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A Simple Stability Indicating Method Development and Validation of Famciclovir in Bulk drug by RP-HPLC

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Abstract

A simple, reliable, sensitive and isocratic stability indicating reversed phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the determination of assay of Famciclovir in Famciclovir drug substance. The paper describes method development, optimization and validation of an isocratic HPLC method for the assay of Famciclovir. The separation was achieved on Zorbax SB C8, 250mm×4.6mm, 5µm particle diameter column. The mobile phase consisted of phosphate buffer (pH: 4.0±0.05 with dilute orthophosphoric acid) and Acetonitrile 65:35 (v/v)); with flow rate of 1.0 mL min-1 at ambient temperature. The analyte was monitored by photodiode array detector at 220 nm. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolysis, thermal and humidity degradation. The peak purity was determined by PDA detector using waters empower pro software. A linear response was observed over the concentration range from 20 - 30 µg mL-1 with correlation coefficient value 0.9999. The average recovery is 99.9%. The relative standard deviation (R.S.D) for intra-day and inter-day was 0.2% and method is robust in all varied conditions. The sample solution was stable for 24 hours at ambient temperature. The results proves that method was suitable for the determination of assay of Famciclovir and successfully applied for routine analysis of Famciclovir drug substance.

Keywords: Famciclovir, Assay method, HPLC, Stability indicating method.

Contents

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1. Introduction

According to an FDA guidance document, a stability-indicating method is "a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability-indicating method accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities."[1]. Chemically Famciclovir is 2-Amino-9-[4-acetoxy-3-(acetoxymethyl)but-1-yl]purine. The molecular formula is C14H19N5O4 and molecular weight is 321.33. Famciclovir, a synthetic acyclic guanine derivative and a prodrug has no antiviral activity, which after oral administration, is rapidly metabolized to highly bioavailable antiviral compound Penciclovir. Penciclovir is active in vitro against the herpes virus's herpes simplex types 1, 2 (HSV-1 and HSV-2), varicella zoster virus (VZV), Epstein-Barr virus, and hepatitis B. Famciclovir is used an effective treatment of immunocompetent patients with acute herpes zoster (shingles) caused by VZV [2-3]. Famciclovir is also used for the treatment of ophthalmic zoster [4]. Famciclovir belongs to a class of drugs called nucleoside analogs that mimic one of the building blocks of DNA. It stops the spread of herpes virus in the body by preventing the replication of viral DNA that is necessary for viruses to multiply [5]. Famciclovir is available 125mg, 250mg and 500mg tablets for oral administration and marketed under the brand name Famvir [6]. In literature, few analytical methods have been reported for the quantification of impurities and assay of Famciclovir.

In literature, few analytical methods have been reported for the quantification of impurities and assay of famciclovir [7-16]. An ion pair RP-HPLC method development, validation and stability indicating assay for famciclovir [7]. Stability indicating LC method was developed and validated for the determination of famciclovir in bulk drug and pharmaceutical dosage form [7].UV- Spectrophotometric determination of Famciclovir [9]. RP-HPLC method developed for the estimation of famciclovir in tablet dosage form [10]. Development and validation of a stability-indicating RP-LC method for the determination of purity of famciclovir in presence of its impurities and degradation products, this method is also suitable for the assay of famciclovir monitored at 215 nm [11].

