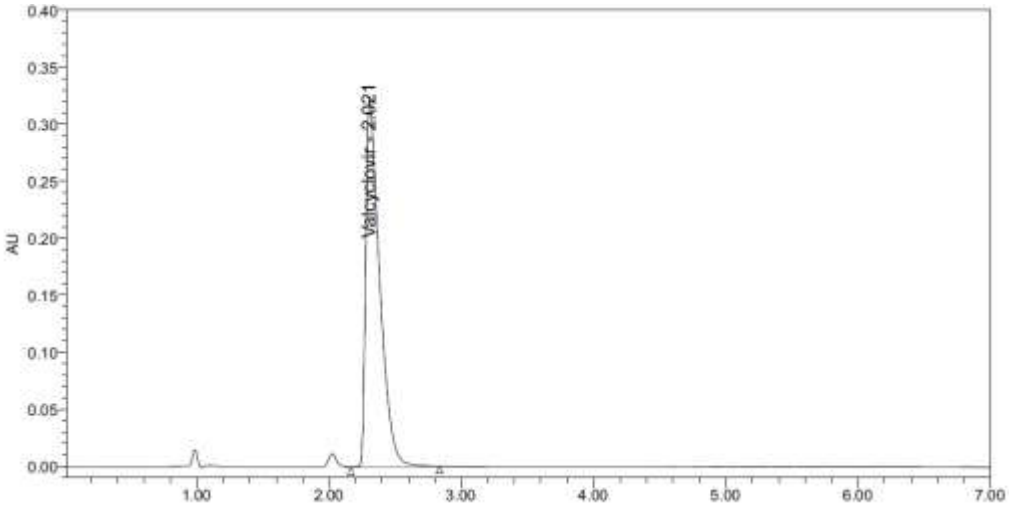


Fig 22. Chromatogram for Accuracy 150% 3rd inj

Table: 5.Recovery studies for the proposed RP-HPLC method

Drug	% Level	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery**
Valacyclovir	50	50	101	99.8	99.7
	50	100	148	99.5	
	50	150	201	99.8	

**Average of three determinations

The RP-HPLC method developed in the present study has been used to quantify valacyclovir in tablet formulations. valcivir(500mg) film coated tablets were analyzed as per the procedure described above. The mean recoveries were found in the range of 99.5-99.8%. The results are presented in **Table 5**. No interfering peaks were found in the chromatogram indicating that Excipients usually present in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

III. PRECISION

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or Relative standard Deviation

