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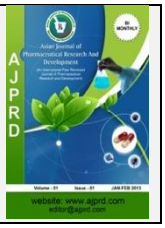

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Research Article

Development of New Sensitive Spectrophotometric Method for Estimation of Famciclovir Using 3-Amino Phenol

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A B S T R A C T

A new simple, sensitive and specific method has been developed for determination of famciclovir in bulk and pharmaceutical dosage forms using 3 amino phenols as a chromogenic reagent. The purpose of this analytical validation procedure is to validate it by laboratory experiments to prove that the method meets the minimum standards for laboratory use. In this reaction drug reacts with sodium nitrite at 0-5˚C in acidic conditions, it forms diazonium salt which is highly reactive and readily couples with 3-amino phenol and leads to formation of a coloured complex measured spectrophotometrically in visible region (*i.e.*, 400-800nm) shows a maximum absorbance at 441nm. This method can be successfully applied for the determination of drug content in pharmaceutical formulations. The results of analysis have been validated statistically.

Key Words: Famciclovir,3-amino phenol, Validation, UV Spectroscopy, ICH guidelines.

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INTRODUCTION:

Famciclovir is a prodrug of penciclovir, is a nucleoside analogue DNA polymerase inhibitor[1]. Famciclovir is chemically known as 2-[2-(2-amino-9H-purin-9-yl) ethyl] -1,3-propanediol diacetate[2]. Its molecular formula is $C_{14}H_{19}N_5O_4$ with molecular weight of 321.3. It is a synthetic acyclic guanine derivative and has the following structure.

Famciclovir tablets are available in three strengths. The tablets of strength 125mg, 250mg are white, round film-coated while tablets of strength 500mg are white, oval film-coated. Famciclovir is a white to pale yellow in colour and is freely soluble in ethanol and isopropanol. Famciclovir is freely soluble (>25% w/v) at 25°C in water.Itprecipitates quickly as sparingly soluble (2%-3% w/v) monohydrate. Its melting point is 102-104^{o}C[3,4].

Literature reviewrevealed that several spectrophotometric[5-20] and liquid chromatographic (LC)[21-24] methods were reported for the determination of famciclovir in bulk drug and pharmaceutical dosage forms.Drug of a particular composition is marketed with various brand names. Minimal changes in the chemical composition and standard of the drug will have a profound effect on the physiological and biological activities of the patient. Spectrophotometric technique has been found to be one of the best versatile analytical techniques for the quantitative estimation of micro quantities of substances with greater amount of accuracy.

The objective of the work is to develop new spectrophotometric method for estimation of famciclovir in bulk and tablet dosage form which is visible, sensitive, economic, and useful for routine quality control of famciclovir in pharmaceutical formulations.In the present study, famciclovir reacts with sodium nitrite at 0-5^{o}C, it forms a diazonium salt which on addition of 3-amino phenol forms yellow colored complex having λ_{max}at 441nm.