Development and validation of spectrophotometric method for the determination of famciclovir in its dosage forms based on redox followed by complex formation with potassium ferricynide-Fe(III) reagent and oxidation followed by complex formation with 2,2-Bipyridyl-Fe(III) to form bluish green colored chromogen exhibiting absorption maximum at 500 nm. Validated spectrophotometric estimation of famciclovir in tablet dosage form based on the condensation reaction of famciclovir with carbonyl reagent such as *p*-dimethylaminocinnamaldehyde (PDCA) in acidic condition to form orange red colored chromogen with absorption maxima at 510 nm [13]. Development and validation of RP-HPLC method for the determination of famciclovir in pharmaceutical formulation using an experimental design [14]. Validated spectrophotometric estimation of famciclovir in tablet dosage form based on the condensation reaction of famciclovir with carbonyl reagent such as *p*-dimethyl amino cinnam aldehyde (PDAB) and vanillin in acidic condition to form orange yellow colored chromogen with absorption maxima at 480 and 470 nm respectively [15]. Spectrophotometric estimation of antiviral drugs (valacyclovir and famciclovir) in bulk and pharmaceutical dosage forms based on extraction with Tpooo analytical reagent [16]. Subsequently, an alternative simple, sensitive RP-HPLC method with photodiode array detector was developed and optimized to determine the assay of famciclovir in famciclovir drug substance.

2. Materials and Methods

Reagents:

HPLC grade Acetonitrile (HPLC Grade, Merck), Potassium dihydrogen orthophosphate (AR, Rankem), Hydrochloric Acid (AR, Rankem) Sodium hydroxide (AR, Rankem), Hydrogen peroxide (AR, Rankem), Ortho phosphoric acid(AR,Rankem),Water (Milli Q water),. Famciclovir pure drug substance, and known related substances of fmaciclovir such as 4-(Dimethylamino)pyridine [DMAP], 9-[4-Hydroxy-3-(hydroxymethyl)but-1-yl]guanine [Penciclovir], 2-Amino-9-(4-hydroxy-3-hydroxymethybut-1-yl)purine [Desacetyl famciclovir (or) 6-Deoxy Penciclovir], 2-Amino-9-[4-acetoxy-3-(hydroxymethyl)but-1-yl]purine [Monoacetyl famciclovir], 2-Amino-7-[4-acetoxy-3-(acetoxymethyl)but-1-yl]purine [N-7 Isomer of famciclovir], 2-N-Acetyl-9-[4-acetoxy-3-(acetoxymethyl)but-1-yl]purine [N-Acetyl famciclovir], 2-Amino-9-[4-acetoxy-3-(chloromethyl)but-1-yl]purine [Chloro impurity of famciclovir], 2-Amino-9-[4-acetoxy- 3-(propionyloxy methyl)but-1-yl]purine [Monopropionyl analog of famciclovir], 2-Amino-9-[4-acetoxy-3-(acetoxymethyl)but-1-yl]-6-chloro purine[6-Chloro famciclovir], 9-[4-Acetoxy-3-acetoxymethylbut-1-yl]-6-n-(4-acetoxy-3 acetoxymethylbut-1-yl]-2,6-diamino purine[6-Amino derevative of famciclovir] was kindly supplied by Aurobindo pharma Limited, India.

Instrumentation:

A liquid chromatography system with Waters 2695 separations module and 2996 Diode array detector (Waters alliance or equivalent)equipped with an injection valve (Rheodyne), & PDA detector. The HPLC system was well equipped with Empower 2 software for data processing. Other instruments like Sartorius Analytical Balance,

Metrohm pH Meter and Biotechnics sonicator were used in sample and standard preparations and for forced degradation studies.

Methodology

Chromatographic conditions:

The analytical column used was Zorbax SB C8,5µ (250mm×4.6mm).The mobile phase was potassium dihydrogen ortho phosphate, adjusted to pH 4.0±0.05 with dilute ortho phosphoric acid and Acetonitrile in the ratio of 65:35v/v..It has a flow rate of 1.0mL/min, injection volume of 10µL with ambient column oven temperature with isocratic elution& UV detection at 220nm & a run time of 15 min.

Standard, sample, mobile phase and diluent preparation:

Diluent: water is used as diluents.

Preparation of phosphate buffer p^H 4.0:

Dissolve 2.72g of potassium dihydrogen orthophosphate in 1000ml of water. Adjust p^H to 4.0±0.05 with dilute orthophosphoric acid. Filter through 0.45µ or finer porosity membrane filter.

