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Simple and reliable stability indicating RP-HPLC method for the determination of assay of famciclovir in Famciclovir drug substance

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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 Symmetry C_{18}, 150mm x 4.6mm, 5µm particle diameter column. The mobile phase consisted of phosphate buffer (0.02M, pH:5.0±0.05 with dilute ortho-phosphoric acid) and acetonitrile 80:20 (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; Development; Assay; Validation; Chromatography.

INTRODUCTION

Chemically famciclovir is 2-Amino-9-[4-acetoxy-3-(acetoxymethyl)but-1-yl]purine. The molecular formula is $C_{14}H_{19}N_5O_4$ and molecular weight is 321. Famciclovir, a synthetic acyclic guanine derivative and a prodrug has no antiviral activity, which after oral administration, is rapidly metabolised to highly bioavailable antiviral compound penciclovir. Penciclovir is active in vitro against the herpes viruses 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 [1-2]. Famciclovir is also used for the treatment of ophthalmic zoster [3]. 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 [4]. Famciclovir is available 125mg, 250mg and 500mg tablets for oral administration and marketed under the brand name Famvir [5]. In literature, few analytical methods have been reported for the quantification of impurities and assay of famciclovir [6-15]. An ion pair RP-HPLC method

development, validation and stability indicating assay for famciclovir [6]. 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 [8]. RP-HPLC method developed for the estimation of famciclovir in tablet dosage form [9]. 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 [10]. 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 [11]. 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 [12]. Development and validation of RP-HPLC method for the determination of famciclovir in pharmaceutical formulation using an experimental design [13]. Validated spectrophotometric estimation of famciclovir in tablet dosage form based on the condensation reaction of famciclovir with carbonyl reagent such as *p*-dimethylaminocinnamaldehyde (PDAB) and vanillin in acidic condition to form orange yellow colored chromogen with absorption maxima at 480 and 470 nm respectively [14]. Spectrophotometric estimation of antiviral drugs (valacyclovir and famciclovir) in bulk and pharmaceutical dosage forms based on extraction with Tpooo analytical reagent [15].

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.

MATERIALS AND METHODS

Chemicals, reagents and samples

The standard and samples of famciclovir and known related substances of famciclovir, 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 peniciclovir*], 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*] were procured from APL Research Centre-II (A division of Aurobindo Pharma Ltd., Hyderabad). Analytical reagent (AR grade) potassium dihydrogen orthophosphate, *o*-phosphoric acid (88 %w/w), hydrochloric acid, sodium hydroxide, hydrogen peroxide (30 %w/v), potassium hydroxide and HPLC grade acetonitrile, triethylamine were procured from E Merck India. Highly purified water obtained from Millipore purification system.

High performance liquid chromatography (HPLC)

Chromatographic separation was performed on HPLC, alliance 2695 separations module system equipped with 2996 photodiode array detector with Empower Pro data handling system was used [Waters Corporation, USA]. The mobile phase consisted of phosphate buffer (0.02M, pH:5.0±0.05 with dilute *ortho*-phosphoric acid) and acetonitrile 80:20 (v/v). To prepare the phosphate buffer (0.02M, pH:5.0), 2.72g of dibasic potassium dihydrogen orthophosphate salt was weighed and dissolved in 1000 mL of purified water. To this 2.0 mL of triethylamine added, mixed well then pH was adjusted to 5.0 ±0.05 with dilute *ortho*-phosphoric acid (Diluted 5.0 mL *ortho*-phosphoric acid to 10 mL with water) then filtered through the 0.45 µ porous membrane. The analysis was carried out on Symmetry C_{18}, 150 mm long, 4.6 mm i.d., 5 µm particle diameter column (Waters Corporation Ltd.,) at ambient temperature. The mobile phase was delivered in an isocratic mode at a flow rate of 1.0 mL min^{-1}. The injection volume was 10 µL. The acquisition time for the standard and sample was 10 min. The analyte was monitored with photodiode array detector at 220 nm. Water used for diluting all the solutions of famciclovir. The retention time of famciclovir is about 5 minutes. The column efficiency as determined from the famciclovir peak is not less than 5000 USP plate count and USP tailing for the same peak is not more than 2.0. Relative standard deviation for sum of the peak areas of famciclovir for five injections of the standard solution is not more than 1.0%.

Standard and sample solutions

Preparation of standard solution

Accurately weigh and transfer about 50 mg of famciclovir reference standard into a 100 mL clean, dry volumetric flask, add 70 mL of water and sonicate to dissolve. Make up to volume with water. Dilute 5 mL of this solution to 100 mL with water. Filter through 0.45 µ or finer porosity membrane filter.

