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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF VALACYLOVIR HYDROCHLORIDE BY UV AND HPLC

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ABSTRACT

A Ultra-Violet (UV) and High Performance Liquid Chromatography (HPLC) have been developed for the estimation of Valacyclovir Hydrochloride in bulk drug and pharmaceutical dosage form. The method is carried out using C18 column 150×4.6mm i.e. particle size 5µm and mobile phase consisting of Methanol: Water (70:30), at flow rate of 0.8ml/min. The column temperature is ambient. Eluents were monitored by UV detector set at 252nm. The method was statistically validated in terms of linearity, accuracy, precision 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 10µgm/ml To 50µgm/ml and the correlation coefficient is 0.9998. Literature survey reveals analytical methods for the estimation of Valacyclovir Hydrochloride 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 Hydrochloride in bulk drug and tablet.

KEYWORDS: Valacyclovir hydrochloride, Method Development, UV and HPLC validation.

1. INTRODUCTION

Valacyclovir was approved for medical use in 1995. It is marketed by GlaxoSmithKline under the trade names Valtrex & Zelitrex. Valacyclovir has been available as a generic drug in the U.S. since Nov.25 2009.

Valacyclovir hydrochloride is a HCL salt of L-valyl ester of Acyclovir. It is [(s)-2-[(2-amino-6-oxo-6, 9-dihyro-3H-purin-9yl) methoxy] ethyl-2-amino-3-methylbutanoate. It is an Anti-

viral drug used in the treatment of herpes simplex & herpes zoster. It inhibits viral DNA synthesis. It is a prodrug intended to increases the bioavailability of acyclovir by increasing lipophilicity. Valacyclovir converted by esterase to active drug acyclovir via hepatic first pass metabolism.

Extensive literature survey reveals that only HPLC & LC-MS Method for the determination of Valacyclovir in plasma samples has been reported.

The objective of this study was to develop a rapid & sensitive RP-HPLC Method for the analysis of Valacyclovir in bulk drug & in its Tablet formulation using the most commonly employed RP-C_{18} column with UV-Detection.

The aim of the present work was to develop a simple & economic liquid chromatographic method that would be suitable for determination of Valacyclovir & its impurities in bulk & dosage form. The proposed method is found to be simple, accurate, reproducible & suitable for routine determination of Valacyclovir from its pharmaceutical dosage form.

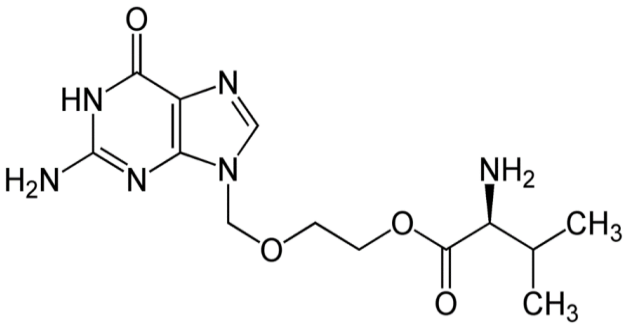

Fig. no. 1: Structure of the Valacyclovir hydrochloride.

Molecular formula: $C_{13}H_{20}N_6O_4.HCL$
Molecular weight: 360.80

Solubility & Description: White to off white powder with a maximum solubility of water of 174 mg/ml at 25^{o}C.

Melting Point: Valacyclovir Hydrochloride has no distinct melting point. It undergoes rapid decomposition above 200^{o}C.

Pharmacokinetic Data:

1	Bioavailability	55%
2	Protein Binding	13-18%
3	Metabolism	Hepatic to (acyclovir)
4	Biological half life	< 30 min. (Valacyclovir) 2.5-3.6 hrs. (acyclovir)

Storage: stored under cool & dry place.

2. MATERIALS AND METHODS

The drug sample of Valacyclovir HCL obtained from Swapnroop Pharmaceuticals, Aurangabad. The formulation (Valcivir-500mg tablet, Cipla pharmaceutical Ltd., Goa, India.) used was purchased from local pharmacy. Merck Ltd. Mumbai, India, supplied HPLC grade methanol and water. An Isocratic High Pressure Liquid Chromatograph (Agilent 1100 & software is Chemstation) with auto sampler injector, variable wavelength programmable UV-Vis detector Analytical technologies limited 2080 system & operating software UV-Vis Analyst was used. The chromatography column used was a reversed phase C_{18} column (150×4.6 mm i.e. particle size 5µm). A mixture consisting of methanol: water (70:30) was used as mobile phase and was filtered before use through 0.45µ membrane filter. The flow rate of mobile phase was maintained at 0.8ml/min. Detection was carried out at 252nm ambient temperature.

3. EXPERIMENTAL WORK

3.1 Drug Identification Test

A) Melting Point

Melting point of the drug was determined by Melting point apparatus with the help of capillary tube.

B) Solubility Test

10mg of Valacyclovir hydrochloride was weighed accurately and transfer into a 10ml volumetric flask. Few drops of water were added and shake thoroughly.

C) Determination of wavelength (λ max)

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 the drugs to be detected. A UV spectrum of Valacyclovir was recorded between 200-400nm.

3.2 UV Method Development

A) Preparation of working solution (for Determination of wavelength)

a) 10 mg of Valacyclovir hydrochloride was weighed accurately and transfer into a 10 ml volumetric flask. 5ml of diluents (methanol: water i.e. 50:50) was added, sonicated to dissolve and the volume was made up to mark with mobile phase to get the concentration of 1000µg/ml (STOCK 1). Take 0.1ml stock I solution and transfer into a 10ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 10µgm/ml.

b) 10 mg of Valacyclovir hydrochloride was weighed accurately and transfer into a 10ml volumetric flask. 5ml of diluents (methanol: water i.e. 70:30) was added, sonicated to dissolve and the volume was made up to mark with mobile phase to get the concentration of 1000µg/ml (STOCK 1). Take 0.1ml stock I solution and transfer into a 10ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 10µgm/ml.

c) 10 mg of Valacyclovir hydrochloride was weighed accurately and transfer into a 10 ml volumetric flask. 5ml of diluents (methanol: water i.e. 90:10) was added, sonicated to dissolve and the volume was made up to mark with mobile phase to get the concentration of 1000µg/ml (STOCK I). Take 0.1ml stock I solution and transfer into a 10ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 10µgm/ml.

B) Preparation of standard stock solution for U.V.

10mg of Valacyclovir hydrochloride was weighed accurately and transfer into a 50ml volumetric flask. 30ml of diluents was added, sonicated to dissolve and the volume was made up to mark with mobile phase to get the concentration of 1000µgm/ml (STOCK I).

C) Preparation of working solutions for U.V.

1. Take 0.1 ml STOCK I solution and transfer into a 10ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 2µgm/ml.
2. Take 0.2ml STOCK 1 solution and transfer into a 10 ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 4µgm/ml.
3. Take 0.3ml STOCK 1 solution and transfer into a 10 ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 6µgm/ml.
4. Take 0.4ml STOCK 1 solution and transfer into a 10 ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 8µgm/ml.

5. Take 0.5ml STOCK 1 solution and transfer into a 10 ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 10µgm/ml.

D) Sample Preparation for assay (U.V.)

