Research Article **Analytical Chemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

STABILITY-INDICATING HPTLC METHOD FOR ESTIMATION OF VALACYCLOVIR HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM IN PRESENCE OF ITS ALKALINE HYDROLYSIS DEGRADATION PRODUCT

PATIL PALLAVI M,[*1,3] WANKHEDE SAGAR B [2] AND CHAUDHARI PRAVEEN.D[3]

[1]*Centre For Research and Development,Prist University,Vallam,Thanjavur,Tamil Nadu 613403,India*
[2] *Padm. Dr.D.Y.Patil Institute of Pharmaceutical Sciences & Research, Pimpri, Pune, Maharashtra, India.*
[3]*P.E Soceity's Modern College of Pharmacy,Nigdi, Pune, Maharashtra, India.*

ABSTRACT

A novel and quick high-performance thin-layer chromatographic method -densitometric method was developed and validated for quantitative determination of Valacyclovir Hydrochloride. Chromatographic separation of the drugs was performed on precoated silica Tab 60 F_{254} Merck plates using Toluene: Methanol: Diethylamine (8:1:1 v/v/v).as a mobile phase. A TLC scanner set at 254 nm was used. Valacyclovir Hydrochloride and degradant were satisfactorily resolved with Rf values of 0.28 ± 0.05, 0.65 ±0.05. The method had an accuracy of 99.85% of Valacyclovir Hydrochloride was validated according to ICH guidelines. The percentage recovery ranges from 99-101%. Force degradation of drugs in hydrolysis,oxidation photolysis and thermal stress as per ICH guideline.The drug showed instability in oxide and Heat and Oxide while it remained stable in neutral conditions. The proposed HPTLC method was utilized to investigate of alkaline degradation of VAL.

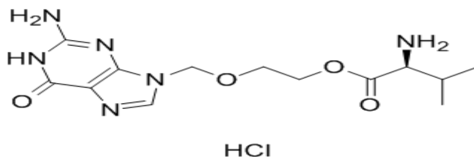
KEYWORDS:Valacyclovir Hydrochloride (VAL), HPTLC, Force Degradation Studies, Investigate Degradant Product (DP).

PATIL PALLAVI M
P.E.Society's Modern College of Pharmacy
Nigdi, Pune-411044.Maharashtra, India.

INTRODUCTION

Valacyclovir Hydrochloride (Fig.1.) is chemically (S)-2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]ethyl-2-amino-3-methylbutanoate) the molecular formula C13H20N6O4 with a molecular weight of 324.336 g/mol. Valacyclovir Hydrochloride is freely soluble in water and sparingly soluble in methanol DMF, DMSO and DMA, practically insoluble in ethanol, toluene and acetone. ValacyclovirHydrochlorideis an anti-viral, prodrug an esterified version of ValacyclovirHydrochloridethat has greater oral bioavailability (about 55%) than acyclovir (10–20%) (P1)[1]. It is a prodrug intended to increase the bioavailability of acyclovir by increasing lipophilicity. ValacyclovirHydrochlorideconverted by esterase to active drug acyclovir via hepatic first pass metabolism [2]. It inhibits viral DNA synthesis [3-6]. Acyclovir that exhibits activity against herpes simplex virus types,1(HSV–1) and 2(HSV–2) and vericellazoster virus. The mechanism of action of acyclovir involves the highly selective inhibition of virus DNA replication, via enhanced uptake in herpes virus-infected cells and phosphorylation by viral thymidine kinase. The substrate specificity of acyclovir triphosphate for viral, rather than cellular, DNA polymerase contributes to the specificity of the drug[7-8]. ValacyclovirHydrochlorideis rapidly converted to acyclovir and further phospharilated to acyclovir triphosphate. The incorporation of acyclovir tri phosphate into the growing chain of viral DNA results in chain termination [9-15]. Acyclovir is a synthetic purine nucleoside analogue derived from guanine and is used for treatment of viral infections and in vitro has been shown to play a role in the suppression of cytomegalovirus infections (Balfour et al., 1989;). Beside in vitro activity against herpes virus and favorable toxicity profile, many potential applications of acyclovir are limited by its poor absorption. Oral bioavailability is reported to be between 15 to 30% (Fletcher and Bean, 1985). Addition of valine moiety to acyclovir results in a substrate for active transport mechanisms in human intestine. The enhancement in oral ValacyclovirHydrochloridebioavailability has been attributed to its enhanced permeation across intestine compared with acyclovir. Following absorption ValacyclovirHydrochlorides rapidly and nearly completely hydrolysed to acyclovir and L-valise, an essential amino ValacyclovirHydrochloridefirst pass metabolism. This hydrolysis is mediated by the enzyme ValacyclovirHydrochloridehydrolase, and occurs in the liver.The oral bioavailability is defined as the function of the fraction of drug administered that reaches the systemic circulation metabolized. Kinetically, oral availability can be described as the product of the fraction of drug absorbed from the gastrointestinal lumen, the intestinal presystemic metabolism and the hepatic first pass availability[16-17].In previous studies, few assayshave been reported for the simultaneous determination of Valacyclovir Hydrochloride and acyclovir in human serum and urine by UV detection[18]. Recently, the chemical and enzymatic stability of Valacyclovir has been investigated by HPLC with UV detection [19]. ValacyclovirHydrochlorides also been quantified in pharmaceutical preparation, human serum and biological fluids by HPLC with UV detection and enantioselective HPLC with UV detection [20-22] Literature survey also reveals that HPLCmethod for the determination of Valacyclovir Hydrochloride plasma samples has been reported [23-24]. The objective of the proposed method is to develop simple and accurate method for the estimation of ValacyclovirHydrochloridein pharmaceutical dosage forms by HTPLC.The aim of the present work is to develop simple, sensitive and selective stability-indicating methods for the quantitative determination of Valacyclovir Hydrochloride in the presence of its alkaline degradation product and in pharmaceutical formulations.

Figure no.1
Chemical Structure ofValacyclovir Hydrochloride



MATERIALS AND METHODS

Toluene: Methanol: Diethylaminefrom S. D. Fine chemicals, Mumbai Reference standard Valacyclovir Hydrochloride was procured from Cipla Ltd., Kumrek, Rangpo, Sikkim, India) for the gift sample for whole experiment Valtovala commercial formulation containing a combination of VAL (500 mg) was purchased from local firms.

Instrumentation and chromatographic conditions are given in the following table

Sr.no.	Instruments	Descriptions
1	HPTLC system	Camag HPTLC system
2	Sample application	Camaglinomate IV automatic sample
3	Scanner	
4	Software	Camag TLC scanner
5	Saturated chamber	Camagwincats software
6	HPTLC plate	Camag Twin trough chamber (10x10) and (20x20)
7	Syringe	Merck HPTLC plate coated with silica Tab 60 F 254(0.2mm thickness)
8		on aluminium sheet
		Hamilton syringe (100µl)

Preparation of Solution
Standard Solution of VAL Standard Solution

Accurately weighed quantity (100 mg) of Valacyclovir Hydrochloride was transferred to 100.0 mL volumetric flask, dissolved and diluted up to the mark with methanol. From this solution, 5.0 mL was transferred to 50.0 mL volumetric flask and diluted to the mark with methanol. (Concentration 100 µg/mL). The solution was mixed and filtered through 0.2 µ membrane filter.

