Current Pharma Research
ISSN: 2230-7842
CPR 1(3), 2011, 227-231.

Development and validation of an RP-HPLC method for estimation of famciclovir in bulk and pharmaceutical formulations.

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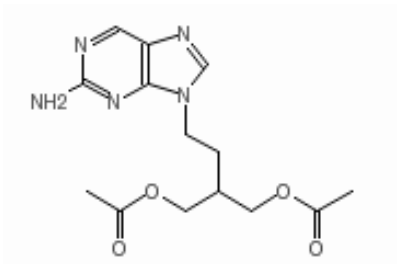
Abstract
A reverse phase HPLC method is described for the determination of famciclovir in bulk and tablets. Chromatography was carried on a C_{18} column using a mixture of acetonitrile, and glacial acetic acid (70:30 v/v) as the mobile phase at a flow rate of 1 ml min^{-1} with detection at 310 nm. The retention time of the drug was 3.56 min. The detector response was linear in the concentration of 1-40 µg/ml. The limit of detection and limit of quantification was 0.335 and 1.014 µg ml^{-1} respectively. The method was validated by determining its sensitivity, linearity, accuracy and precision. The proposed method is simple, economical, fast, accurate and precise and hence can be applied for routine quality control of famciclovir in bulk and tablets.

Key Words
Famciclovir, RP-HPLC, validation.

Introduction
Chemically, famciclovir (FCV), known as 2-[2-(2-amino-9H-purin-9-yl) ethyl]-1, 3-propanediol diacetate (Fig. 1) is a novel antiviral drug, which is highly efficient in treatment of acute uncomplicated herpes zoster.



It was reported that FCV dosed at 250 mg three times daily for 7 days was effective as 800 mg acyclovir dosed five times daily for 7 days in the treatment of the acute signs and symptoms of herpes zoster.[1] This drug is also used for the treatment of the ophthalmic zoster.[2] FCV is a synthetic guanine derivative, which is metabolized to penciclovir having potent antiviral activity. Penciclovir is active against herpes simplex virus type 2, vericella zoster virus I, Epstein-Barr virus and hepatitis B.[3] Like acyclovir, penciclovir is selectively phosphorylated in virus-infected cells to a monophosphate ester by thymidine kinase, followed by further phosphorylation to triphosphate ester, which inhibits virus DNA polymerases.[4] Compared with acyclovir, penciclovir administration leads to higher triphosphate ester concentrations in virus-infected cells and consequently its antiviral activity persists for a longer time after removal of the compound.[4-6] FCV is absorbed rapidly and extensively after oral administration, and total systemic availability of penciclovir is 77%,[7] which is about four times higher than that of acyclovir.[8] Metabolism of FCV involves sequential hydrolysis of both acetyl groups to give 6- deoxypenciclovir, which is subsequently oxidized to penciclovir,[9] penciclovir determination in plasma was developed by F.Schenkel *et al*,[10] which was synthesized by Briony Brand *et al*.[11] FCV stability in different buffer solutions was studied by Zhang *et al*.[12] Since FCV is widely used in the antiviral therapy, it is important to develop and validate analytical methods for its determination in pharmaceutical dosage form. Few RP-LC methods were found in literature for the determination of famciclovir in pharmaceutical formulations.[13-16] The present work reports the development of a more rapid, economic and sensitive HPLC method with UV detection for estimation of FCV, useful for routine quality control of FCV in pharmaceutical formulations. The method was validated by parameters such as linearity, accuracy, precision and robustness.

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Experimental

Apparatus

The HPLC used consisted of Hitachi chromatographic system equipped with a Hitachi pump L-7110, Rheodyne universal injector 7725 and Hitachi L-7400 UV-visible detector. The chromatographic studies were performed using Varian® Microsorb-MV 100 C_{18}, 5 µm, 250 mm x 4.6mm i.d. column, at ambient temperature. Peak area integration was performed using Winchrom software. A Shimadzu model 1700 double beam UV-visible spectrophotometer with a pair of 10mm matched quartz cells was employed for determination of absorption maximum.

Reagents and chemicals

HPLC grade acetonitrile and glacial acetic acid were obtained from Rankem (Mumbai, India). Pure sample of 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.

