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UV spectrophotometric method for estimation of famciclovir in pharmaceutical formulation

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Abstract

A simple, rapid, sensitive and accurate UV-spectrophotometric method has been developed for the estimation of Famciclovir in pharmaceutical formulation. The method was developed by using 0.1 N HCl as a solvent and absorbance was measured at 312 nm. The drug exhibited the linearity in the concentration range of 2-12 µg/ml with a correlation coefficient of 0.9978. The % recovery of the drug was found to be 98.62 % - 100.5 %. The method was validated as per ICH guidelines. The proposed method is economical and sensitive for the estimation of Famciclovir in bulk and tablet dosage form.

Keywords: Famciclovir; 0.1 N HCl; UV-spectrophotometric method; ICH guidelines

1. Introduction

Famciclovir is chemically 2-{2-(2-amino-9H-purin-9-yl) ethyl} trimethylene diacetate (Fig-1). It is an acyclic guanine nucleoside analog and a new generation antiviral drug. This new generation antiviral drug is orally administered in the treatment of herpes zoster and genital mucocutaneous herpes. Famciclovir is a prodrug of the antiviral agent penciclovir. Its molecular weight is 321.3 g/mol and molecular formula C₁₄H₁₉N₅O₄. It is freely soluble in acetone and methanol, and sparingly soluble in ethanol and isopropanol. It is a white to pale yellow solid. It is marketed as a white, film-coated tablet. 125 mg and 250 mg tablets are round, whereas 500 mg tablets are oval in shape. Inactive ingredients consist of hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, polyethylene glycols, sodium starch glycolate and titanium dioxide. Since famciclovir is widely used in the antiviral therapy, it is important to develop and validate analytical methods for its determination in pharmaceutical dosage forms.

Literature survey reveals analytical methods reported for estimation of Famciclovir in API, pharmaceutical dosage form and biological fluid includes spectrophotometry [1-4] and RP-HPLC [5-7]. The HPLC and LC-MS analytical techniques are quite expensive and not easily available in every lab. So the alternate choice is the spectrophotometric technique in which UV spectrophotometric methods [8-15] are widely used because of its cost effectiveness and easy availability. Here authors attempt to develop and validate a simple, rapid and sensitive zero order UV spectrophotometric method for the estimation of Famciclovir in API and pharmaceutical formulation. The developed method is validated as per the ICH guidelines [16] and results are statistically interpreted.

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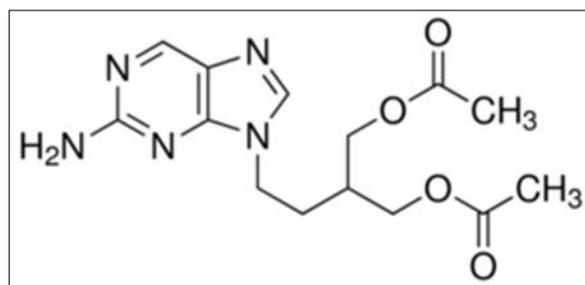


Figure 1 Structure of Famciclovir

2. Material and methods

2.1. Instrumentation

All the absorbance and spectral measurements were made by using ELICO (Hyderabad, India) double beam model SL 244 UV/VISIBLE Spectrophotometer. One cm matched quartz cells were used for absorbance measurements. The pH measurements were performed with an LI 1120 (Elico, India). All weighing were carried out on AUX220 (Shimadzu, Japan). Ultra sonicator (Citizen ultra sonicator) were used for solubilisation purpose.

2.2. Reagents and materials

Famciclovir raw material was obtained from Yarrow Chem. products, Mumbai, India. Analytical grade Hydrochloric acid was obtained from SD Fine Chemicals. Tablet formulation VIROVIR (FDC Limited, India) containing Famciclovir 500 mg was purchased from local pharmacy. All reagents and solvents used were analytical grade. Double distilled water was obtained from a water purification unit.

2.3. Preparation of standard solution

A standard stock solution was prepared by weighing 10 mg of Famciclovir pure, dissolved with little quantity of 0.1 N HCl and transferred into 10 ml volumetric flask. The volume was made up to the mark with 0.1 N HCl. This solution produces 1000 µg/ml Famciclovir concentration. Working standard solution equivalent to 100 µg/ml of Famciclovir was obtained by appropriate dilution of stock solution with 0.1 N HCl.

2.4. Preparation of working standard solution

From the standard stock solution 0.4 ml was transferred to 10 ml volumetric flask and diluted to 10 ml with 0.1 N HCl to get a concentration of 4 µg/ml. Working standard solution of Famciclovir was scanned between 200–400 nm. The wavelength maximum exhibited for Famciclovir was at 312 nm (Fig.2).