Stock Solution of degradant

Accurately weighed quantity (100 mg) of degradant was transferred to 100.0 mL volumetric flask, dissolved and diluted up to the mark with methanol. From this solution, 5.0 mL was transferred to 50.0 mL volumetric flask and diluted to the mark with methanol. (Concentration 100 µg/mL). The solution was mixed and filtered through 0.2 µ membrane filter.

Sample Application

The standard and formulation samples of VAL were spotted on Pre coated TLC plates in the form of narrow bands of lengths 6 mm, with 10 mm from the bottom and left margin and with 9 mm distance between two bands.

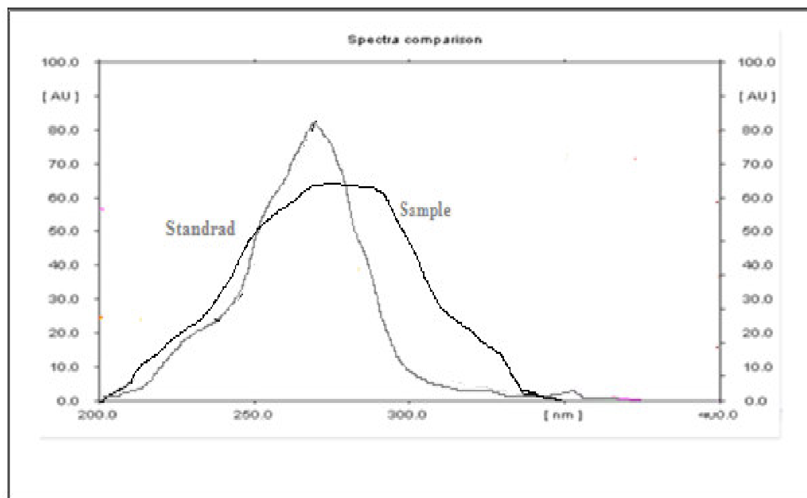
Analytical Techniques
Selection of Mobile Phase

Aliquot portion of standard stock solutions D (5 µL each) were applied on TLC plates in the form of band (band size: 6mm). Different mobile phase system like methanol:diethyl amine,methanol:toluene,toluene:diethyl amine.ethyl acetate:toluene were initially tried in order to determine best condition for separation of drugs and degradant.

Selection of wavelength for densitometric evaluation of separated bands

Standard stock solution D was applied on TLC plate with the help of CAMAG LINOMAT-V automatic sample applicator, the plate was chromatographed in twin-through glass chamber saturated with mobile phase for 30 minute. After chromatographic development, the plate was removed and air dried. The separated bands on the TLC plate were scanned over the wavelength range of 200-700 nm. The wavelength 254 nm was selected for densitometric evaluation of separated bands. The overlain spectrum obtained is depicted in Fig. No 2.

Figure No.2
Spectrum of VAL standard and sample measured from 200 to 400nm



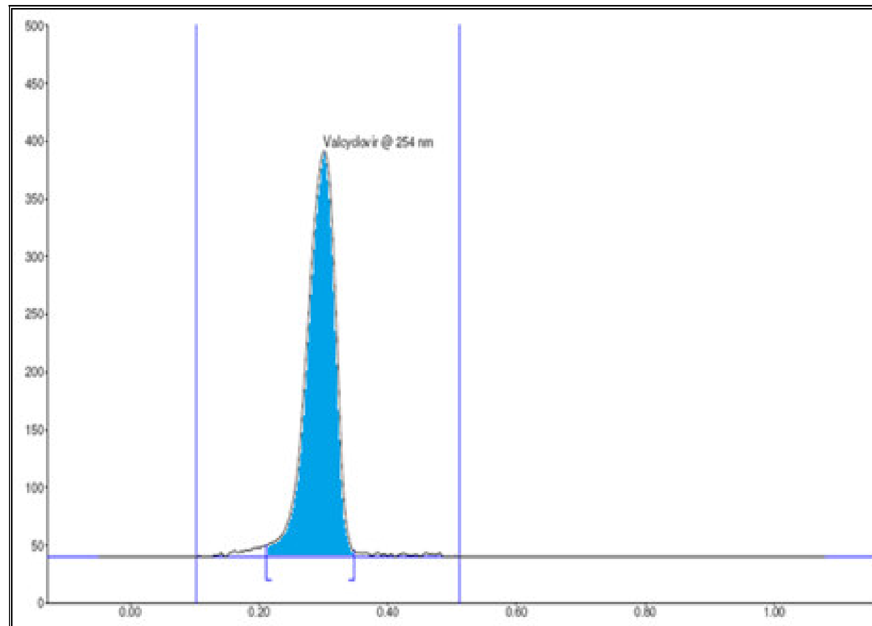
Optimum Chromatographic conditions

Stationary phase : Aluminium plates precoated with silica Tab 60 F_{254} (Merck)
Mobile phase : Toluene: Methanol: Diethylamine (8:1:1 v/v/v).
Plate size : 10 cm X 10 cm,
Mode of application : Band
Band size : 6 mm (Distance between two bands: 5.5 mm)
Sample volume : 5 µL
Development chamber: Twin-through glass chamber, 10 cm X 10 cm with (20 X20) Stain less steel lid.
Saturation time : 30 minutes
Séparation technique : Ascending
Migration distance : ≈ 80 mm
Temperature : 20 ± 5^0c
Scanning mode : Absorbance/Reflectance
Slit dimensions : 5 X 0.45 mm
Scanning wavelength : 254 nm

The retention factors of VAL and Degradant were 0.28 ± 0.05, 0.65 ±0.05

Densitogram of VAL is shown in Fig.No.3.

