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Research Article

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A rapid RP-HPLC method for the estimation of famciclovir in tablet dosage forms

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ABSTRACT

A rapid and simple RP-HPLC chromatographic method has been developed for the estimation of famciclovir in API and tablet dosage form. Chromatographic separation was achieved on Phenomenex C18 column (250 x 4.6 mm; 5µ) using 0.1% ortho - phosphoric acid buffer (pH 2.6) and methanol in the ratio of 50:50 v/v as mobile phase at a flow rate was 1mL/min. The detection wavelength was set as 220nm. The retention time of the drug was found to be 3.822 min. The method was applied to tablet dosage forms, without any interference from excipients. The calibration curve was linear over the range of 10 – 125 µg/mL. The performance of the method was validated according to ICH guidelines and it was found suitable for the the analysis of famciclovir in tablet dosage forms.

Keywords: Famciclovir, RP-HPLC, Method Development, Validation, ICH guidelines.

INTRODUCTION

Famciclovir (Fig 1.), a prodrug of penciclovir, is a guanine analogue antiviral drug used for the treatment of various herpes virus infections. Chemically it is 2-[(acetyloxy) methyl]-4-(2-amino-9H-purin-9-yl) butyl acetate. Famciclovir is indicated for the treatment of herpes zoster (shingles) **[1]**, herpes simplex virus 2 (genital herpes) **[2]**, herpes labialis (cold sores) in immunocompetent patients **[3]** and for the suppression of recurring episodes of herpes simplex virus 2. It is also indicated for treatment of recurrent episodes of herpes simplex in HIV patients.

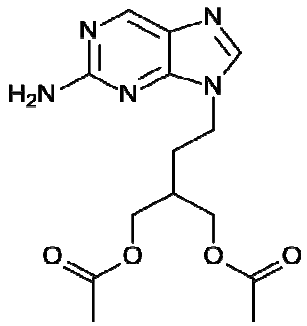

Fig 1. Structure of famciclovir

Literature survey revealed that several analytical methods have been reported for the quantitative estimation of famciclovir in tablet dosage forms by HPLC and UV spectrophotometric techniques.**[4-14]** We tried to develop and

validate RP-HPLC method with short retention and run times in bulk and tablet dosage forms. Conformation of the applicability of the developed method was validated according to ICH guidelines. **[15]**

EXPERIMENTAL SECTION

Chemicals, solvents and drugs:
Ortho - phosphoric acid, triethylamine GR grade and methanol HPLC grade was purchased from Merck Chemicals Limited. HPLC grade water was prepared using Millipore Milli-Q system. Famciclovir working standard was obtained from Aurobindo Pharma Ltd. (Hyderabad, India) as gift sample.

Equipment and chromatographic conditions:
The chromatographic system consisted of Shimadzu HPLC fitted with Prominence LC 20 AD Series pump and SPD 20A UV detector using LC Solutions software as data handling system. Phenomenex C18 (250 x 4.6 mm, 5µm) was used for this method. All chromatographic runs were carried out in isocratic mode with a flow rate of 1.0 mL/min. *Ortho* – phosphoric acid buffer was prepared by dissolving 0.1mL of *ortho* – phosphoric acid in 1000mL of water. pH was adjusted to 2.6 using 10% v/v triethylamine. It was filtered through 0.45µ filter and sonicated. The mobile phase consisted of buffer and methanol (HPLC grade) in the ratio of 50:50%v/v. The detector wavelength was set at 220 nm. The injection volume was 20µL.

Table 1. Optimized chromatographic conditions

Stationary Phase	Phenomenex C18 (250 x 4.6 mm, 5µm)
Mobile Phase	*O* - Phosphoric acid buffer : Methanol =50:50 v/v
Diluent	*O* - Phosphoric acid buffer : Methanol =50:50 v/v
Flow Rate	1.0 mL/min
Column Temperature	26°C
Injection Volume	20 µL
Detection Wavelength	220 nm
Run Time	10 min

Preparation of diluent:
Mobile phase was used as diluent.

Preparation of working standard solution of famciclovir:
100 mg of famciclovir was accurately weighed and transferred into a 100 mL volumetric flask. 70mL of diluent was transferred into it and sonicated to dissolve. The volume was made up with further quantity of diluent and mixed well. This is used as standard stock solution. 10 mL of this solution was transferred into a 100mL volumetric flask, diluted to volume with diluent and mixed to get concentration of 100µg/mL of famciclovir. This was used as working standard solution.