A. System Precision or System suitability

B. Method Precision

A. System Precision or System Suitability

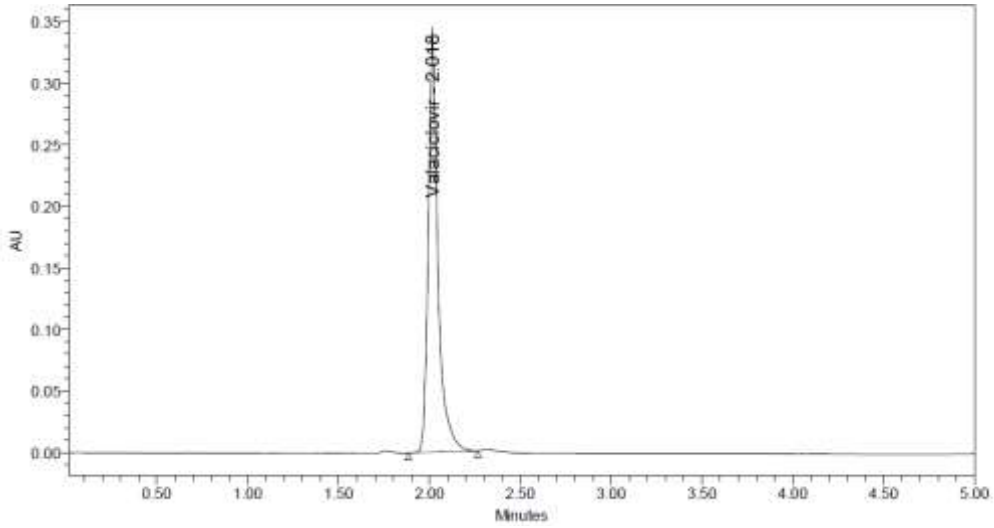


Fig: 23. Chromatogram of System Precision

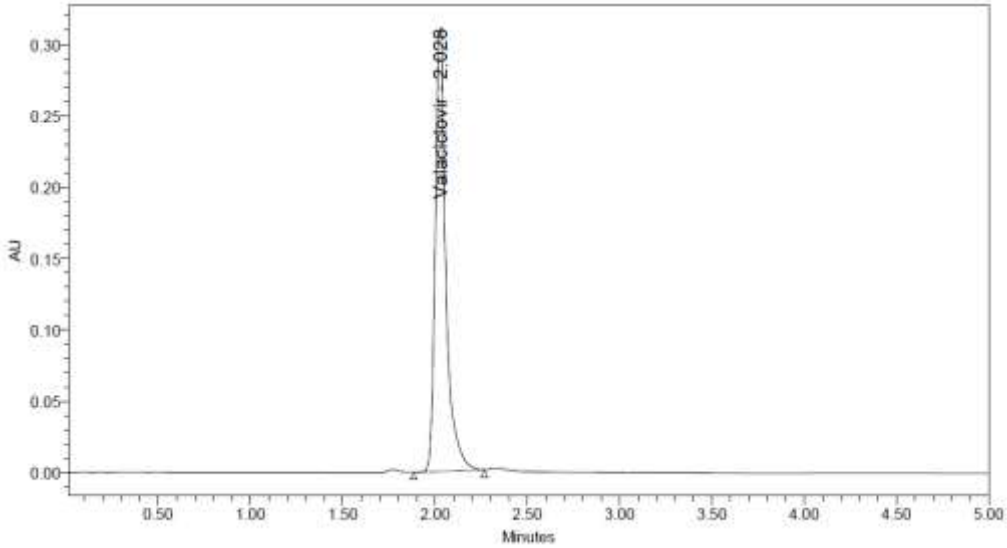


Fig 24.Chromatogram for System Precision

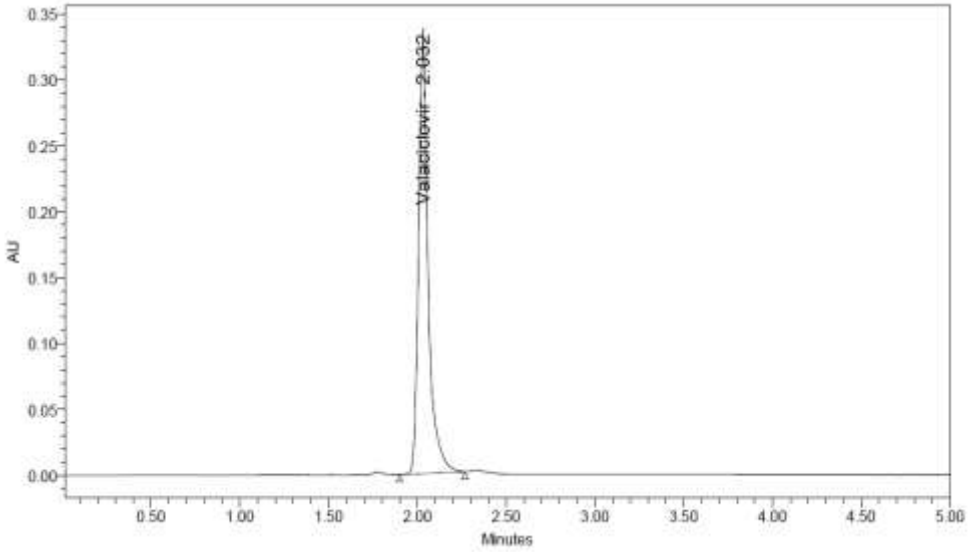


Fig 25.Chromatogram for System Precision

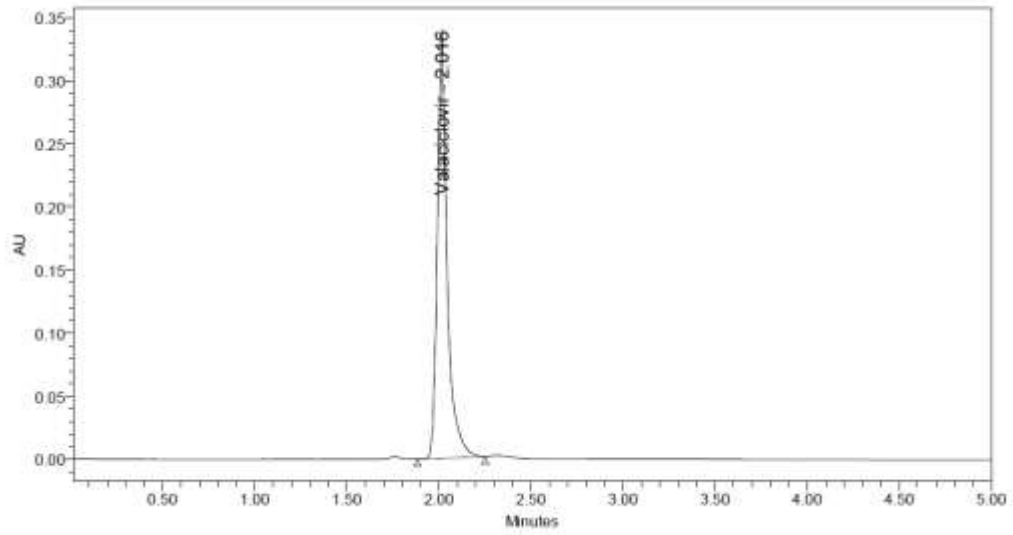


Fig 27.Chromatogram for System Precision

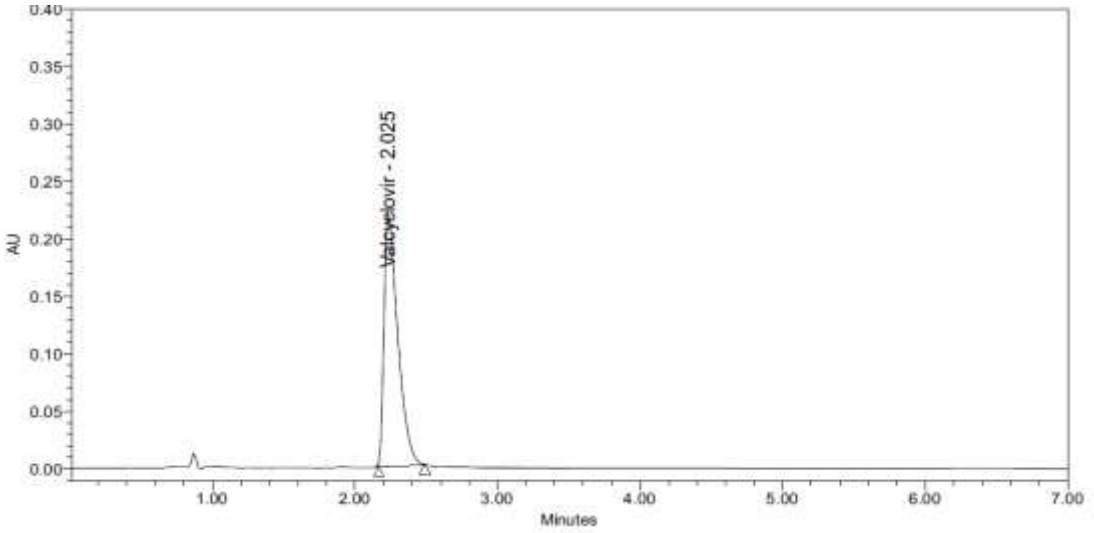


Fig 28.Chromatogram for System Precision