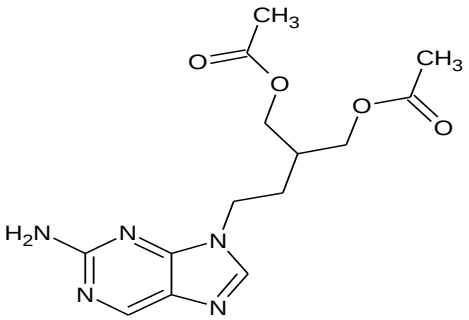

Figure 1: Molecular structure of Famciclovir

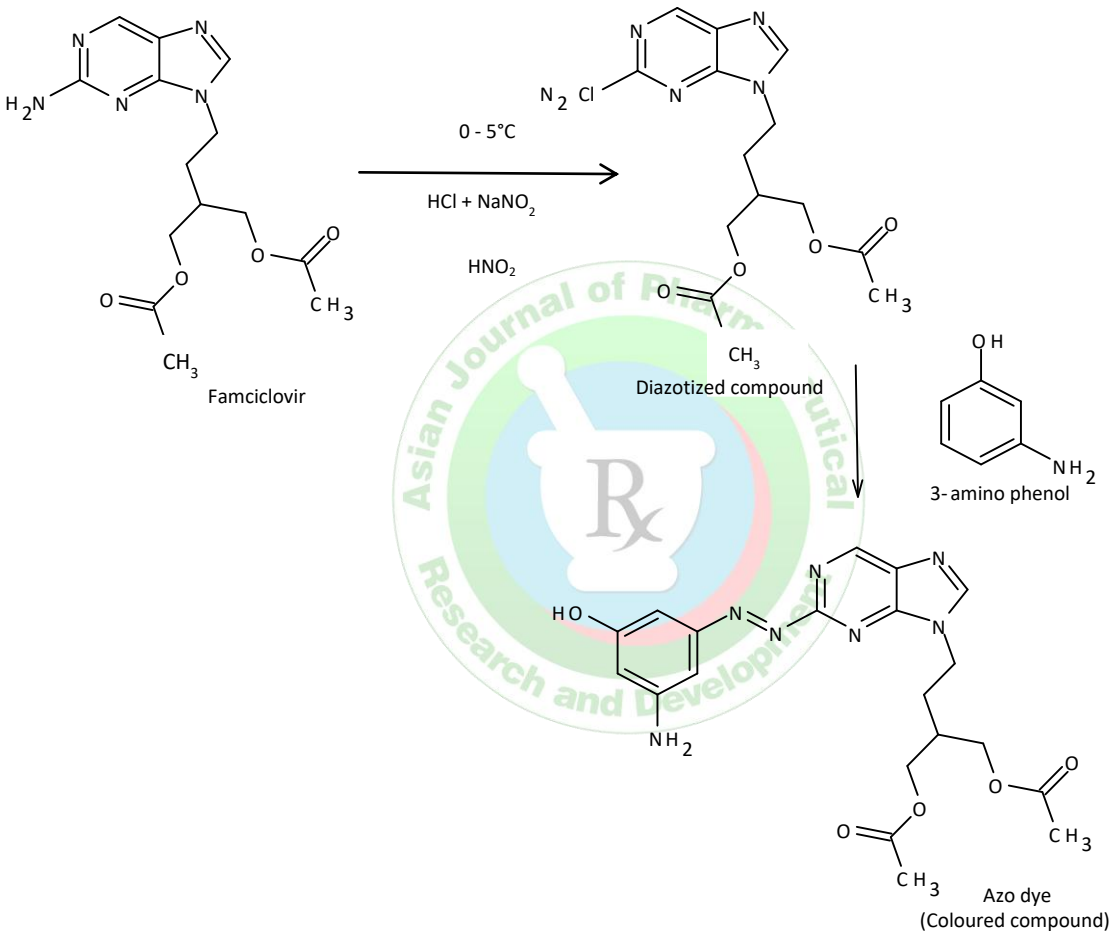

Figure 2: Reaction scheme of famciclovir with 3-amino phenol reagent

MATERIALS AND METHODS:

Chemicals and reagents:

3aminophenol, Sodium nitrite, Hydrochloric acid, ammonium sulphamate were obtained from Sd fine chemicals limited (SDFCL), Mumbai. All chemicals used were of analytical grade. Solutions were prepared using double distilled water. Famciclovir standard was gift from Hetero labs, Hyderabad. Penvir 250mg film coated tablets (Famciclovir), were manufactured by Hetero healthcare limited (Assam) and were purchased from the local drug store.

Instruments:

UV-visible spectrophotometer (Shimadzu 1800), pH meter (Elico LI-127), Digital balance (Shimadzu BL220H), Ultrasonic bath Sonicator (PCI Analytics 6.5li200H), Hot Air Oven (Tempo Equipment Private Limited).

Preparation of reagent and chemical solutions:

Preparation of 3-amino phenol solution (0.15%):

Solution was prepared by dissolving 150mg of 3-amino phenol in 100mL distilled water.

Preparation of sodium nitrite solution (0.1%):

Solutionwas prepared by dissolving 100mg of sodium nitrite in 100mL of distilled water.

Preparation of ammonium sulphamate solution (0.1%):

Solutionwas prepared by dissolving 100mg of ammonium sulphamate in 100mL of distilled water.

Preparation of stock solution:

Standard Famciclovir, 10mg was weighed and transferred to 10mL volumetric flask and dissolved in 0.1N HCl. The flask was shaken and was made up to the mark with 0.1 N HCl to give a solution of 1000µg/mL. From this stock solution 1mL was pipetted out into another 10mL volumetric flask and the volume was made up to 10mL with 0.1N HCl to give 100µg/mL. From this, 1mL was pipetted out into another 10mL volumetric flask, and the volume was made up to 10mL with 0.1N HCl to give 10µg/mL.