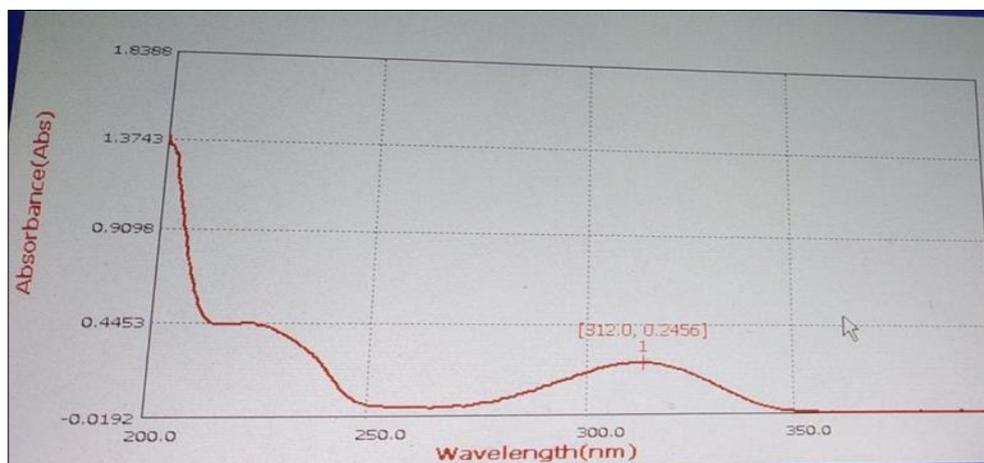


Figure 2 UV spectra of Famciclovir

2.5. Procedure for Calibration Curve

Appropriate volume of aliquot (0.2-1.2 ml) from standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with 0.1 N HCl to give solutions concentrations in the range of 2-12 µg/ml. The absorbance measurements of these solutions were carried out against 0.1 N HCl as blank at 312 nm. Calibration curve was constructed by plotting absorbance versus concentrations. Linear regression equation was obtained from this calibration curve.

2.6. Procedure for Pharmaceutical Formulation

Ten tablets (VIROVIR) were weighed and average weight was calculated. Tablets were triturated to a fine powder in a mortar and pestle. Tablet powder equivalent to 10 mg Famciclovir was accurately weighed and transferred to 100 ml volumetric flask. To this 60 ml of 0.1 N HCl was added and sonicated for 10 min. The flask was shaken and volume was made up to the mark with 0.1 N HCl. The above solution was filtered through whatmann filter paper No.41. From the above solution 0.6 ml was transferred to 10 ml volumetric flask. Volume was made up to the mark with 0.1 N HCl to give a solution containing 6 µg/ml. The content in the tablet was calculated from the calibration curve.

3. Results and discussion

3.1. Method Validation

The developed method was validated in terms of Linearity, precision, accuracy, Limit of detection (LOD) and Limit of Quantitation (LOQ), robustness and ruggedness.

3.1.1. Linearity

Six point calibration curve was obtained in a concentration range from 2-12 µg/ml for Famciclovir. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was $y=0.0575x+0.0005$ with correlation coefficient 0.9978 (Table 1 and 2, Fig. 2).

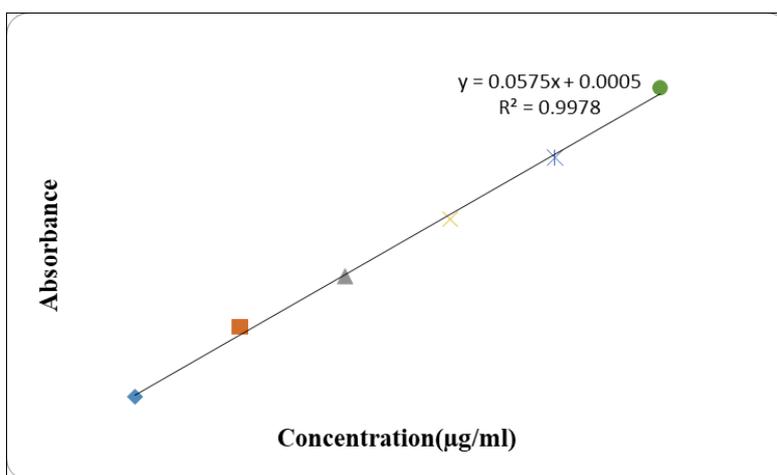


Figure 2 Calibration curve of Famciclovir

Table 1 Linearity Data for Famciclovir

S. No	Concentration (µg/ml)	Absorbance
1	2	0.112
2	4	0.245
3	6	0.342
4	8	0.451
5	10	0.568
6	12	0.702

Table 2 Optical Characteristics of Famciclovir

S. No	Parameters	Method
1	λ_{\max} (nm)	312
2	Beers law limit ($\mu\text{g/ml}$)	2-12
3	Sandell's sensitivity ($\mu\text{g/cm}^2/0.001 \text{ A.U}$)	0.0034
4	Molar absorptivity ($\text{L mol}^{-1} \text{ cm}^{-1}$)	2.5×10^4
5	Correlation coefficient (r)	0.9978
6	Regression equation($Y=mX+c$)	$Y=0.0575X+0.0005$
7	Slope(m)	0.0575
8	Intercept(c)	0.0005
9	LOD ($\mu\text{g/ml}$)	0.14
10	LOQ ($\mu\text{g/ml}$)	0.44
11	Standard error of mean of Regression line	0.00294

3.1.2. Precision

Precision was checked in terms of repeatability, inter and intraday precision. It was expressed in percentage RSD.