20 tablets weigh accurately and the average weight was calculated. Tablets were crushed to fine powder and equivalent to 15.3mg of Valacyclovir was weighed and transfer into 50ml volumetric flask. 30ml of mobile phase was added and sonicated for 30min. with intermediated shaking. Volume was made up with mobile phase to get the concentration of 1000µgm/ml (STOCK II). Take 0.4ml stock 2 solution and transfer into a 10ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 8µgm/ml.

E) Preparation of solution for accuracy (UV)

Take 2µgm/ml tablet solution for accuracy (2×80%=1.6µgm/ml, 2×100%=2µgm/ml, 2×120%= 2.4µgm/ml).

01) Take 0.02ml tablet solution and transfer into a 10ml volumetric flask and add 1.6µgm/ml and make up the volume up to 10ml with mobile phase to get the %recovery of 80%.
02) Take 0.02ml tablet solution and transfer into a 10ml volumetric flask and add 2µgm/ml and make up the volume up to 10ml with mobile phase to get the %recovery of 100%.
03) Take 0.02ml tablet solution and transfer into a 10ml volumetric flask and add 2.4µgm/ml and make up the volume up to 10ml with mobile phase to get the %recovery of 120%.

3.4 Validation of UV method According to ICH guideline

A) Linearity

For quantitative analysis of Valacyclovir Hydrochloride, the calibration were plotted for each concentration ranges. The linearity ranges for Valacyclovir Hydrochloride found to be 2µgm/ml to 10µgm/ml respectively.

B) Accuracy

The accuracy was determined by standard addition method. Three different levels (80%, 100%, and 120%) of standards were spiked to commercial tablets in triplicate. The mean of percentage recoveries and the % RSD was calculated.

C) Precision

The reproducibility of proposed method was determined by performing tablet assay at different time intervals (3 hour interval) on same day (Intra-day precision) and on three different days (Inter-day precision) Valacyclovir Hydrochloride.

D) Robustness

The robustness study was carried out by determining the effect of small variation in mobile phase composition and in ruggedness sample was analyzed by two different analysts.

E) Limit of detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ of Valacyclovir Hydrochloride by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as 3.3 SD/ S and 10 SD/S respectively, where S is the slope of the calibration curve and SD is the standard deviation.

3.5 HPLC Method Development:

A) Trails

Table no. 1: Trails.

Sr.no.	Trails	Observation	Remarks
1	Mobile phase - methanol: water (50:50) Flow Rate – 1.0ml/min. Column – C18 (150×4.6mm, 5µm).	Peak not sharp.	Not Satisfactory
2	Mobile phase - methanol: water (70:30) Flow Rate – 1.0ml/min. Column – C18 (150×4.6mm, 5µm).	No. of theoretical plate is less	Not Satisfactory
3	Mobile phase - methanol: water (70:30) Flow Rate – 0.8ml/min. Column – C18 (150×4.6mm, 5µm).	Peak sharp, No. theoretical plate is greater than 2000, retention time is than other.	Satisfactory
Acceptance Criteria: Tailing factor < 2, Theoretical Plate Count >2000, %RSD - NMT 2			

B) Preparation of standard stock solution for HPLC

10 mg of Valacyclovir hydrochloride was weighed accurately and transfer into a 10 ml volumetric flask. 5ml of diluents was added, sonicated to dissolve and the volume was made up to mark with mobile phase to get the concentration of 1000µg/ml (STOCK I).

C) Preparation of working solutions for HPLC

1. Take 0.1 ml STOCK I solution and transfer into a 10ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 10µg/ml.
2. Take 0.2ml STOCK 1 solution and transfer into a 10 ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 20µg/ml.
3. Take 0.3ml STOCK 1 solution and transfer into a 10 ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 30µg/ml.
4. Take 0.4ml STOCK 1 solution and transfer into a 10 ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 40µg/ml.
5. Take 0.5ml STOCK 1 solution and transfer into a 10 ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 50µg/ml.

D) Sample Preparation for assay (HPLC)

20 tablets weigh accurately and the average weight was calculated. Tablets were crushed to fine powder and equivalent to 15.3mg of Valacyclovir was weighed and transfer into 10ml volumetric flask. 5ml of mobile phase was added and sonicated for 30min.with intermediated shaking. Volume was made up with mobile phase to get the concentration of 1000µgm/ml (STOCK II). Take 0.4ml stock 2 solution and transfer into a 10ml volumetric flask and make up the volume up to mark with mobile phase to get the concentration of 40µgm/ml.

E) Preparation of solution for accuracy (HPLC)

Take 10µgm/ml tablet solution for accuracy (10×80%=8µgm/ml, 10×100%=10µgm/ml, 10×120%= 12µgm/ml).

1. Take 0.1ml tablet solution and transfer into a 10ml volumetric flask and add 8µgm/ml and make up the volume up to 10ml with mobile phase to get the %recovery of 80%.
2. Take 0.1ml tablet solution and transfer into a 10ml volumetric flask and add 10µgm/ml and make up the volume up to 10ml with mobile phase to get the %recovery of 100%.
3. Take 0.1ml tablet solution and transfer into a 10ml volumetric flask and add 12µgm/ml and make up the volume up to 10ml with mobile phase to get the %recovery of 120.

3.6 Validation of HPLC method According to ICH guideline

A) System Suitability

System suitability was done to verify the repeatability of the HPLC method. Theoretical plate and repeatability of retention time and peak area were determined and compared.

B) Linearity

For quantitative analysis of Valacyclovir Hydrochloride, the calibration were plotted for each concentration ranges. The linearity ranges for Valacyclovir Hydrochloride found to be 10-50µg/ml respectively.

C) Accuracy

The accuracy was determined by standard addition method. Three different levels (80%, 100%, and 120%) of standards were spiked to commercial tablets in triplicate. The mean of percentage recoveries and the % RSD was calculated.

D) Precision

The reproducibility of proposed method was determined by performing tablet assay at different time intervals (3 hour interval) on same day (Intra-day precision) and on three different days (Inter-day precision) Valacyclovir Hydrochloride.

E) Robustness

The robustness study was carried out by determining the effect of small variation in mobile phase composition and in ruggedness sample was analyzed by two different analysts.

F) Limit of detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ of Valacyclovir Hydrochloride by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as 3.3 SD/ S and 10 SD/S respectively, where S is the slope of the Calibration curve and SD is the standard deviation.

4. RESULTS AND DISCUSSION

4.1 Drug Identification Test

A) Melting Point

Melting Point was not found because Valacyclovir hydrochloride has no distinct melting point. It undergoes rapid decomposition above 200^{o}C.

B) Solubility Test

Valacyclovir Hydrochloride was soluble in water.

4.2 Determination of wavelength (λ max)

Using appropriate dilution of standard stock solution the solutions were scanned in order to get good results. The wavelength selected should be such that at wavelength the absorptive of components should be as large as possible. So the wavelength choose was 252 nm for Valacyclovir Hydrochloride.

