RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FAMCICLOVIR IN TABLET DOSAGE FORM

V. S. MANNUR[1]*, B. SHRAVAN KUMAR[2], V. S. MASTHIHOLIMATH[3]

Department of Quality Assurance, KLEU's College of Pharmacy, Belgaum-10, Karnataka, India. Email: vsmannur@gmail.com[1], Shravan_1786@yahoo.com[2]
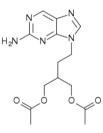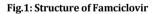
ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method developed for the estimation of Famciclovir in tablet dosage form. A Hypersil-BDS RP C-18 column (250x4.6mmx5µm) with mobile phase consisting of 20mM of Ammonium formate buffer: methanol (Ammonium formate pH adjusted to 4.5 with 10%v/v acetic acid) 70:30% v/v was used. The flow rate was 0.9ml/min and the effluents were monitored at 305 nm. The retention time was 20.40min. The detector response was linear in the concentration of 100-300µg/ml. The limit of detection and limit of quantification was found to be 0.085µg/ml and 0.38µg/ml respectively. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Famciclovir in bulk drug and in its pharmaceutical dosage form.

Keywords: Famciclovir, HPLC, Tablet formulation.

INTRODUCTION

Chemically Famciclovir[1] is a 2-[2-(2-amino-9*H*purine-9-yl) ethyl]-1, 3-propane dioldiacetate Fig.1 is a guanine analogue antiviral drug used for the treatment of various herpes virus infections. This drug is also used for the treatment of the ophthalmic zoster [2]. Famciclovir is indicated for the treatment of herpes zoster (shingles). It is a prodrug of penciclovir with improved oral bioavailability. Literature survey revealed that HPLC[3-6] and spectrophotometric[7-9] methods were carried out for the estimation of Famciclovir in tablet dosage form as well in pure form .The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Famciclovir in bulk drug samples and in pharmaceutical dosage form.

Fig.1: Structure of Famciclovir

MATERIALS AND METHODS

Reagents and chemicals

Standard bulk drug sample of Famciclovir was provided by Star tech Labs pvt. Limited (Hyderabad, India).Methanol (HPLC grade), Ammonium formate (AR grade) was obtained from Qualigen Fine Chemicals, Mumbai, India. Ultra-pure water was obtained from a Milli-Q® UF-Plus apparatus (Millipore) and was used to prepare all solutions for the HPLC method. Acetic acid (AR grade) was obtained from Ranbaxy Fine Chemicals Ltd, New Delhi, India. Tablet formulation (Famvir) was procured from a local market, with labelled amount 250 mg per tablet.

Apparatus and chromatographic conditions

HPLC method development and validation was done on a Shimadzu (Japan) Liquid chromatograph equipped with (LC-10 ATvp pump), SPD-10Avp UV detector, (rheodyne) 7725i injection with 20µL loop and class-LC solution software. Stationary phase used was Hypersil-BDS C-18 (250×4.6 mmx5µm) i.d. and the mobile phase was 20mM Ammonium formate (pH adjusted to 4.5 with 10%v/v acetic acid):

methanol (70:30% v/v). The mobile phase was filtered using 0.45µ membrane filter (Rankem Nylon membranes, New Delhi, India). The mobile phase flow rate was 0.9ml/min and injection volume was 20µL. All weighing were done on Shimadzu electronic balance, BL-220 H (Shimadzu Corporation, Japan).

Preparation of mobile phase

Prepare accurately a mixture of 700 ml of 20mM ammonium formate buffer and 300 ml of methanol and degas.

Preparation of Standard Stock solution

A stock solution of Famciclovir was prepared by accurately weighing 50 mg of drug, transferring to 100 ml volumetric flask, Add about 30 ml of mobile phase and sonicate to dissolve it completely and make up volume up to mark with mobile phase to get stock solution of 500µg/ml.

