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Research Article

To Develop And Validate Stability Indicating Assay Of Famciclovir By Using UV And HPLC Method For Pharmaceutical Dosage Form

Pavan Patil*[1], Shailesh B. Patil

[1]*DCS's ARA College of Pharmacy, Nagaon, Dhule-424002, Maharashtra, India.*
[2] *DCS's ARA College of Pharmacy, Nagaon, Dhule-424002, Maharashtra, India*

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ABSTRACT

The study aimed to develop and validate a stability-indicating assay for Famciclovir using UV and HPLC methods in pharmaceutical formulations. Famciclovir samples were confirmed for identity via UV-visible wavelength scan and FT-IR spectra, showing characteristic peaks for its chemical structure. UV-Visible spectra exhibited absorption at 312 nm, with a strong linear calibration curve (R^2 = 0.999) across concentrations. Method development involved optimizing chromatographic conditions, resulting in satisfactory separation on an Eclipse Plus column with a mobile phase of Acetonitrile, methanol, and glacial acetic acid (50:20:30 v/v/v) at 1 ml/min flow rate. Method validation demonstrated high linearity, accuracy, precision, and robustness, with system suitability tests confirming adequate chromatographic performance. Assay methods for Famciclovir in API and pharmaceutical formulations showed consistent accuracy and precision over three consecutive days. Forced degradation studies under various stress conditions, including acid/base hydrolysis, oxidation, and thermal and photo degradation, confirmed the method's stability-indicating capability. Despite degradation, Famciclovir was distinguished from its degradation products, indicating method specificity.

INTRODUCTION

Famciclovir is a potent antiviral medication primarily used to treat infections caused by herpes viruses, including herpes simplex virus (HSV) and varicella-zoster virus (VZV). It belongs to the nucleoside analogue class of drugs and is structurally similar to acyclovir. Famciclovir is available in oral dosage forms and marketed under various brand names worldwide.

***Corresponding Author:** Pavan Patil
Address: *DCS's ARA College of Pharmacy, Nagaon, Dhule-424002, Maharashtra, India.*
Email : pavanpatil10498@gmail.com
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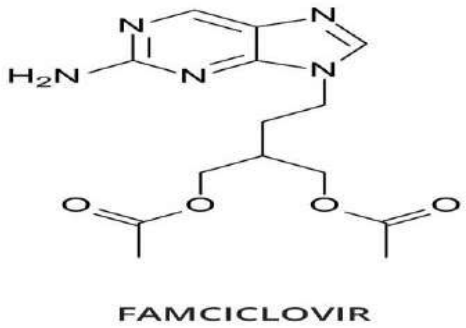

Figure 01: Structure and IUPAC name: 2-Amino-9H-purine in which the hydrogen at position 9 is substituted by a 4-acetoxy-3-(acetoxymethyl) but-1-yl group.

Overview of Herpes Viruses:

Herpes viruses are a group of DNA viruses that infect humans and other animals, causing a wide range of diseases, including oral and genital herpes, shingles (herpes zoster), and chickenpox (varicella). These viruses establish lifelong infections in the host, with periodic reactivation leading to recurrent outbreaks of symptoms.

Herpes Simplex Virus (HSV):

HSV exists in two primary types: HSV-1 and HSV-2. HSV-1 commonly causes oral herpes, characterized by cold sores or fever blisters around the mouth, while HSV-2 is primarily associated with genital herpes, leading to painful sores in the genital area.

Varicella-Zoster Virus (VZV):

VZV causes chickenpox during primary infection, and later in life, it can reactivate to cause shingles, characterized by a painful rash and blisters along a specific dermatome.

Clinical Manifestations:

The clinical manifestations of herpes virus infections vary depending on the site and type of infection. While some individuals may experience mild symptoms or asymptomatic infection, others may suffer from severe discomfort, pain, and complications. Effective antiviral therapy is essential for managing these infections and reducing their associated morbidity and transmission.

Mechanism of Action:

Famciclovir acts by inhibiting the replication of herpes viruses through its conversion to penciclovir, an active metabolite, by the viral enzyme thymidine kinase. Penciclovir then competitively inhibits viral DNA polymerase, preventing the elongation of viral DNA and thus halting viral replication. This mechanism of action is similar to that of acyclovir.

Development and Advantages:

The development of Famciclovir as a therapeutic agent stemmed from the need for more potent and bioavailable antiviral drugs with improved pharmacokinetic properties compared to acyclovir. Famciclovir offers several advantages over acyclovir, including higher oral bioavailability, longer intracellular half-life, and greater activity against certain herpes virus strains. These attributes contribute to its efficacy in treating herpes infections and preventing recurrent outbreaks.

Pharmacokinetics:

Famciclovir involves rapid and extensive absorption following oral administration, with peak plasma concentrations achieved within 1 to 2 hours. The drug undergoes extensive first-pass metabolism in the liver, primarily mediated by hepatic esterases, leading to the formation of penciclovir. Penciclovir exhibits high protein binding and is primarily eliminated unchanged in the urine via renal excretion.

Clinical Efficacy:

Clinical studies have demonstrated the efficacy of Famciclovir in treating various herpes virus infections, including genital herpes, herpes labialis (cold sores), and herpes zoster. In randomized controlled trials, Famciclovir has been shown to reduce the duration of viral shedding, accelerate lesion healing, and alleviate symptoms associated with herpes outbreaks. Additionally, suppressive therapy with Famciclovir has been effective in

reducing the frequency and severity of recurrent genital herpes episodes.

Prophylactic Use:

Famciclovir has been investigated for its potential prophylactic use in certain populations, such as Immuno compromised individuals at risk of herpes virus reactivation. Prophylactic administration of Famciclovir has been shown to reduce the incidence of herpes zoster and herpes simplex virus infections in these high-risk populations, thereby improving their quality of life and reducing healthcare burden.

Safety Profile:

The safety profile of Famciclovir is generally favorable, with most adverse effects being mild to moderate in severity and transient in nature. Common side effects include headache, nausea, diarrhea, and abdominal pain. Rare but serious adverse reactions, such as allergic reactions, thrombotic thrombocytopenic purpura (TTP), and central nervous system effects, have been reported with the use of Famciclovir, although causality is often difficult to establish.

Contraindications: The use of Famciclovir may be contraindicated in certain patient populations, including those with known hypersensitivity to the drug or its components. Caution is advised in patients with renal impairment, as dosage adjustment may be necessary to prevent drug accumulation and potential toxicity. Additionally, Famciclovir should be used with caution during pregnancy and lactation, as limited data are available regarding its safety in these populations.