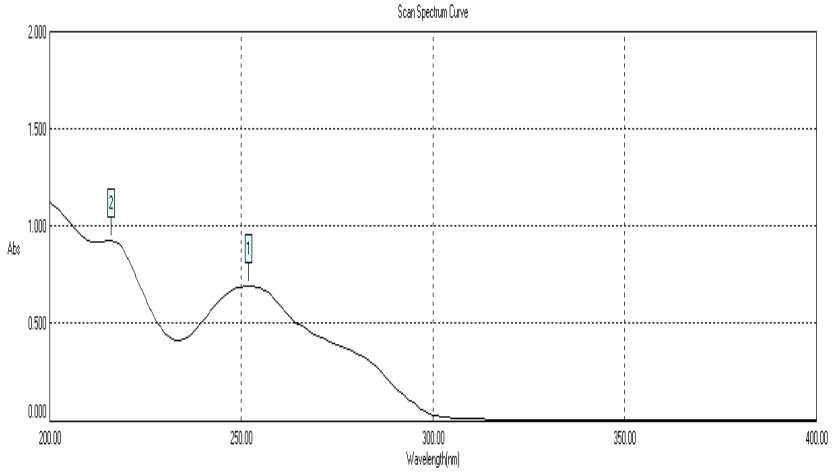

Fig. No. 2: UV spectra for Valacyclovir Hydrochloride (Methanol: Water i.e. 70:30).

Sr. no.	P/V	Wavelength(nm)	Absorbance
1	Peak	252	0.693
2	Peak	216	0.926

4.3 Ultra Violet Spectroscopy

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.

1) Linearity

The linearity of the method was demonstrated over the concentration range of 2µgm/ml - 10µgm/ml of the target concentration. Aliquots of 2,4,6,8 and 10µgm/ml were prepared from stock solution. The correlation coefficient for the peak area at each level versus concentration of analyte was calculated and the calibration parameters of Valacyclovir were showed in Table no. 8.

Table no.2: Linearity results for Valacyclovir.

Sr.no.	Conc. (µgm/ml)	Absorbance
1	2	0.04205
2	4	0.0985
3	6	0.1645
4	8	0.228
5	10	0.2802

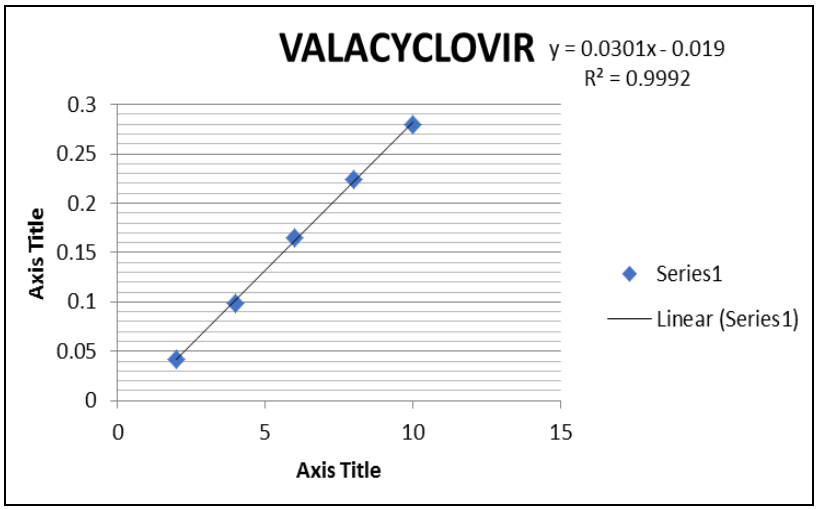

Fig no. 3: Calibration curve of Valacyclovir Hcl at 252nm.

Table no. 3: Calibration parameters of Valacyclovir.

Slope	0.0301
Intercept	0.019
Correlation coefficient	0.9992

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. The intra-day and inter-day precision study (Table no. 04 &05) of the developed method confirmed adequate sample stability and method reliability where all the RSDs were <2%.

Table no. 4: Precision results for Valacyclovir (intraday).

Sr.no.	Conc. (µgm./ml)	Absorbance 1	Absorbance 2	Mean	SD	%RSD
1	2	0.097	0.098	0.0975	0.00070	0.72523
2	6	0.163	0.164	0.1635	0.00070	0.43248
3	10	0.219	0.22	0.2195	0.00070	0.32214

Table no. 5: Precision results for Valacyclovir (interday).

Sr.no.	Conc. (µgm./ml)	Absorbance 1	Absorbance 2	Mean	SD	%RSD
1	2	0.098	0.099	0.0985	0.00070	0.717875
2	6	0.162	0.163	0.1625	0.00070	0.435143
3	10	0.216	0.218	0.217	0.00141	0.651711

Repeatability

Table no.6: Repeatability results for Valacyclovir.

Sr.no.	Conc. (µgm./ml)	Absorbance 1	Absorbance 2	Mean	SD	%RSD
1	2	0.0421	0.042	0.04205	7.07107	0.168159

Absorbance and %RSD was calculated and reported in Table no. 04 & 05. The %RSD values were within 2 and the method was found to be precise.

03) Accuracy and recovery

Accuracy of the method was determined by recovery experiments. Recovery studies were carried out by adding known amount of standard drug solution to the sample solution. The % recovery was calculated and reported in Table no. 07.

Table no.7: Recovery studies for the proposed UV method.

Sample solution Conc.	%Level	Amount added (µgm./ml)	Amount found	%Recovery	Mean recovery
2(µgm./ml)	80%	1.6	3.5747	98.42	
2(µgm./ml)	100%	2	4.0025	100.16	99.09
2(µgm./ml)	120%	2.4	4.3685	98.698	

The HPLC method developed in the present study has been used to quantify Valacyclovir in tablet formulations. Valcivir (500mg) film coated tablets were analysed as per the procedure described above. The mean recoveries were found in the range of 98.42-100.16%. The results are presented in Table no. 12.

04) LOD and LOQ

The LOD and LOQ were calculated for all methods and mentioned below:

LOD = 3.3× average SD/ slope

LOD = 3.3×0.00089681/ 0.0301

LOD = 0.098µgm/ml

LOQ = 10×average SD/ slope

LOQ = 10×0.00089681/0.0301

LOQ = 0.297µgm/ml

The limit of detection and limit of quantification for this method were 0.098µg/ml and 0.297µg/ml, respectively. Sensitive method can be detected.

5) Assay of Valacyclovir Hydrochloride in tablets

The concentration of tablet solution was determined using linear regression equation (using slope and Y Intercept) and amount of drug in tablet was determined. The results of assay in tablets are summarized in Table no. 08.

Table no.8: Results of assay in tablets.

Sr.no.	Conc. (µgm./ml)	Absorbance	Amt Fd	%Amt Fd
1	8	0.224	8.073	100.91
2	8	0.221	7.973	99.66

Labelled claim (mg)	500mg
Amount Found (mg)	8.023
%labelled claim	100.285
%RSD	0.88135

4.4 High performance or pressure liquid chromatography (HPLC) Method

1. Trails

According to acceptance criteria choose the composition because of its theoretical plate greater than 2000 peak was sharp and also retention time was less than the other trials.

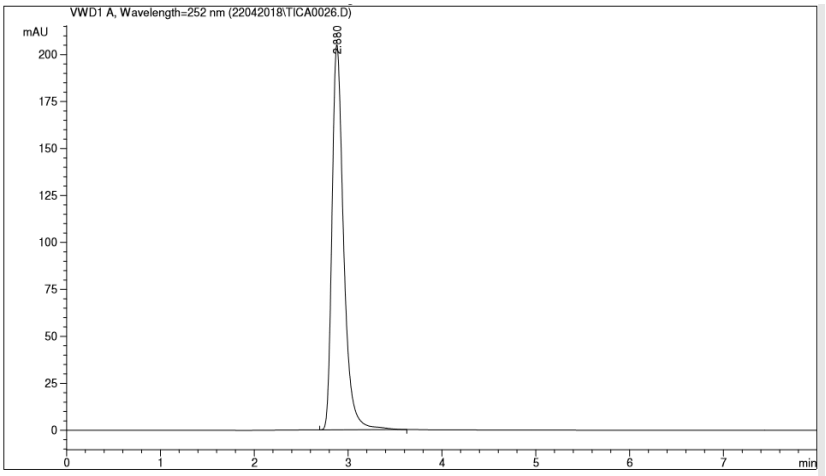

Fig. No. 4: Chromatogram of Trial (Methanol: water i.e.70:30, flow rate 0.8ml/min.).