Working Standard solution

20 ml of the above stock solution was taken in 50 ml volumetric flask and thereafter made up to 50 ml with mobile phase to get a concentration of 200µg/ml.
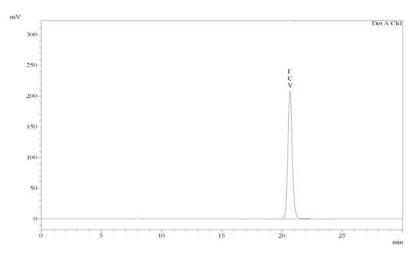
Preparation of Sample solution

Twenty tablets (Famvir 250mg) were weighed, and then powdered. Quantity equivalent to 50 mg was weighed transferred to 50 ml volumetric flask. Add about 20ml of mobile phase and sonicate. Make volume up to the mark with mobile phase and mix. Filter the solution through 0.45µm filter, Dilute 5.0 ml of the resulting solution to 25 ml with mobile phase to obtain standard solution of 200µg/ml.

RESULTS AND DISCUSSION

HPLC method

For the RP-HPLC, chromatographic conditions were optimized to get best resolution and peak shape. The selection of mobile phase was based on peak parameters; (symmetry, theoretical plates, capacity factor and tailing factor) ease of preparation and cost. A symmetrical peak with good separation (Rt of FCV 20.40 min) was obtained with C-18 column and mobile phase consisting 20mM of Ammonium formate: methanol (Ammonium formate pH adjusted to 4.5 with 10%v/v acetic acid) 70:30% v/v at a flow rate of 0.9ml/min. Chromatogram of standards and formulation are given in fig. 2 and 3 respectively. The optimum wave length for detection and quantification was 305 nm, at which good detector response was obtained with symmetrical peaks.
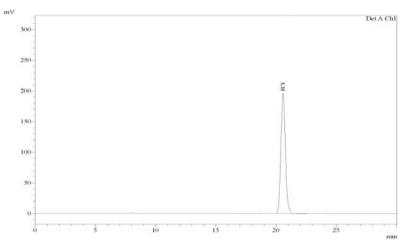
Fig. 2: Chromatogram of Famciclovir Standard (200µg/ml)

Fig. 3: Chromatogram of Famciclovir Formulation (200µg/ml)

Method validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures [10-11].

Linearity and Range

Aliquots of standard Famciclovir stock solution were taken in different 10 ml volumetric flasks and diluted up to then mark with the mobile phase such that the final concentrations of Famciclovir are in the range of 100-300 µg/ml. Each of these drug solutions (20µL) was injected three times into the column, and the peak areas and retention times were recorded. Calibration graph was obtained by plotting peak area versus concentration of Famciclovir shown in fig.4. The plot of peak area of each sample against respective concentration of Famciclovir was found to be linear in the range of 100-300 µg/ml with correlation coefficient of 0.9998. Linear regression least square fit data obtained from the measurements are given in Table 1. The respective linear regression equation being Y= -1143.6000x + 26616.4340.
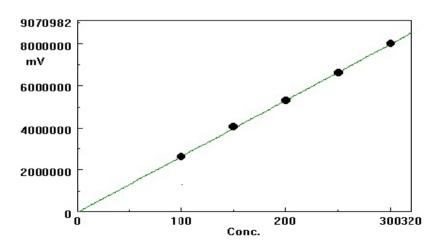

Fig. 4: Calibration curve of Famciclovir

Table 1: Calibration data of Famciclovir by RP-HPLC method

Concentration (µg/ml)	Peak area
100	2630847.00
150	4030976.00
200	5294033.00
250	6605633.00
300	7997627.00

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.085µg/ml and 0.38µg/ml respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

Accuracy

Accuracy was determined by recovery studies of Famciclovir, known amount of standard was added to the preanalyzed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 2. The study was done at three different concentration levels.