Calibration curve:
Calibration curve was performed by preparing solutions of famciclovir at different concentration levels including working concentration mentioned in experimental condition. Twenty microlitres of each concentration was injected into the HPLC system. The response was read at 220nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plot of concentration over the mean peak area was constructed.

Estimation of the drugs from tablet dosage forms:
Three Famcimac-250 tablets (Macleods Pharma Ltd.) were separately weighed and ground to fine powder. An amount equivalent to 100 mg of famciclovir was transferred into a 100 mL volumetric flask and to it 70mL of diluent was added and sonicated for 20min. The diluent was further added to make up the volume and mixed. A portion of the above solution was filtered through 0.22µm membrane filter (discarding the first few mL of the filtrate). 10 mL of this filtrate was transferred into a 100mL volumetric flask containing about 30mL diluent. The volume was made up to mark with diluent and mixed well. The above solution was then chromatographed six times. The mean peak area of the drug was calculated and the drug content in the formulation was calculated by the regression equation of the method.

RESULTS AND DISCUSSION

During initial method optimization studies C18 columns with different column lengths were tried. Finally below mentioned chromatographic conditions were finalized after evaluating column efficiency parameters like theoretical

plates and tailing. Wavelength was selected by scanning standard solution of the drug in diluent, over 200nm to 400nm. Using mobile phase, base line separation for the famciclovir peak was achieved. Under these conditions, the retention time for famciclovir was found to be 3.822 min. The proposed method was also applicable to tablet formulations.

Specificity:

A good analytical method should be able to measure the analytes accurately in the presence of suspected interferences such as blank, excipients, and degradation products. Fig. 2 shows chromatographic base-line separation of famciclovir. Fig. 3 demonstrates that no interferences were found at the retention time of famciclovir in its dosage form due to excipients.

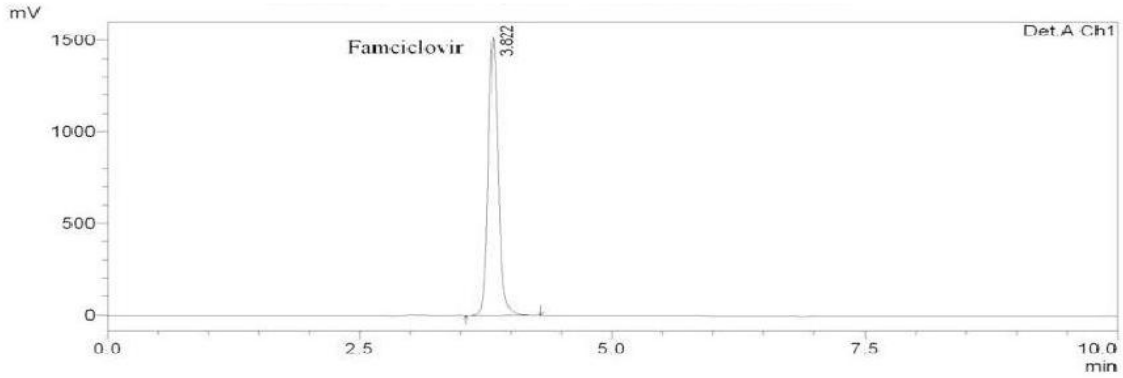

Fig 2. Representative chromatogram obtained from the analysis of famciclovir from working standard solution