Retention time	Area	Theoretical plate
2.880	1766.33289	3001

2. System Suitability

Table No.9: Chromatographic Conditions

Flow Rate	**0.8ml/min**
Wavelength	252nm
Injection volume	20µl
Run Time	5 to 6 min.
Mobile Phase	Methanol:Water (70:30)
Column Oven Temperature	Ambient Temperature
Detector	UV

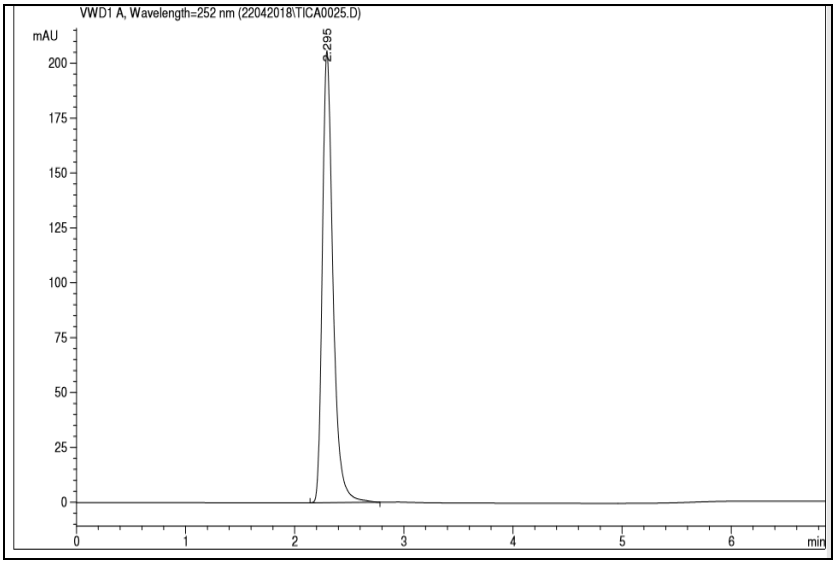

Table No.5: chromatogram of System suitability.

Retention time	**Area**	**Tailing factor**	**Theoretical plate**
2.295	1396.45691	0.65	2996

Table No. 11 System suitability Parameters.

Sr. No.	**Parameter**	**Mean**	**Limit**
1	Area	1396.45691	%RSD (<2%)
2	RT (min)	2.295	< 10-5
3	Theoretical plates	2996	>2000
4	Tailing factor	0.65	<2

3. 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.

1. Linearity

The linearity of the method was demonstrated over the concentration range of 10µgm/ml to50µgm/ml of the target concentration. Aliquots of 10,20,30,40 and 50µgm/ml were prepared from stock solution. A Calibration curve was produced by analyzing different concentrations of the pure drug from the chromatogram in Fig no. 6. The correlation coefficient for the peak area at each level versus concentration of analyte was calculated and reported in Table no. 12. And the calibration parameters of Valacyclovir were showed in Table no. 13.

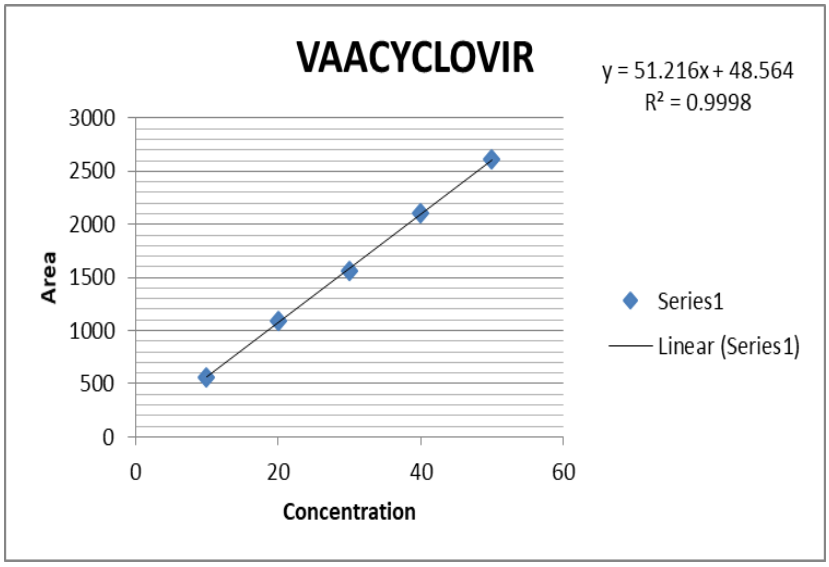

Fig no.6: Calibration curve of Valacyclovir Hcl at 252nm.

Table no. 12: Linearity results for Valacyclovir.

Sr.no.	Conc.(µgm./ml)	PEAK AREA
1	10	556.205
2	20	1089.11
3	30	1567.80
4	40	2101.07
5	50	2611.01
Retention time	Area	Theoretical plate
2.666	1083.21240	3070

Table no. 13: Calibration parameters of Valacyclovir.

Parameters	Results
Slope	51.216
Intercept	48.564
Correlation coefficient	0.9998

2) Accuracy

Retention time	Area	Theoretical plate
2.661	1169.30603	3060

Accuracy of the method was determined by recovery experiments. Recovery studies were carried out by adding known amount of standard drug solution to the sample solution. The % recovery was calculated and reported in Table no. 14.

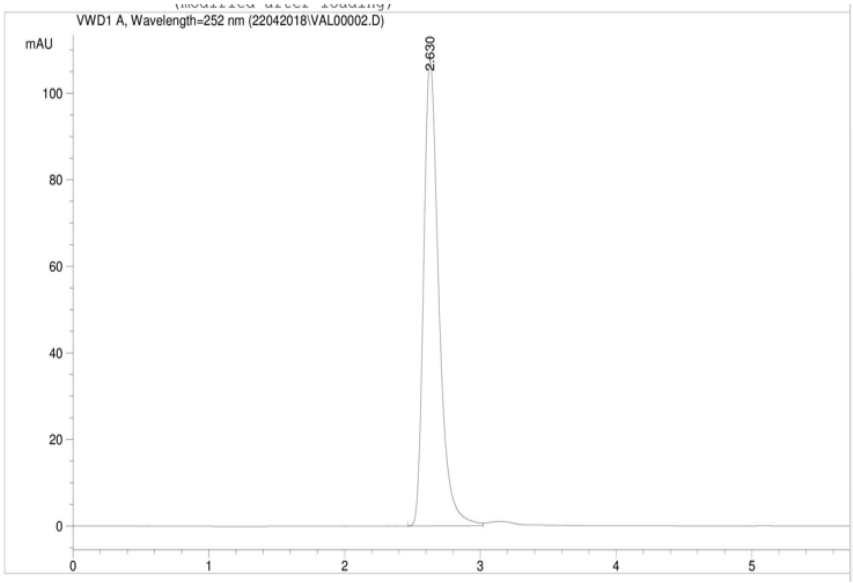

Fig no. 7: Chromatogram for accuracy 80% of Valacyclovir Hcl.

