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RP-HPLC METHOD FOR THE ESTIMATION OF FAMCICLOVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A rapid and reproducible RP-HPLC chromatographic method has been developed for the estimation of famciclovir in its pure form as well as in pharmaceutical formulation. Chromatography was carried out on an C_{18} column using a mixture of methanol and acetonitrile as the mobile phase at flow rate of 0.5 mL/min and detection was done at 222 nm. The retention time of the drug was 1.63. The results obtained with the proposed methods are in good agreement with labeled amounts, when marketed pharmaceutical preparations are analyzed. The recovery in the present method is in the range of 99.24 - 99.56. Results obtained are found to be reproducible.

Key words: RP-HPLC, Famiclovir

INTRODUCTION

Famciclovir is chemically 2-[2-(2-aino-9H-purin-9-yl)ethyl] trimethylene diacetate[1, 2]. It is an acyclic guanine nucleoside analog and a new generation antiviral drug, which is active *in vitro* and *in vivo* against herpes simplex virus types 1 and 2 and against varicella-zoster virus[3-6]. It is not official in any Pharmacopoeia. A few analytical methods have been reported for its quantitative estimation in pharmaceutical formulations that include estimation in plasma and urine by HPLC[7] and UV spectrophotometric[8, 9] estimation in methanol.

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EXPERIMENTAL

Instrumentation

An isocratic high performance liquid chromatography (Knauer HPLC) with Wellchrom HPLC - Pump K 501 and with software C2000 version 1.7 and UV/vis detector K 2501(Knauer). Column used was C-18, 250 x 4 mm i. dl; particle size 5 μm and packing material was eurosphere - 100.

Chemical and reagents

Famciclovir was the gift sample obtained from FDA Limited Goa, India. Acetonitrile (HPLC grade) and methanol (HPLC grade) are from Merck. Bombay, India.

Chromatographic conditions

The chromatographic column used was a 250 × 4 mm stainless steel with 5 μm particles. The HPLC equipment was operated at ambient temperature. The flow rate of the mobile phase was maintained at 0.5 mL/min in the ratio of 90 : 10 (Methanol : Acetonitrile). Detection was carried out by UV detector at 222 nm and the injection volume was 20 μL.

Working standard of drug solution

About 100 mg of famciclovir was weighed accurately and dissolved in 50 mL of methanol (AR grade) in a 100 mL volumetric flask and diluted up to the mark with methanol (1 mg/mL). 10 mL of this solution was diluted to 100 mL in a volumetric flask with methanol to get a final concentration 100 μg/mL.

Procedure

The solution was prepared on a weight basis and volumetric flasks were used to minimize solvent evaporation. Stock solution of drug was prepared by dissolving 100 mg of famciclovir in 100 mL volumetric flask containing 70 mL of methanol sonicated for 15 min by using Bandelin sonoplus HD2070 Sonicator and then made upto volume with methanol daily

Working standard solution of famciclovir was prepared by suitable dilution of the stock solution with mobile phase.

Five sets of the famciclovir solution were prepared in mobile phase at

concentration of 0.2, 0.4, 0.6, 0.8, 10 and 20 µg/mL. Each of these samples (20 µL) was injected five times into the column and the peak area of the drug was recorded.

Assay of famciclovir tablets

Twenty tablets of famciclovir each containing 250 mg were weighed, finely powered and an accurately weighed sample of powered tablets equivalent to 60 mg of famciclovir was placed in a 100 mL volumetric flask. 70 mL of methanol : acetonitrile were added and the flask was allowed to stand for 7 hrs with intermittent sonication to ensure complete solubility of drug. The mixture was then made up to 100 mL with mobile phase, thoroughly mixed and filtered through a 0.45 µm membrane filter. An aliquot of the filtrate (1 mL) was transferred to a volumetric flask and made up to volume with mobile phase to give an expected concentration 10 µg/mL of famciclovir. All determinations were conducted in triplicate.

RESULTS AND DISCUSSION

The run time of the method was set at 5 min and famciclovir appeared on the chromatogram at 1.63 min (Fig. 1).