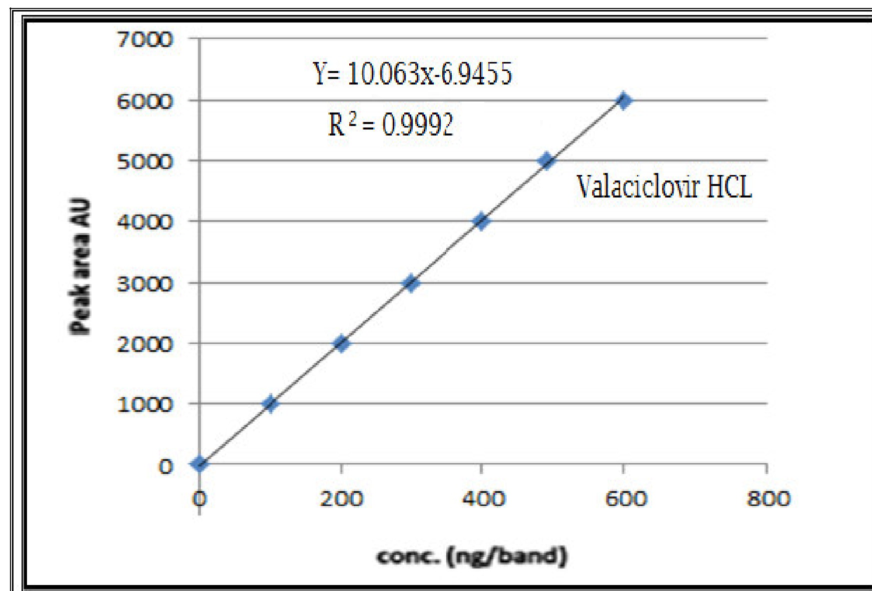
Figure No.3
Typical Densitogram of VAL



Calibration Curve for HPTLC Method

The standard stock solution containing ValacyclovirHydrochloridewas applied on the TLC plate in the range 1-6 µL with the help of micro syringe using LINOMAT-V automatic sample applicator. The plate was then developed and scanned under the above mentioned chromatographic conditions. Rf was recorded for each drug concentration and the calibration curves of the concentration vs. Rf were constructed for both the drugs. The calibration curves for Valacyclovir Hydrochloride are depicted in Fig. No. 4

Figure No.4
Standard Calibration Curve for VAL

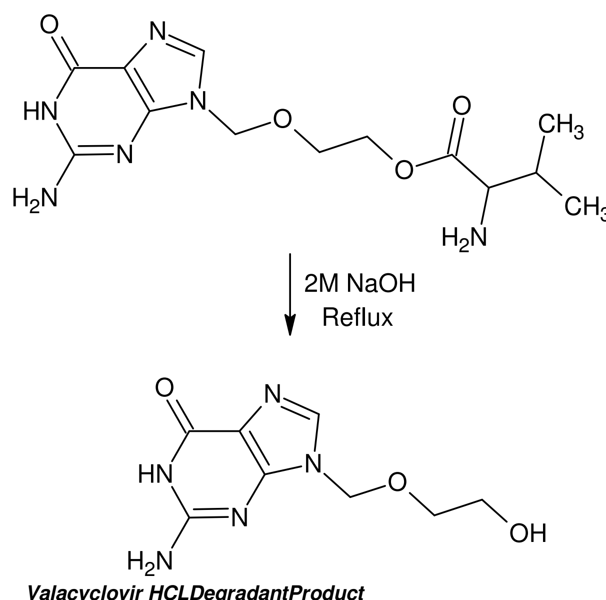


Linearity and Calibration Curve

Linearity of the method was evaluated by constructing calibration curves at six concentration levels. Calibration curves were plotted over a concentration range of 100-600 ng/spot. Aliquots of standard working solution of VAL were applied to the plate (1, 2, 3, 4, 5; and 6 µl/spot). The calibration curves were developed by plotting peak area versus concentrations (n =6) with the help of the win CATS software.

Preparation of Alkali-Induced DegradationProduct

Accurately weighed 100 mg of VAL was dissolved in 25 mL acetonitrile. Subsequently, 25 ml 1 M sodium hydroxide was added and the solution was heated in a temperature controlled oven at 50 ^{0}C for 12h. The solution was concentrated nearly to dryness under vacuum, cooled to room temperature (25^{0}C), then quantitatively transferred into a 100-mL measuring flask and the volume was completed with methanol. Complete alkaline degradation of the studied drug was confirmed by the proposed HPTLC method, where no peaks corresponding to intact drug were detected in case of the degraded samples. Scheme of Alkaline Degradation of VALare depicted below.

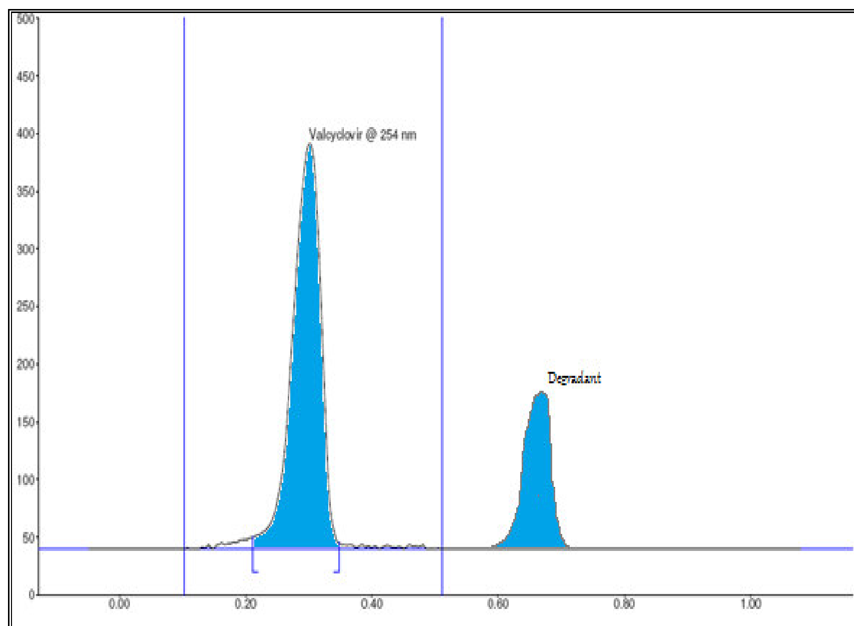


Valacyclovir HCLDegradantProduct

Studies on the Alkaline Degradation of VAL by Proposed HPTLC Method

Into a series of 10 test tubes, 2.5 mL of VAL working solution 100ug/ mL was transferred and mixed with 2 mL of 1.0 M sodium hydroxide. The test tubes were allowed to stand in a thermostatically controlled oven at 100 ^{0}C and then were removed from the oven, one by one at 15 min time intervals up to 180 min. The test tubes were immediately inserted into an ice-bath to terminate the degradation reaction and then were put in another water bath set at room temperature. The contents of the test tubes were transferred into 10 mL volumetric flasks and diluted to volume with methanol. The proposed HPTLC method was applied for the determination of the remaining intact VAL at each time interval from its corresponding regression equation. A plot of log of the remaining concentration versus time in minutes was then performed to determine of alkaline degradation process. Structuralinterpreted of the obtained degradation product was achieved by IR Spectrophotometry.Chromatogram of Valacyclovir HCL and degradant are depicted in Fig No.10.