Retention time	Area	Theoretical plate
2.630	970.15997	2946

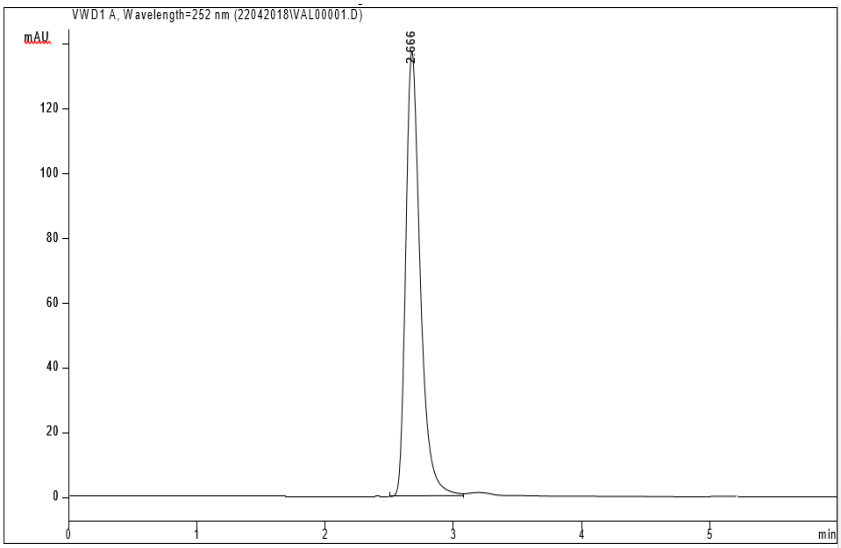

Fig. no.8: chromatogram for accuracy 100% of Valacyclovir Hcl.

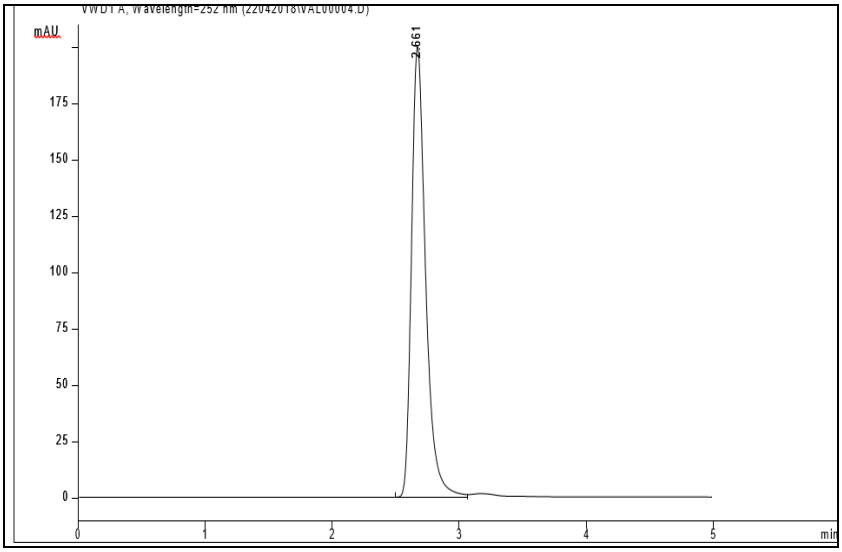

Fig. No.9: Chromatogram for accuracy 120% of Valacyclovir Hcl.

Table no. 14: Recovery studies for the proposed HPLC method.

Conc. (µgm./ml)	%Level	Amount added (µgm./ml)	Amount found	%Recovery	Mean recovery
10	80%	8mg	18.005	100.06	
10	100%	10mg	20.245	102.47	100.47
10	120%	12mg	21.86	98.88	

The HPLC method developed in the present study has been used to quantify Valacyclovir in tablet formulations. Valcivir (500mg) film coated tablets were analysed as per the procedure described above. The recoveries were found in the range of 98.88-102.47%. The results are presented in Table no.14.

3) 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.

Table no. 15: Precision results for Valacyclovir (intraday).

Sr.no.	Conc.	Area 1	Area 2	Mean	SD	%RSD
1	10	544.3	548.39	546.345	2.89207	0.52935
2	30	1548	1559.97	1553.99	8.46407	0.54467
3	50	2651.19	2655.98	2653.59	3.38704	0.12764

Table no. 16: Precision results for Valacyclovir (interday).

Sr.no	Conc.	Area 1	Area 2	Mean	SD	%RSD
1	10	544.3	548.39	546.345	2.89207	0.52935
2	30	1548	1559.97	1553.99	8.46407	0.54467
3	50	2651.19	2655.98	2653.59	3.38704	0.12764

Repeatability

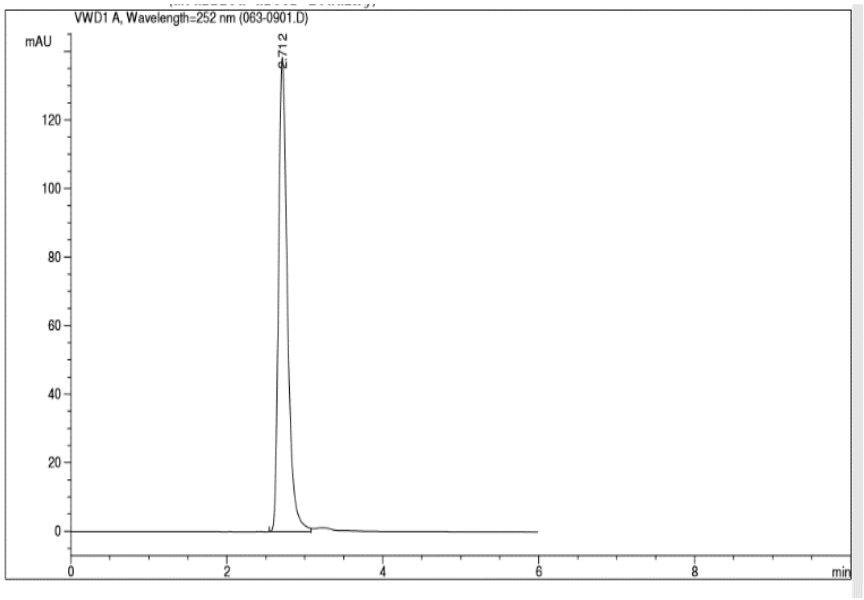

Fig. No.10: chromatogram of repeatability 20µgm/ml.

Table no.17: Repeatability results for Valacyclovir.

Sr.no	Conc	Area1	Area2	Mean	SD	%RSD
1	20	1092.31	1093.91	1093.11	1.13137	0.1035

The peak area and % RSD was calculated and reported in Table no.15 & 16. The %RSD values were within 2 and the method was found to be precise.