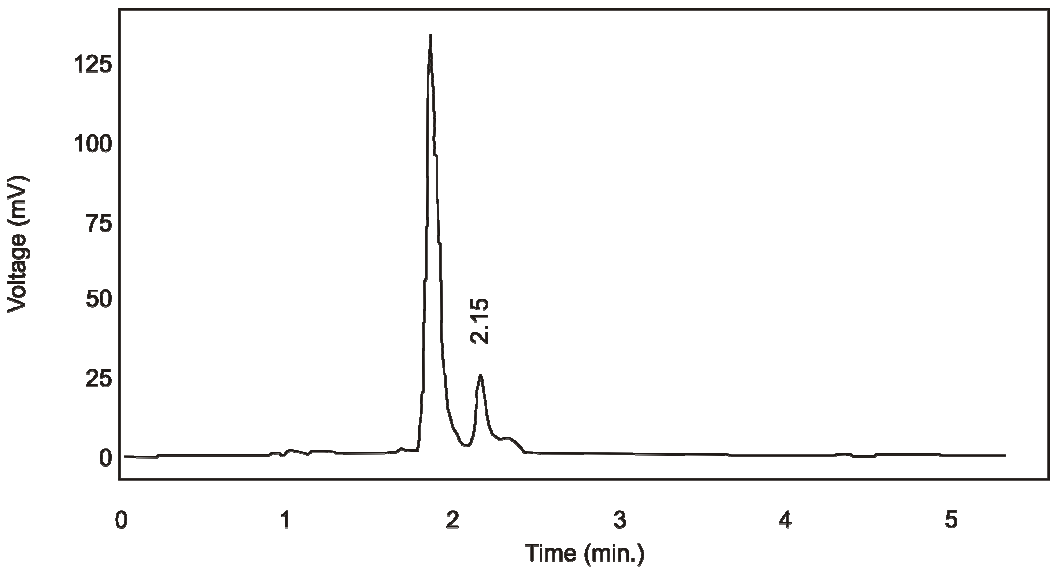

Fig. 1 : Typical chromatogram of famciclovir standard for proposed method

When the same drug solution was injected 5 times, the retention time of the drug was same. The peak area of famciclovir was calculated and the average of five such determinations were given in Table 1.

Table 1. Calibration of the HPLC method for the estimation of famciclovir

Concentration of famciclovir (μg/mL)	Peak area*	C. V. (%)
0	0.00	0.00
2	250.09	1000.36
4	482.12	1920.48
6	701.32	2801.28
8	930.12	3720.48
10	1145.82	4583.28
20	2160.19	8640.76

*Mean of five determinations

Regression equation :

Y = -1121.51 + 0.4717.14 X (r = 0.9998)

Table 2. Inter-and intra-day precision for famciclovir assay in pharmaceutical dosage forms by the proposed HPLC method

Concentration of famciclovir (μg/mL)	Conc. of famciclovir found on			
	Intra-day		Inter-day	
	Mean (n = 5)	C. V. (%)	Mean (n = 5)	C. V. (%)
10	10.11	0.9988	10.22	1.127
20	20.09	1.285	20.10	2.167

When the concentration of famciclovir and its respective peak area were subjected to regression analysis by least square method, a high correlation coefficient was observed (r = 0.9998) in the range of 2-20 μg/ mL. The regression of famciclovir concentration over its peak area was found to be Y= - 1121.51 + 0.4717.14 X, where Y is the peak area and X

is the concentration of famciclovir.

The proposed method was also validated for intra-and inter-day variation. When the solutions containing 10-20 µg/ mL of famciclovir were repeatedly injected on the same day, the coefficient of variation (CV) in the peak area of the drug for five replicate injection was found to be less than 2%. Also intra-day variation (3 and 5 injections) was found to be less than 2% (Table 2), thus, the results have shown that the proposed method is highly reproducible.

When a known amount of drug solution (10 or 20 µg/ mL) was added to a known concentration of drug solution (20 µg/ mL), there was a high recovery (99.24 -97.5) of famciclovir (Table 3), which indicates that the proposed method is accurate.