Figure No.10
Chromatogram of ValacyclovirHydrocholride and Degradant



Identification of the Degradation Product
Valacyclovir Hydrochloride is smoothly hydrolysed with 1 M sodium hydroxide after 2.5 h at 100^{0}C, through the splitting of the ester group. The expected major degradation product (DP) is obtained according to the suggested mechanism for the alkaline degradation process of ValacyclovirHydrocholride, 15–18 (Fig. 1). The assignments and structural elucidation of the degradation product were predicted by the IR. The IR spectrum (KBr) of DP was characterized by the absorption frequency of NH2-band as a doublet at 3323.71 cm_1 and OH band as 3204.15cm^{-1} ,C-H at 3204.15 cm-. Identified VAL and degradant product are depicted in Fig No.11, 12,

Figure No.11
Standard IR of Valcyclovir HCL

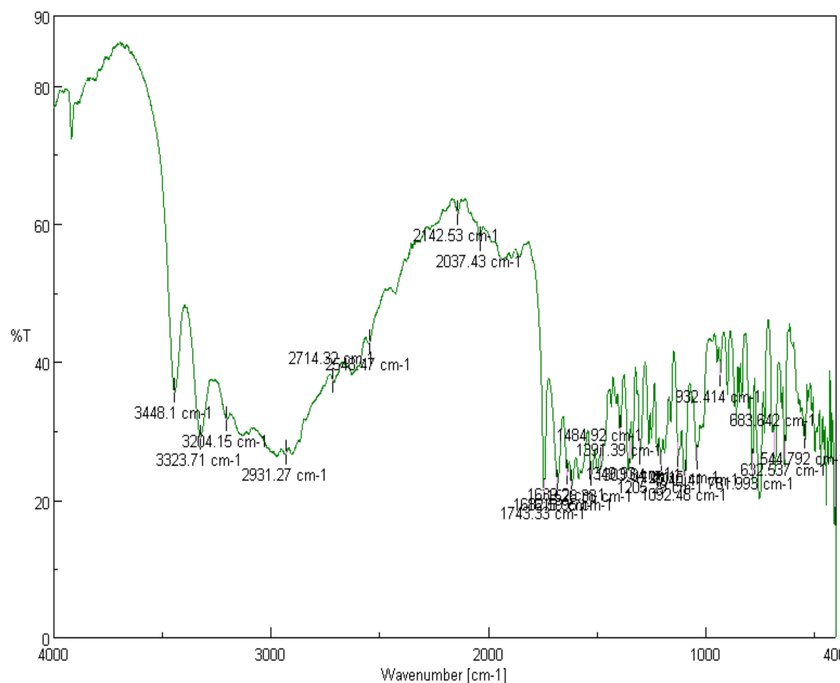
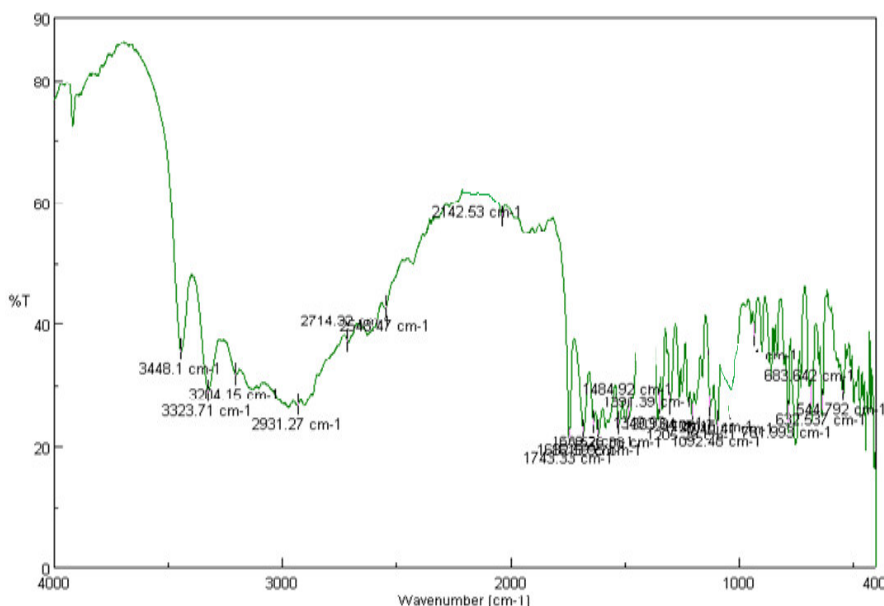


Figure No.12
IRDegradant of Valcyclovir HCL



HPTLC Method Developments and Optimization

The quantitative determination of VAL in the presence of its alkaline degradation product of the conventional simultaneous equation method is completely hindered due to the strong spectral overlap throughout the wavelength range). The suggested methods used to resolve a complex mixture of such compounds are mainly HPTLC

System Suitability

The standard and formulation samples of VAL were spotted on Precoated TLC plates in the form of narrow bands of lengths 6 mm, with 10 mm from the bottom and left margin and with 9 mm distance between two bands. The developed HPTLC method has been applied for the separation and determination of VAL in the presence of its alkaline degradation product. To optimize the HPTLC assay parameters, the mobile phase composition was studied. A satisfactory separation was obtained the solvent system containing Toluene: Methanol: Diethylamine (8:1:1 v/v/v). at an ambient temperature followed by UV detection at 254 nm. All chromatogram of resolution efficiency was calculated to ensure that the system is working correctly during the analysis. Valacyclovir Hydrochloride and degradant were satisfactorily resolved with Rf values of, 0.28 ± 0.05, 0.65 ±0.05 HPTLC chromatogram of a mixture of intact and degraded VAL sample is shown in Fig. 3, where complete Rf separation of VAL and its degradation product was noticed. The average Rf for VAL and DP, were found to be 0.28 ± 0.05, 0.65 ±0.05, respectively for 6 replicates. Chromatogram of ValacyclovirHCL and degradant are depicted in Fig No.10

Method Validation

Solution Stability

The solution stability of Valacyclovir Hydrochloride was evaluated by the suggested HPTLC method, where the studied compound solutions exhibited no chromatographic changes for 48 h when kept at room temperature and for 4 days when stored at 5 ^{0}C, where the chromatogram plots in all samples showed only one peak corresponding to pure ValacyclovirHydrochloride with no detectable peaks for degradation products or impurities.

Linearity and Range

The standard stock solution containing Valacyclovir Hydrochloride and degradant stock solution was applied on the TLC plate in the range 1-6 µL with the help of micro syringe using LINOMAT-V automatic sample applicator. The plate was then developed and scanned under the above mentioned chromatographic conditions. Rf was recorded

for each drug concentration and the calibration curves of the concentration vs. Rf were constructed for both the drugs. The calibration curves for Valacyclovir Hydrochloride are depicted in Fig No .4

Detection and Quantization Limits (Sensitivity)

The LOD and LOQ were separately determined which is based on the standard deviation of response of the calibration curve. The standard deviation of y- intercept and slope of the calibration curves were used to calculate the LOD and LOQ. Results are shown in Table No.7.

Table No.7
LOD and LOQ

Parameter	VAL
Limit of Detection (ng/band)	14.49
Limit of Quantification (ng/band)	39.17

Precision

Intra-day Precision

Intraday precision was determined by analysing Tab sample solutions at different time intervals on the same day. Tab sample solution was prepared and analysed in the similar manner as described under analysis of the Tab formulation.

Inter-day Precision

Inter-day precision was determined by analysing Tab sample solutions on three different days. Tab sample solution was prepared and analysed in the similar manner as described in analysis of the Tab formulation. Results of intra-day precision and inter-day precision are shown in Table No. 5 and 6, respectively.