Dilute orthophosphoric acid: Dilute 5.0ml of orthophosphoric acid to 50ml of water.

Preparation of mobile phase: Prepare a degassed mixture of phosphate buffer p^H 4.0 and Acetonitrile in the ratio of 65:35v/v.

Preparation of standard solution:

Accurately weigh and transfer about 50mg of Famciclovir working standard into a clean, dry 100ml volumetric flask, add 70ml of diluents and sonicate to dissolve. Make up to volume with diluent. Dilute 5ml of this solution to 100ml with diluents. Filter through 0.45µ or fine porosity membrane filter.

Preparation of sample solution:

Accurately weigh and transfer about 50mg of sample into a clean, dry 100ml volumetric flask, add 70ml of diluents and sonicate to dissolve. Make up to volume with diluents. Diluents 5ml of this solution to 100ml with diluents. Filter through 0.45µ or fine porosity filter.

3. Results and Discussion

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.Specificity was demonstrated by injecting a blank, placebo and standard solution. No interference was seen at the retention time of analyte. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolysis, thermal and humidity degradation and found that there was no interference observed for Famciclovir peak. Purity angle is less than purity threshold for all the stress conditions. The results are tabulated in Table No.:1. Figures 4-9 represents different stress conditions.

Table 1: Evaluation of forced degradation studies

Degradation mechanism	Degradation condition	Assay (%w/w)	% Degradation	Peak purity	
				Purity angle	Purity threshold
-	Undegraded	98.6	-	0.060	0.256
Acid degradation	5M HCl/ RT/ 10min.	87.7	11.1	0.065	0.262
Base degradation	0.05M NaOH/Initial	90.2	8.5	0.057	0.247
Peroxide degradation	30%/H_2O_2/85 ºc/ 30min.	79.9	19.0	0.054	0.252
Thermal degradation	80ºC/ 120 Hours	99.3	Nil	0.068	0.254
Photolytic degradation	10K Lux/ 120 hours	100.5	Nil	0.071	0.258
Humidity degradation	92%RH/25ºC / 120Hours	99.4	Nil	0.064	0.261

System suitability Testing:

System suitability testing is used to verify that the reproducibility of the system is adequate for the analysis to be performed. System suitability is done by preparing and injecting the standard solution 5 times and calculating its RSD. Other parameters like tailing and theoretical plates should also be taken in to consideration. Results are tabulated in Table No.:3

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample[11]. The linearity of the test method

was performed by plotting a graph between concentration of the test solution on X-axis and response of the corresponding solutions on Y-axis from 80% to 120% of test concentration and calculated the correlation coefficient, it was found to be 0.999.The results are tabulated in Table No.:2 and the graphs are represented as Fig No:10.

Table 2: Statistical data of linearity

Statistical analysis	
Slope	42.790
Intercept	5.183
%Y-Intercept	0.5
Residual sum of squares	0.565
Correlation coefficient	1.0000

Solution stability

The sample solution was prepared as per test methodology. The stability of sample solution was tested by recording the chromatograms freshly prepared and at different intervals with the gap of every one hour up to 24 hours by keeping, sample cooler temperature at 25°C. The % difference in the peak areas of famciclovir from freshly to different time interval was found 0.0. From the results, it is concluded that sample solution was stable for 24 hours at ambient temperature (25°C).