Experimental:

Chemicals and reagents: HPLC grade Acetonitrile, Methanol, and glacial acetic acid were obtained from Rankem (Mumbai, India). Pure sample of Famciclovir drug was obtained from FDC Limited, Goa, India. Ultra pure water obtained from Milli-Q academic system (Millipore Pvt. Ltd., Bangalore, India) was used to prepare all solutions for the method. The procured samples were tested to confirm their identity and this included UV-visible wavelength scan, and recording of FT-IR spectra. FT-IR spectra were recorded at ARACOP, Dhule. The sample was prepared as a KBr pellet for recording the spectra. The UV-Visible spectra of Telmisartan were recorded using methanol as solvent was recorded using water as solvent on SICAN 1900 Instrument shown in figure 02.

Chromatographic conditions:

Table 01: HPLC methods for the quantitative analysis of Famciclovir in the pharmaceutical dosage form:

Parameter	Description
Column	Eclipse Plus column (250 x 4.6 mm i.d. x 5 micron)
Detector	Photo-diode array detector
Detection Wavelength	312 nm
Mobile Phase Composition	Acetonitrile : Methanol : Glacial Acetic Acid (50:20:30 v/v/v)
Flow Rate	1 ml/min
Mobile Phase Preparation	Filtered through a 0.42 micron Nylon filter before use

a Design and optimize UV and HPLC methods for the quantitative analysis of Famciclovir in the pharmaceutical dosage form.

b Experiment with different chromatographic conditions, including column type, mobile phase composition, flow rate, and detection wavelength for HPLC method.

- The process was carried out on C18 column (5µm, 250 x 4.6mm, i.d) using the mobile phase consisting of Acetonitrile, methanol, and glacial acetic acid in the ratio 50:20:30 v/v/v respectively at a flow rate of 1 ml min-1. Wavelength was fixed at 310 nm. The

mobile phase was filtered through 0.45µm membrane filter and degassed.

c. Determine the optimal conditions for UV analysis, including wavelength selection and sample preparation technique.

- Stock standard solution of the pure drug was prepared by dissolving 100 mg of FCV in 100 ml volumetric flask using water. Then the volume was made up to the mark with the same solvent to give a final concentration of 1000 µg ml-1. Standard solutions of FCV (1.0, 5.0, 10.0, 20.0, 30.0, and 40.0 µg ml-1) were prepared by subsequent dilution with mobile phase using Acetonitrile, methanol, and glacial acetic acid in the ratio 50:20:30 v/v/v.

Preparation of Standard Solutions:

a Prepare stock solutions of Famciclovir of known concentrations to be used for method calibration and validation.

b Ensure the accuracy and precision of the stock solutions through appropriate dilutions and analytical techniques.

Validation of the method:

The proposed analytical method was validated as per the International Conference on Harmonization (ICH) guidelines Q2 (R1)25: Linearity Precision Robustness Specificity Accuracy Linearity Precision Robustness. Validation of the proposed method Validation of the proposed methods was performed in accordance with the International Conference on Harmonization (ICH) guidelines (2005).

System Suitability Testing:

Perform system suitability tests for the HPLC method to ensure adequate chromatographic performance and resolution. Evaluate parameters such as retention time, peak symmetry, resolution, and column efficiency to ensure the reliability of the method.

Resolution:

The ability of the chromatographic system to separate adjacent peaks. Resolution is determined by the peak width and baseline separation between peaks.

Retention Time:

The time taken for a compound to travel through the chromatographic column and reach the detector. Retention time should be consistent and reproducible for each analyte.

Peak Symmetry:

The shape of chromatographic peaks should be symmetrical, indicating efficient and uniform elution of analytes. Peak asymmetry can indicate issues such as overloading, column degradation, or improper mobile phase composition.

Peak Area:

The area under each peak corresponds to the amount of analyte present in the sample. Peak area should be consistent and proportional to the concentration of analyte injected.

Selectivity:

The ability of the chromatographic method to separate analytes of interest from potential interferences or impurities. Selectivity ensures accurate quantification and identification of target compounds.

Method Validation:

Conduct validation studies according to the developed protocol.

Linearity:

Calibration curves were constructed using three series of standard FCV solutions in the range of 1.0 - 40.0 µg ml-1. The equation of linear regression and statistical data are shown in the table. The linearity of the calibration curve was validated by the high value of the correlation coefficient.

Limit of detection (LOD) and limit of quantification (LOQ):

The limit of detection and the limit of quantification are defined as LOD and LOQ respectively, where σ denotes standard deviation of y-intercepts of regression lines and s denotes slope of the corresponding calibration curve.17

Precision:

The assay was investigated with respect to system suitability test, method precision and intermediate precision. The system suitability test and method precision were carried out to monitor repeatability and reproducibility. In order to measure repeatability of the system (system suitability test), five consecutive injections were made and the results were evaluated by considering peak area values of FCV. The precision values with their R.S.D. are shown in Table 7.2. The results in Table 2 indicate that the R.S.D. (%) is less than 2%. Three different concentrations of FCV were analyzed in three independent series in the same day (intra-day precision) and three consecutive days (inter-day precision), within each series every sample was injected six times. The R.S.D. values of intra- and inter-day studies varied from 0.17 to 0.57 % showing that the intermediate precision of the method was satisfactory.

Accuracy and recovery studies:

The accuracy of a method is expressed as the closeness of agreement between the value found and the value that is accepted as a reference value. It is determined by calculating the percent difference (bias %) between the measured mean contents and the corresponding nominal contents.18 Shown in the table results obtained for intra- and inter-day accuracy. The accuracy of the proposed method was also tested by recovery experiments. Recovery experiments were performed by taking different sample concentrations and spiking with FCV at two different concentration levels (50% and 100% FCV). Six samples were prepared for each recovery level. Samples were treated as described in the procedure for sample preparations. The results obtained are shown in Table from which it is clear that both the recoveries and repeatabilities are excellent.

Robustness:

Robustness relates to the capacity of the method to remain unaffected by small but deliberate variations introduced into the method parameters. Influences of small changes in the mobile phase composition (±10%) and flow rate (±10%) were studied to determine robustness of the method. Peak areas and retention time changes were observed. Peak area values and retention time values varied by less than 2 %. Despite the changes in retention time there was no problem for quantification.

Assay method:

a. API
b. Pharmaceutical formulation.