Table No. 5
Intra-Day Precision Data

Drug	% Mean*	S. D.	C. V.
VAL	99.91	± 0.4540	0.2748

* ***Average of six determinations.***

Table No.6
Inter-Day Precision Data

Drug	% Mean*	S. D.	C. V.
VAL	99.93	± 0.4470	0.2776

**** Average of six determinations***

Specificity

The specificity of the developed method was established analysing the sample solutions containing VAL sample and marketed tablets in relation to interferences from formulation ingredients. The spot for VAL in the sample was confirmed by comparing retardation factor (Rf) values of the spot with that of the standard.

Application to Commercial Tablet

Twenty tablets were weighed and finely powdered. Quantity equivalent to 100 mg of drug was weighed accurately and dissolved in 50 ml methanol. The solution was sonicated for 15 min and then filtered through Whatman filter paper no. 41. The residue was washed thoroughly with methanol. The filtrate and washings were combined and diluted suitably with methanol to obtain a 1 mg/mL

concentration of VAL. An aliquot of this solution (1.0 ml) was further diluted to 10 ml with methanol to obtain a solution containing 100 ug/mL of VAL. On plates, 5 uL of these solutions were spotted and analysed for VAL content using the proposed method as described earlier. The possibility of interference from other components of the tablet formulation in the analysis was studied.

Accuracy

To ascertain the accuracy of the proposed method, recovery studies were carried out by standard addition method, as per ICH guidelines.

Preparation of Sample Solutions

An accurately weighed quantity of pre-analysed Tab equivalent to about 100 mg valacyclovir was transferred individually in nine different 100.0 mL volumetric flasks. To each of the flask following quantities of Valacyclovir Hydrochloride were added.

Flask No.1 toFlask No 9 each content 100 mg ValacyclovirHydrochloride

Then 100 mL methanol was added to each flask and content of the flask were ultrasonicated for 10 minutes, volume was then made up to the mark with methanol. The solution was individually mixed and filtered through Whatman filter paper No. 42. From the filtrate, 1.0 mL solution was diluted to 10.0 mL with methanol. The diluted solution was filtered through 0.2 µ membrane filter.

On the TLC plate two bands of standard stock Valacyclovir Hydrochloride solution and one bands of sample solution, 5.0 µL each, were applied and the plate was developed and scanned under the optimum chromatographic condition. After chromatographic development the peak obtained for standard and sample bands were integrated. The amount of Valacyclovir Hydrochloride present in an applied volume of standard solution was fed to computer. Amount of drug present in an applied volume of sample solution was obtained by comparing Rf of sample bands with that of standard bands. Amount of Valacyclovir Hydrochloride in the sample was calculated by comparing the mean Rf for standard and sample solution by formula no.1Amount of Valacyclovir Hydrochloride in the sample (mg) was calculated by following formula.

Mean amount estimated

$$\text{Amount of drug Valacyclovir Hydrochloride estimated (mg)} = \frac{\text{(µg) in applied volume}}{\text{Volume of sample solution applied (µL)}} \times \text{Volume of stock solution (mL)} \quad \text{......................... (1)}$$

Amount of the drug recovered (mg) and % recovery was calculated and Results of recovery studies and statistically are shown in Table No.1and3

Robustness Conclusion

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of change in solvent ratio and pH of solvent on Rf value was studied. The solution containing 100 µg/mL of Valacyclovir Hydrochloride was injected (in triplicate) into the sample injector of HPTLC three times under the varied conditions. Robustness data are given in Table No. 8

Table No.8
Result of Robustness Study

Chromatographic Changes		
Factor	**Level**	**R_fValue**
Mobile phase composition (± 0.1 mL)		**VAL**
7.9:0.5:0.5	– 0.1	0.28
8:1:1 v/v/v	0	0.28
8.5:1.5:1.5	+ 0.1	0.28
Amount of Mobile Phase(v/v)(± 1mL)		**VAL**
9	– 1.0	0.28
10	0	0.28
11	+1.0	0.28

Force degradation studies
To evaluate the stability indicating property of the developed HPTLC method, VAL was subjected to forced degradation conditions according to following procedures like VAL base hydrolysis, oxidation, wet heat, temperature/humidity, dry heat and photo-degradation followed by development and scanning under optimized chromatographic conditions. The forced degradation in VAL base and hydrogen peroxide was performed in the dark, in order to exclude the possible degradative effect of light on the drugs.

(a) 1000 mg/ml solution of VAL was refluxed at 50 ^{0}C for 45 min in different conditions: VAL hydrolysis 0.1M hydrochloric VAL separately), base hydrolysis (0.1 M sodium hydroxide separately), oxidation (3% hydrogen peroxide separately) and wet heat degradation were done. (b) 10 mg of VAL powder was subjected to various conditions: dry heat degradation (in an oven at 50 ^{0}C for 24 h), photo-degradation (exposed to UV light in UV chamber and sunlight for 24 h) data is given in Table No. 9.

Table No.9
Results of Degradation Study

Sr. No.	Stress Condition	Percent assay of active substance (VAL)	Rf Value of degraded product
1.	Acid (0.1 M HCL)	98.19	0.72
2.	Alkali(0.1MNaOH)	98.76	0.73
3.	Oxide(3% H_2O_2)	99.66	0.63, 0.78
4.	Heat (60^{0}C)	97.17	0.63,0.80,0.88
5.	UV (240nm)	99.74	0.58

Degradation investigation
To study the alkaline induced degradation of ValaciclovirHydrochloride, final concentration of Valacyclovir Hydrochloride standard solution was 2 mg/ml in 1 M Sodium hydroxide. This flask was refluxed at 70, 80, 90 and 100 ^{0}C temperatures. At specified time intervals, the content of the flask was quantitatively transferred to 25 ml volumetric flask and further neutralized to give a final concentration of 1 mg/ml. From this neutralized solution, (2000 ng/band) was applied on HPTLC plate and development was carried out under optimized chromatographic conditions. Furthermore, in situ UV densitometric scanning of both Valacyclovir Hydrochloride and degradation product was performed on HPTLC plates. Moreover, DP was also separated by scrapping from the HPTLC plate and IR spectra of both Valacyclovir Hydrochloride and DP were obtained and show in Fig No. 11, 12

