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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR FAMCICLOVIR

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ABSTRACT

A high-performance liquid chromatographic method for the determination of famciclovir, a highly active antiviral agent, was developed in the present work. The various parameters, such as linearity, precision, accuracy, specificity, robustness, limit of detection and limit of quantitation were studied according to International Conference on Harmonization guidelines. HPLC was carried out by using the reversed-phase technique on an RP-C18 column with a mobile phase composed of methanol and water (75 : 25 v/v), adjusted to pH 3.05 with orthophosphoric acid. The mobile phase was pumped at a flow rate of 1 mL/min and detection was made at 221 nm with UV dual absorbance detector. The proposed methods are highly sensitive, precise and accurate and therefore can be used for its intended purpose.

Key words: Famcivclovir, HPLC, Validation, Reverse phase, Harmonization.

INTRODUCTION

Famciclovir, an anti-viral agent (acyclic guanine derivative), chemically it is 2-[2-(2-amino-9H purin-9-yl) ethyl]-1, 3-propanediol diacetate. The structure of Famciclovir is shown in Fig. 1, having molecular formula $C_{14}H_{19}N_5O_4$ and molecular weight 321.3. It undergoes rapid biotransformation to the active antiviral compound penciclovir, which has inhibitory activity against herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and varicella zoster virus (VZV). In cells infected with HSV-1, HSV-2 or VZV, viral thymidine kinase phosphorylates penciclovir to a monophosphate form that, in turn, is converted to penciclovir triphosphate by cellular kinases. *In vitro* studies demonstrate that penciclovir triphosphate inhibits HSV-2 DNA polymerase competitively with deoxyguanosine

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triphosphate. Consequently, herpes viral DNA synthesis and, therefore, replication are selectively inhibited.

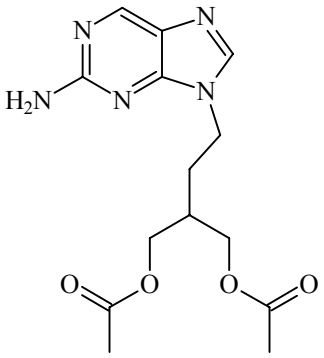

Fig. 1: Chemical structure of Famciclovir

Famciclovir is widely used in the antiviral therapy, it is important to develop and validate analytical methods for its determination in pharmaceutical dosage forms. Extensive literature survey revealed that the determination of the drug in pure and tablet dosage form is not official in any pharmacopoeia and there fore, requires much more investigation. The literature suggested and reported a few analytical methods till now reported for its quantitative estimation in plasma and urine by HPLC[1], spectrophotometric[2-6], electrophoretic techniques[7-8] and liquid chromatography[9-10] in biological fluids and pharmaceutical formulations. Famciclovir has also been reported to be quantified in combination by HPLC spectrophotographic method.

In the present investigation, an attempt has been made to develop simple, sensitive, specific, economic and an alternative HPLC method for the estimation of Famciclovir.

Material and methods

Instrumentation

Isocratic HPLC system (CYBER LAB 22 HPLC Model-LC-100 with Chrom work station Software) containing C_{18} (SHISEIDO 250 x 4.6 mm, 5 µm) column with UV detection.

Chemicals and reagents

Famciclovir was a gift from Hetero Drugs Ltd. (Hyderabad, India). All other chemicals and reagents were of analytical-grade and were purchased from Merck (Worli, Mumbai, India).

Chromatographic condition

Glasswares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. The mobile phase consisted of methanol and water in ratio 75:25%v/v. The contents of the mobile phase were filtered before use through a 0.45 μm membrane and degassed for 20 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 mL/min and the injection volume was 25 μL. The column temperature was maintained at ambient temperature. The eluents were monitored at 221 nm using UV lamp.

Preparation of standard stock solutions

Accurately weighed 10 mg of Famciclovir was transferred to separate 10 mL volumetric flask and dissolved in 100 mL mobile phase. The flasks were shaken and volume was made up to the mark with methanol to give solutions containing 1000 μg/mL Famciclovir. From this solution, 1 mL was transferred to volumetric flask of 10 mL capacity. Volume was made up to the mark to give a solution containing 100 μg/mL of Famciclovir.

RESULTS AND DISCUSSION

Calibration of drug

The standard calibration curve was constructed for Famciclovir different volumes of stock solutions of each were accurately transferred in to 10 mL volumetric flasks and diluted to mark to yield a concentration range of 10-35 μg/mL solutions of Famciclovir solutions. The calibration line was obtained by plotting the peak area against concentration of drug. The results were shown in Table 1 and Fig. 2.