Twenty tablets were weighed, crushed and an amount of powder equivalent to100 mg of FCV was accurately weighed, transferred to a 100 ml volumetric flask, made up to volume with water and placed in an ultrasonic bath for 20 min. After filtration through a 0.45µm membrane filter, the solution was suitably diluted with mobile phase to obtain the required concentration. 20µL of solution was injected into the HPLC system to obtain the chromatograms for the standard drug solution and the sample solution. A steady baseline was recorded with the optimized chromatographic conditions. The standard solution of FCV was injected and the chromatogram recorded. The retention time of FCV was found to be 3.56 min. The sample solution prepared from the tablets was then injected and the amount of drug present was calculated from the calibration curve.

Results and discussion:

Result of Procurement and Confirmation of Identity of Famciclovir:

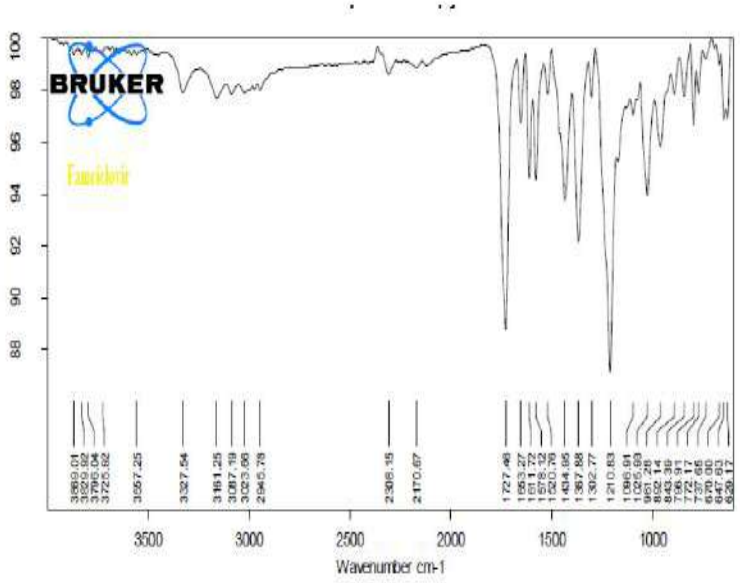

Figure 02: FT-IR spectra of Famciclovir.

Table 02: FT-IR spectra values of Famciclovir

Sr. No.	Functional Group	FT-IR Peaks cm^{-1}
1	Aromatic rings	1600-1500 (C=C stretching vibrations)
2	Amide group	1650 (C=O stretching), 3300-3400 (N-H stretching)
3	Ether group	1100-1000 (C-O stretching)
4	Aliphatic groups	3000-2800 (C-H stretching)

Famciclovir is an antiviral drug used in the treatment of herpes simplex virus infections. Its chemical structure contains several functional groups that would produce characteristic peaks in the FT-IR spectra above table and figure. The UV-Visible spectra of Famciclovir were recorded using methanol as solvent was recorded using water as solvent on SICAN 1900 Instrument.

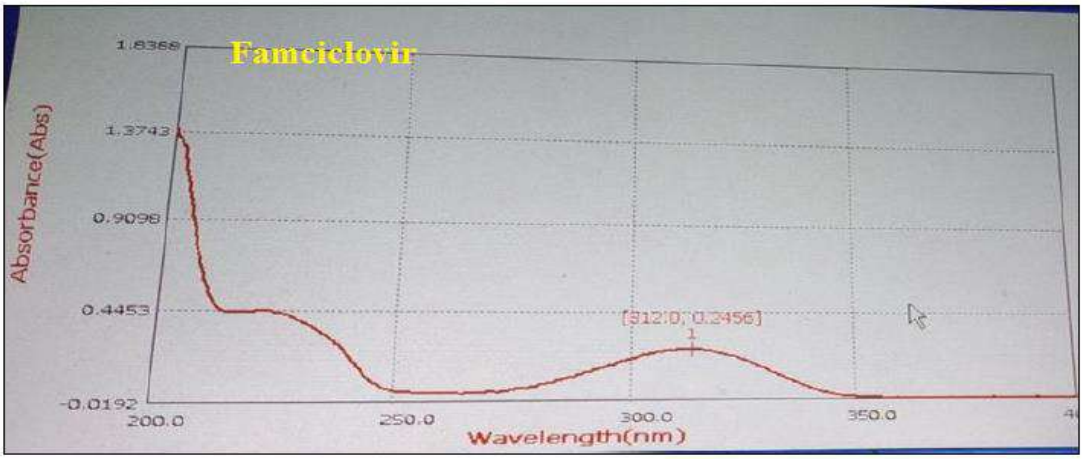

Figure no. 03: UV-Visible spectra of Famciclovir at 312 nm.

Average of six determinations

Table 03: Calibration curve for Famciclovir:

Concentration µg/ml	Absorbance
00	0.00
10	0.122
20	0.224
30	0.486
40	0.648
50	0.850
60	1.208

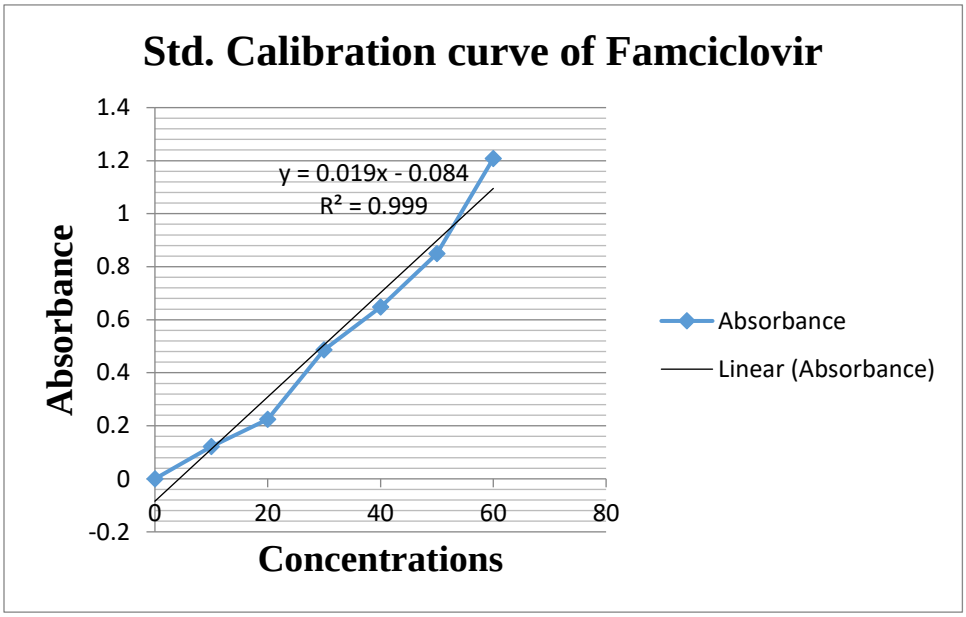

Figure no. 04: Std. Calibration curve of Famciclovir r2 =0.999

UV-Visible spectra of Famciclovir exhibit absorption at 312 nm. The standard calibration curve for Famciclovir demonstrates strong linearity with an R^2 value of 0.999, indicating reliable quantitative analysis capability.