Chromatographic conditions

The process was carried out on C_{18} column (5µm, 250 x 4.6mm, i.d) using the mobile phase consisting of acetonitrile and glacial acetic acid in the ratio 70:30 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.

Preparation of solutions

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, 40.0 µg ml^{-1}) were prepared by subsequent dilution with mobile phase.

Validation

Three series of standard calibration solutions in the range of 1.0 – 40.0 µg ml^{-1} were prepared and analyzed as described above. Calibration curves were constructed using three series of standard FCV solutions in the range of 1.0 - 40.0 µg ml^{-1}. Peak area was recorded for all the peaks and a calibration graph was obtained by plotting peak area *versus* concentration of FCV (Figure 2). To establish the accuracy and intra-day and inter-day precision of the method, six replicate solutions at three different concentrations (5.0, 10.0, 20.0 µg ml^{-1}) were assayed on single day and three separate days.

Assay method

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

Chromatographic conditions:

Various compositions of mobile phase consisting of acetonitrile and glacial acetic acid (50:50 to 80:20) were used in the study and the composition of 70:30 was selected as it gave best elution, reasonable retention time and least tailing. The typical chromatogram obtained for famciclovir is presented in Figure 3.

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 presented in Table 1. The linearity of the calibration curve was validated by the high value of the correlation coefficient (r=0.9999).

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

The limit of detection and the limit of quantification are defined as LOD = 3.3σ/s and LOQ = 10σ/s respectively, where σ denotes standard deviation of y-intercepts of regression lines and s denotes slope of the corresponding calibration curve.[17]

The limit of detection was determined as 0.335 μg ml^{-1}. The limit of quantification was determined as 1.014 μg ml^{-1}.

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 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 (Table 2) 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] Table 2 shows the 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 3, 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.

Analysis of pharmaceutical formulation:

In order to evaluate the applicability and reliability of the proposed methodology, it was applied to the determination of FCV in tablets. Satisfactory results were obtained and were found to be in agreement with label claims (Table 4). On comparison of the results obtained by the proposed method and the reference method, no significant difference was found (Table 4).

Conclusion

The proposed high performance liquid chromatographic method has been evaluated for linearity, precision, accuracy, specificity and proved to be convenient and effective for the quality control of famciclovir in given application. The measured signal was shown to be precise, accurate, and linear over the concentration range tested (1.0–40.0 μg ml^{-1}) with a correlation coefficient better than 0.9999. Moreover, the lower solvent consumption leads to a cost effective and environmentally friendly chromatographic procedure. Thus, the proposed methodology is rapid, selective, requires a simple sample preparation procedure, and represents a good procedure of famciclovir determination in pharmaceutical dosage forms.

Acknowledgment

The authors thank M/s FDC Limited, Goa, India for providing the pure drug to develop the method and Dr. B.G.Desai, Principal of K.L.E University's College of Pharmacy, Bangalore for providing support and necessary research facilities.

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Figure 2. Standard curve for FCV.

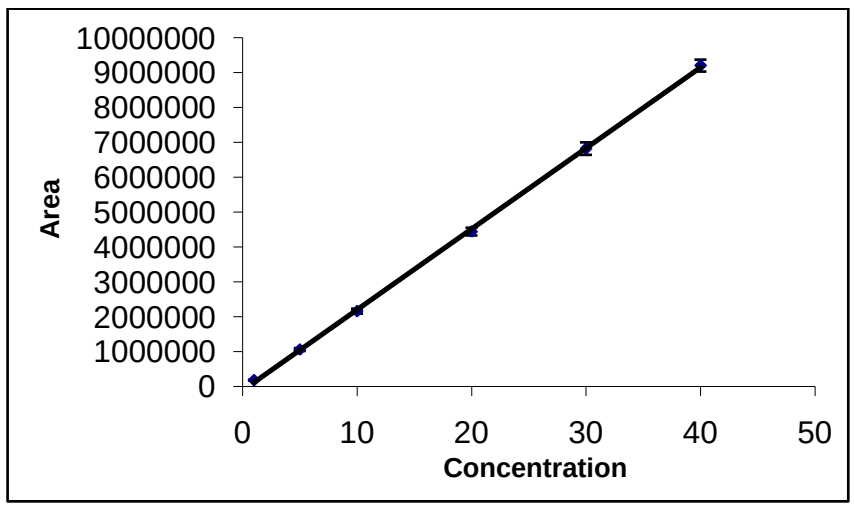

Figure 3. Chromatogram of FCV.

