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# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FAMCICLOVIR IN BULK AND TABLET DOSAGE FORM BY USING RP-HPLC METHOD

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**ABSTRACT**

A simple and reliable reverse phase high-performance liquid chromatography method was developed and validated for Famciclovir in pure and pharmaceutical dosage form. The method was developed on Hypercil BDS C18 (4.6 x 150mm, 5 µm, Make: Agilent), with a mobile phase of phosphate buffer ($P_H$ 3.0): Methanol (65:35) %v/v at a flow rate of 1.2 ml/min with UV detection at 310nm. The effluent was monitored by: Shimadzu HPLC, variable wavelength prominence UV/ VIS detector. Calibration curve was linear over the concentration range of 30 –110µg/ml. For inter–day and intra–day precision % relative standard deviation values were found to be 0.07% and 0.3% respectively. Recovery of Famciclovir was found to be in the range of 99.80 -101.40%. The retention time and run time was very short; hence it is cost effective, making it more economical and rapid. Hence, this method can be used for the analysis of large number of samples.

**Key Words:-** Famciclovir, RP-HPLC, Validation, Pharmaceutical dosage form.

## INTRODUCTION

Famciclovir is a antiviral drug which is used in the treatment of acute uncomplicated herpes zoaster. It shows inhibitory activity against herpes simplex-1,herpes simplex-2 and vercilla zoaster virus.It is the oral form of Penciclovir, a nucleoside analogue which shares the same antiviral spectrum as Aciclovir for herpes virus and has similar potency. Chemically Famciclovir is 2-[2-(2-amino-9H-purin-yl)ethyl],diacetate ester.

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Famciclovir is used to treat antiviral infections. Literature survey reveals a few spectrophotometric and chromatographic methods for the estimation of Famciclovir as a single component (Syed nizzamudin *et al.*, 2009; Jose gnana babu C *et al.*, 2009; Vanitha S *et al.*, 2011). The objective of present work is to develop simple, rapid, and precise RP-HPLC method for the estimation of Famciclovir using phosphate buffer (3.0 pH): Methanol (65: 35) v/v.

## MATERIALS AND METHODS

### Materials

HPLC grade Water, Methanol procured from Merck, India. Potassium dihydrogen orthophosphate (AR

grade) Rankem Company, O-phosphoric acid (AR grade) Rankem Company, Mumbai. Formulations of Famciclovir are purchased from local market. Standard drugs of Famciclovir procured from pharma labs.

**Equipment**

The instrument used was Shimadzu Class-gradient LC system HPLC instrument. The instrument is equipped with a LC -7000 pump and variable wavelength programmable UV detector and a 10µL Rheodyne Inject port. The other equipment used is UV-2301 UV-Visible spectrophotometer. Sonication was done using Loba ultrasonic bath sonicator. Weighing was done on Denwar weighing balance.

**Chromatographic Conditions**

Hypersil BDS C18, 150 mm x 4.6 mm, 5µ) was used for separation. The mobile phase containing Buffer: Methanol in the ratio of 65:35(v/v) was delivered at a flow rate 1.2 ml/min and the elution was monitored at 310 nm. Injection volume was 10µl and the analysis was performed at 35ºC temperature.

**Standard stock solution**

50mg of Famciclovir working standard was weighed accurately and transferred into a 100ml volumetric flask and diluted to the volume with diluent. Further diluted 3ml of the above solution to 20ml with diluent. The above solution was filtered through 0.45 µm nylon filter.

**Preparation of Sample solution**

20 tablets were weighed and powdered. Weighed accurately a quantity equivalent to 50mg of Famciclovir and transferred into a 100ml volumetric flask and diluted to the volume with diluent. Further diluted 3ml of the above solution to 100ml with diluent and mixed. The above solution was filtered through 0.45µm nylon filter.

**Method validation** (ICH guidelines 1994 and 1996, USP 1995)

The method was validated as per ICH guidelines14, 15.