4) 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

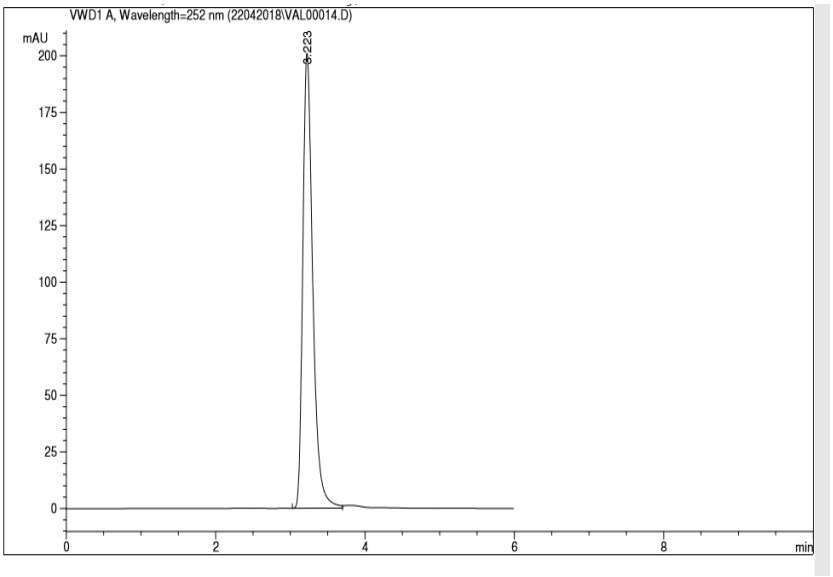

Fig. No. 11: Chromatogram of Robustness (Less Flow rate i.e.0.7ml).

Retention time	Area	Theoretical plate
3.223	1862.67468	3117

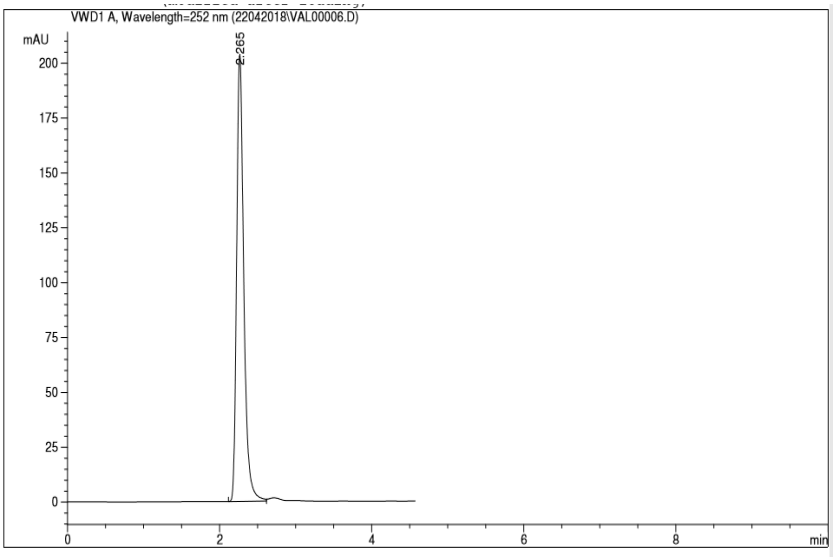

Fig no.12: Chromatogram of Robustness (More flow rate i.e. 0.9ml).

Retention time	Area	Theoretical plate
3.223	1862.67468	3117

Table no.18: Robustness results for Valacyclovir when Flow Rate change.

Sr.no.	Flow Rate change	Conc.	Area 1	Area 2	Mean	SD	%RSD
1	0.7ml	30µgm/ml	1862.67	1870.17	1866.42	5.3033	0.28414
2	0.9ml	30µgm/ml	1326.35	1325.63	1325.99	0.50912	0.0384

B) Effect of composition change

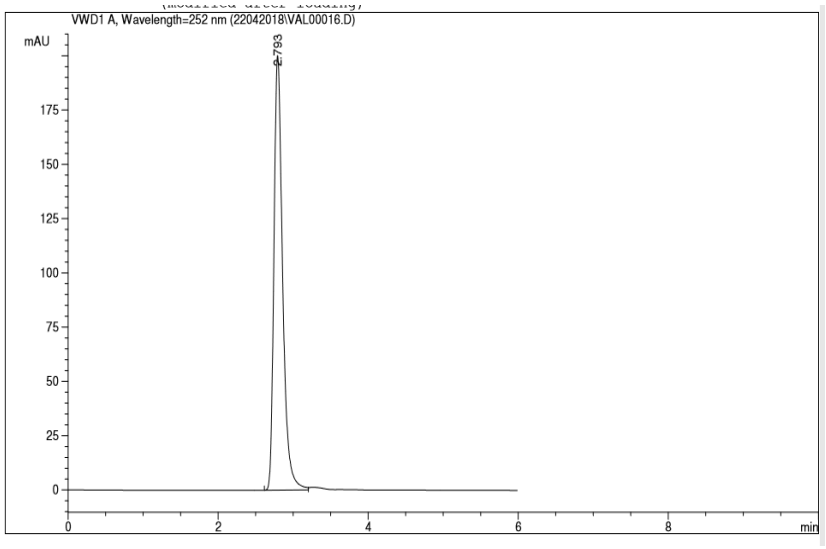

Fig. no.13: Chromatogram of Robustness (Composition change 69:31).

Retention time	Area	Theoretical Plates
2.793	1608.84521	3186

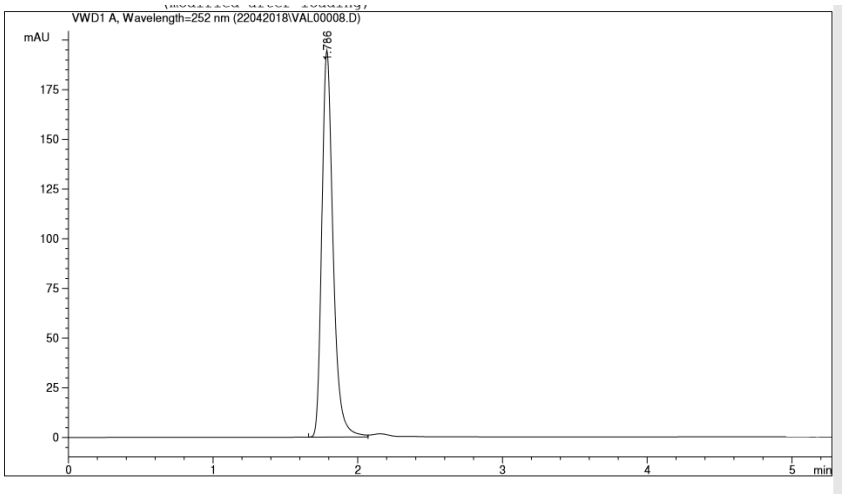

Fig. no. 14: Chromatogram of Robustness (Composition change 71:29).

Retention Time	Area	Theoretical plates
1.786	1579.92273	2858

Table no.19: Robustness results for Valacyclovir when compositions change.