Repeatability

The repeatability was evaluated by assaying six times of sample solution prepared for assay determination. Percentage RSD was calculated (Table 3).

Table 3 Repeatability

Concentration($\mu\text{g/ml}$)	Absorbance
6	0.341
6	0.345
6	0.344
6	0.343
6	0.345
6	0.345
Mean	0.343
Standard Deviation	0.0016
% RSD	0.466

Interday and Intraday precision

The intraday and interday precision study of Famciclovir was carried out by estimating different concentrations of Famciclovir six times on the same day (intraday precision) and on different days (interday precision) and the results were reported in terms of Percentage RSD (Table 4). The developed method was found to be précised as the average % RSD values for intraday and inter-day precision study was found to be 1.68 % and 1.51 % respectively (Table 4).

Table 4 Results for precision

Precision	Intraday		Interday	
	Morning	Evening	Day 1	Day 2
Mean (n=6)	0.342	0.346	0.332	0.347
SD	0.007305	0.006735	0.007305	0.006802
RSD	0.016878	0.015096	0.016878	0.015194
%RSD	1.68	1.50	1.68	1.51

3.1.3. Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the known amount of marketed formulation at three different concentration levels 80, 100 and 120 % taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed three times and average recoveries were measured (Table 5). Results of recovery study were within the range of 92.62 %-100.5 % indicating that the developed method is an accurate method for determination of Famciclovir. The results are summarized in Table 3.

Table 5 Results for Accuracy

Recovery level (%)	Concentration ($\mu\text{g/ml}$)		%Mean Recovery* \pm SD	%RSD
	Test Con.	Std. added		
80	6	4.8	99.12 \pm 0.99	1.01
100	6	6	99.62 \pm 0.28	0.29
120	6	7.2	100.51 \pm 0.16	0.17

*Average of three determinations

3.1.4. Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ± 2 nm. The effect of variation in solvent strength was studied at ± 0.02 N HCl. For changes of conditions, the sample was assayed in triplicates. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values. Assay for all deliberate changes of conditions should be within 98.0–102.0 % for the proposed method (Table 6).

Table 6 Robustness Studies

Formulation	Amount of drug taken from tablet(mg)	At 310 nm	At 314 nm
		(n=3) %Assay \pm %RSD	(n=3) % Assay \pm %RSD
VIROVIR (Tablets)	500	99.73 \pm 0.313	99.91 \pm 0.224
		At 0.08 N HCl	At 0.12 N HCl
		(n=3)%Assay \pm %RSD	(n=3)% Assay \pm %RSD
		99.32 \pm 0.498	99.67 \pm 0.602

3.1.5. Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogeneous slot by two analysts using same operational and environmental conditions (Table 7).

Table 7 Ruggedness Studies

Formulation	Amount of drug taken from tablet(mg)	Analyst 1	Analyst 2
		(n=3) %Assay±%RSD	(n=3)%Assay±%RSD
VIROVIR (Tablets)	500	99.83±0.243	99.86±0.324

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated according to below equation given by

$$\text{LOD} = 3.3 \sigma/s$$

$$\text{LOQ} = 10 \sigma/s$$

Where σ is the standard deviation of y intercepts of regression lines and s is the slope of the calibration curve (Table 2). The low value of LOD and LOQ indicate that the method is sensitive.

3.2. Application of method to formulation

The proposed was applied to pharmaceutical formulation of Famciclovir (Table 8).

Table 8 Assay of VIROVIR

Formulation	Labeled Amount(mg)	Amount* Obtained(mg)	%Purity ± SD
VIROVIR(Tablets)	500	499.86	99.97%± 0.685

*Average of three determinations

The % purity of Famciclovir presented in the table indicates that there is a good recovery of Famciclovir in tablet formulation by the developed UV spectrophotometric method.

4. Conclusion

A simple, economic, precise, accurate and sensitive UV spectrophotometric method for estimation of Famciclovir in bulk and in formulation was developed. This developed method was validated according to ICH guidelines. Beer's law was obeyed in concentration range of 2-12 $\mu\text{g/ml}$. The correlation coefficient (r^2) for Famciclovir was found to be 0.9978. The % recoveries were found to be in the range of 99.84-100.18 % for Famciclovir. The precision of method was determined by repeatability, intraday and interday precision whose % RSD < 1% indicates the precision of the method. The Limit of detection for Famciclovir was found to be 0.14 $\mu\text{g/ml}$. Limit of quantitation for Famciclovir was found to be 0.44 $\mu\text{g/ml}$. The proposed method was found to be simple, accurate, precise, reproducible and gave acceptable recovery of the analyte which can be applied to analysis of bulk and pharmaceutical capsule formulation of Famciclovir. Additionally, the short analysis time and low cost is the other advantages of these methods for routine analysis. Its advantages over other existing methods are simplicity, speed and low cost.

Compliance with ethical standards

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Disclosure of conflict of interest

None

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