Method Development:

The process was carried out on C18 column (5µm, 250 x 4.6mm, i.d) using the mobile phase consisting of Acetonitrile, methanol, and glacial acetic acid in the ratio 50:20:30 v/v/v respectively at a flow rate of 1 ml min-1. Wavelength was fixed at 312 nm. The mobile phase was filtered through 0.45µm membrane filter and degassed

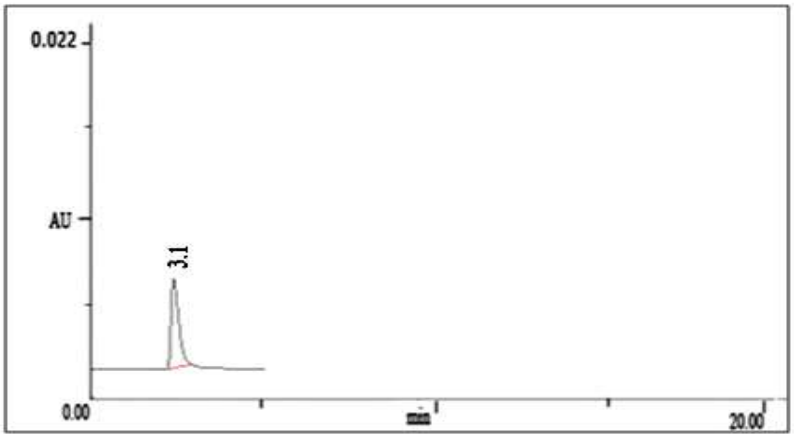

Figure 05: Mobile phase: Acetonitrile, methanol, and glacial acetic acid in the ratio 50:20:30 v/v/v for Famciclovir determination.

Table no.05 : Different composition of mobile phase:

Sr. No.	Mobile Phase Composition	Ratio
1	Acetonitrile, methanol, and glacial acetic acid	60:10:30 v/v/v
2	Acetonitrile, methanol, and glacial acetic acid	50:20:30 v/v/v
3	Acetonitrile, methanol, and glacial acetic acid	40:30:30 v/v/v

Result of Preparation of Standard Solutions:

Figure 06: Std. curve for Famciclovir by HPLC method.

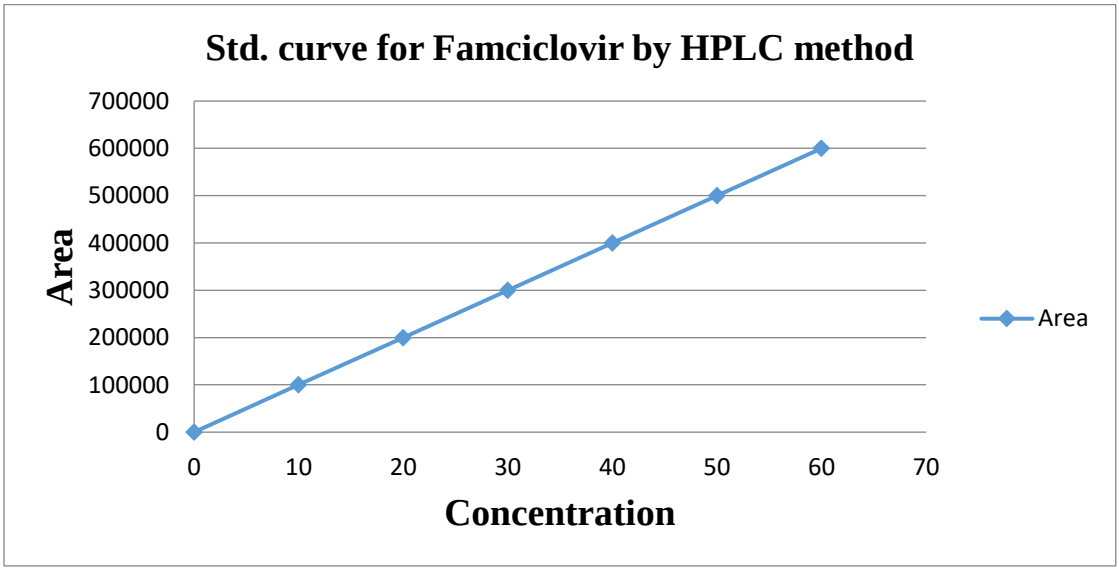

Table 06: Std. curve for Famciclovir by HPLC method:

Concentration µg/ml	Area
0	0
10	100000
20	200000
30	300000
40	400000
50	500000

Result of Method Validation:

Linearity:

Calibration curves were constructed using three series of standard FCV solutions in the range of 0.0 - 40.0 µg ml-1. The equation of linear regression and statistical data are presented in Table 06 and figure 06.

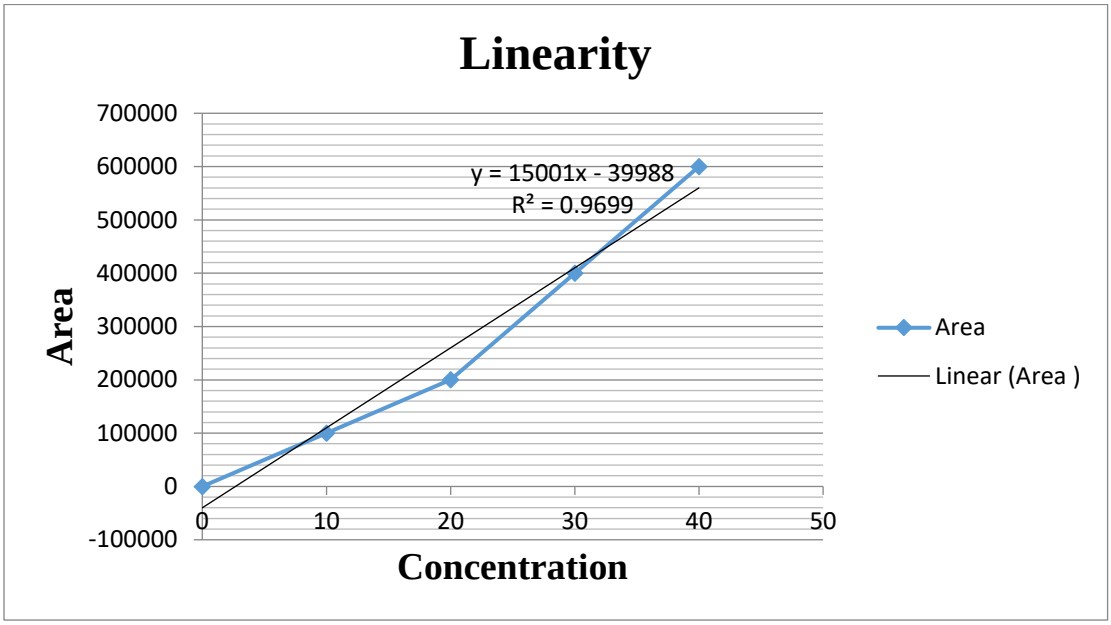

Figure no. 06: Linearity of Famciclovir.