Table: 6. System precision results for valacyclovir

S,No	Rt	Peak area	Tailing factor	Theoritical plates
1	2.018	1256384	1.3	4591
2	2.018	1265378	1.3	4557
3	2.025	1256384	1.3	4705
4	2.028	1245681	1.3	4594
5	2.032	1247911	1.3	4565
6	2.016	1244209	1.3	4648
Mean	2.021	1251158.83	1.3	4610
SD	0.006623192	8150.615		
%RSD	0.0032771	0.0065144		

Acceptance Criteria: Tailing factor < 2
Plate Count >2000
%RSD - NMT 2

B. Method Precision

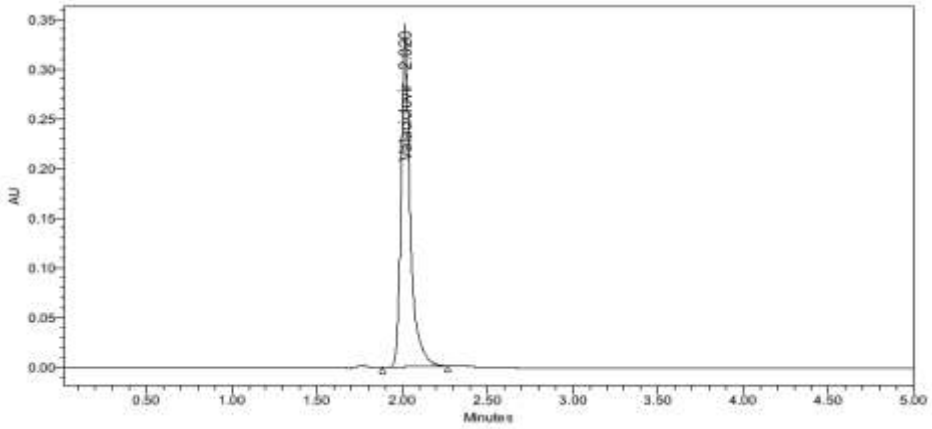


Fig: 29. Chromatogram of Method Precision (Intraday)