Preparation sample solution

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

RESULTS AND DISCUSSION

Method development and optimization

The objective of this work is, to develop a simple and rapid method for the determination of assay of famciclovir in famciclovir drug substance by using HPLC system. Method development was initiated with famciclovir drug substance solubility study, based on that water was chosen as diluent for diluting all the solutions of famciclovir. From the molecular formula of famciclovir, it was observed that famciclovir is polar in nature, based on that non-polar symmetry C_{18} stationary phase column was selected for developing RP-HPLC method. Famciclovir solution pH was 6.3 (C=1 %w/v, in water, at 25°C), based on that buffer was chosen pH 5.0 (pH about ±2.0 with respect to observed pH 6.3). As there is chromophore present in famciclovir, there is possibility for UV-Visible detection, based on the colour absorbance experiment 220 nm was chosen for monitoring the response of famciclovir. Preliminary experiment was carried out by using Symmetry C_{18}, 150 mm x 4.6 mm, 5.0 µm particle diameter column with dibasic potassium dihydrogen ortho phosphate buffer (0.02M, pH:5.0) as mobile phase, delivered in an isocaratic mode with a flow rate of 1.0 mL min^{-1} at ambient temperature and analytes were monitored with PDA detector, no any peak was eluted upto 40 minutes. Elution of analyte was achieved**,** with the combination of phosphate buffer (0.02M, pH:5.0) and acetonitrile in the ratio of 70:30% (v/v). In this trial famciclovir peak was eluted at about 2.3 min and interfering with unknown peak eluted at about 2.0 min and also broad peak shapes were observed. For better resolution, trial was made with phosphate buffer (0.02M, pH:5.0) with acetonitrile in the ratio of 80:20% (v/v), peaks were well resolved from each other and famciclovir peak was eluted at about 5.0 min and unknown peak eluted at about 2.0 min. For better peak shapes, again trial was made with 1000 mL of phosphate buffer (0.02M), 2.0 mL of triethylamine added and then pH was adjusted to 5.0. This phosphate buffer with acetonitrile in the ratio of 80:20% (v/v), excellent peak shape was achieved.

Finally, satisfactory separation with better peak shape was achieved, on chromatographic conditions which have been mentioned in high performance liquid chromatography (HPLC), was used for validation study to evaluate its performance characteristics.

Method validation

The method was validated as per the ICH guidelines [16], in terms of specificity, forced degradation studies (stability indicating nature), linearity, stability of sample solution, robustness, precision (system precision, method precision and intermediate precision or ruggedness) and accuracy.

Specificity

For chromatographic methods, developing a separation involves demonstrating specificity, which is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. The response of the analyte in test mixtures containing the analyte and all potential sample components (synthesis intermediates, degradation products, process impurities) is compared with the response of a solution containing only the analyte. For specificity determination, diluent, all related substances of famciclovir solutions were prepared individually as per methodology and injected into HPLC to confirm the retention times. After that solutions of famciclovir drug substance prepared in triplicate (consided as control samples) and famciclovir drug substance spiked with all related substances prepared in triplicate (considered as spiked samples) as per methodology and injected into HPLC to confirm any co-elution with famciclovir peak from any of related substance peak and diluent. The peak homogeneity was verified for famciclovir peak in control sample and spiked sample using waters empower pro software and found to be pure (purity angle should be less than purity threshold). The specificity results are shown in Table 1 and an overlay chromatogram of control sample and spiked sample chromatograms is shown Fig. 1.

Table 1: Statistical data of Specificity

Sample	Purity Angle	Purity Threshold	Mean assay (%w/w)	% Difference
Control Sample	0.213	0.420	99.7	0.0
Spiked Sample	1.015	2.165	99.7	