Determination of Absorption maximum:

1mL of drug solution is taken into a 10mL volumetric flask. 1mL of 0.1N hydrochloric acid solution is transferred into a 10mL standard flask. To this solution 1mL of 0.1% sodium nitritesolution is added, 1mL of ammonium sulphamate and 3-amino phenol is added. The resultant solution is diluted to the mark of the 10mL with distilled water.

The optical absorbance is measured in the wavelength range from 300 to 700nm against the blankwhich shows a maximum absorbance at 441nm and the absorption spectrum is shown in figure 3.

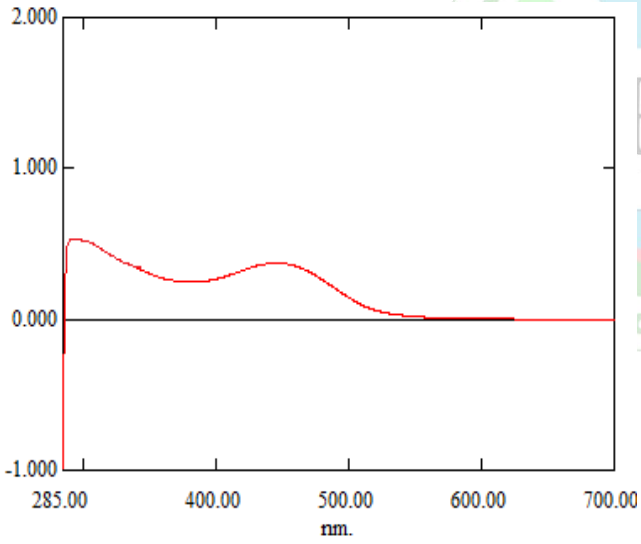

Figure 3: Spectrum of famciclovir with 3-amino phenol

RESULTS AND DISCUSSION:

Calibration curve for Famciclovir with 3-amino phenol (1000-5000ng/mL):

From 10µg/mL stock, aliquots of 1, 2, 3, 4, 5mL was taken in 10mL test tubes followed by addition of 1mL of 0.1N hydrochloric acid solution, 1mL of sodium nitrite solution and allowed to stand for five minutes at 0-5°C temperature. 1mL of ammonium sulphamate was added, and the contents are thoroughly mixed and allowed to stand for five minutes at 0-5°C temperature for complete diazotisation. Then 1mL of 3-amino phenol solution was added and allowed to stand for 5 minutes at room temperature, yellow colour is formed. Then the volume was made up to 10mL with distilled water to give a solution of 1000, 2000, 3000, 4000 and 5000ng/mL. The absorbance of the resulting-coloured solution was measured against respective blank solution (i.e., without drug) in the visible region. The proposed methods were validated as per the ICH guidelines[25].

Table 1: Calibration curve data of famciclovir

Concentration(ng/mL)	Absorbance
1000	0.044
2000	0.076
3000	0.111
4000	0.145
5000	0.184

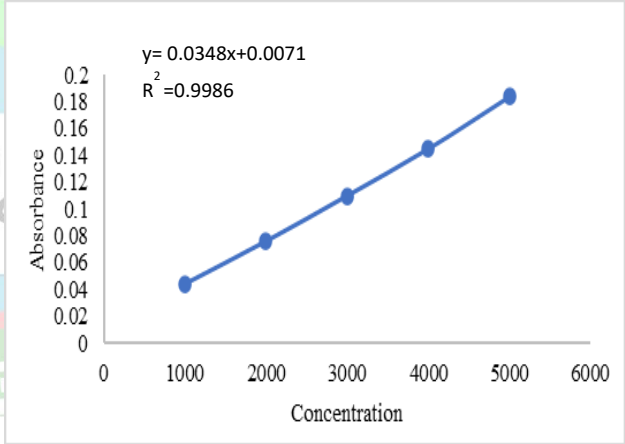

Figure 4: Calibration curve for Famciclovir at 431nm with 3-amino phenol

Precision:

The precision of the developed analytical method was assessed by checking repeatability, intra-day precision and inter-day precision for famciclovir drug using 3-amino phenol as reagent.