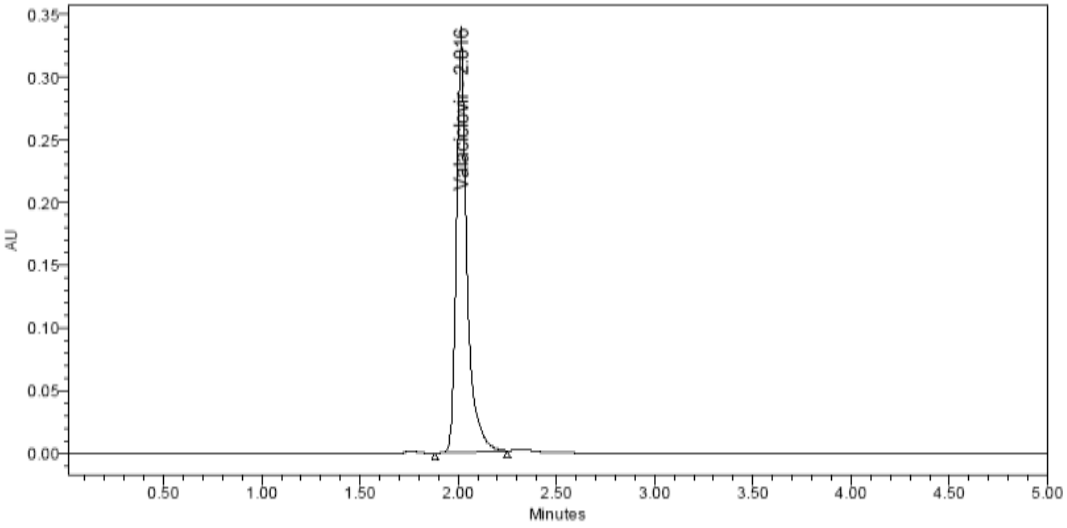


Fig: 30. Chromatogram of Method Precision (Interday)

Table: 7. Method Precision results for valacyclovir

SL.No.	Conc	Intra day	interday
1	100	1258290	1233072
2	100	1267124	1204813
3	100	1255318	1243218
4	100	1242958	1230047
5	100	1262952	1245832
6	100	1262575	1254460
Avg		1258202.833	1235240.333
SD		8508.730162	17336.18845
%RSD		0.6762	1.40

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intraday studies were performed by injecting six (6) repeated injections of 100% concentration from tablet solution within a day. Peak area and %RSD were calculated and reported in **Table7-.** The chromatograms of intra-day precision studies were shown in **Fig29.** Inter-day precision studies, was done by injecting six (6) repeated injections of 100% concentration from tablet solution for six consecutive days. Peak area and %RSD were calculated and

reported in **Table-7.** The chromatograms of inter-day precision studies were shown in **Fig.30.** The % RSD values were within 2 and the method was found to be precise.

