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RESEARCH ARTICLE

STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE QUANTITATIVE DETERMINATION OF VALGANCICLOVIR IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A selective, specific and sensitive stability-indicating high-performance liquid chromatographic method was developed and validated for the determination of Valganciclovir in tablet dosage forms. Reversed-phase chromatography was performed on Shimadzu Model CBM-20A/20 Alite, equipped with SPD M20A prominence photodiode array detector (Isocratic mode) using C18 column (250 mm × 4.6 mm, 5 µm) with a flow rate of 0.8 mL/min. UV detection was carried at 254 nm. Linearity was observed in the concentration range of 1.0–200 µg/mL with regression equation y = 50968 x + 86374 with correlation coefficient of 0.999. The LOQ and LOD were found to be 0.8641 µg/mL and 0.2813 µg/mL respectively. Valganciclovir was subjected to stress conditions such as acidic, alkaline, oxidation, photolysis and thermal degradations. The developed method was validated as per ICH guidelines and it can be applied for the determination of Valganciclovir in pharmaceutical dosage forms.

Keywords: Valganciclovir, Isocratic mode, RP-HPLC, Validation, Stability-indicating, LOD, LOQ.

INTRODUCTION

Valganciclovir HCl (VGC), chemically L-Valine, 2[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]-3-hydroxypropyl ester, monohydrochloride. (Figure 1) is a white to off-white crystalline powder with a molecular formula of $C_{14}H_{22}N_6O_5 \cdot HCl$ and a molecular weight of 390.83 It is an antiviral medication used to treat cytomegalovirus infections. As the L-valyl ester of ganciclovir, it is actually a prodrug for ganciclovir. After oral administration, it is rapidly converted to ganciclovir by intestinal and hepatic esterases. The mechanism of action of Valganciclovir is that it is a prodrug of ganciclovir that exists as a mixture of two diastereomers. After administration, these diastereomers are rapidly converted to ganciclovir by hepatic and intestinal esterases. In cytomegalovirus infected cells, ganciclovir is initially phosphorylated to the monophosphate form by viral protein kinase, then it is further phosphorylated via cellular kinases to produce the triphosphate form. This triphosphate form is slowly. Very few methods are reported in the literature including liquid chromatographic methods[2-4], spectrophotometric techniques[5] and LC/MS/MS[6-8] methods for the determination of Valganciclovir in tablet dosage forms and in biological fluids. Suresh Kumar et al., have studied the Impurity profile and related substances[9] of Valganciclovir and Stefanidis et al., studied the reactivity[10] of Valganciclovir in aqueous solution. In the present work the authors have proposed a simple, rapid, robust, precise and accurate reverse phase liquid chromatographic method for the determination of Valganciclovir in tablet dosage forms.

MATERIALS AND METHODS

Chemicals and Reagents

Valganciclovir standard (purity ≥ 99.98%) was obtained from Roche, India. Methanol (HPLC grade), sodium hydroxide and hydrochloric acid, Glacial acetic acid and hydrogen peroxide were purchased from Merck (India).

Valganciclovir is available as tablets with brand name VALCYT® as tablets (Label claim: 450 mg). All chemicals were of analytical grade and used as received.

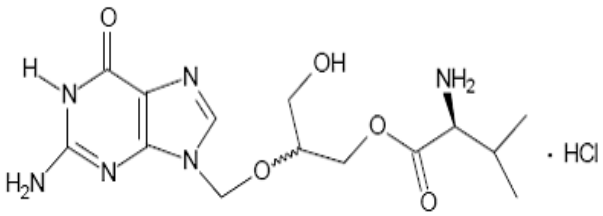

Figure 1: Chemical structure of Valganciclovir (VGC)

Instrumentation and Chromatographic Conditions

Chromatographic separation was achieved by using a Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector (250 mm × 4.6 mm, 5 µm particle size) maintained at 25 ºC. Isocratic elution was performed using a mixture of methanol, water and glacial acetic acid (45:55:0.1,v/v) with flow rate 0.8 mL/min. 20 µL of each solutions were injected into the HPLC system.