**Accuracy**: The accuracy of the method was determined by recovery experiments. The recovery studies were carried out three times and the percentage recovery were calculated and presented in Table 1.

**Precision**

The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, six repeated injections of standard solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter day variation studies, six repeated injections of standard solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated.

**Linearity**

The linearity of the method was determined at concentration levels ranging from 50-150%levels. The calibration curve was constructed by plotting response factor against concentration of drugs.

**Robustness**

Robustness of the method was determined by creating variations in flow rate, temperature and changes in wavelength and determining percentage of change in assay

**RESULTS**

In order to achieve elution of the component, initial trails were performed with the objective to select adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and their proportions, detection wavelength, optimum pH, different columns and concentration of the standard solutions were carefully studied. Several solvents were tested by using different proportions. Buffer: Methanol in the ratio of 65:35(v/v) was selected as the optimum mobile phase and a flow rate of 1.2 mL/min. Under these conditions, the analyte peak was well resolved and was free from tailing. The tailing factor was <2.0 for the analytes. The retention times of Famciclovir was found to be 3.3min, respectively.

**Optimized Chromatographic conditions**

**Mobile phase** : Buffer: MeOH (65:35) v/v.
**Diluent** : Buffer
**Flow rate** : 1.2ml/min
**Column** : Hypersil BDS, $C_{18}$ column (150 x 4.6 mm, 5 µ)
**Detector wave length** : 310 nm
**Column temperature** : 35ºC
**Injection volume** : 10 µl

**Accuracy**

The recovery studies yielded the mean results within 97 to 102 % of true concentration of drug indicating that the test method has an acceptable level of accuracy.

**Linearity**

The calibration plot was constructed by plotting response factor (RF) versus concentration (µg/ml) of

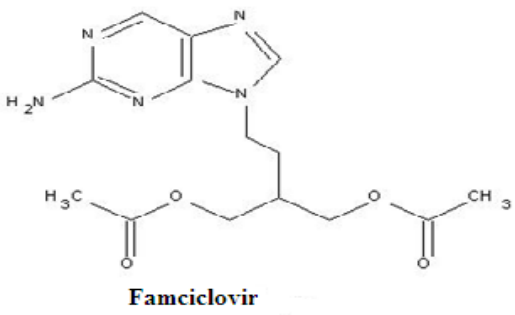
Famciclovir which was found to be linear in the range of 30-110 µg/ml with correlation coefficient of 0.9995.

**Robustness**

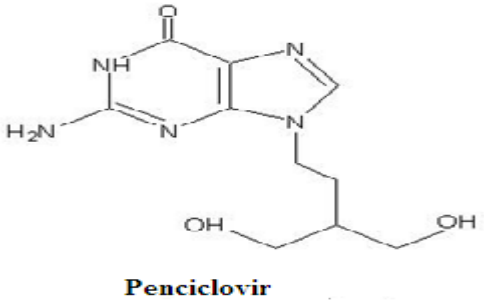
% of change and relative standard deviation was calculated and found to be less than 2%.

**Assay**

The proposed method was applied to the estimation of Famciclovir in tablets. The assay results show that the proposed method was selective for the determination of Famciclovir without interference from the excipients used in the tablet dosage form. The values were shown in Table 6.



Famciclovir



Penciclovir

**Table 1. Recovery studies**

| Sample (%) | Area | mg added | mg found | % Recovery | %RSD |
|---|---|---|---|---|---|
| **50%** | 371324 | 1249.90 | 1254.12 | 100.3 | 0.6 |
| **75%** | 565916 | 1875.92 | 1911.68 | 101.9 | 0.9 |
| **100%** | 750896 | 2500.89 | 2536.11 | 101.4 | 0.8 |
| **125%** | 929019 | 3125.52 | 3137.70 | 100.4 | 0.5 |
| **150%** | 1085395 | 3751.44 | 3665.85 | 97.7 | 0.1 |