Table 06: Linearity of Famciclovir

Concentration µg/ml	Area
00	00
10	100020
20	200045
30	400056

Average of six determinations

Limit of detection (LOD) and limit of quantification (LOQ):

The limit of detection and the limit of quantification are defined as LOD and LOQ respectively, where σ denotes standard deviation of y-intercepts of regression lines and s denotes slope of the corresponding calibration curve.17

LOD =3.3 σ/s = 0.310, LOQ=10 σ/s = 1.022

Table 07: Optical Characteristics of Famciclovir

Sr. No	Parameters	Method
1	λmax (nm)	312
2	Beers law limit (µg/ml)	0-40
3	Sandell's sensitivity (µg/cm2/0.001 A.U)	0.0034
4	Molar absorptivity (L mol–1 cm–1)	2.5 x 104
5	Correlation coefficient (r)	0.999
6	Regression equation(Y=mX+c)	y = 15001x – 39988
7	Slope(m)	0.0575
8	Intercept(c)	0.0005
9	LOD (µg/ml)	0.310
10	LOQ (µg/ml)	1.022
11	Standard error of mean of Regression line	0.00294

Precision:

The R.S.D. values of intra- and inter-day studies (Table 08) varied from 0.16 to 0.50 % showing that the intermediate precision of the method was satisfactory.18

Table 08 Precision: Intra- and inter-day precision of Famciclovir.

Concentration of drug (µg/ml)	Observed concentration of drug (µg/ml)			
	Intra-day		Inter-day	
	Mean	%RSD	Mean	%RSD
10	9.98	0.37	9.99	0.5
15	15.01	0.16	14.9	0.2
20	19.99	0.16	19.86	0.4

***Average of six determinations. R.S.D. (%):** relative standard deviation; bias (%): [(found – taken)/taken] x 100.

Accuracy and recovery studies: The results obtained are shown in Table 9 and, 10 from which it is clear that both the recoveries and repeatabilities are excellent.

Table 09: Precision: Intra- and inter-day precision of Famciclovir.

Concentration of drug (µg/ml)	Accuracy	
	Intra-day	Inter-day
10	0.3	0.42
15	0.2	0.025
20	0.3	0.016

***Average of six determinations.**

Table no. 10: Recovery Data for the Proposed RP-HPLC method. Studies of Famciclovir:

Concentration of drug (µg/ml)	Amount (µg/ml)		% Recovery ± R.S.D.
	Taken + Added	Found* ± S.D.	
10	10 + 05	15.01 ± 0.038	100.06 ± 0.378
15	15 + 05	19.97 ± 0.052	99.80 ± 0.347
20	20 + 10	29.97 ± 0.036	99.86 ± 0.178
Pharmaceutical formulations (PF)	**Amount (µg/ml)**		**% Recovery ± R.S.D.**
	Taken + Added	**Found* ± S.D.**	
Tablets I	10 + 05	14.01 ± 0.038	98.06 ± 0.378
Tablets II	15 + 05	19.07 ± 0.032	97.80 ± 0.347
Tablets III	20 + 10	29.10 ± 0.03	98.86 ± 0.178

***Average of six determinations. R.S.D. (%): relative standard deviation; bias (%): [(found – taken)/taken] x 100.**

Robustness: Results from each condition, along with their respective RSD values, are detailed in Table 11. Notably, there were no significant deviations in retention times or peak areas. Additionally, both drugs' content remained close to 100%, with RSD values consistently below 2% across all tested conditions. 19, 20.

Table 11: Famciclovir content and their respective RSD values following each Chromatographic condition variation:

Condition		Retention time (min)	Content (%)	
	Famciclovir	PF	Famciclovir	PF
No changes	3.1	3.7	101.2	99.01
Flow 0.9 mL.min−1	3.0	3.4	103.1	104.2
Flow 0.7 mL.min−1	3.1	3.6	102.1	101.2
Column temperature 32.5ºC	3.3	3.6	104.1	104.4
Column temperature 37.5ºC	3.3	3.7	103.1	101.2
Mobile phase ratio 50:20:30	3.1	3.7	100.1	104.2
Mobile phase ratio 60:10:30	3.6	3.8	98.11	103.2
Mean			**100.1**	**101.2**
RSD			**1.26**	**1.46**

***Average of six determinations. R.S.D. (%): relative standard deviation; bias (%): [(found – taken)/taken] x 100.**

System Suitability Testing:

Perform system suitability tests for the HPLC method to ensure adequate chromatographic performance and resolution. Evaluate parameters such as retention time, peak symmetry, resolution, and column efficiency to ensure the reliability of the method.

Table no.12: System Suitability Test Parameters for Famciclovir:

Analyte	RSD of Replicate Injections	Tailing Factor	No. of Theoretical Plates	Capacity factor
	(< 2)	(< 2)	(> 2000)	(>0.5)
Famciclovir	0.8549	1.0272	8363.83	1.87
PF	0.9549	1.2272	9363.83	1.97

Assay method:

API and Pharmaceutical formulation. 20µL of solution was injected into the HPLC system to obtain the chromatograms for the standard drug solution and the sample solution. A steady baseline was recorded with the optimized chromatographic conditions. The standard solution of FCV was injected and the chromatogram recorded. The retention time of FCV was found to be 3.56 min. The sample solution prepared from the tablets was then injected and the amount of drug present was calculated from the calibration curve.