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The recovery study for the assay method was evaluated in triplicate at three different concentration levels ranging form 80% to 120% i.e 80%, 100% and 120% of the test concentration (25.0 µg mL-1). These samples were prepared as per test method, analyzed in triplicate and the percentage recoveries were calculated. The average recovery values ranged from 99.7% - 100.0% and the average recovery of three levels (nine determinations) was 99.8%.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Method precision:

Determined the precision of the test method by preparing & injecting 6 test solutions of Famciclovir drug substance in to the chromatograph and recorded the results. The average % assay was found to be 99.7with % RSD of 0.3. The results are tabulated in Table No.:5

Intermediate precision:

Performed the assay of Famciclovir drug substance by following the same procedure as that of Method precision but on a different day, different column and by a different analyst. The average % assay was found to be 99.6%with % RSD of 0.30.Overall RSD when compared with Method precision is 0.3. The results are tabulated in Table No.:5

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [11].Robustness was performed by injecting the Famciclovir standard solution in to the HPLC by altering the Flow rate, wavelength, Column oven temperature and also by changing the pH of the buffer & composition of the organic solvent from the normal chromatographic conditions. The results are tabulated in Table No.:6

Calculation:

%Assay:

$$\frac{At}{As} \times \frac{Ds}{Dt} \times P \times \frac{100}{100-L}$$

Where

A_T = Average area counts of sample solution
D_T = Dilution factor for the sample solution (weight/dilution)
P = Percentage potency of Famciclovir working standard used
A_S = Average area counts of standard solution
D_S = Dilution factor for the standard solution (weight/dilution)
L = Loss on drying of sample(%w/w)

Table 3: Evaluation of forced degradation studies

Degradation mechanism	**Degradation condition**	**Assay (%w/w)**	**% Degradation**	**Peak purity**	
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Photolytic degradation	10K Lux/ 120 hours	100.5	Nil	0.071	0.258
Humidity degradation	92%RH/25ºC/ 120Hours	99.4	Nil	0.064	0.261

Table 4

% Concentration	**Concentration (µg/ml)**	**Average area**	**Statistical analysis**	
80	19.96	859.594	Slope	42.790
90	22.45	965.165	Intercept	5.183
100	24.95	1072.797	%Y-Intercept	0.5
110	27.44	1179.924	Residual sum of squares	0.565
120	29.94	1286.017	Correlation coefficient	1.0000

Table 5: Evaluation of system suitability

Famciclovir System Suitability									
Injection No.	**1**	**2**	**3**	**4**	**5**	**Mean**	**STDEV**	**RSD**	***Limits***
Standard Area:	1144673	1144698	1155834	1143369	1144282	1146571	5206	0.5	RSD NMT 1.0%

Table 6: Evaluation of stability of sample solution

Room temperature (~25ºC)		
Time in hours	**Area**	**% Diference**
Initial	1089.765	-
1	1087.324	0.2
2	1089.702	0.0
3	1090.003	0.0
4	1088.998	0.1
5	1088.064	0.2
6	1089.916	0.0
7	101.484	0.2
8	1089.28	0.0
9	1090.377	0.1
10	1088.418	0.1
11	1092.06	0.3
12	1092.482	0.2

Table 7: Evaluation of precision

Precision (System precision, Method precision and Ruggedness)				
Injection Identification	**System precision Area (µV*sec)**	**Method precision Assay (%w/w)**	**Ruggedness Assay (%w/w)**	
			SET-I	**SET-II**
1	1074500	99.8	99.8	99.2
2	1074572	99.1	99.1	99.8
3	1073451	99.8	99.8	99.5
4	1075028	99.7	99.7	99.3
5	1069949	99.7	99.7	99.1
	1070674	99.9	99.9	99.7
Mean		99.7	99.6	
SD		0.29	0.30	
%RSD		0.3	0.3	

Table 8: Evaluation of robustness

Parameter	**Variation**	**System suitability**		
		Theoretical plates	**Asymmetry**	**% RSD**
STP	-	11912	1.0	0.3
Flow rate	-10%	12729	1.0	0.2
	+10%	11157	1.0	0.1
Wavelength	-5nm	8362	1.2	0.2
	+5nm	8367	1.2	0.2
% organic phase	-2% absolute	12262	1.0	0.2
	+2% absolute	11578	1.0	0.3
Column oven temperature	-5ºC	11705	1.0	0.3
	+5ºC	12322	1.0	0.1
p^H	-0.2 units	8154	1.1	0.5
	+0.2 units	8318	1.1	0.4