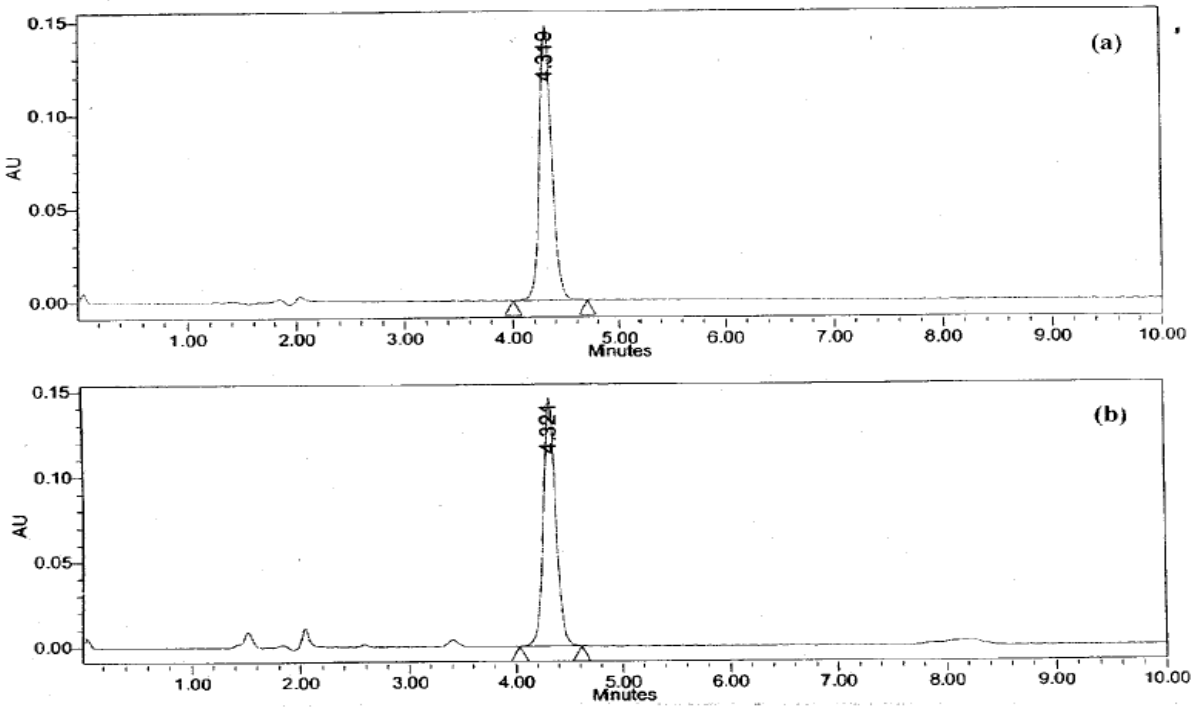

Fig. 1. A typical representative overlay chromatogram of (a) Control sample (b) Spiked sample

The stability indicating nature of the method was further evaluated by performing the forced degradation studies. As per International Conference on Harmonization (ICH), stress testing is to be carried out to identify the likely degradation products or to elucidate the inherent stability characteristics of the active substance [17]. 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. The stress study experiment results are shown in Table 2.

Linearity and Range

The linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Range is the interval between the upper and lower levels of analyte been demonstrated to be determined with accuracy and linearity. The range is normally expressed in the same units as the test results obtained by the method. The ICH guidelines specify a minimum of five concentration levels, along with certain minimum specified ranges. For assay method, linearity specified range is from 80% to 120% of the test concentration (25.0 µg mL^{-1}). Linearity was evaluated based on the residual standard deviation of a regression line and slope was adopted. Standard solutions were injected into HPLC chromatograph, five concentration levels form 20.0 µg mL^{-1} - 30.0 µg mL^{-1} (80% to 120% of test concentration). A plot of peak area (µV*sec) versus concentration (µg mL^{-1}) was drawn and data was subjected to statistical analysis using a linear-regression model. The statistical parameters slope, intercept, residual standard on deviation response and correlation coefficient values are calculated and shown in Table 3.