Preparation of Valganciclovir Stock Solution

Valganciclovir stock solution (1000 µg/mL) was prepared by accurately weighing 25 mg of Valganciclovir in a 25 mL amber volumetric flask and making up to volume with mobile phase. Working solutions for HPLC injections were prepared from the stock solution mobile phase. Solutions were filtered through a 0.45 µm membrane filter prior to injection.

Assay of marketed formulations

Twenty tablets from each brand (VALCYT®) were procured, weighed and crushed to a fine powder. Powder equivalent to 25 mg Valganciclovir was accurately weighed into a 25 ml volumetric flask and made up to volume with mobile phase. The contents of the volumetric flask were sonicated for 30 min to enable complete dissolution of Valganciclovir. The solution was filtered and the filtrate was diluted with mobile phase. 20 µL of these solutions were injected into the system and the peak area was recorded from the respective chromatogram.

Forced Degradation Studies/Specificity

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method [11]. All solutions for use in stress studies were prepared at an initial concentration of 1 mg/mL of Valganciclovir and refluxed for 30 min at 80 ºC. All samples were then diluted in mobile phase to give a final concentration of 100 µg/mL and filtered before injection.

Acidic and Alkaline Degradation

Acid decomposition was carried out in 0.1 M HCl at a concentration of 1.0 mg/mL Valganciclovir and after refluxation for 30 min at 80 ºC the stressed sample was cooled, neutralized and diluted with mobile phase to give a final concentration of 100 µg/mL and filtered before injection. Similarly stress studies in alkaline conditions were conducted using a concentration of 1.0 mg/mL in 0.1 M NaOH and refluxed for 30 min at 100 ºC. After cooling the solution was neutralized and diluted with mobile phase to give a final concentration of 100 µg/mL and filtered before injection.

Oxidative Degradation

Solutions for oxidative stress studies were prepared using 3% H_2O_2 at a concentration of 1 mg/mL of Valganciclovir and after refluxation for 30 min at 100 ºC on the thermostat the sample solution was cooled and diluted with the mobile phase to give a final concentration of 100 µg/mL and filtered before injection.

Thermal Degradation

For thermal stress testing, the drug solution (1 mg/mL) was heated in thermostat at 100 ºC for 30 min, cooled and diluted with the mobile phase to give a final concentration of 100 µg/mL and filtered before injection.

Method Validation

The method was validated for the following parameters: system suitability, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and robustness[12].

Linearity

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels of the analyte (1.0-200 µg/mL). 20 µL of each solution was injected in to the HPLC system and the peak area of the chromatogram obtained was noted.

Precision

The intra-day precision of the assay method was evaluated by carrying out 9 independent assays of a test sample of Valganciclovir at three concentration levels (10, 20 and 50 µg/mL) (n=3) against a qualified reference standard. The %RSD of three obtained assay values at three different concentration levels was calculated. The interday precision study was performed on three different days i.e. day 1, day 2 and day 3 at three different concentration levels (10, 20 and 50 µg/mL) and each value is the average of three determinations (n=3). The % RSD of three obtained assay values on three different days was calculated.

Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Valganciclovir in the drug product. The study was carried out in triplicate at 18, 20 and 22 µg/mL. The percentage recovery in each case was calculated.