IV. Linearity:

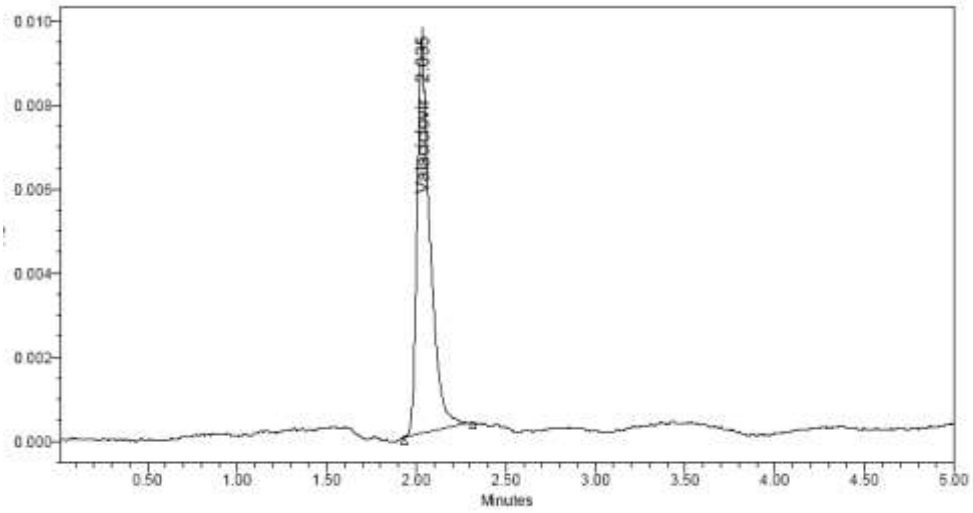


Fig: 31. Chromatogram of 20 µg/ml of valacyclovir

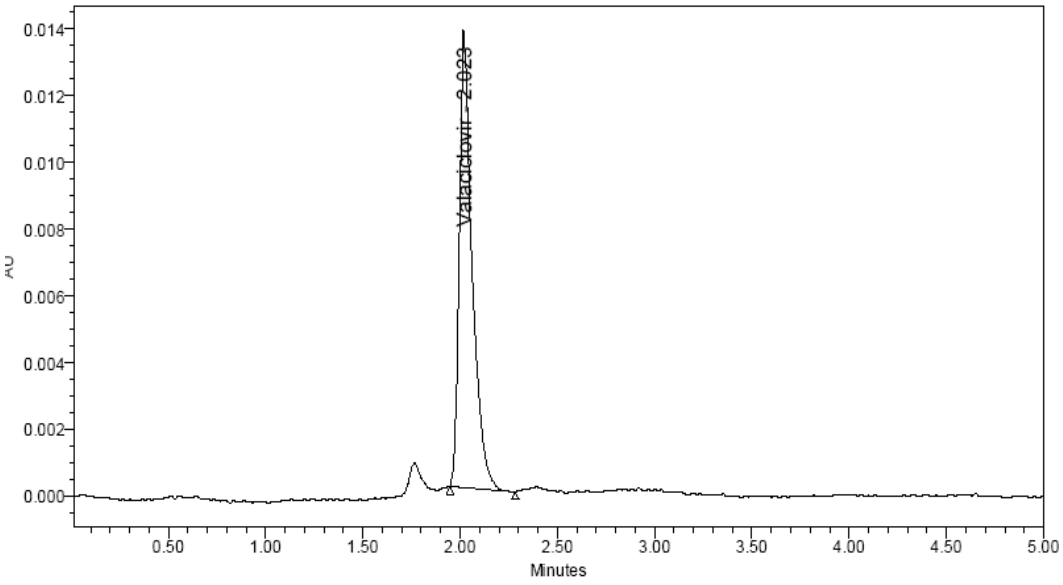


Fig: 32. Chromatogram of 50 µg/ml of valacyclovir

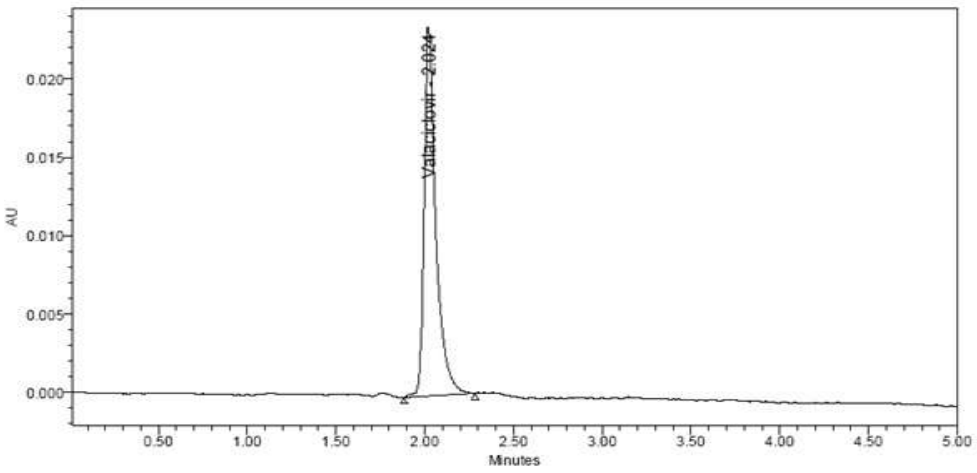


Fig: 33. Chromatogram of 100 µg/ml of valcyclovir

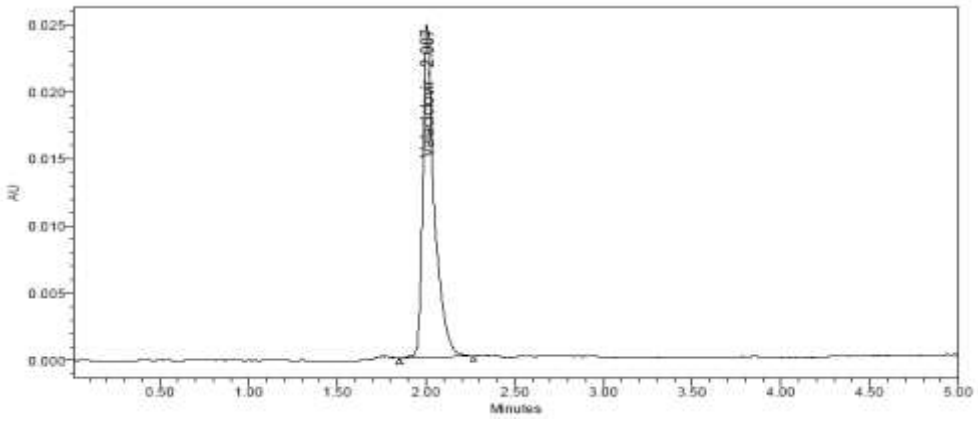


Fig: 34. Chromatogram of 120 µg/ml of valacyclovir

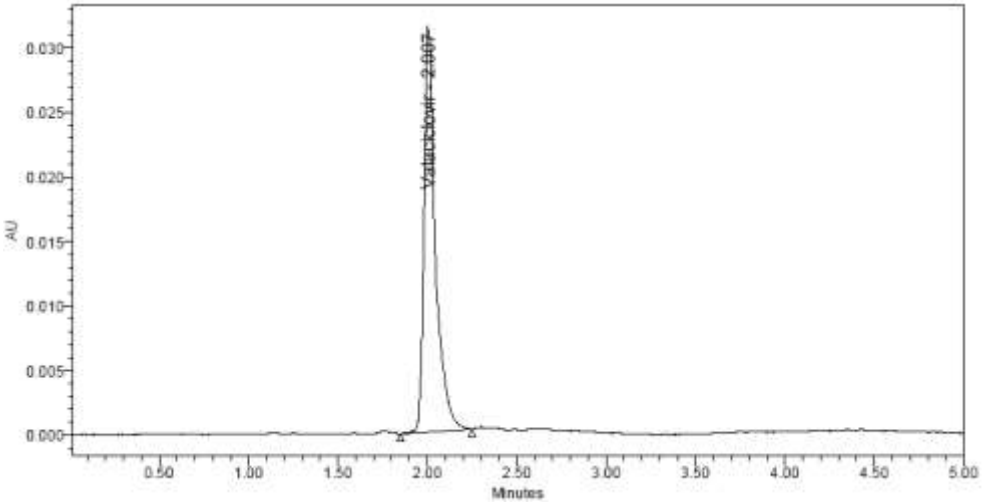


Fig: 35. Chromatogram of 150 µg/ml of valacyclovir