Table 2: Evaluation of forced degradation studies

Type of Degradation	Degradation Condition	Famciclovir assay (% w/w)	Degradation (% w/w)	Purity angle	Purity threshold
Undegraded sample	-	99.8	Nil	0.099	0.309
Acid degradation	5.0M HCL/Initial	25.1	74.8	-	-
	5.0M HCL/RT/5min	31.0	68.9	-	-
	5.0M HCL/RT/10min	30.6	69.3	-	-
	5.0M HCL/RT/20min	14.3	85.6	-	-
	0.5M HCL/Initial	82.8	17.0	-	-
	0.5M HCL/RT/5min	82.4	17.4	-	-
	0.5M HCL/RT/10min*	82.1	17.7	0.129	0.324
	0.5M HCL/RT/20min	73.6	26.2	-	-
	0.05M HCL/Initial	99.6	0.2	-	-
	0.05M HCL/RT/10min	98.1	1.7	-	-
	0.05M HCL/RT/20min	97.4	2.4	-	-
Alkaline degradation	5.0M NaOH/Initial	0.0	99.9	-	-
	0.05M NaOH/Initial*	77.5	22.3	0.200	0.333
	0.005M NaOH/Initial	98.0	1.8	-	-
	0.005M NaOH/RT/5min	69.2	30.6	-	-
	0.005M NaOH/RT/10min	62.6	37.2	-	-
	0.005M NaOH/RT/20min	48.1	51.8	-	-
Peroxide degradation	30% H_2O_2/Initial	99.9	-0.1	-	-
	30% H_2O_2/90°C/10min	89.8	10.0	-	-
	30% H_2O_2/90°C/20min*	74.8	25.0	0.616	1.438
	30% H_2O_2/90°C/30min	65.2	34.6	-	-
Thermal degradation	80°C/120 Hrs*	99.2	0.6	0.122	0.308
Photolytic degradation	10 K Lux/120 Hrs*	99.6	0.2	0.121	0.321
Humidity degradation	92% RH/25°C/120 Hrs*	99.5	0.3	0.127	0.351

** Upto 25%w/w, degradation is considered for reporting in all types of degradation conditions.*

Table 3: Statistical data of linearity

Statistical parameters	Famciclovir
Concentration range (µg mL^{-1})	20 - 30
Slope	43737
Intercept	-8471
Residual standard on deviation response	1555
Correlation coefficient	0.9999

Accuracy

Accuracy is the closeness of the test results obtained by the analytical method to the true value. Accuracy of the method was performed by recovery experiments using standard addition technique. Accuracy criteria for an assay method (FDA) is that the mean recovery will be 100 ± 2% at each concentration over the range of 80 - 120% of the test concentration. To document accuracy the ICH guideline on methodology recommends collecting data form a minimum of nine determinations over a minimum of three concentration levels covering the specified range. 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 99.9%. The completely validated accuracy results are shown in Table 4.

Table 4: Statistical data of Accuracy

Sample Identification	Famciclovir				
	Amount Added (mg)	Amout Found (mg)	Recovery (%)	Statistical analysis	
80% Level sample-1	40.26	40.21	99.9	Mean*	100.0
80% Level sample-2	40.32	40.34	100.0	SD*	0.1
80% Level sample-3	40.40	40.45	100.1	%RSD*	0.1
100% Level sample-1	50.03	49.99	99.9	Mean*	99.9
100% Level sample-2	50.07	49.97	99.8	SD*	0.10
100% Level sample-3	50.01	50.03	100.0	%RSD*	0.1
120% Level sample-1	60.54	60.61	100.1	Mean*	99.7
120% Level sample-2	60.29	60.13	99.7	SD*	0.40
120% Level sample-3	59.63	59.21	99.3	%RSD*	0.4
Overall statistical analysis	Mean[a]	99.9			
	SD[a]	0.25			
	%RSD[a]	0.3			

** : Average of three replicates*
a : Average of nine replicates

Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation (RSD) for a statistically significant number of samples. The performance of the method was evaluated with replicate injections of standard and sample solutions. Standard solution was analyzed six times for checking the performance of the HPLC system under the chromatographic conditions on the day tested (System precision). The relative standard deviation for famciclovir standard is 0.4%. Repeatability and reproducibility of the method was studied by analyzing six sample solutions separately. Repeatability was the intra-day variation (Method precision), demonstrated by preparing six sample solutions individually using a single batch of famciclovir drug substance as per methodology and assay was determined. The relative standard deviation for the assay of famciclovir is 0.2%. The intermediate precision was the inter-day variation (Ruggedness), was defined as the degree of reproducibility obtained by following the same procedure as mentioned for method precision experiment. The analysis of the same sample (which is used in the Method precision) under a variety of conditions using different system, column, with different analyst on different day by preparing new standard, new mobile phase and assay was determined. The relative standard deviation for the assay of famciclovir is 0.2%. The completely validated precision (System precision, Method precision and Ruggedness) results are shown in Table 5.