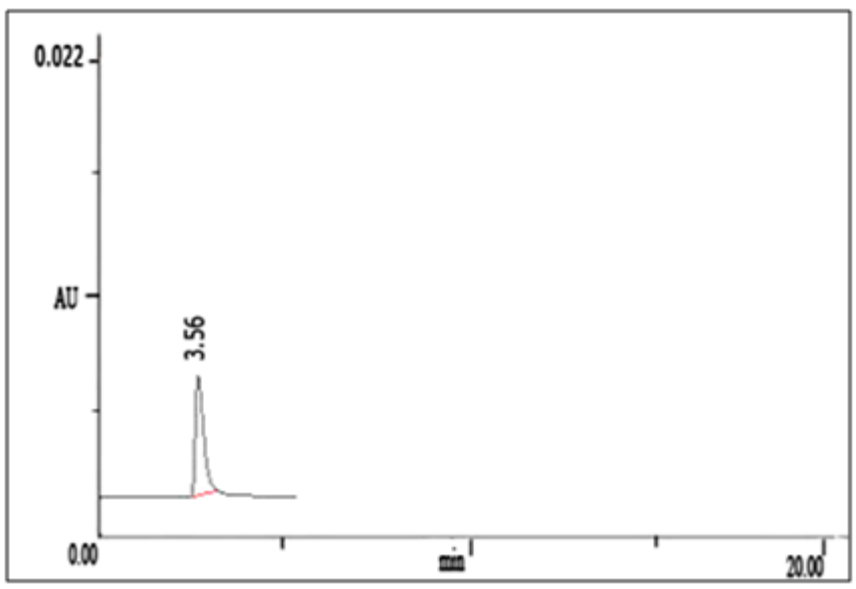

Table 1: Statistical data of calibration curves of famciclovir.

Parameters	Famciclovir
Linearity	1 – 40 µg/ml
Regression equation	Y = 231469X - 108246
Standard deviation of slope	4659.90
Relative standard deviation of slope (%)	2.01
Standard deviation of intercept	23481.25
Correlation coefficient (r)	0.9999
Limit of Detection (LOD) (µg/ml)	0.335 µg/ml
Limit of Quantitation (LOQ) (µg/ml)	1.014 µg/ml

Table 2: Intra- and inter-day precision in pharmaceutical dosage forms containing FCV.

Concentration of drug (µg/ml)	Observed concentration of drug * (µg/ml)				Accuracy# (bias %)	
	Intra-day		Inter-day			
	Mean	%RSD	Mean	%RSD	Intra-day	Inter-day
5	5.00	0.38	4.98	0.39	0.3	0.6
10	9.97	0.17	10.01	0.57	0.2	0.05
20	19.92	0.17	19.95	0.29	0.3	0.125

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

Table 3: Recovery Data for the Proposed RP-HPLC method.

Pharmaceutical formulations	Amount (µg/ml)		% Recovery ±R.S.D.
	Taken + Added	Found* ± S.D.	
Tablets I	05 + 05	10.01 ± 0.038	100.06 ± 0.378
Tablets II	10 + 05	14.97 ± 0.052	99.80 ± 0.347
Tablets III	10 + 10	19.97 ± 0.036	99.86 ± 0.178
Tablets IV	20 + 10	30.03 ± 0.086	100.1 ± 0.287

*****Average of six determinations; n=6.

Table 4: Analysis of famciclovir from pharmaceutical formulations by proposed method.

Sample	Labelled amount (mg)	Amount found* ± S.D.	Reference method	% Recovery ± R.S.D.
Tablets I	250	249.83 ± 0.970 t = 0.009, F = 1.44	249.83 ± 0.807	99.93 ± 0.388
Tablets II	250	249.88 ± 0.967 t = 0.058, F = 2.75	249.85 ± 0.583	99.95 ± 0.387
Tablets III	250	248.33 ± 0.204 t = 0.112, F = 1.06	248.35 ± 0.210	99.33 ± 0.082
Tablets IV	250	249.96 ± 0.781 t = 0.021, F = 1.24	249.97 ± 0.870	99.98 ± 0.312

*****Average of six determinations