Sensitivity/Limit of quantification (LOQ) and limit of detection (LOD)

The limit of quantification (LOQ) and limit of detection (LOD) were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1) [12]. Sensitivity of the method was established with respect to limit of detection (LOD) and LOQ for Valganciclovir. LOD and LOQ were established by slope method as mentioned below. LOD and LOQ were experimentally verified by injecting six replicate injections of each impurity at the concentration obtained from the above formula.

$$\text{LOD} = \frac{3.3 \times \text{standard deviation of y-intercept}}{\text{Slope of the calibration curve}}$$

$$\text{LOQ} = \frac{10 \times \text{standard deviation of y-intercept}}{\text{Slope of the calibration curve}}$$

Robustness

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (252 and 256 nm), percentage of methanol in the mobile phase (53 and 57) and flow rate (0.7 and 0.9 mL/min). Robustness of the method was studied using six replicates at a concentration level of 20 µg/mL of Valganciclovir.

RESULTS AND DISCUSSION

A reversed-phase liquid chromatographic technique was developed to quantitate Valganciclovir in pharmaceutical dosage forms. No stability indicating liquid chromatographic method was reported earlier in the literature. A detailed comparative study of the previously published methods with the present method was given in Table 1.

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Table 1 Comparison of the performance characteristics of the present method with the published methods

S. No.	Method /Reagent	λ (nm)	Linearity (µg/mL)	Remarks	Ref
1.	(HPLC) Acetonitrile:methanol:KH_2PO_4 (pH 5.0) (40:20:40,v/v)	255	10-3000	human serum	2
2.	(HPLC) Acetonitrile: Potassium dihydrogen phosphate (pH 4.0) (60:40,v/v)	254	0.01-60	Low linearity range	3
3	(HPLC) n-Hexane: ethanol: isopropyl alcohol: tri-fluoro acetic acid (98: 1.5: 0.5: 0.1,v/v/v/v)	215	–	Chiral purity	4
4	(Spectrophotometry) Methanol	254	5-39	Very narrow linearity range	5
5	LC/MS	-	-	Human plasma	6
6	LC/MS/MS	-	-	Plasma and its active metabolites	7
7	LC/MS/MS	-	-	Plasma and its active metabolites	8
8	(HPLC) Methanol: trifluoro acetic acid (Gradient mode)	-	-	Impurity profile and related substances	9
9	(HPLC) Methanol: water:glacial acetic acid (55:44:0.1,v/v)	254	1.00-200	Stability indicating method Wide linearity range (PDA detector)	Present work

A reversed-phase chromatographic technique was developed for the determination of Valganciclovir in tablets. Satisfactory resolution was achieved with use of a mixture of methanol, water and glacial acetic acid (55:45:0.1,v/v) with UV detection at 254 nm (Figure 2). C8 and C18 columns were first evaluated as stationary phase for the separation of Valganciclovir but C18 column was adopted for the analysis as it has provided a better separation of the analytes.

HPLC Method Development and Optimization

Initially the stressed samples were analyzed using a mobile phase consisting of water: acetonitrile (70:30, v/v) at a flow rate of 1.0 mL/min. Under these conditions, the resolution and peak symmetry were not satisfactory, so the mobile phase was changed to methanol: water (40:60,v/v) with a flow rate of 1.0 mL/min under which peak tailing was observed.

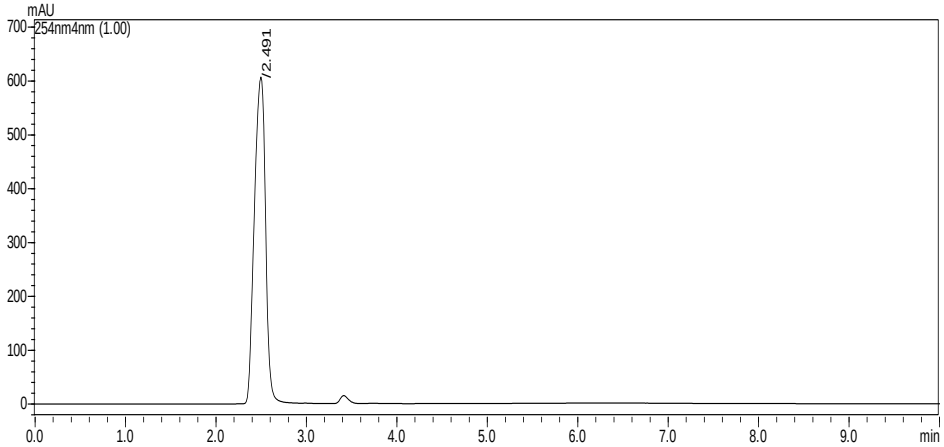


Figure 2: Typical Chromatogram of Valganciclovir (100 µg/mL)