Table 5: Statistical data 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)
1	1075414	99.8	99.3
2	1084519	99.7	99.8
3	1078688	99.8	99.5
4	1085283	99.9	99.6
5	1082837	100.1	99.6
6	1079936	100.0	100.0
Mean	1081113	99.9	99.6
SD	3787	0.15	0.24
%RSD	0.4	0.2	0.2

Robustness

To assess the robustness of the method, experimental conditions were deliberately altered. The study was carried out with respect to pH ± 0.2, wavelength ± 5 nm, organic variation in mobile phase ± 2% and column flow ± 10%. In each robustness condition remaining chromatographic conditions are same as per test method. In each robustness condition, standard solution was prepared as per methodology and injected single time in HPLC system as system suitability and again standard solution was injected five replicates in HPLC system. From the system suitability it was observed that there is no much variation in retention time, USP plate count and USP tailing for famciclovir peak, obtained at different deliberately varied robustness conditions from the test method. Hence the test method is robust for all varied conditions. The completely robustness results are shown in Table 6.

Table 6: Statistical data of Robustness

Robustness condition	System suitability (Single Injection)			Standard solution (Five replicate injections)		
	Variation	USP plate count	USP tailing	Mean area (µV*sec)	SD	%RSD
As per test method	-	6571	1.0	1081348	4185	0.4
pH (± 0.2)	- 0.2	6501	1.0	1081340	1118	0.1
	+ 0.2	6343	1.1	1083199	1989	0.2
Wavelength (± 5 nm)	- 5 nm	6454	1.1	784103	1783	0.2
	+ 5 nm	6431	1.1	1011255	4142	0.4
% of Organic variation in mobile phase (± 2%)	- 2%	6805	1.1	1083905	2154	0.2
	+ 2%	5911	1.1	1082798	1283	0.1
Flow (± 10%)	- 10%	6594	1.1	1201947	2637	0.2
	+ 10%	6047	1.1	984546	2026	0.2

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).

CONCLUSION

A simple isocratic stability indicating reverse phase liquid chromatography (RP-HPLC) method was developed and validated for the determination of assay of famciclovir in famciclovir drug substance. The results of various validation parameters demonstrated that the method is specific, stability indicating, linear, precise, solution stability, robust and accurate. Hence the proposed method is simple, reliable and userfriendly, for the determination of assay of famciclovir in famciclovir drug substance.

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REFERENCES

[1] Perry CM, Wagstaff AJ, *Drugs.*, **1995**, 50(2), 396-415.
[2] Rashidi MR, Smith JA, Clarke SE, Beedham C, *Drug Metab Dispos.*, **1997**, 25(7), 805-13.
[3] Stephen Tyring, Reinhard Engst, Christine Corriveau, Nicole Robillard, Sylvie Trottier, Steven Van Slycken, Rachel A Crann, Leslie A Locke, Robin Saltzman, Alan G Palestine, *Br J Ophthalmol.*, **2001,** 85, 576- 581.
[4] http://www.medicinenet.com/famciclovir/article.htm
[5] http://www.rxlist.com/famvir-drug.htm
[6] K.V. Subrahmanyam, N. Gopal, *Pharmaceutical Reviews.*, **2007**, 5(2).
[7] Ch. B. V. Narasimha Raju, Gunanidhi Panda and G. Nageswara Rao, *Chromatographia.*, **2008**, 68, 837-841.
[8] K.V. Subrahmanyam, V.S. Saravanan, P. Mohan raj, N. Gopal, *Asian Journal of Chemistry.*, **2007**, 19, 4911-4913.
[9] V.S. Mannur, B. Shravan Kumar, V.S. Masthiholimath, *Int J Pharm Pharm Sci.*, **2011**, 3, 198-200.
[10] N V V S S Raman, K A Harikrishna, A V S S Prasad, K Ratnakar Reddy, K Ramakrishna, *Journal of Pharmaceutical and Biomedical Analysis.*, **2009**, 50(5), 797-802.
[11] Tulasamma P, Venkateswarlu P, *International Journal of ChemTech Research.*, **2011**, 3, 574-579.
[12] B. Anil Reddy, A. Srikar, *Journal of Pharmaceutical Science and Technology.*, **2009**, 1(1), 36-39.
[13] Srinivas Vishnumulaka, Narasimha Rao Medicherla, Allam Appa Rao, Gedela Srinubabu, *E-Journal of Chemistry.*, **2008**, 5, 58-67.
[14] C. Jose Gnana Babu, G. Vijaya Kumar, *International Journal of ChemTech Research.*, **2009**, 1, 1368-1371.
[15] P. Venkata Reddy, B. Sudha Rani, *E-Journal of Chemistry.*, **2006**, 3, 154-158.

[16] International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonized tripartite guideline, Validation of analytical procedures: Text and methodology Q2(R1), step 4 **2005**.
[17] International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonized tripartite guideline, Stability testing of new drug substances and products Q1A(R2), step 4 **2003**.