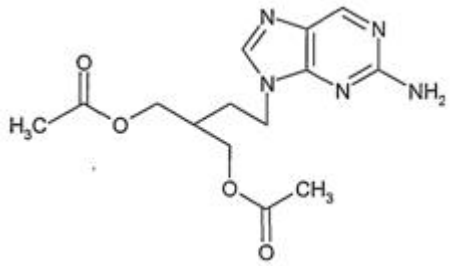

Figure 1: Famciclovr

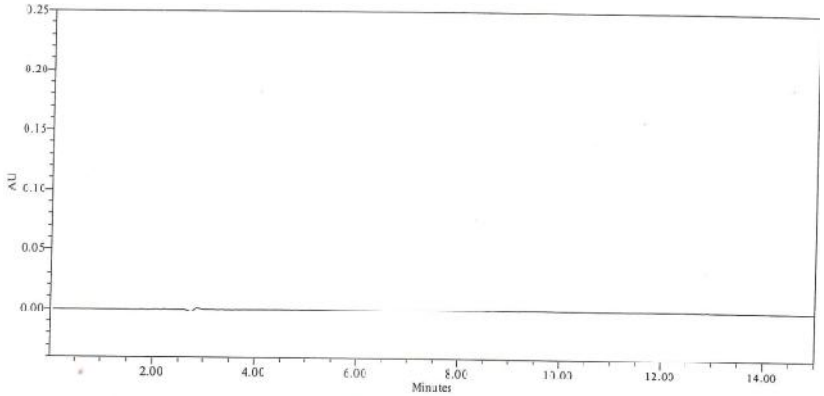

Figure 2: Blank-Diluent

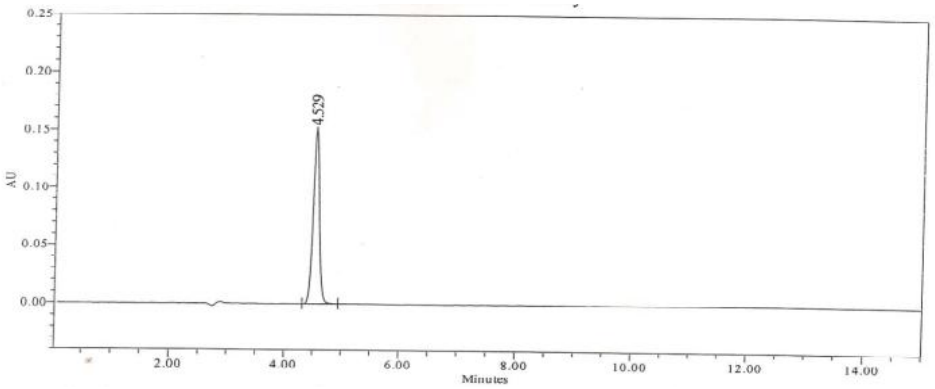

Figure 3: Standard

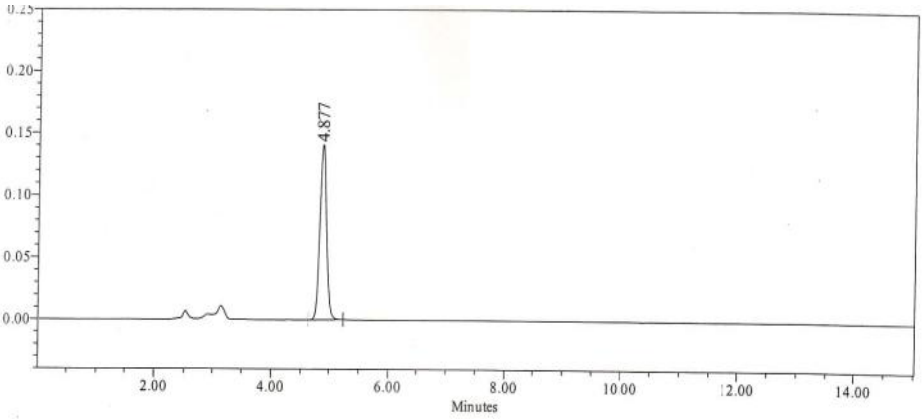

Figure 4: Acid Stressed Sample

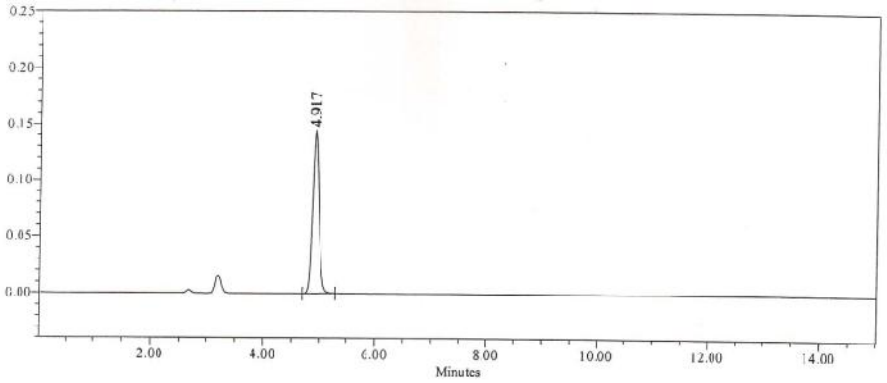

Figure 5: Alkali Stressed Sample in