Finally the mobile phase containing methanol: water: glacial acetic acid (45:55: 0.1,v/v) was chosen as the best chromatographic response for the entire study where a sharp peak was observed around 2.5 mins.

Method Validation

Linearity

The calibration curve for Valganciclovir was linear over the concentration range of 1.00–200 µg/mL. The data for the peak area of the drug in corresponds to the concentration was treated by linear regression analysis (Table 2) and the regression equation for the calibration

curve (Figure 3) was found to be y = 50968 x + 86374 with correlation coefficient of 0.999.

Limit of Detection and Limit of Quantification

The LOQ and LOD were found to be 0.8641 µg/mL and 0.2813 µg/mL respectively.

Table 2: Linearity of Valganciclovir

Conc. (µg/mL)	*Mean peak area ± SD	RSD (%)
1	124567 ± 361.24	0.29
5	417982 ±1379.34	0.33
10	517992 ±2538.16	0.49
20	1195971 ±7893.41	0.66
50	2645833 ± 14022.31	0.53
100	5177187 ± 19673.31	0.38
150	7778901 ± 63786.99	0.52
200	10237618 ± 38902.95	0.38

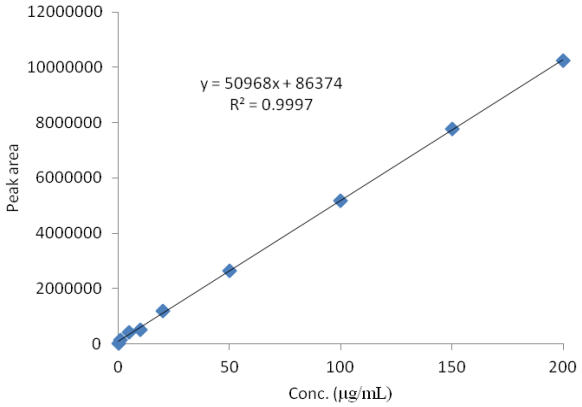


Figure 3: Calibration Curve of Valganciclovir

Precision

The inter-day precision was calculated by assaying three samples of each at three different concentration levels (10, 20 and 50 µg/mL) on three different days. The % RSD range was obtained as 0.28-0.61 and 0.35-0.89 for intra-day and inter-day precision studies respectively (Table 3).

Accuracy

The method accuracy was proven by the recovery test. A known amount of Valganciclovir standard (10 µg/mL) was added to aliquots of samples solutions and then diluted to yield total concentrations as 18, 20 and 22 µg/mL as described in Table 3. The assay was repeated over 3 consecutive days. The resultant % RSD was 0.29-0.92 (<2.0 %) with a recovery 98.78-98.82 %.

Table 3: Precision and accuracy study of Valganciclovir

Conc. (µg/mL)	Intra-day precision	Inter-day precision	
	*Mean peak area ± SD (% RSD)	*Mean peak area ± SD (% RSD)	
10	517987 ± 1450.36 (0.28)	517889 ± 1812.6115 (0.35)	
20	1195891 ± 5859.86 (0.49)	1196981 ± 6703.09 (0.56)	
50	2644929 ±16134.06 (0.61)	2645032 ± 23540.78 (0.89)	
Accuracy			
Conc. (µg/mL)	*Mean peak area ± SD (% RSD)	*Drug found (µg/mL)	% Recovery*
18	698456.00 ± 20255.24 (0.29)	17.78	98.79
20	1088191.00 ± 7073.24 (0.65)	19.96	99.82
22	1745889.00 ± 16062.17 (0.92)	21.73	98.78