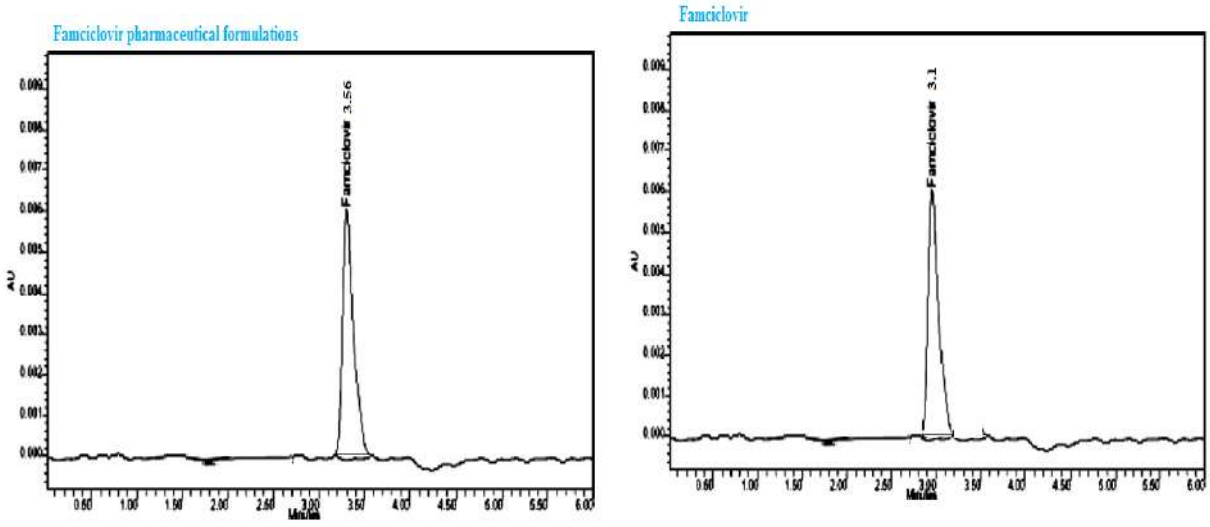

Figure 07: Result of Assay method API and Pharmaceutical formulation for Famciclovir.

Table 13: Analysis of Famciclovir from API and pharmaceutical formulations by proposed method.

Concentration of drug (µg/ml)	Sample	Labelled amount (mg)	Amount found± S.D.	Reference method	% Recovery ± R.S.D.
Day 1	FCV	250	249.83 ± 0.970 t = 0.009, F = 1.44	249.83 ± 0.807	99.93 ± 0.388
	FCV	250	249.88 ± 0.967 t = 0.058, F = 2.75	249.85 ± 0.583	99.95 ± 0.387
	FCV	250	248.33 ± 0.204 t = 0.112, F = 1.06	248.35 ± 0.210	99.33 ± 0.082
Pharmaceutical formulations (PF)	**Sample**	**Labelled amount (mg)**	**Amount found* ± S.D.**	**Reference method**	**% Recovery ± R.S.D.**
Day 1	PF	250	248.83 ± 0.870 t = 0.009, F = 1.44	246.83 ± 0.807	99.93 ± 0.388
	PF	250	249.77 ± 0.968 t = 0.058, F = 2.75	248.85 ± 0.583	99.96 ± 0.387
	PF	250	247.33 ± 0.204 t = 0.113, F = 1.06	247.25 ± 0.210	99.23 ± 0.082

***Average of six determinations. R.S.D. (%): relative standard deviation; bias (%): [(found – taken)/taken] x 100.**

These results illustrate the findings from the analysis conducted on the consequential three days. The amounts found closely align with the reference method, indicating high accuracy, while the percentage recoveries demonstrate the method's reliability with low relative standard deviation (R.S.D.) values.

Forced Degradation Studies:

Conduct forced degradation studies to assess the stability-indicating capability of the developed methods. Subject Famciclovir samples to various stress conditions such as heat, light, acid/base hydrolysis, oxidation, and photolysis. Monitor the degradation products using UV and HPLC methods to ensure that the method can accurately quantify Famciclovir in the presence of degradation products.

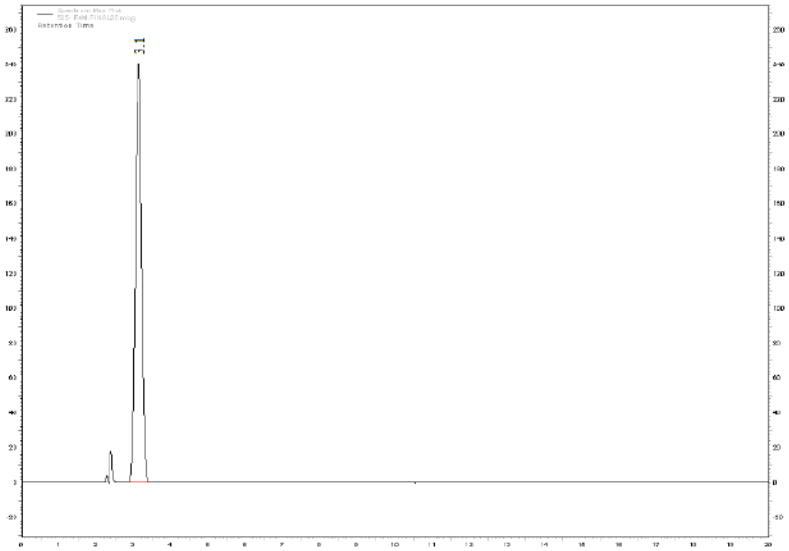

Figure 8: Result of normal condition of Famciclovir.

Acid degradation studies:

To 10 mL of Famciclovir solution (2mg/mL in distilled water) added 10 mL of 1N HCL and was kept at room temperature for 3 hour. The solution was neutralized. Further dilutions were made with mobile phase to obtain concentration 20g/mL for HPLC analysis.

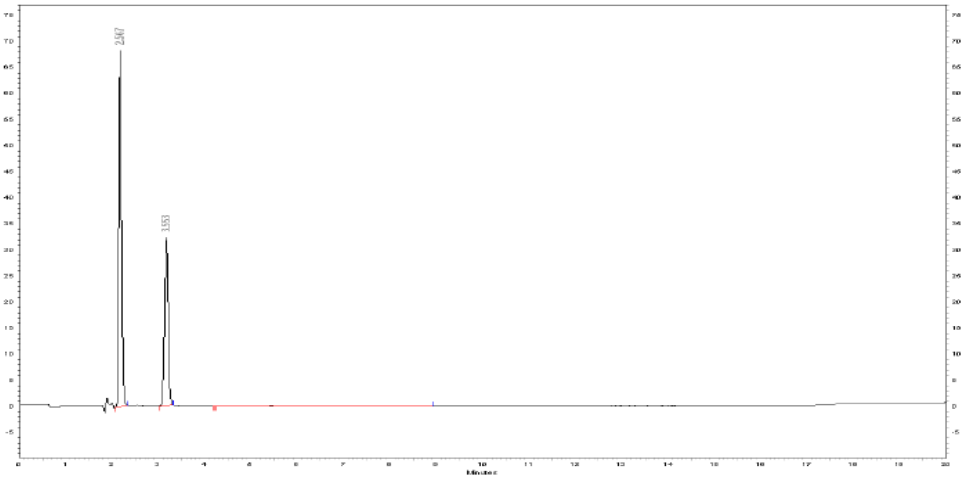

Figure 09: Result of Acid hydrolysis (1M HCL, 3 hr., RT) of Famciclovir.