RESULTS AND DISCUSSION

To develop HPTLC method of analysis for VAL for routine analysis, selection of solvents were

carried out on the basis of polarity. A solvent system that would give dense and compact spots with appropriate and significantly different Rf value for VAL was desired. Various solvent systems such as chloroform-methanol, methanol-toluene, n-Butanol methanol-ethyl acetate, toluene-ethyl acetate, toluene-chloroform-methanol, n-butanol, chloroform-methanol-formic VAL were evaluated in different proportions. Among these, the solvent system comprising of Toluene: Methanol: Diethylamine (8:1:1 v/v/v). gave good separation of VAL from its matrix with an R*f* value of 0.28. It was also observed that chamber saturation time and solvent migration distance are crucial in chromatographic separation as chamber saturation time of less than 15min and solvent migration distances greater than 70 mm resulted diffusion of analyse spot. Therefore, Toluene: Methanol: Diethylamine (8:1:1 v/v/v). Proportion with chamber saturation time of 20min at 25°C and solvent migration distance of 70 mm was used as mobile phase. These chromatographic conditions produced a well-defined compact spot of VAL and degradantwith optimum migration at R_f = 0.28± 0.05, 0.65 ± 0.05 at 265 nm Fig. 2 & 3. It also gave a good resolution of analyse from excipients used in marketed tablet formulations.Under the experimental conditions employed, the lowest amount of drug that could be detected was found to be 100 ng/spot and the lowest amount of drug that could be quantified was found to be 300 ng/spot, with RSD <5%.Specificity is the ability of an analytical method to assess unequivocally the analyse in the presence of sample matrix. VALand degradant were separated from excipients with an R_f of 0.54 ± 0.05 and 0.65 ± 0.05 Fig. 11. Linearity of an analytical method is its ability, within a given range, to obtain test results that are directly, or through a mathematical transformation, proportional to concentration of analyse. Method was found to be linear in a concentration range of 100-600 ng/spot (n = 6), with respect to peak area. The regression data as shown in Table 1 reveal a good linear relationship over the concentration range studied demonstrating its suitability for analysis. No significant difference was observed in the slopes of standard curves (ANOVA, P>.05).Accuracy of an analytical method is the closeness of test results to true value. It was determined by the application of analytical procedure to recovery studies, where known amount of the standard is spiked in preanalyzed samples solutions. Results of accuracy studies were shown in Table 3 recovery values demonstrated the accuracy of the method in the desired range was shown in Table 4 The precision of an analytical method expresses the degree of scatter between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Intraday precision refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment whereas Interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts. In all instances, %RSD values were less than 5% confirming the precision of the method.Ten-microliter aliquots of samples containing 100, 300, and 600 ng of VAL were analysed according to proposed method. In order to control scanner parameters, that is, repeatability of measurement of peak area, one spot was analysed without changing position of plate (n = 7). By spotting and analysing the same amount several times (n =7), precision of automatic spotting device was evaluated. %RSD was consistently less than 5%, which was well below the instrumental specifications, ensuring repeatability of developed method as well as proper functioning of the HPTLC system.The low values of %RSD obtained after introducing small deliberate changes in the developed HPTLC method confirmed the robustness of the method.The VAL content of the marketed formulations were found to be within the limits (±5% of the theoretical value) The low %RSD value indicated the suitability of this method for routine analysis of VAL in various formulations. IR spectra of both Valacyclovir Hydrochloride and DP were obtained.

Table No.1
Results of Analysis Tablet

Marketed tablet	Average Weight: 4.000mg		
Sr.No.	**Weight of Tab taken (mg)**	**Amount of drug estimated (mg/Tab taken)**	**% Label Claim**
		VAL	**VAL**
1.	100	100.05	100.88
2.	100	99.68	99.61
3.	100	98.48	98.57
4.	100	99.10	99.11
5.	100	100.00	100.00
6.	100	101.16	100.17

Table No.2
Statistical Validation for Analysis of Tablet

Sr.no	Drug	Amount of drug estimated (mg/Tab)*	% Label Claim*	S.D	C.V	S.E
1.	**VAL**	99.83	99.79	±1.2943	±1.3278	0.2081

****mean of six determinations***

Table No. 3
Results of Recovery Studies

Level of recover	Weight of Tab taken (mg)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery
		VAL	**VAL**	**VAL**
80 %	100	80	79.77	99.69
	100	80	80.03	100.03
	100	80	80.11	100.24
100 %	100	100	99.15	99.77
	100	100	99.68	99.78
	100	100	100.11	100.18
120 %	100	120	120.18	100.24
	100	120	120.01	100.04
	100	120	119.78	99.94

Table No.4
Statistical Validation for Recovery Study

Level of recovery	%Mean Recovery*	Standard Deviation	% R.S.D.	S.E
	VAL	**VAL**	**VAL**	**VAL**
80 %	99.97	± 1.5892	± 1.3765	0.5519
100 %	99.95	± 1.3453	± 1.3290	0.4280
120 %	100.02	±1.2372	± 1.2871	0.3847