Mean of three replicates

Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for routine analysis [12]. The robustness of the method was evaluated by assaying the same sample under different analytical conditions deliberately changing from the original condition. The detection wavelength was set at 252 and 256 nm (± 2 nm), the ratio of percentage of water: methanol in the mobile phase was applied as 53:47 and 57:43 (± 2 %, v/v), the flow rate was set at 0.7 and 0.9 mL/min (± 0.1 mL/min). The results obtained from assay of the test solutions were not affected by varying the conditions and were in accordance with the results for original conditions. The % RSD value of assay determined

for the same sample under original conditions and robustness conditions was less than 2.0% indicating that the developed method was robust (Table 4).

Table 4: Robustness study of Valganciclovir

Parameter	Condition	*Mean peak area	*Mean ± SD (% RSD)	% Assay*
Flow rate (mL/min)	0.7	519835	517805.33 ± 3003.27 (0.58)	99.98
	0.8	517992		
	0.9	515589		
Detection wavelength (nm)	252	510881	512087.33 ± 3943.07 (0.77)	99.80
	254	517992		
	256	507389		
Mobile phase composition (Water: Methanol) (v/v)	53:47	510831	516255.00 ± 4956.04 (0.96)	98.94
	55:45	517992		
	57:43	519942		

Mean of three replicates

Analysis of Commercial Formulations (Tablets)

The proposed method was applied to the determination of Valganciclovir tablets and VALCYT® and the result of these assays yielded 99.81 % respectively with RSD < 2.0 %. The result of the assay (Table 5) indicates that the method is selective for the assay of Valganciclovir without interference from the excipients used in these tablets. The typical chromatograms for Valganciclovir obtained from the extracted marketed formulations were shown in Figure 4.

Table 5: Analysis of Valganciclovir commercial formulation (Tablets)

Sample No.	Formulation	Labeled claim (mg)	*Amount found (mg)	*Recovery (%)
1	VALCYT®	450	388.41	99.81

Mean of three replicates

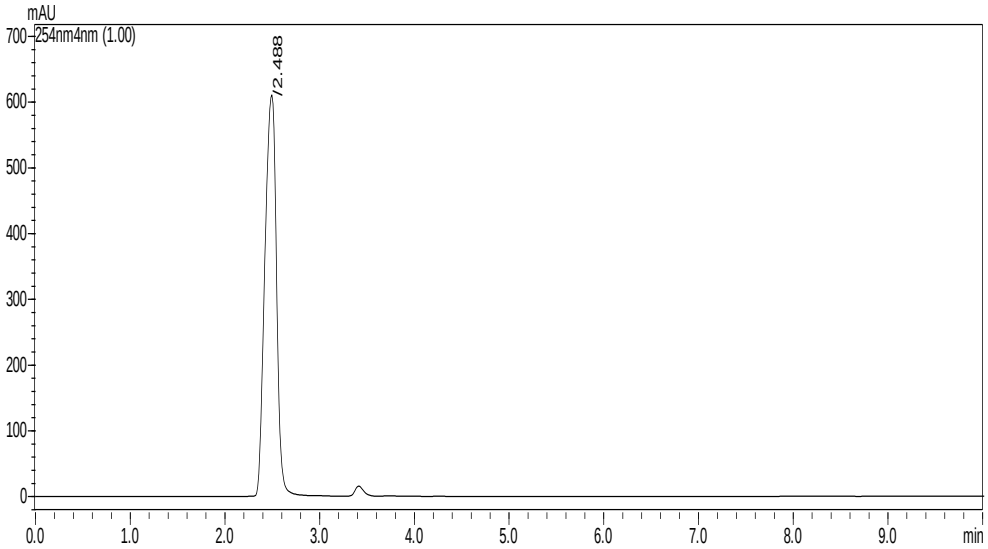


Figure 4: Typical Chromatogram of Valganciclovir (100 µg/mL) VALCYT® (450 mg)

Selectivity/Specificity

The specificity of the developed method was determined by injecting sample solutions (100 µg/mL) which were prepared by forcibly degrading under such stress conditions as heat, light, oxidative agent, acid and base under the proposed chromatographic conditions. The stability indicating capability of the method was established from the separation of Valganciclovir peak from the degraded samples derived from the software. The degradation of Valganciclovir was found to be very similar for both the tablets and standard.