Table: 8. Linearity results for valacyclovir

SL.NO	Linearity Level (%)	Peak Area
1	20	25027
2	50	49999
3	100	94684
4	120	112000
5	150	138046

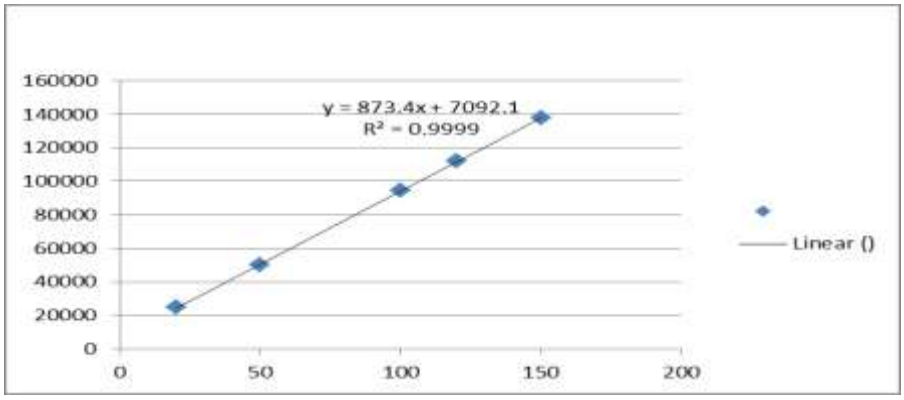


Fig: 36. Calibration curve of Val acyclovir at 253 nm

Table: 9. Calibration parameters of valacyclovir

Parameters	Results
Slope	873.4
Intercept	7092.1
Correlation co-efficient	0.9999

V. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

(A) Effect of flow Rate:

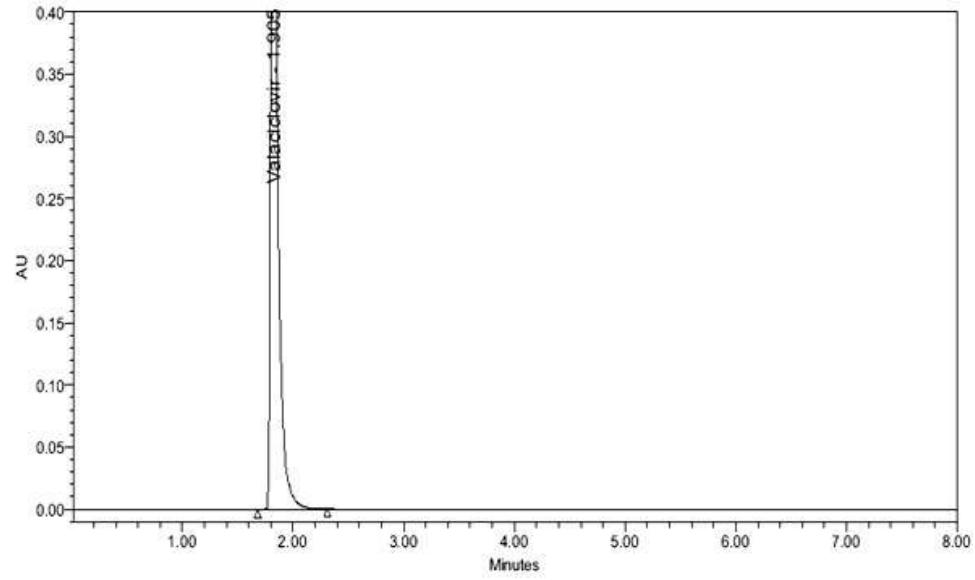


Fig.37. Chromatogram of Robustness (More flow rate)

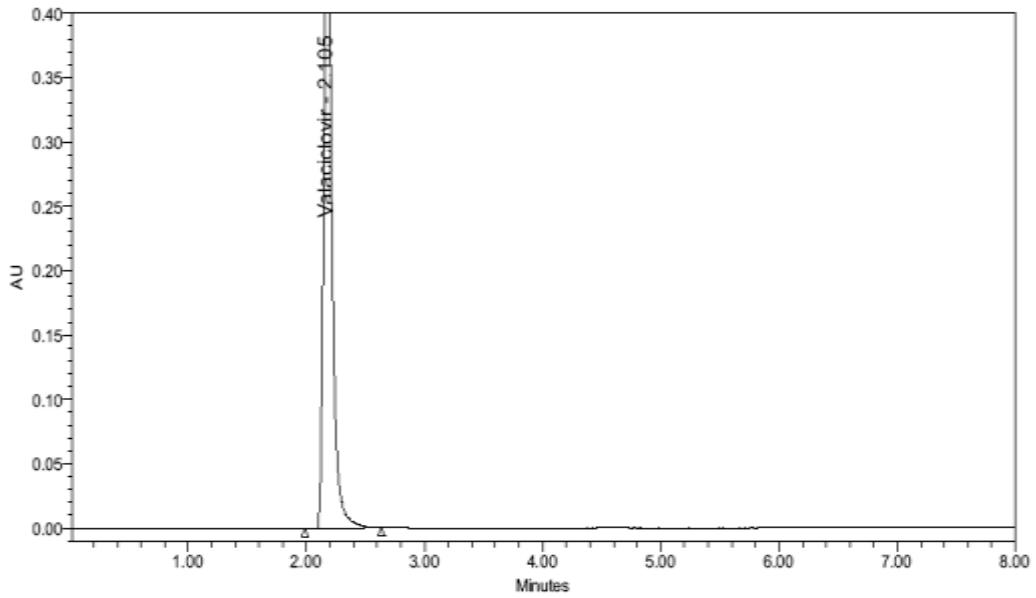


Fig.38.Chromatogram of Robustness (Less Flow rate)

Table: 10. Robustness Flowrate results for valacyclovir

Flowrate(ml/min)	Retention Time	Tailing Factor	USP Plate Count
0.8	3.342	1.3	4874
1	2.105	1.3	4827
1.2	1.915	1.3	4435

(B) Effect of Temperature:

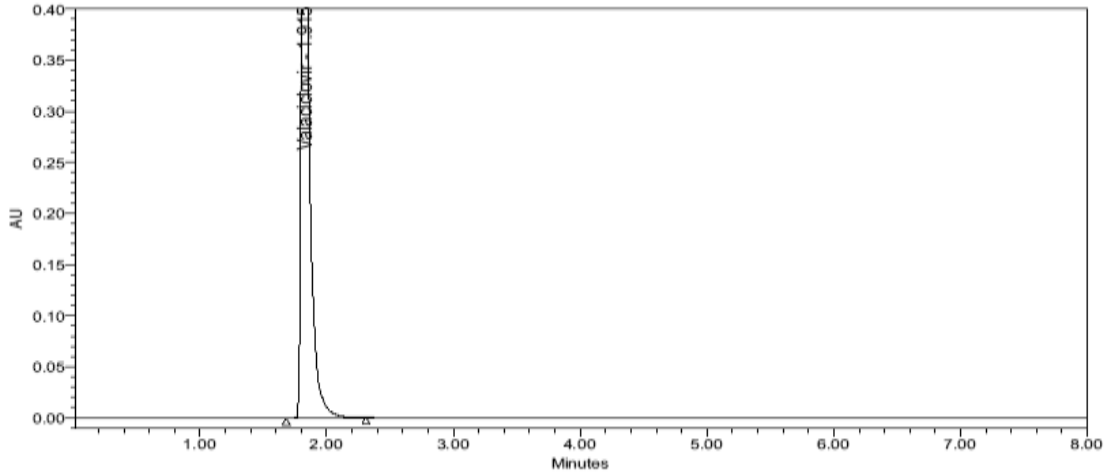


Fig: 39.Chromatogram of Robustness (temperature 30 º C)