**** Mean of three determinations***

Figure No.5
Chromatogram of 0.1 M NaOH Tablet

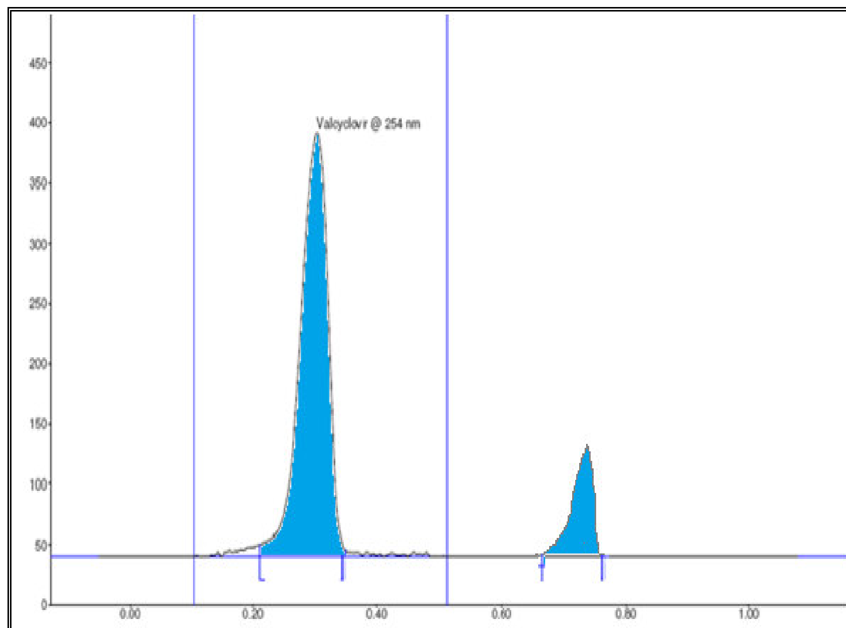


Figure No.6
Chromatogram of 0.1 M HCl Tablet

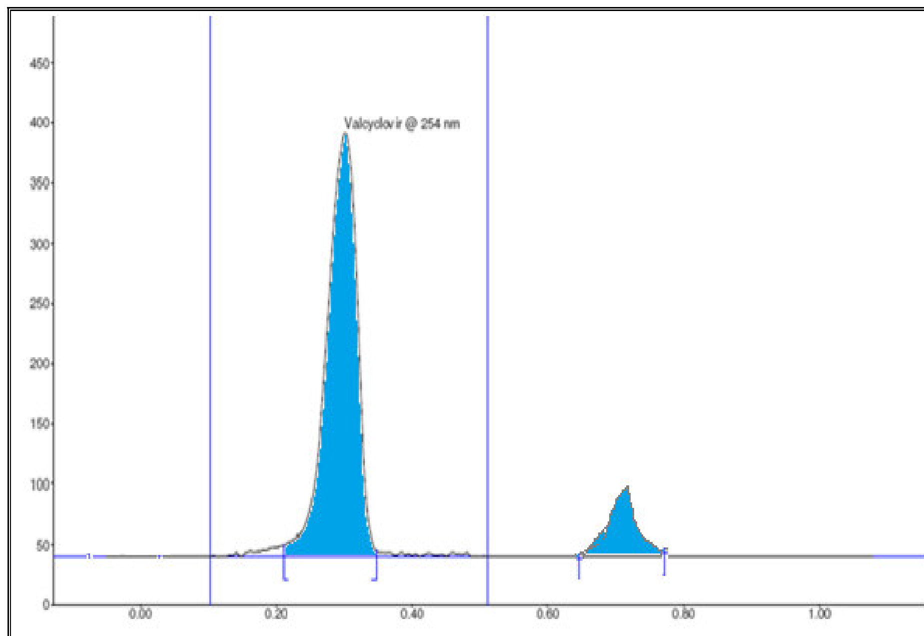


Figure No.7
Chromatogram of H_2O_2(3 %) Tablet

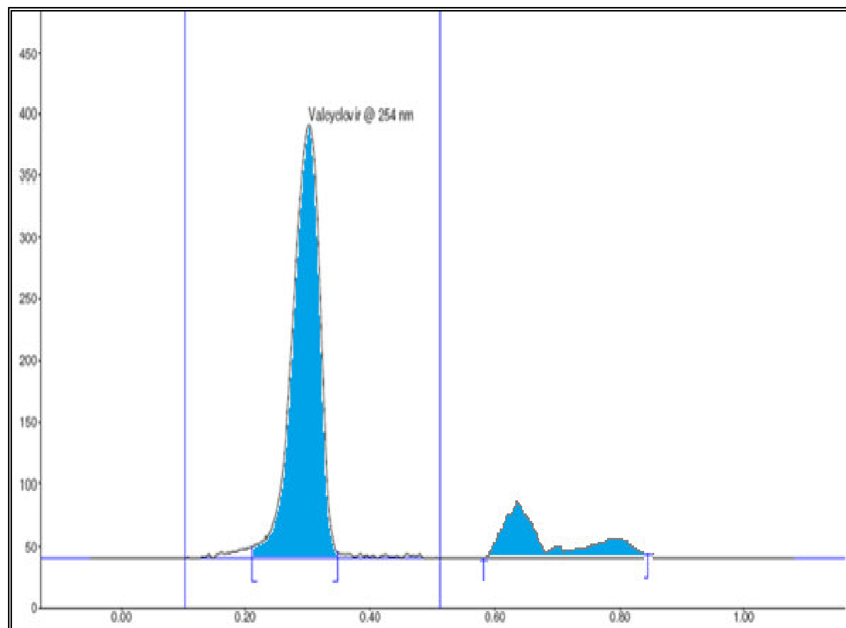


Figure No.8
Chromatogram of Dry Heat Tablet

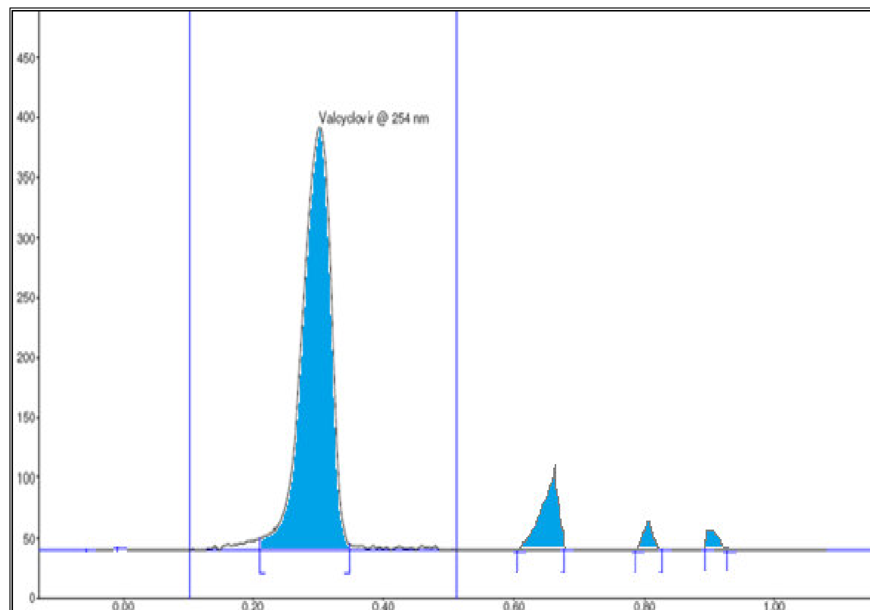
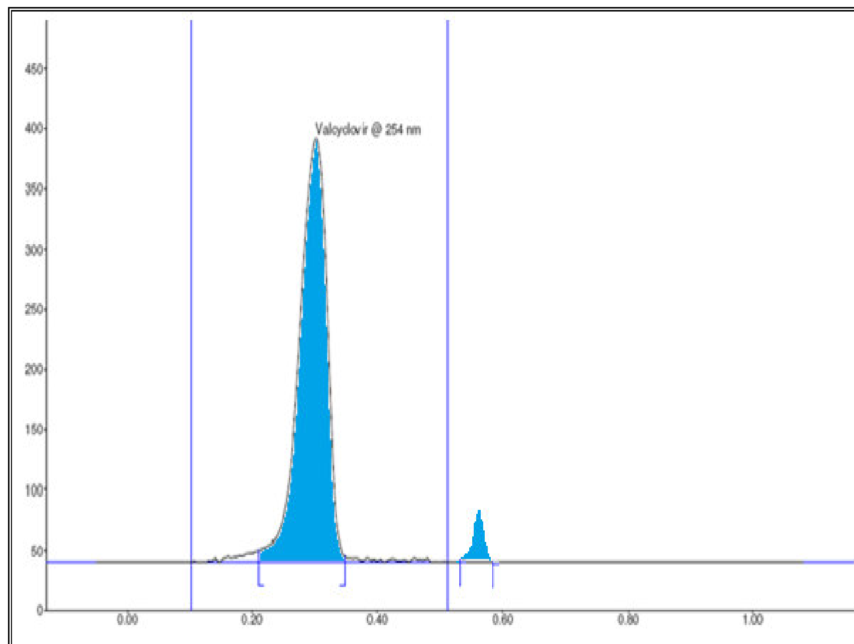


Figure No.9
Chromatogram of UV Radiation Tablet



CONCULSION

A new HPTLC method has been developed for the identification and quantification of ValaciclovirHydrochloride. Low cost, faster speed, and satisfactory precision and accuracy are the main features of this method. Method was successfully validated as per ICH guidelines and statistical analysis proves that method is sensitive, specific, and repeatable. It can be conveniently employed for routine quality control analysis of Valacyclovir Hydrochloride as bulk drug and in marketed formulations without any interference from excipients.From the previous discussion and results obtained in this work, we can conclude with 95% of confidence that suggested methods are simple, sensitive, and selective and can be applied for quality control and routine analysis of Valacyclovir Hydrochloride in pure form, in the presence of its alkaline degradation product and in the available dosage form without any interference from the excipients. In addition, the suggested HPTLC method has an advantage over the reference one7 of being used to investigate the of alkaline degradation process of ValacyclovirHydrochloridein a detailed study.