Table 1: Calibration data of Famciclovir

Conc.	Peak area 1	Peak area 2	Average	SD	% RSD	
10	77413	77589	77501	124.4508	0.16058	Slope = 8946.6
15	133647	133543	133595	73.53911	0.055046	Intercept = -6316.5
20	174729	174824	174776.5	67.17514	0.038435	
25	219529	219318	219423.5	149.1995	0.067996	
30	256785	256880	256832.5	67.17514	0.026155	$R^2 = 0.9971$
35	307627	307891	307759	186.6762	0.060657	

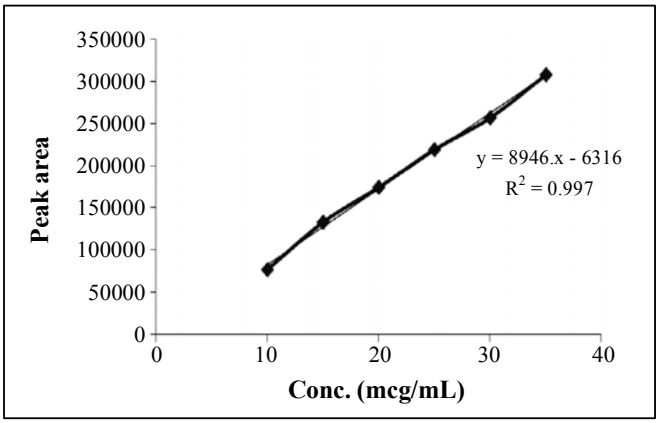

Fig. 2: Calibration curve of Famciclovir

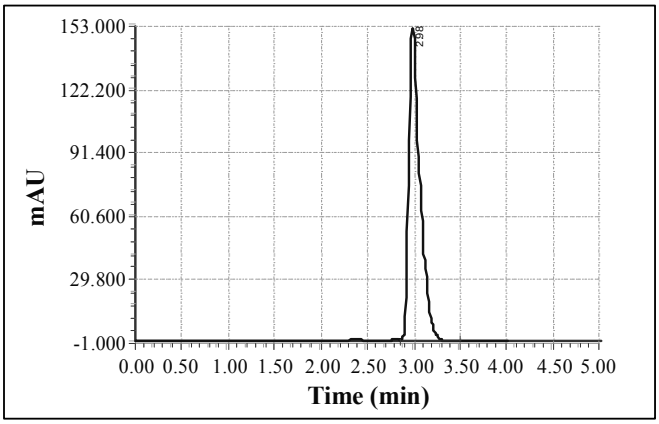

Fig. 2: Chromatogram Curve of Famciclovir

Method validation

Method was validated as per ICH guidelines[11,12] with respect to linearity, accuracy, precision, specificity, and robustness, limit of detection and limit of quantification. The precision was calculated by percentage relative standard deviation. The accuracy was expressed in terms of percent recovery of the know amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. Various validation parameters are perfomed[13].

System suitability

It is defined as tests to measure the method that can generate result of acceptable accuracy and precision. The system suitability was carried out after the method development

and validation have been completed. The system suitability was assessed by triplicate analyses of the drugs at a concentration of 20 µg mL^{-1} of Famciclovir for this, parameters like plate number, tailing factor, peak asymmetry of samples were measured, and shown in Table 2.

Table 2: System suitability

Injection (20 µg/mL)	No of theoretical plates	Tailing factor	Retention time (min)
1	2264.01	1.6	2.98
2	2249.505	2.24	
3	2127.67	2.11	
4	2180.925	2.025	
5	2278.96	1.83	
6	2208.17	1.855	
Average ± S.D	2218.207 ± 90.914	1.943333 ± 0.269	

Specificity

Specificity of the method was evaluated by injecting the blank injection, working standard samples into the chromatograph to check the co-elution, if any, at the retention time of 2.98 min the proposed method was specific for the detection of Famciclovir. There was no peaks at the retention time of Famciclovir.

Linearity

It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope or regression line. Appropriate volume of aliquot from Famciclovir standard stock solution was transferred to volumetric flask of 10 mL capacity. The volumes were adjusted to the mark with mobile phase to give a solutions containing 10-35 µg/mL Famciclovir. The slope, Y-intercept and correlation coefficient were calculated. The regression line relating standard concentrations of drug using regression analysis was calculated. The calibration curves were linear in the studied range and equations of the regression analysis were obtained. The mean and correlation %RSD (N = 6) were calculated. The represented data was shown in below Fig. 2 and Table 1.