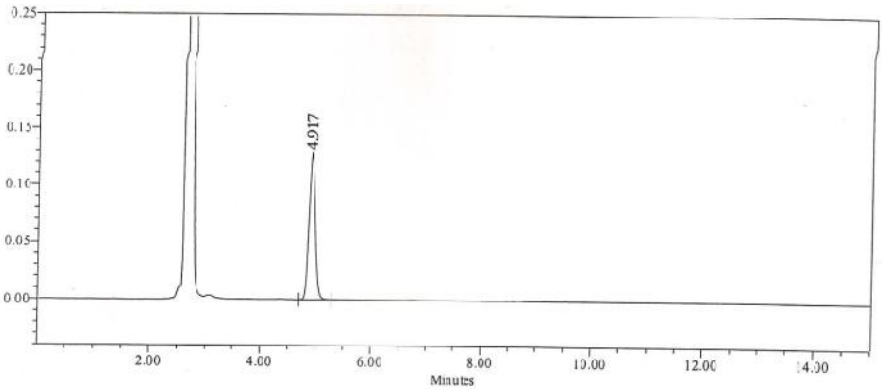

Figure 6: Peroxide Stressed Sample

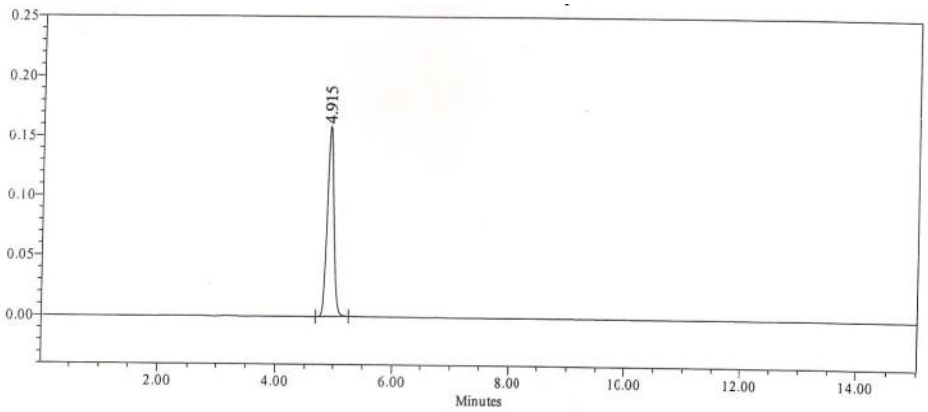

Figure 7: Water Stressed Sample

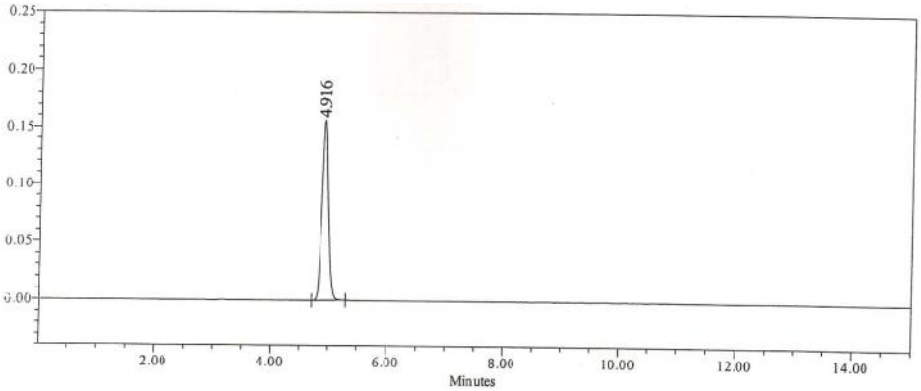

Figure 8: Heat Stressed Sample

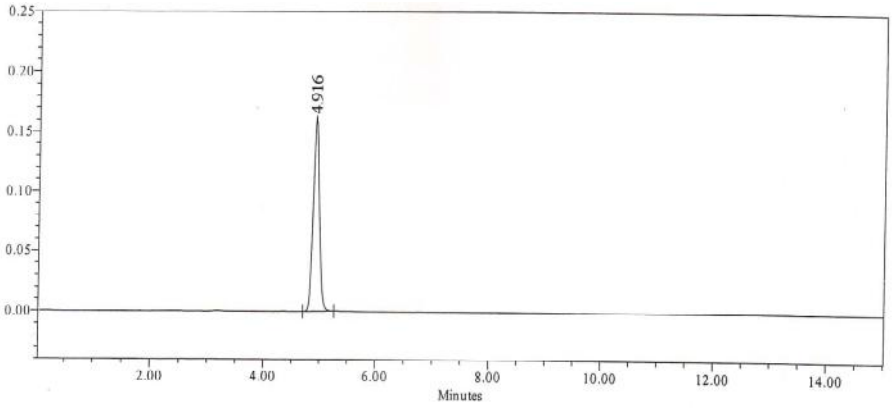

Figure 9: UV Stressed Sample

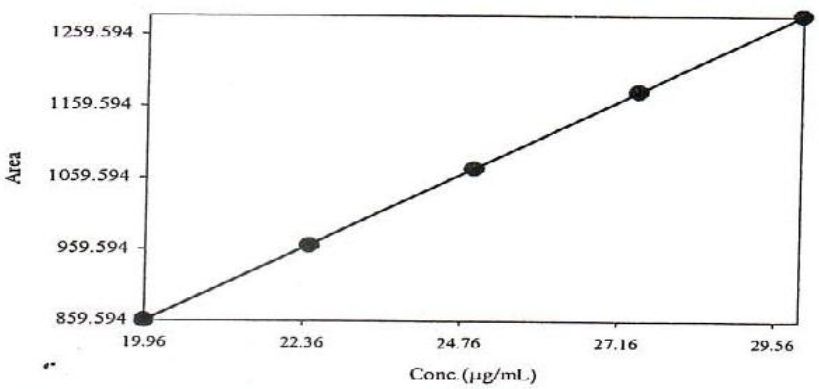