Table 3. Recovery of famciclovir using the proposed HPLC method

Amount of drug added (µg)	Mean (± s. d) amount found (mg) (n = 5)	Mean (± s. d) % of recovery (n = 5)
10	9.90 ± 0.16	99.24 ± 1.7
20	19.50 ± 0.05	97.50 ± 0.1

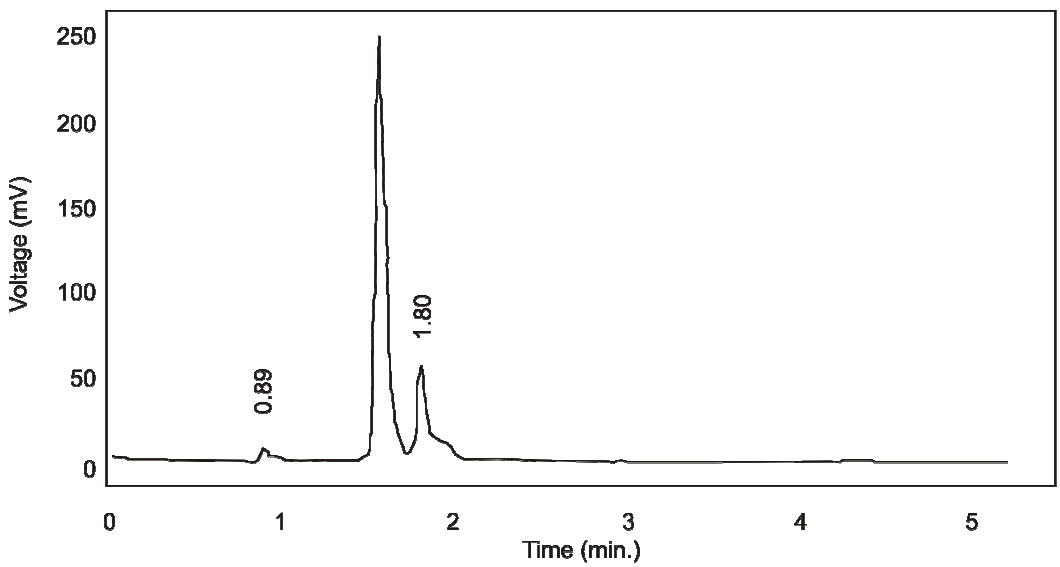

Fig. 2 : Typical chromatogram of famciclovir formulation for proposed method

The proposed method, developed in the present study has been used to quantify famciclovir in tablet dosage forms. Famciclovir (containing 250 mg of the drug) were analyzed as per the procedure described above (Fig. 2). The calibration curve for proposed method is given in Fig. 3. The average drug content was found to be 99% of the labeled amount (Table 4).

Table 4. Mean (± s. d.) amount of famciclovir in tablet dosage forms by proposed HPLC method

Tablets	Labelled amount of drug (mg)	Mean (± s. d) amount found (mg) (n=5)	Mean (±s. d) % purity
T1	250	196.56 ± 0.02	98.62 ± 0.03
T2	250	198.28 ± 0.04	99.26 ± 0.04

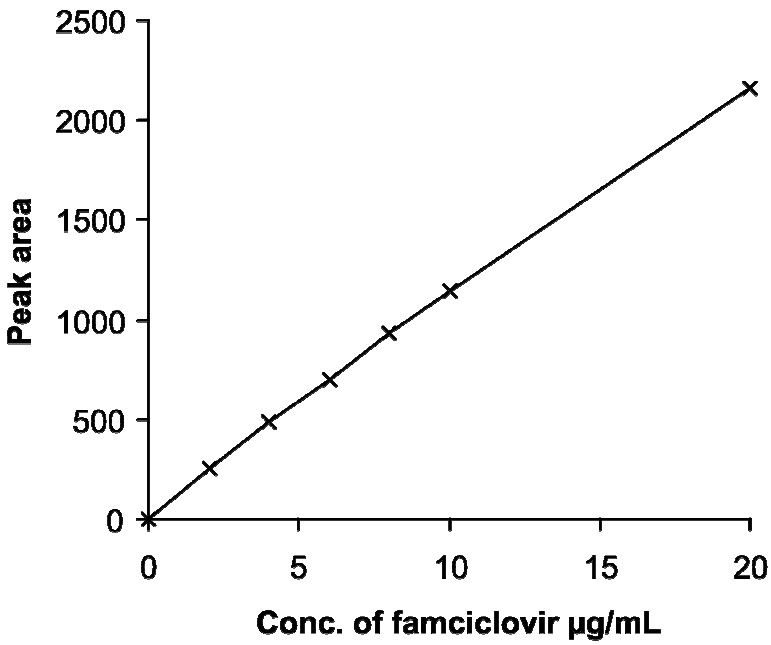

Fig. 3: Calibration curve of famciclovir (RP-HPLC) for proposed method

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