Sr.no.	composition change	Conc.	Area 1	Area 2	Mean	SD	%RSD
1	69:31	30µgm/ml	1608.84	1599.29	1604.07	6.75287	0.42098
2	71:29	30µgm/ml	1579.92	1582.53	1581.23	1.84555	0.11672

C) Effect of wavelength change

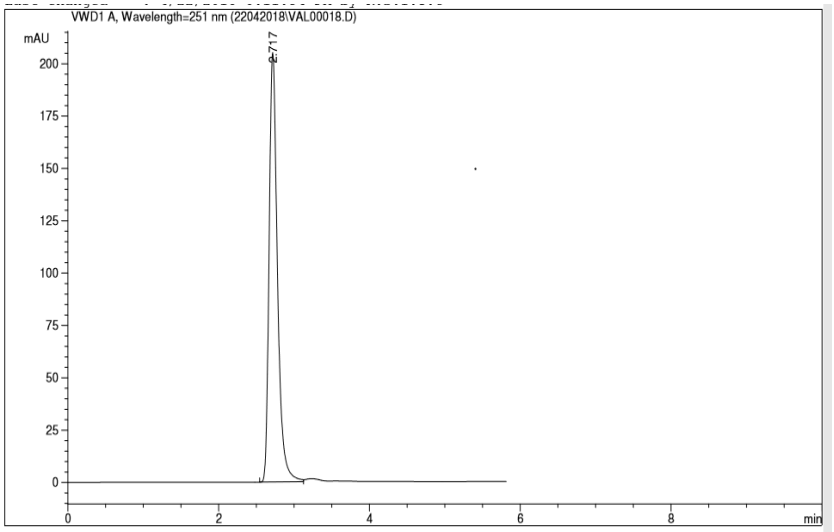

Fig. no.15: Chromatogram of Robustness (wavelength change i.e. 251).

Retention Time	Area	Theoretical Plates
2.717	1570.61877	3379

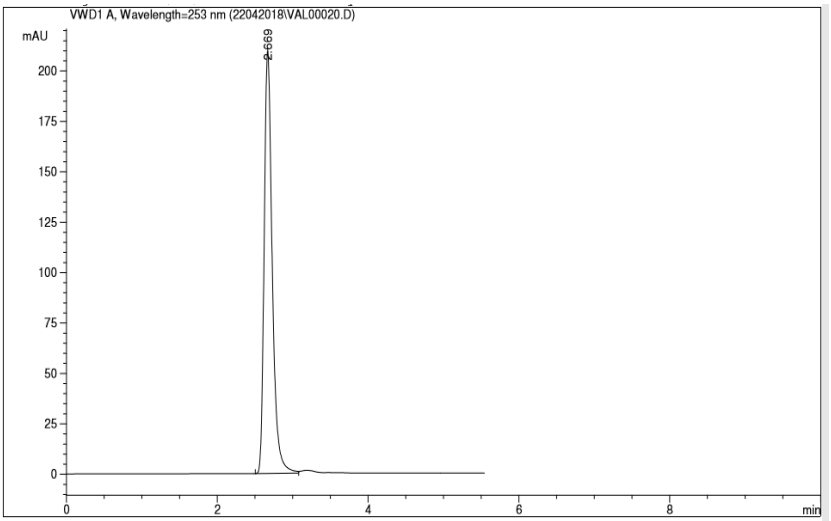

Fig. no. 16: Chromatogram of Robustness (wavelength change i.e.253).

Retention Time	Area	Theoretical Plates
2.669	1554.27014	3461

Table no.21: Robustness results for Valacyclovir when wavelength changes.

Sr.no.	Wavelength change	Conc.	Area 1	Area 2	Mean	SD	%RSD
1	251	30µgm/ml	1570.61	1564.83	1567.72	4.08708	0.2607
2	253	30µgm/ml	1554.27	1538.96	1546.62	10.8258	0.69997

Keeping the ratio of mobile phase constant and the chromatograms of drug solution were recorded with different flow rates such as 0.7ml/min and 0.9ml/min. At the flow rate of 0.7ml/min and 0.9ml/min, the peaks were sharp with good resolution and found to be satisfactory. The results are presented in Table no.21. Keeping the flow rate constant (0.8ml/min.) and the chromatograms of drug solution were recorded by changing composition (i.e. Methanol: Water 61:31 & Methanol: Water 71:29) and wavelength (i.e. 251 & 253), the peaks were sharp with good resolution and found to be satisfactory. The results are presented in Table no. 22 & 23 respectively.

5) Limit of Detection and Limit of Quantitation

The LOD and LOQ were calculated for all methods and mentioned below:

LOD = 3.3 × Average of S.D./Slope

LOD = 3.3×6.03/51.21

LOD = 0.388µgm/ml.

LOQ = 10 × Average of S.D. /Slope

LOQ = 10 × 6.03/51.21

LOQ = 1.177µgm/ml.

The limit of detection and limit of quantification for this method were 0.388 µg/ml and 1.177 µg/ml, respectively. Sensitive method can be detected.

6) Assay of Valacyclovir Hydrochloride in tablets

The concentration of tablet solution was determined using linear regression equation (using slope and Y Intercept) and amount of drug in tablet was determined. The results of assay in tablets are summarized in Table no.22.

Table no. 22 Results of assay of Valacyclovir Hydrochloride in tablets.

Sr.no.	Conc. (µgm./ml)	Peak Area	Amt Fd	%Amt Fd
1	40	2108.93	40.233	100.58
2	40	2111.62	40.286	100.71

Labelled claim (mg)	500mg
Amount Found (mg)	40.2595
%labelled claim	100.645
%RSD	0.09309

5. SUMMARY AND CONCLUSION

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

A method for the determination of Valacyclovir in the bulk drug & tablet formulation has been developed from the spectrum of Valacyclovir Hydrochloride as shown in Fig. No.7, it was found that the maximum Absorbance is at about 252nm in Methanol: Water (70:30). A good linear relationship (0.9992) was observed between the conc. Ranges of 2 µgm/ml. To 10 µgm/ml. The regression of Valacyclovir Hydrochloride was found to be Y = 0.0301x – 0.019, where 'Y' is the peak area and 'X' is the concentration of Valacyclovir Hydrochloride. The assay of Valacyclovir tablet was found to be % 100.285.

From literature review and solubility analysis initial chromatographic conditions were set and different trials were run to Valacyclovir Hydrochloride get eluted with good peak symmetric properties. Mobile phase methanol: water (70:30), C18 column, flow rate 0.8 ml/min detection wave length 252 nm, column temperature ambient and diluents. 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 10 to 50% levels concentration range of 10 µgm/ml To 50µgm/ml, the regression of Valacyclovir was found to be Y = 51.21x +48.56, where 'Y' is the peak area and 'X' is the concentration of Valacyclovir Hydrochloride. The regression equation was used to estimate the amount of Valacyclovir, R^2 value found 0.9998. The assay of Valacyclovir tablet was found to be % 100.645. 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. This method is suitable for the routine quality control of the tablet dosage forms.

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7. REFERENCES

1. Sharma B.K**.** "Instrumental methods of chemical analysis", 23rd Edition, Goel Publishing House, 2004; 4.
2. Skoog, D. A., Holler, F. J., Crouch S. R. "Principle of Instrumental Analysis", Eastern Press, Bangalore, 2004; 13-16,378-385,901-905,893-900.
3. Chatwal, G. R.; Anand, S. K. **"**Instrumental Methods of Chemical Analysis"**,** 5th Edition, Himalaya Publishing House, New Delhi, 2004; 2.149-2.184, 2.566-2.587 and 2.624-2.639.
4. https://Www.slideshare.net/sagarsavale1/analyticalmethod-development.
5. Potadar M.A. "Pharmaceutical Quality Assurance", 2nd edition, Nirali Prakashan, 2010; 8.1 - 8.108.
6. Nash R.A, Watcher A.H, "Pharmaceutical process validation", 3rd edition, Macchel Dekker, Inc.: New York, 2005; 507-522.