Precision

It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits (Table 3).

Table 3: Precision studies of Famciclovir (Intra day)

Conc.	Retention time (min)	Peak area	% RSD
10 ug/mL	2.98	75003 ± 2667.555	0.035
20 ug/mL		176372.3 ± 2403.395	0.013
30 ug/mL		251371.2 ± 14259.13	0.056

Values are expressed in % RSD of the three replicate samples

Intermediate precision/ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method. Precision was performed on different day by using same column of same dimensions. The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits. (Table 3 & 4) Acceptance Criteria: The % RSD for all the five standard injections results should not be more than 2%.

Table 4: Ruggedness

Conc. (µg/mL)	Day	Peak area	% RSD
10	1	76001 ± 1996.87	2.627
20		174776.5 ± 67.175	0.038
30		253284 ± 3870.703	1.528
10	2	68572 ± 923.481	0.013
20		177385 ± 3159.353	0.017
30		257718 ± 1763.524	0.006

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Conc. (µg/mL)	Day	Peak area	% RSD
10	3	73085 ± 1992.627	2.726
20		177678 ± 1516.037	0.853
30		253284 ± 3870.703	1.528

Values are expressed in % RSD of the three replicate samples

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 value found. Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified placebo preparation at 3 different concentration levels 50, 100 and 150%, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured. Results of assay and recovery were presented in the Table 5.

Table 5: Accuracy of the Famciclovir

S. No.	Conc of sample (µg)	Avg conc of sample	% Recovery
1	20	20.38263 ± 1.420774	101.913
2	30	29.74894 ± 0.002819	99.163
3	40	40.10939 ± 0.124412	100.273

Values are expressed in % recovery of the three replicate samples

Robustness

As part of the Robustness, deliberate change in the Flow rate was made to evaluate the impact on the method

The flow rate was varied at 0.8 mL/min to 1.2 mL/min

The standard solution of Famciclovir was prepared and analysed using the varied flow rates along with method developed flow rate. On evaluation of the above results, it was concluded that the variation in flow rate does not affected the method significantly. Hence it was indicated that the method was robust even by change in the flow rate (Table 6).

Table 6: Peak results for Robustness for Famciclovir

Flow rate	Conc (µg/mL)	Average area	(% RSD)
0.8 mL/min	10	80005.45	2.359
	20	166000.4	1.106
	30	246835	4.816
1 mL/min	10	76001.05	2.627
	20	171027	2.648
	30	253483.3	1.895
1.2 mL/min	10	78479.2	2.108
	20	167713.1	4.716
	30	252865.9	1.117

Values are expressed in % RSD of the three replicate samples

LOD and LOQ

LOD and LOQ were calculated from the formula 3.3 x (σ/S) and 10 x (σ/S), respectively where, σ is standard deviation of intercept and S is the mean of slope. The LOD and LOQ can also be determined by S/N. The value for LOD should be 3-5 whilst for LOQ 10-15. Results of LOQ and LOD studies are shown in Table 7.

Table 7: LOQ, LOD data for Famciclovir

Component	LOD (µg/mL)	LOQ (µg/mL)
Famciclovir	0.041	0.139

Table 8: Summary of validation parameters

S. No.	Parameters	Results
1	Limit of linearity	10-35 µg/mL
2	Regression equation	y = 8946.6x – 6316.5
	Slope	8946.6
	Intercept	6316.5
	Correlation coefficient	0.997

Cont…

S. No.	Parameters	Results
3	Accuracy	99-101%
4	Precision:	
	Intraday	(0.01-2.72)
	Interday	(0.006-2.726)
5	LOD (µg/mL)	0.041
6	LOQ (µg/mL)	0.139

CONCLUSION

A new, sensitive, and specific assay has been established for analysis of Famciclovir in bulk drug sample. The method validation results indicate the method is sufficiently accurate and precise for routine analysis of the drug. In addition to assay it may be used to detect related substance or other impurities, which are formed during storage conditions and the analyte of interest could be estimated without any interferences. The use of C18 column in the present work has shown better elution of analytes with good resolution, improved plate count, capacity factor, reduced tailing. So the C18 column can be used to achieve high specificity in shorter time of analysis of Famciclovir and in injectables as per ICH Q2 (R1) guidelines.

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