Repeatability:

Repeatability assessment of an analytical method was performed by analysing six replicates of single concentration of 3000ng/mL. Absorbances of samples were recorded at 441nm. The % relative standard deviation (RSD) was calculated arepresented in **table 2.**

Table 2: Repeatability data of famciclovir using 3-amino phenol at 441nm

S. No	Concentration (ng/mL)	Absorbance	Mean ± SD	% RSD
1	3000	0.109	0.111 ± 0.002	1.5
2	3000	0.109		
3	3000	0.111		
4	3000	0.112		
5	3000	0.111		
6	3000	0.113		

Intermediate Precision:

Intra-day precision: The intra-assay precision of the proposed method was determined on samples of drug solutions at varying concentration levels (32000ng/mL, 4000ng/mL, 4800ng/mL) by analysing three replicates of each sample as a batch in a single assay run at 441nm and results were reported in terms of relative standard deviation. The results arepresented in **table 3.**

Table 3: Intra-day precision data of famciclovir using 3-amino phenol reagent at 441nm

S. No	Concentration(ng/mL)	Absorbance		Mean ± SD	%RSD
1	3200	Day-1	0.119	0.120 ± 0.02	1.3
		Day-2	0.120		
		Day-3	0.122		
2	4000	Day-1	0.140	0.142 ± 0.02	1.5
		Day-2	0.143		
		Day-3	0.144		
3	4800	Day-1	0.165	0.165 ± 0.02	0.9
		Day-2	0.163		
		Day-3	0.166		

Inter-day precision:

The Inter-assay precision was determined by analysing the same samples (32000ng/mL, 4000ng/mL, 4800ng/mL) in three consecutive days at 441nm and results were reported in terms of relative standard deviation. The results arepresented in **table 4.**

Table 4: Inter-day precision data of famciclovir using 3-amino phenol reagent at 441nm

S. No	Concentration (ng/mL)	Absorbance			Mean ± SD	%RSD
		Day-1	Day-2	Day-3		
1	3200	0.119	0.120	0.122	0.120 ± 0.02	1.3
2	4000	0.140	0.143	0.144	0.142 ± 0.02	1.5
3	4800	.0.165	0.163	0.166	0.165 ± 0.02	0.9

Accuracy:

The analytical accuracy is the nearness of the results obtained against the real values at each level of famciclovir concentration. The results obtained for accuracy studies for the drug substance and drug product were reported in terms of % RSD and % recovery respectively.

For drug substance:

Accuracy for drug substance was determined on samples of drug solutions at varying concentration levels in the range of 80%-120% (2400ng/mL, 3000ng/mL, 3600ng/mL) by analysing three replicates of each sample as a batch in a single assay.

For drug product (recovery study):

To study the accuracy of drug product, 10 tablets were weighed, powdered and estimation was carried out. Recovery studies were carried out by adding known amount of standard drug (2400 ng/mL, 3000 ng/mL, 3600ng/mL) to the sample solution (3000ng/mL). The results are presented in **table 5.**

Table 5: Accuracy data of famciclovir (pure drug) using 3-amino phenol at 441nm

Tablet used	Levels	Amount of sample added (mL)	Amount of standard added (ug/mL)	Amount recovered (µg/mL)	% Recovery ± SD
Famciclovir	80%	2.4	3.0	2.5	96.66±0.058
	100%	3.0	3.0	3.1	95.4±0.058
	120%	3.6	3.0	3.7	97.7±0.058

Assay:

Twenty tablets of famciclovir (Penvir - famciclovir tablets IP 250mg manufactured by Hetero labs Limited) were weighed and finely powdered. The powder equivalent to 10mg was weighed and transferred to 10mL volumetric flask. The flask was shaken, and volume was made up to mark with 0.1N HCl to obtain a solution of 1000µg/mL. From the above solution 1mL was pipetted out into 10mL graduated tube and volume was made up to mark with 0.1N HCl to obtain a solution of 100µg/mL. From the above solution (100µg/mL) of standard drug solution, 0.1mL was pipetted out followed by addition of 1.0 mL of 0.1N HCl solution, 1.0 mL of sodium nitrite solution, 1.0 mL of ammonium sulphamate solution, 1mL of 3-amino phenol solution. The absorbance of the yellow colour solution is read at 441nm, against reagent blank. The amount of the drug present in an unknown sample is estimated from the calibration graph.The obtained results are presented in **table 6.**

Table 6: Assay data of tablet formulation

Tablet used	Label Claim(mg)	Amount found	% Purity
Famciclovir with 3-amino phenol	250	244.1	97.64%

CONCLUSION:

The proposed visible spectrophotometric 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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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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