7. ICH Q2B, "Validation of Analytical Method Procedures", Methodology, November, 1996.
8. International Conference on Harmonization (ICH), Validation of analytical procedures: Text & methodology, In ICH Harmonized Tripartite Guidelines Q2 (R1), November, 2005.
9. P.S. Kumbhar, "Development and validation of a simple UV spectrophotometric and fluorometric method for the determination of Valacyclovir hydrochloride both in bulk and marketed dosage form", International Journal of Pharma and Chemical Research, Apr–Jun, 2018; 4(2): 99-105.
10. Nabil A. F. Alhemiary, Ahmed N. Al-Hakemi, **"**A simple assay methods development and validation of Valacyclovir in blood, urine and pharmaceutical dosage forms by spectrophotometry", Indo American Journal of pharmaceutical sciences, 2016; 3(6): 586 to 593.
11. Pavani Jagu, "development of enantio-selective reverse phase capillary electrophoresis direct separation method for the determination of Valacyclovir D-isomer", World Journal of Pharmaceutical Research, 2016; 5(9): 361 to 376.
12. D. Sathis Kumar, "method development and validation of Valacyclovir Hydrochloride and ritonavir in tablet dosage form using RP-HPLC", jurnal Teknologi (Sciences & Engineering), 2015; 76(1): 39 to 45.
13. Girija B. Bhavar, Sanjay S. Pekamwar, kiran B. Aher, Sanjay R. Chaudhary, "Development and Validation of RP-HPLC Method for the Determination of Valacyclovir Hydrochloride and its Related Substances in Tablet Formulation", International Journal of Pharmaceutical Sciences Review and Research, March –April 2014; 251(08): 53-58.
14. B. Bhagyalaxm**i, "**Method Development & Validation of related substances by HPLC for analysis of Valacyclovir Hcl in Valacyclovir Hcl tablet formulation",
15. International journal of pharmacy and analytical research (IJPAR), April to June, 2014; 3(2): 192-201.
16. Sena Caglar, "determination of Valacyclovir Hydrochloride in tablets and spiked plasma samples by Spectrofluorimetry", Journal of Analytical Chemistry, 2014; 69(4): 362 to 366.
17. Dilip Kumar Sahoo, "Stability indicating RP-HPLC method for the determination of Valacyclovir Hydrochloride in bulk and pharmaceutical dosage forms", Chemical Science Transactions, 2014; 3(2): 510 to 517.

18. Patil Pallavi M, "Stability indicating HPTLC method for estimation of Valacyclovir Hydrochloride in bulk and pharmaceutical dosage form in presence of its alkaline hydrolysis degradation product", International Journal of pharmaceutical and bio sciences, 2014; 5(4): 186 to 203.
19. Janhavi Gulimi, "Visible spectrophotometric determination of Valacyclovir by using ara dimethyl amino cinnam aldehyde reagent", International Journal of Pharm Tech Research, July–Aug, 2014; 6(3): 1036 to 1039.
20. Janhavi Bandla, Ashok Gorja, "Method development and validation of Valacyclovir HCL assay by RP-HPLC in pharmaceutical dosage form", International Journal of Advanced Research in Pharmaceutical & Bio-sciences, 2013; 3(1): 33-41.
21. Sheetal Ramya Lahari. N.A., "Method development and validation of Valacyclovir in bulk and tablet dosage form by RP-HPLC method", IOSR Journal of Pharmacy and Biological Sciences, Jan –Feb 2013; 5: 56-75.
22. Sriharia G, Rami Reddy N, Ngarajasetty Kchakravarthic IE, "Spectrophotometric Determination of Valacyclovir in Pharmaceutical Formulations", chemical Science Transactions, 2013; 2: 61-64.
23. Sathis Kumar Dinakaran, **"**Spectrophotometric method development and validation for Valacyclovir Hydrochloride Monohydrate and Ritonavir in bulk and Tablet dosage form using absorption ratio method", Malaysian Journal of Pharmaceutical Sciences, 2013; 11(2): 21 to 29.
24. Yasmeen Sultana, "Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Valacyclovir in Pharmaceutical Dosage Forms", International Journal of Pharmaceutical and Clinical Research, 2013; 5(1): 7-12.
25. VMK Gowtham Potnuru, **"**Accelerated stability studies on Valacyclovir hydrochloride by RP-HPLC", International Journal of pharmaceutical and chemical sciences, Jan-Mar, 2013; 2(1): 110 to 117.
26. Radhika Devi M R, "Method Development and Validation for the estimation of Valacyclovir in pharmaceutical formulation by RP-HPLC", Indo American Journal of pharmaceutical sciences, 2013; 3(4): 3080 to 3088.
27. Siva Ramakrishna V, Ravi Kumar D, Malleswara Rao NVN, Ram Babu C, "Method development & determination of Valacyclovir HCL in pharmaceutical dosage forms by visible spectrophotometry", International Journal of pharm Tech Research, 2012; 4(3): 1009-1014.

28. Ravi Kumar konda, "Development and Validation of analytical methods for new chemical entities and their dosage forms by using HPLC", Hindawi Publishing Corporation, Journal of Analytical methods in chemistry, 2012, article ID 101249 8 pages.
29. M Sugumaran, V Bharathi, R Hemachander and M Lakshmi, "RP-HPLC Method for the Determination of Valacyclovir in bulk and Pharmaceutical formulation", Scholars Research Library, Der Pharma Chemicals, 2011; 3(4): 190-194.
30. T. Anil Kumar, "Selective and validated Spectrophotometric method for determination of Acyclovir and Valacyclovir using N-Bromosuccinimide", Journal of Pharmacy Research, 2011; 4(1): 24 to 27.
31. Sugumaran M, Jothieswari D, "Development & validation of spectroscopic method for estimation of Valacyclovir in tablet dosage form", Oriental Journal of Chemistry, 2010; 26: 163-165.
32. M. Ganesh, C.V. Narasimharao, A. Saravana Kumar, Kamalakannan, M.Vinoba, H.s.mahajan and Sivakumar, "UV Spectrophotometric Method for the Estimation of Valacyclovir HCL in Tablet Dosage form", E-Journal of Chemistry, 2009; 6(3): 814-818.
33. K. Srinivasa Rao and M Sunil, "Stability indicating liquid chromatographic method for Valacyclovir Hydrochloride", International journal of ChemTech research, July-Sept. 2009; 1(3): 702-708.
34. Bhavar GB, Gosavi SA, Shewale PB, Shinde MA, "Development & validation of spectroscopic method for estimation of Valacyclovir in tablet dosage form", Analytical chemistry ; An Indian Journal, 2008; 8(7): 1-3 h. 2013; 5(1): 7-12.
35. Rao PN, Rjayathirtharao K, Vseshagirirao JV, "RP-HPLC Estimation of Valacyclovir in tablets", Asian Journal of chemistry, 2006; 18: 2552-2556.
36. Palacios ML, demasiG, Pizzorno MT, Segall AI, "Validation of an HPLC method for the determination of Valacyclovir HCL in p'ceutical dosage", Journal of liquid chromatography & related technologies, 2005; 28(5): 751-762.
37. V.S. Kulkarni and S.P. Pathak," A Laboratory Hand-Book of Organic Qualitative Analysis and Separations", Dastane Ramchandra and co, Pune, 2008; 4&5: 12 – 14.