**Table 2. Intraday Precision**

| Sample Id | Average Area | Assay(mg/tablet) | % Labelled Amount |
|---|---|---|---|
| **1** | 593337 | 493.79 | 98.8 |
| **2** | 597916 | 496.83 | 99.4 |
| **3** | 599042 | 499.88 | 100.0 |
| **4** | 593189 | 494.18 | 98.8 |
| **5** | 595118 | 495.43 | 99.1 |
| **6** | 602549 | 500.68 | 100.1 |
| **AVG** | 596858.5 | 496.80 | 99.41 |
| **STD** | 3665.06 | 2.91 | 0.58 |
| **%RSD** | 0.6 | 0.6 | 0.6 |

**Table 3. Interday precision**

| Sample Id | Average Area | Assay(mg/tablet) | % Labelled Amount |
|---|---|---|---|
| **1** | 601895 | 500.91 | 100.2 |
| **2** | 600691 | 500.29 | 100.1 |
| **3** | 602144 | 500.82 | 100.2 |
| **4** | 601738 | 501.48 | 100.3 |
| **5** | 600028 | 499.34 | 99.9 |
| **6** | 599757 | 500.64 | 100.1 |
| **AVG** | 600903 | 500.41 | 100.1 |
| **STD** | 1077.96 | 0.81 | 0.17 |
| **%RSD** | 0.18 | 0.2 | 0.2 |

**Table 4. Linearity results**

| S.No | Concentration(µg/ml) | Peak Area |
|---|---|---|
| 1 | 37.31 | 370910 |
| 2 | 49.75 | 493345 |
| 3 | 67.16 | 672807 |
| 4 | 74.62 | 745982 |
| 5 | 92.4 | 926354 |
| 6 | 99.50 | 1011603 |
| 7 | 109.45 | 1084027 |

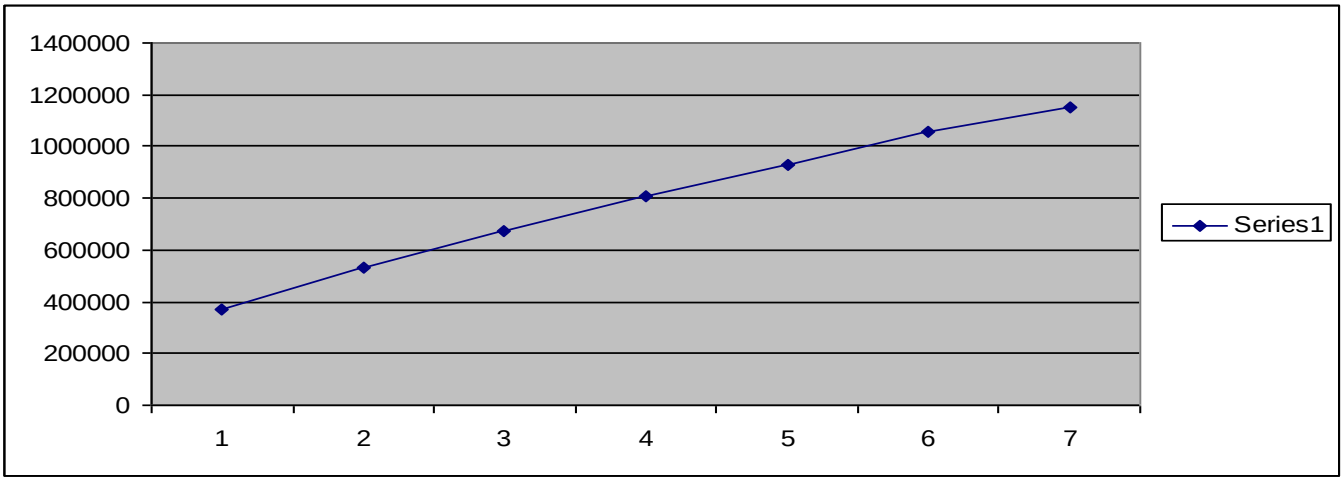
**Table 5. Robustness**

| S.No | Condition | Modification | Peak area | Rt |
|---|---|---|---|---|
| 1 | Flow Rate (ml/min) | 0.9 | 801368 | 3.722 |
| | | 1.1 | 659964 | 3.064 |
| 2 | Wave length | 305nm | 734379 | 3.358 |
| | | 315nm | 658080 | 3.358 |
| 3 | Temperature | 30ºC | 718205 | 3.449 |
| | | 40ºC | 725513 | 3.244 |