Table 2: Recovery studies

Drug	Level (%)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	%RSD
Famciclovir	100	100	99.34	98.34	0.550
(200µg/ml)	200	200	197.30	98.65	0.315
	300	300	297.91	99.30	0.524

Precision

The intra-day precision was determined by analysing standard solution of concentration 200µg/ml for 6 times on the same day while inter-day precision was determined by analysing corresponding standards daily for 6 day over a period of one week. The values of percentage relative standard deviation (% RSD) for intra-and inter-day variation are given in Table 3.

Table 3: Precision Studies

Injections	Concentration (µg/ml)	Intraday (peak area)	Interday (peak area)
1		5503750	5325598
2		5504214	5387258
3		5506028	5384616
4	200	5505452	5387076
5		5503868	5396748
6		5505229	5395872
Mean		5504757	5379528
Std deviation		940.2626	26887.59
%RSD		0.017	0.499

System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), number of theoretical plates (N), tailing factor (T), and resolution were evaluated for six replicate injections of the drug at a concentration of 200µg/ml .The results given in Table 4.

Table 4: System Suitability

Property	Values	Acceptance limit
Retention time	20.40	% RSD < 2
Theoretical Plates (N)	15176	> 3000
Tailing Factor	1.10	< 1.5
Resolution	1.63	> 1.5

Robustness

The robustness of the proposed method was evaluated by slight modification in the organic composition and pH values of aqueous phase of the mobile phase and flow rate. During these studies it was found that there was not much change retention time, area and symmetry of peak.

The developed method was used for the assay of commercially available tablets and six replicate determinations were performed. Experimental values obtained for the determination of tablets are given in Table 5. The interference of excipients was studied by comparing the chromatography of standards and formulations. The same shape and retention times of peaks showed that there was no interference from the excipients.

Table 5: Analysis of formulation

Labeled amount, mg /tablet	Amount found, mg/ tablet	%RSD*
250	249.23	0.4132

* RSD of six observations

CONCLUSION

The developed RP-HPLC method was simple, sensitive, precise and accurate, hence can be used in routine for the determination of Famciclovir in bulk as well as pharmaceutical preparations.

REFERENCES

1. Sweetman SC. Martindale, The complete drug reference. 34th ed. London: The Pharmaceutical Press; 2002. p. 620.
2. Tyring S, Engst R, Corriveau C, Robillard N, Trottire S, VanSlycken S et al. *Br.* J.Ophthalmol.2001; 85: 576-581.
3. Ramakrishna k, Raman NV, Harikrishna KA Prasad AV, Reddy R. Development and validation of a stability- indicating RP-LC method for famciclovir. J. Pharm. Biomed. Anal. 2009; 50: 797–802.
4. Narasimha Raju BV, Gunanidhi Panda, Rao NG. Stability indicating LC assay method for the determination of famciclovir in bulk drug and pharmaceutical dosage forms. Chromatographia. 2008; 68:837-41.
5. Gedela S, Vishnumulaka S, Medicherla N, Allam A. Development and validation of LC method for the determination of famciclovir in pharmaceutical formulation using an experimental design. E-Journal of Chemistry 2008; 5(1):58-67.
6. Gedela S, Batchu S, Lade S, Rao S. Development and validation of Liquid Chromatographic and UV derivative spectrophotometric methods for the determination of famciclovir in pharmaceutical dosage forms. Chem. Pharm. Bull. 2006; 54(6):819-22.
7. Reddy A, Srikar A. Validated spectrophometric estimation of famciclovir in tablet dosage form. J Pharm Sci and Tech 2009; 1(1):36-9.
8. Gnana Babu C, Vijaya G. Validated Spectrophotometric Estimation of Famciclovir in Tablet Dosage Form. Int J Chem Tech Res 2009; 4(1): 1368-71.
9. Gouda A, Shafey Z, Hossny N, Azzazy R. Spectrophotometric determination of hyoscine butylbromide and famciclovir in pure form and in pharmaceutical formulations. Spectrochim. Acta, Part A 2008; 70: 785–92.
10. ICH, Q2A Text on Validation of Analytical Procedures, International Conference on. Harmonization, Oct, 1994.
11. ICH, Q3B Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Nov, 1996.