ACKNOWLEDGMENT

The authors are thankful to Cipla Ltd., Kumrek, Rangpo, Sikkim, India) for the gift sample of ValacyclovirHydrochloridepure powder, Anchrom Test lab Pvt. Ltd., Mumbai for providing facilities for carrying out analytical work.

REFERENCES

1. Y. Sultana, N. Agrawal, S. Khanam; Development And Validation Of Stability Indicating Rp-Hplc Method For Estimation Of Valcyclovir In Pharmaceutical Dosag Forms. International Journal Of Pharmaceutical And Clinical Research 2013; 5(1): 7-12.
2. M. Sugumaran, V. Bharathi, M. Lakshmi; Rp-Hplc Method For The Determination Of Valcyclovir In Bulk And

Pharmaceutical Formulation. Der Pharma Chemical 2011, 3(4):190-194.
3. Sweetman, S.C., Eds., In; Martindale, 33rdEdn. The Pharmaceutical Press. Inc., London,Volume*1* Pages *1*-2190 2002, 612.
4. D. A. Williams, T.L. Lemke, Foye's Principle Of Medicinal Chemistry. Wolters, Wuwer, Health Pvt Ltd, New Delhi, 2006, 29-51
5. Betram, Katzung, Basic And Clinical Pharmacology. Mc Raw Hill Companies, New York, 2004, 8,2435-89
6. Hp Range, Mm Deal, Jm Ritter, Pk Moore; Pharmacology, Churchill Livingstone, The *Pharmacological* Basis of Therapeutics 2003,5,210-57
7. Brien J JCampoli-Richards, Acyclovir. An Updated Review Of Its Antiviral Activity, Pharmacokinetic Properties And Therapeutic Efficacy.D M Drugs, 1989, 37,233.
8. Landowski C P, Sun D, Foster D R, Menon S, Barnett J L, Welage L S, Ramchandran C And Amidon G L, J PharmacolExpTher., 2003, 306, 778.
9. Beutner Karl R:Valacyclovir: A Review Of Its Antiviral Activity, Pharmacokinetic Properties, And Clinical Efficacy Antiviral Research 1995; 28: 281-290.
10. V.Siva Ramakrishna, D.Ravi Kumar, N.V.N.MalleswaraRao, And C. Ram Babu* Method Development And Determination Of ValacyclovirHcl In Pharmaceutical Dosage Forms By Visible Spectrophotometry Analytical Chemical Acta 2006; 555: 341-347.
11. Maria Kasiari, EvagelosGikas, Sofia Georgakakou, Michael Kazanis, IrenePanderi: Selective And Rapid Liquid Chromatography/Negative-Ion Electrospray Ionization Mass Spectrometry Method For The Quantification Of Valacyclovir And Its Metabolite In Human Plasma Journal Of Chromatography 2008: 864: 78-76.
12. VmkGowtamPotnuru, Krishna Reddy Y, ArjunCh, Prasanthi P, Ramya Krishna M, And Chandra Sekhar E* Spectrophotometric Method Development And Validation Of Valacyclovir Hydrochloride In Bulk And Pharmaceutical Dosage Form 1 ,1 ,13-18, 2012
13. Gerda Marie Friedrichsn, WeigingChem, Mikael Begtrup, Chao-Pin Lee, Philip L. Smith S Ynthesis Of Analogs Of L-Valacyclovir And Determination Of Their Substrate Activity For The Oligopeptide Transporter In Caco-2 Cells European Journal Of Pharmaceutical Sciences 2002; 16: 1-13.
14. Dillip Kumar Sahoo*1 AndPrafulla Kumar Sahu Stability-Indicating Rp-Hplc Method For The Determination Of Valacyclovir Hydrochloride Hydrochloride In Bulk And Pharmaceutical Dosage Forms Chemical Science Transactions.714 2014, 3(2), 510-517.
15. KeshavaNavin Kumar Reddy1, MurugullaAdharvana Chari2*, Mayur D. Khunt1, V. V. N. K. V. Prasada Raju1, BojjaRamachandra Reddy1, Vurimidi Himabindu3 New Method Of Palladium Metal Trapping Through Resins In Antiviral Drug: ValacyclovirHcl International Journal Of Organic Chemistry, , 3, 251-255 2013.
16. Balfour, H.H., Chace, B.A., Stapleton, J.T., Simmons, R.L., Fryd, D.S., A Randomized Placebo-Controlled Trial Of Oral Acyclovir For The Prevention Of Cytomegalovirus Disease In Recipients Of Renal Allogrfts 1989; 320, 1381-1387.
17. Fletcher, C., Bean, B., Evaluation Of Oral Valacyclovir Hydrochloride Therapy. Drug Intell. Clin. Pharm 1985; 19, 518-524.
18. G. Srihari, N. Rami Reddy*, K. NagarajaSetty And I.E. Chakravarthi Spectrophotometric Determination Of Valacyclovir In Pharmaceutical Formulations ChemSci Trans., 2013, 2(1), 61-64.
19. Granero G E AndAmidonGl, Stability Of Val Acyclovir: Implications For Its Oral BioavailabilityInt J Pharm., 2006, 317(1), 14.
20. K.Srinivasa Rao*and M. Sunil Stability-indicating liquid chromatographic method for Valacyclovir International Journal of ChemTech Research,2009 1,(3), 702-708

21. M. Ganesh, C.V.Narasimharao, A.Saravana Kumar, K.Kamalakannan, M.Vinoba, H. S. Mahajan And T. Sivakumar. Uv Spectrophotometric Method For The Estimation Of ValacyclovirHcl In Tablet Dosage Form, 6(3), 814-818. 2009
22. Shingare M S, Jadhav A S AndPathare D B, Development And Validation Of Enantioselective High Performance Liquid Chromatographic Method For Valacyclovir, An Antiviral Drug In Drug Substance. J Pharm Biomed Anal., 43(43), 1568, 2007
23. Mani Ganesh, Margandan Bhagiyalakshmi, Pushparaj Hemalatha,C. Varaga Narasimha Rao, Hyun Tae Jang Krishnan Rajasekar.Rp-Hplc Estimation Of ValacyclovirHcl In Tablet Formulation Asian Journal Of Chemistry; Vol. 23, No. 3 1317-1320., 2011.
24. Anand B S, Katragadda S And Mitra A K,Pharmacokinetics Of Novel Dipeptide Ester Prodrugs Of Acyclovir After Oral Administration: Intestinal Absorption And Liver Met J PharmacolExpTher 311,(2),659., 2004.

This article can be downloaded from www.ijpbs.net