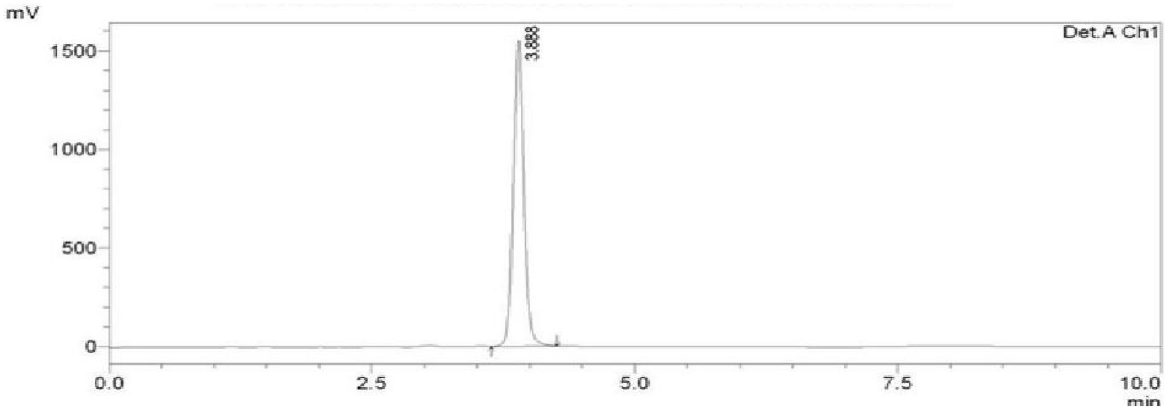

Fig 3. Representative chromatogram obtained from the analysis of famciclovir from sample solution

Linearity:

The regression of the plot was computed by least square regression method and is shown in the Fig. 4. The calibration curve (n=3) constructed for the drug was linear over the concentration range of 10-125 µg/mL. The correlation coefficient is greater than 0.99 and the %RSD for each concentration studied was less than 2. The linearity data is shown in Tab. 2.

Table 2. Linearity data

S.No.	Concentration of famciclovir (µg/ml)	Mean peak area (n=3)
1	10	1179643
2	25	3408556
3	50	6107141
4	75	8904713
5	100	12290885
6	125	15447780

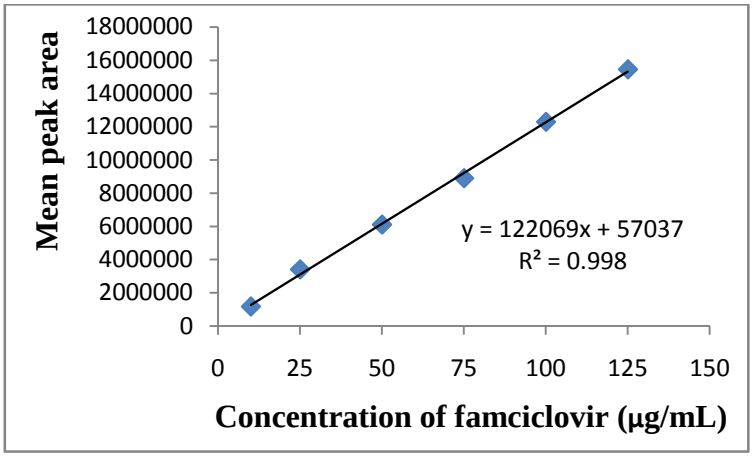

Fig 4. Linearity plot for famciclovir

Accuracy and precision:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery and standard deviation of the percentage recovery were calculated and represented in Tab. 3. The high percentage of recovery indicates that the proposed method is highly accurate. The precision of the method was demonstrated by inter-day and intra-day variation studies. Six replicate injections of sample solutions were made and the percentage RSD was calculated and represented in Tab. 4. From the data obtained the developed RP-HPLC method was found to be precise.

Table 3. Accuracy data of the proposed method

Analyte	Amount of the analyte taken(µg/mL)	Mean recovery (µg/mL) ± SD	% Mean recovery ± SD
Famciclovir	80	80.08 ± 0.34	100.1 ± 0.42
	100	100.22 ± 0.95	100.22 ± 0.95
	120	120.36 ± 1.28	100.30 ± 1.06

Table 4. Precision data for the proposed method

	Intra-day precision	Inter-day precision
Mean peak area	10335487	10373611
SD	26428.27	45066.6
%RSD	0.255	0.43

System suitability parameters:

System suitability parameters were studied with six replicates of standard sample solution and the parameters are presented in Tab. 5.

Table 5. System suitability parameters of the proposed method

Parameter	Value
Retention time (min)	3.822
Tailing factor	1.13
Theoretical plates	6786.6
HETP	3.6837×10^{-2}

Method suitability:

The commercial tablet formulation from Macleods Pharma Ltd., Famcimac-250 tablets was analyzed by the proposed method and the result is shown in Tab. 6. The value was found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of famciclovir in pharmaceutical dosage forms.

Table 6. Recovery of famciclovir from tablet dosage form

Name of the formulation	Amount recovered (n=6) (µg/ml)	% Recovery
Famcimac-250	249.87	99.95

CONCLUSION

The proposed RP -HPLC method is sensitive, precise and accurate and can be used for the routine quality control analysis for the determination of famciclovir in its tablet dosage forms.

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