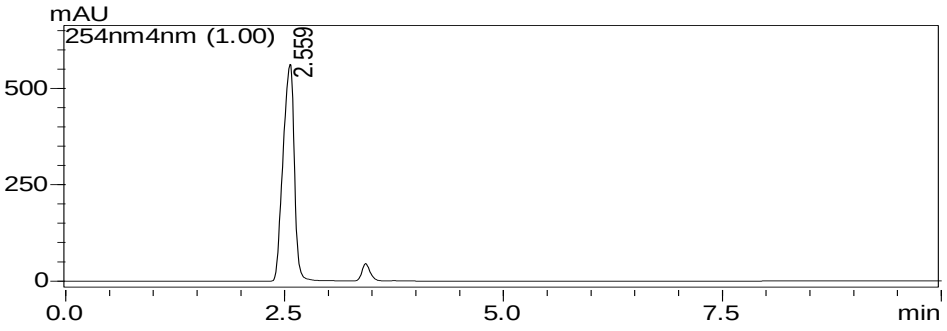
Solution Stability and Mobile Phase Stability

The %RSD of the assay of Valganciclovir from the solution stability and mobile phase stability experiments was within 2%. The results of the solution and mobile phase stability experiments confirm that the sample

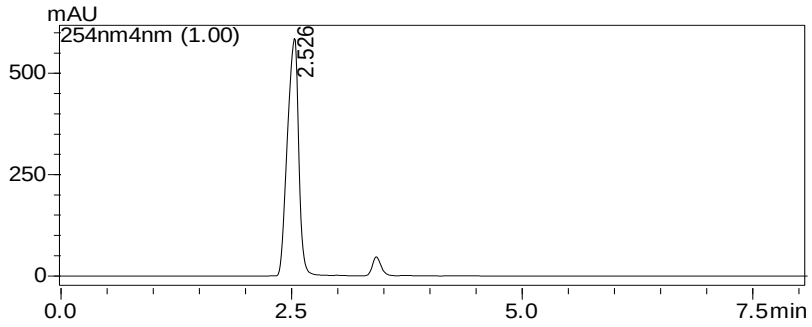
solutions and mobile phase used during the assays were stable up to 48 h at room temperature and up to 3 months at 4ºC.

Forced Degradation Studies

Valganciclovir standard and tablet powder was found to be quite stable under dry heat conditions. Typical chromatograms obtained following the assay of stressed samples are shown in Figure 5 (A-E).



[A]



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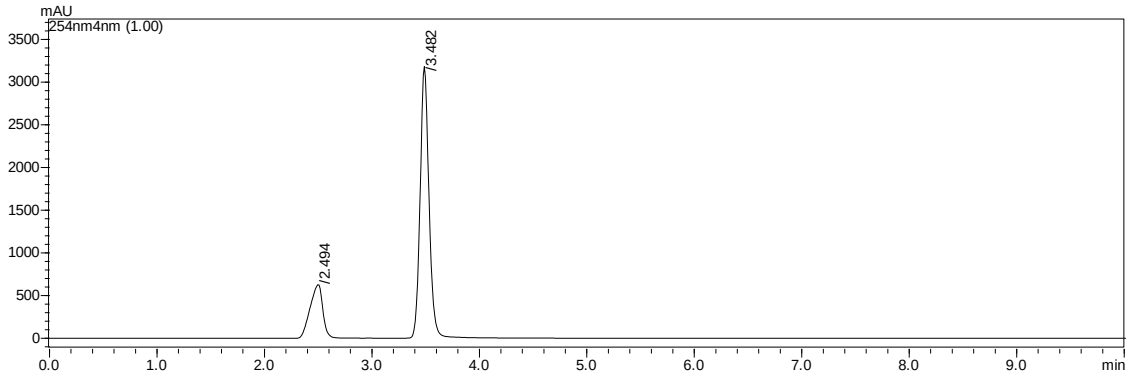