Figure 10: Linearity

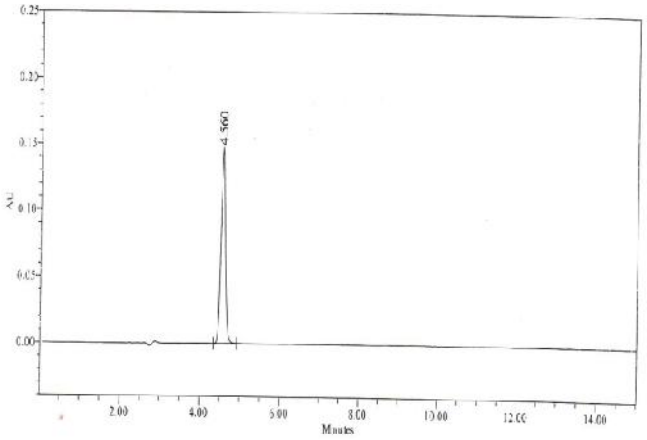

Figure 11: Control sample

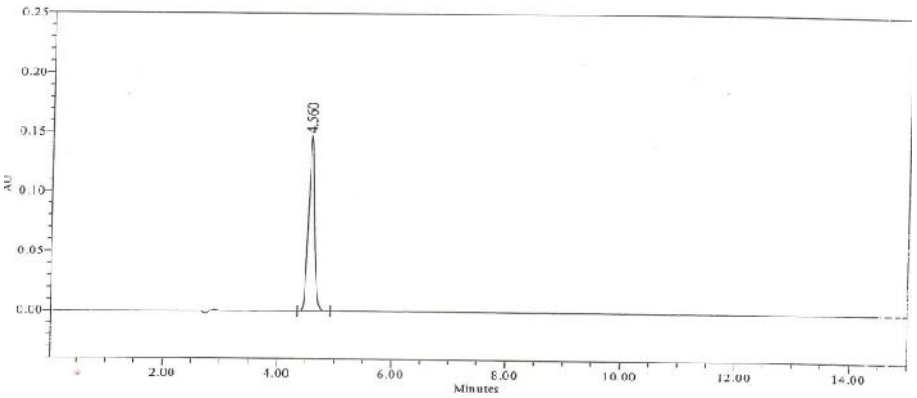

Figure 12: Test sample

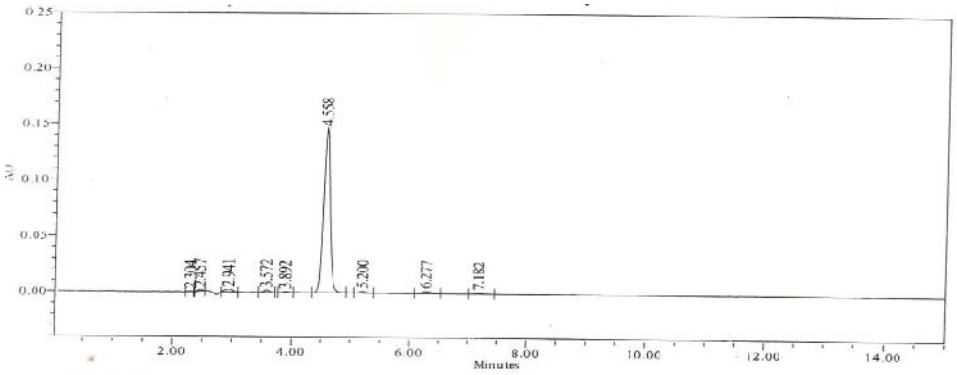

Figure 13: Spiked Sample

4. Conclusion

The reported HPLC method was proved to be simple, rapid with a runtime of 4 min & reproducible. The validation data indicates good specificity, precision, accuracy & reliability of the method. The developed method has many advantages like isocratic mode of elution, easy sample preparation, short run time and can be used for routine quality control analysis of Famciclovir drug.

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