**Table 6. Assay Results**

| Tablet average weight | | Injection | Standard Area | Sample Area |
|---|---|---|---|---|
| Standard weight | 50mg | 1 | 795087 | 601228 |
| Sample weight | 167.74mg | 2 | 800433 | 597826 |
| Label claim | 500mg | 3 | 799714 | 59726 |
| | | AVG | 789155 | 601414.6 |
| Standard purity | 99.9% | SD | 2901.31 | 3224.25 |
| | | % RSD | 1.637 | 0.55 |

**Fig 1. Linearity Graph of Famciclovir**




## DISCCUSSION AND CONCLUSION

The developed RP-HPLC method was accurate, precise, reproducible and robust. The percentage recoveries of Famciclovir were found to be in the range of 97.7-101.9%. The results were shown in Table 1 which indicates that the method is accurate. A precision study has shown the result within acceptance limit, % RSD below 2.0, indicating reproducibility of the method shown in tables 2 and 3. The developed method has been found to be better, because of its wide range of linearity 30-

110µg/ml, robust under the influence of small variations in flow rate, wavelength, temperature as shown in Table 5, use of a readily available mobile phase, lack of extraction procedure and low retention times. The assay results and low %RSD values indicated that the developed method can be used for routine analysis of Famciclovir in pharmaceutical dosage form. All these factors make the proposed method suitable for the quantification of Famciclovir in bulk drugs and in table dosage form. The method can be successfully used for the routine analysis of Famciclovir in pharmaceutical dosage forms without interference.

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**REFERENCES**

Boyd MR, Bacon TH, Sutton D, Cole M. Antiherpesvirus activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine (BRL 39123) in cell culture. *Antimicrob.Agents, Chemother*, 31(8), 1987, 1238-42.

Devi Kusum V, Bhosale UV. Formulation and optimization of polymeric Nano drug delivery system of acyclovir using $3^2$ Full factorial design. *International Journal of Pharm Tech Research.* 1, 2009, 644-653.

Goweri Sankar D, Joy Babu B, Anil Kumar, Mandavilatha PV. *International J.Pharma. Sci*. 14(3), 2006, 481.

Jose Gnana Babu C, Vijaya Kumar G. Validated Spectrophotometric Estimation of Famciclovir in Tablet Dosage Form. *International Journal of Chem Tech Research*, 1(4), 2009, 1368-1371.

Kasture AV, Mahadik KR, Wadodker SG. Instrumental methods of Pharmaceutical Analysis, Pune:nirali *prakshan.* 14th edition, 2006, 2- 21, 48-50.

Nizamuddin S, Gurupadayya BM, Ravi MC, Manohara YN, Raju SA. *Ind.J.Pharm.Science*, 69, 2007, 451-453.

Saltzman R, Jurewicz R, Boon R. Safety of Famciclovir in patients with Herpes Zoaster and Genital Herpes, *Antimicrob.Agents Chemother*, 38, 1994, 24-54.

Syed nizzamudin K C, Prasanth T, Shekshavali B K, Sridhar, Appala Raju S. RP-HPLC Method for the estimation of famciclovir in bulk and pharmaceutical dosage form. *Int. J. Chem. Sci*, 7(1), 2009, 9-15.

Vanita S, Gowri Sankar D. Development and validation of an RP-HPLC method for estimation of famciclovir in bulk and pharmaceutical formulations. *Current Pharma Research*, 1(3), 2011, 227-231.