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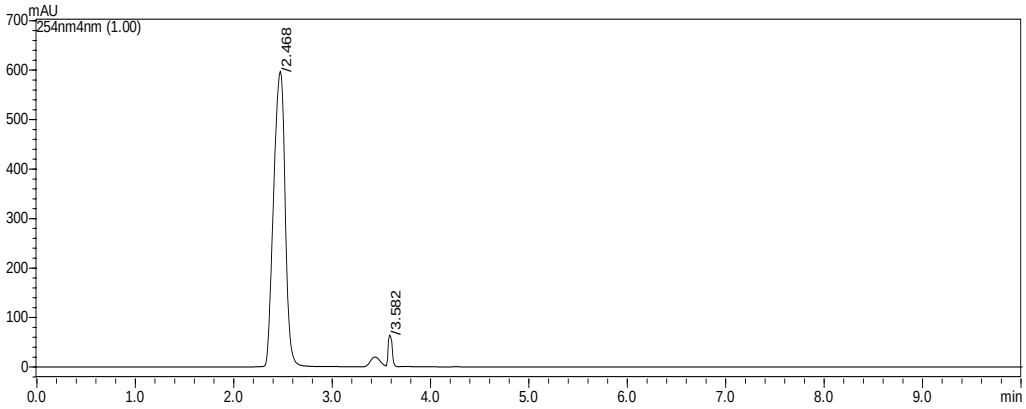

[D]

Figure 5: Typical Chromatograms of Valganciclovir (100 µg/mL)) on *Acidic [A], Alkaline [B], Oxidative [C] and Thermal [D] Degradations*

A very slight decomposition was seen on exposure of Valganciclovir drug solution to acidic (3.23), alkaline (3.21) and oxidation (1.25). During the oxidative degradation a major degradants were observed at 3.482 mins without interfering the elution of drug peak. Valganciclovir has undergone thermal degradation (1.11) very slightly and Table 6 summarises the data of degradation studies.

System Suitability

The system suitability test was performed to ensure that the complete testing system was suitable for the intended application. The parameters measured were peak area, retention time, tailing factor, capacity factor and theoretical plates. In all measurements the peak area varied less than 2.0%, the average retention time was 5.5 ± 0.05 minutes. The capacity factor was more than 2, theoretical plates were more than 2000 and tailing factor was less than 1.5 for the Valganciclovir peak (Table 6).

Table 6: Forced degradation studies of Valganciclovir

Stress conditions	*Mean peak area	% Drug recovered	% Drug decomposed	Theoretical plates	Tailing factor
Standard Drug	5195753	100	-	4261.187	1.012
Acidic degradation	5081089	96.77	3.23	4169.378	1.011
Alkaline degradation	5075503	96.79	3.21	4087.112	1.013
Oxidative degradation	4657716	98.75	1.25	4066.286	1.004
Thermal degradation	5177187	98.89	1.11	4031.448	1.118

Mean of three replicates

CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guidelines and can be applied for the determination of Valganciclovir in pharmaceutical dosage forms. The method was found to be accurate, precise, robust and specific as the drug peak did not interfere with the extra peaks aroused during the forced degradation studies. At the same time the chromatographic elution step is undertaken in a short time (< 3 min). No interference from any components of pharmaceutical dosage form can be successfully applied to perform accelerated stability studies of Valganciclovir formulations.

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