Alkali degradation studies:

To 10 mL of Famciclovir solution (2mg/mL in distilled water) added 10 mL of 0.1M NaOH and was kept at room temperature for 3 hour. The solution was neutralized and diluted with mobile phase for further study.

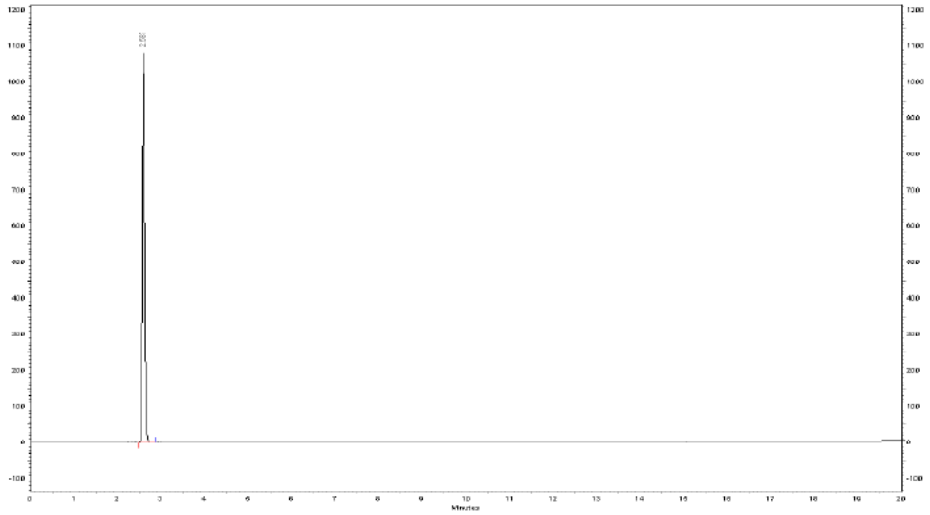

Figure 10: Result of ne hydrolysis (0.1 M NaOH, 3hr., RT) of Famciclovir.

Neutral degradation studies:

To 10 mL of Famciclovir solution (2mg/mL in distilled water) added 10 mL distilled water refluxed at 80 0C for 12 hour. The solution was diluted with mobile phase for further study.

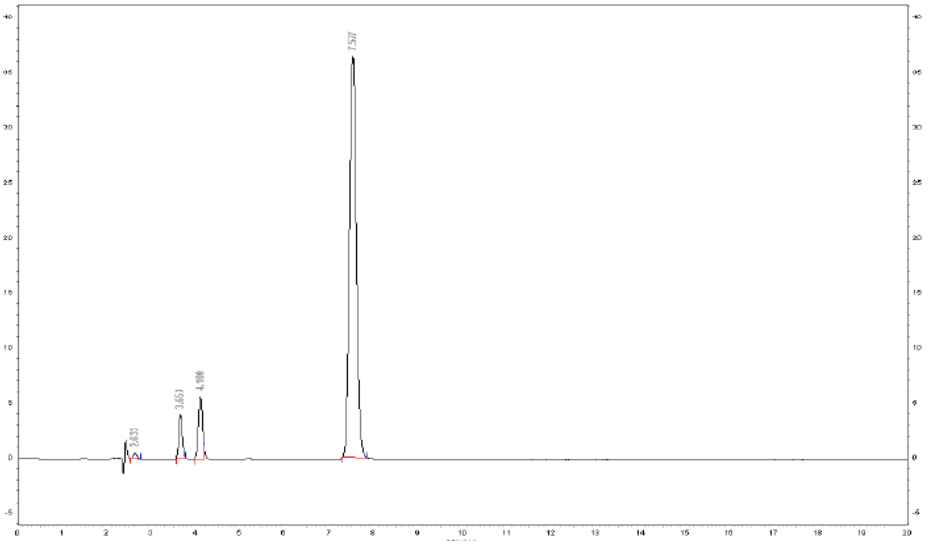

Figure 11: Result of Neutral hydrolysis (12 hr reflux, 800C) of Famciclovir.

Oxidation studies:

To 10 mL of Famciclovir solution (2mg/mL in distilled water) added 10 mL of 30% H2O2 at room temperature for 48 hour.

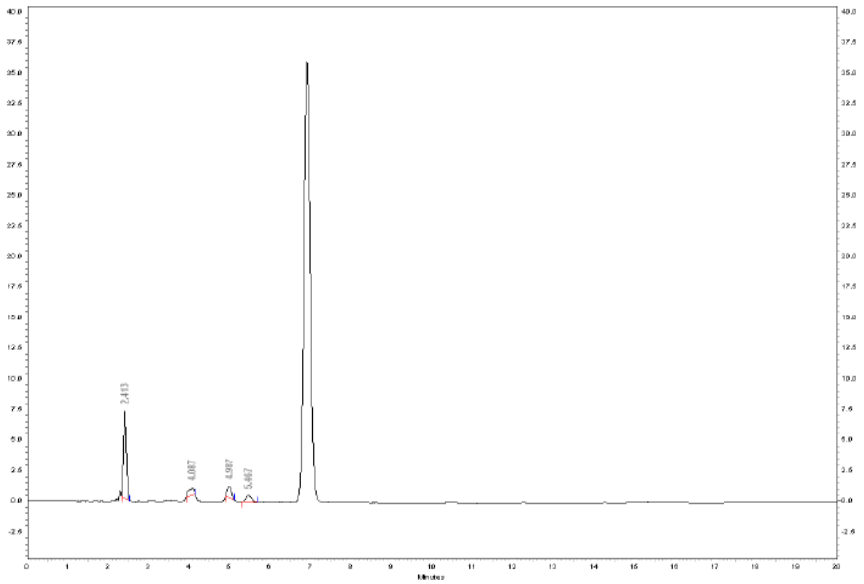

Figure 12: Result of Oxidation studies (30 % H2O2, 48 hr., RT) of Famciclovir.