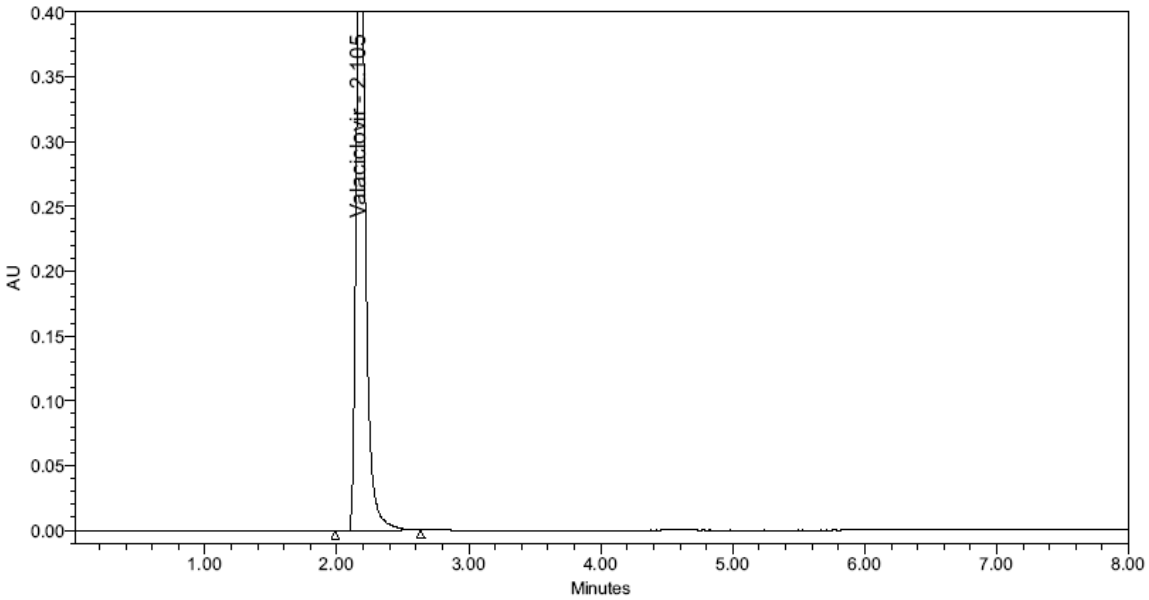


Fig: 40 .Chromatogram of Robustness (temperature 20 º C)

Table: 11. Robustness Temperature results for Val acyclovir

Temperature	Retention Time	Tailing Factor	USP Plate Count
20	2.105	1.3	4827
30	1.915	1.3	4435

Keeping the ratio of mobile phase constant and the chromatograms of drug solution were recorded with different flow rates such as 0.8 ml / min, 1.0 ml / min and 1.2 ml / min. At the flow rate of 1.0 ml / min, the

peaks were sharp with good resolution and found to be satisfactory. So 1.0 ml / min flow rate was kept constant throughout the analysis. The results are presented in **Table 10.** Keeping the flow rate constant (1ml / min) and the chromatograms of drug solution were recorded by changing temperature ,the peaks were sharp with good resolution and found to be satisfactory. The results are presented in **Table 11.**

VI. CONCLUSION

Valacyclovir is the drug used in the treatment of herpes simplex & herpes zoster virus . It is an anti-viral agent.

From literature review and solubility analysis initial chromatographic conditions were set and different trials were run to valacyclovir get eluted with good peak symmetric properties. Mobile phase buffer: water:methanol(50:50), agilent C8 Zorbax column, flow rate 1 ml/min detection wave length 253 nm, column temperature 25^{o}C and diluent Mobile phase, conditions were finalized as optimized method. 0.01M Orthophosphoric acid solution was used as buffer.

System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 20% to 150% levels concentration range of 20mcg/ml to 75mcg/ml, The regression of valacyclovir was found to be Y = 873.4 X +7092.1, where 'Y' is the peak area and 'X' is the concentration ofvalacyclovir . The regression equation was used to estimate the amount of valacyclovir , R^2 value found 0.9999.

It can concluded that the proposed methods show good approach for obtaining reliable results which is simple, precise, accurate ,sensitive, economic and less time consuming and were found to be suitable for the routine estimation of valacyclovir in bulk and Pharmaceutical dosage forms.

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The completion of this dissertation is not only fulfillment of my dreams, but also the dreams of my Parents, who have taken lots of pain for me in completion of my higher studies.

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