Thermal studies:

The pure solid drug substance was spread to about 1mm thickness in petri dish exposed to dry heat at 50 0C for 48 hour in hot air oven. Then, powder equivalent to 25 mg dissolved in 25.0 mL of distilled water. Further dilutions were made in mobile phase to obtain appropriate concentration of Famciclovir.

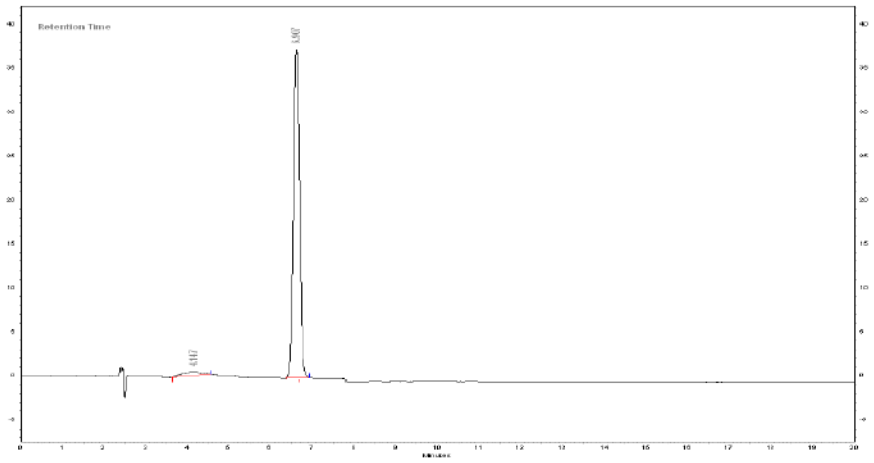

Figure 13: Result of Dry heat (500 C, 24hr.) of Famciclovir.

Photo degradation studies:

The pure solid drug substance was spread to about 1mm thickness in petri dish exposed to sunlight for 12 hours and then powder equivalent to 25 mg dissolved in 25.0 mL of distilled water. Further dilutions were made in mobile phase to obtain appropriate concentration of Famciclovir.

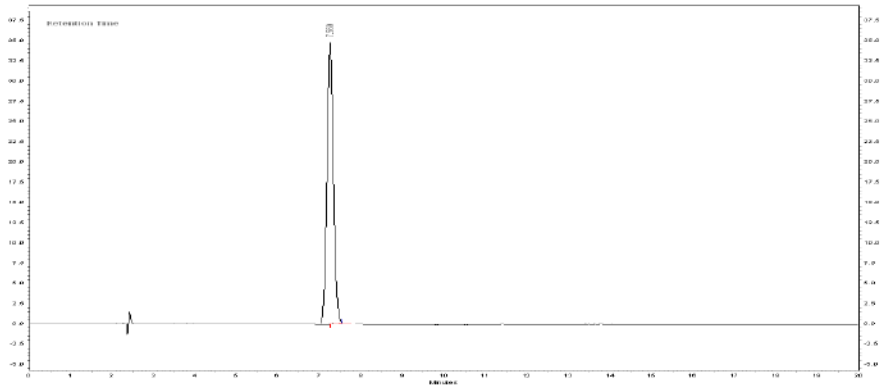

Figure 14: Result of Photo degradation (Sunlight exposure, 12 hr) of Famciclovir.

The results of the stress studies indicate the specificity and stability indicating ability of the method developed. Famciclovir was depredated in 1M HCl and 0.1M NaOH when kept at room temperature for 3 hr. The neutralization of degraded sample is important step in analysis. The degraded products have the good resolution from the Famciclovir main peak. The results of forced degradation studies are given in Table 15. 20, 25.

Table no 15: Results of forced degradation studies:

Analyte (Famciclovir)	Retention time of Famciclovir (min.)	Retention time of Degradation Products (min)
Normal condition	3.15 (100%)	-
Acidic hydrolysis 1M HCL, 3 hr, RT	7.11 (7.77%)	2.56 (56.27%)
Acidic hydrolysis 1M HCL, 3 hr, RT	2.15 (100%)	2.587 (100%)
Neutral hydrolysis, 24 hr reflux at 80^0C	7.52 (86.63%)	2.63 (0.50%)
Oxidative condition 30% H2O2, RT, 48 hr	6.91 (89.46 %)	2.41 (7.65%)
Dry heat studies 50^0C, 48 hr	6.90 (96.22%)	4.14 (3.78%)
Photo degradation study Sunlight, 12 hr.	7.56 (100%)	-

DISCUSSIONS:

A stability-indicating assay for Famciclovir was developed and validated using UV and HPLC methods for pharmaceutical dosage forms. The instrumentation included an Agilent Technologies 1100 series HPLC system with a quaternary pump, diode array detector, and Rheodyne injector, managed by EZ Chrome Elite software.

Chromatographic Conditions:

Column- Eclipse Plus (250 x 4.6 mm, 5 micron), Detection- 312 nm, Mobile Phase- Acetonitrile, methanol, glacial acetic acid (50:20:30 v/v/v), Flow Rate-1 ml/min (filtered through a 0.42 micron Nylon filter)

- Identity Confirmation: UV-visible wavelength scan and FT-IR spectra verified Famciclovir identity.
- UV-Visible Spectra: Absorption recorded at 312 nm using methanol as the solvent.
- Calibration Curve: Showed strong linearity (R^2 = 0.9700) across 0.0 to 60.0 µg/ml concentrations.
- Method Validation: Demonstrated satisfactory linearity, accuracy, precision, and robustness.
- -System Suitability Testing: Confirmed adequate performance with acceptable retention time and peak symmetry.

- Assay Method: High accuracy and precision in analyzing Famciclovir from API and formulations over three days.
- -Forced Degradation Studies: Confirmed stability-indicating capability under stress conditions like acid/base hydrolysis, oxidation, and thermal/photo degradation. Overall, the methods developed are robust and specific for quantitatively determining Famciclovir in pharmaceutical formulations, ensuring reliable quality control in drug manufacturing.

CONCLUSION:

A stability-indicating assay for Famciclovir using UV and HPLC methods was developed and validated, yielding reliable results. The Agilent 1100 series HPLC system provided accurate data. Chromatographic conditions, including an Eclipse Plus column and specific mobile phase, ensured efficient separation. Identity confirmation was achieved using UV-visible and FT-IR spectra. The calibration curve showed excellent linearity (R^2 = 0.999). Method development and validation demonstrated satisfactory linearity, accuracy, precision, and robustness. Forced degradation studies under various stress conditions confirmed the method's stability-indicating capability, making it suitable for routine quality control of Famciclovir in pharmaceutical formulations.

CONFLICT OF INTEREST:

Authors don't have any conflict of interest

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