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Development and Validation of Various UV Spectrophotometric Methods for the Estimation of Famciclovir in Bulk and its Formulation

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Abstract: The present paper described about the development and validation of three different simple, sensitive, rapid, accurate and economical UV Spectrophotometric methods. The developed methods were used for the quantitative estimation of famciclovir in bulk drug and its pharmaceutical dosage form. UV 1800 double beam UV Visible Spectrophotometer with a pair of 10mm path length matched quartz cells were used for the study. Method A (Borate buffer pH9), Method B (0.1N NaOH) Method C (Phosphate buffer pH4) and Method D (Phosphate buffer pH 7) were developed for estimation of famciclovir by zero-order and first-order derivative. Beer's law is valid in the concentration range of 10-80 µg/mL and 10-90 µg/mL respectively and the correlation coefficient was found to be 0.999. The percentage recoveries were found to be 98-102%. The relative standard deviation was found to be <2%. LOD&LOQ were estimated. The statistical analysis proves that the methods are reproducible and selective for the routine analysis of famciclovir in bulk and its pharmaceutical dosage form.
Keywords: Famciclovir, Spectrophotometer, method development, validation.

INTRODUCTION

Famciclovir is chemically 2-{2-(2-amino-9H-purin-9-y1) ethyl} trimethylene diacetate [1] (Fig-1). It is an acyclic guanine nucleoside analog and a new generation antiviral drug [2]. This new generation antiviral drug is orally administered in the treatment of *herpes zoster* and genital mucocutaneous herpes [3]. Famciclovir is a prodrug of the antiviral agent penciclovir. Its molecular weight is 321.3 and molecular formula $C_{14}H_{19}N_5O_4$. 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 where as 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 [4]. Few analytical methods have been reported for the estimation of famciclovir in biological fluids or pharmaceutical formulations include liquid chromatography and UV-visible spectrophotometry [5-9].

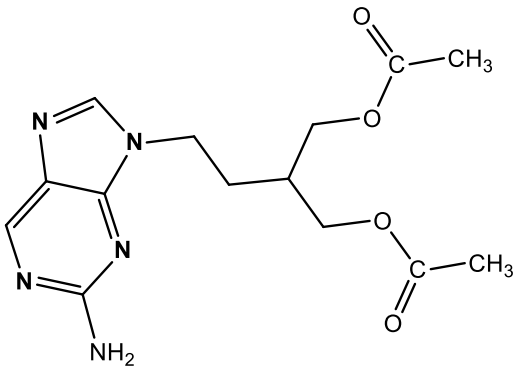


Fig-1: Chemical structure of Famciclovir

Still there was no work was reported for the estimation of famciclovir using zero and first order derivative, hence it is necessary to develop simple, economic, accurate and validated method using UV spectrophotometer for the estimation of famciclovir.

MATERIALS AND METHODS

Chemicals and reagents:

All the chemicals used for the experiment were of analytical grade. Distilled water was used throughout the experiment. The tablet formulations were procured from local pharmacy.

Instrumentation

UV 1800 double beam UV Visible Spectrophotometer with a pair of 10mm path length matched quartz cells were used for the study. The UV solutions 2.42 software was used.

Preparation of standard drug solution

Standard stock solution was prepared by weighing 10mg of pure drug, transferred into 100mL volumetric flask. The contents of the flask were dissolved in 50mL methanol. Then the solution was subjected to ultrasonication for about 10mins. The volume was then adjusted to the mark with methanol.

Preparation of test solution:

Test solution was prepared by taking the tablets contain 250 mg strength. Twenty tablets were weighed and powdered. The tablet powder equivalent to 10 mg weight of famciclovir was transferred into 100 mL volumetric flask containing 50 mL of methanol and flask was kept for ultrasonication for 10 minutes, then it was diluted up to the mark with methanol and then the solution was filtered through Whatmann filter paper No. 41. Working standard was prepared from the above solution. 10 mL was pipetted out into a 100 mL volumetric flask and the volume was made up to the mark with methanol. The final concentration of famciclovir was brought to 100 µg/mL. The prepared solution was scanned in the range of 200-400 nm to determine the wavelength of maximum absorption.

METHOD VALIDATION

The proposed method was optimised using methanol as stock solvent and various buffers as diluted solvents; the present method was validated for the various parameters as per ICH guidelines [10].

Linearity

Different aliquots were taken from working solution and diluted with borate buffer pH 9, 0.1N NaOH, phosphate buffer pH-4, phosphate buffer pH-7 separately to prepare series of concentrations from 10-80 µg/mL and 10-90 µg/mL respectively. Absorbance was measured at 305 nm and the calibration curve was plotted between concentration and absorbance.

LOD and LOQ

Limits of detection (LOD) and quantification (LOQ) were calculated directly from the calibration plot. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, correspondingly, where σ is the standard deviation of intercept and S is the slope of the calibration plot [11].

Assay of Famciclovir in tablet dosage form

Twenty tablets were weighed and powdered. Weight equivalent to 10mg was dissolved in 50 mL methanol in a 100mL volumetric flask. The solution was then filtered and the filtrate was diluted with methanol to get the solution of 100 µg/mL concentration and prepare a working standard of 100µg/mL. From the above solution four unknown concentrations were taken into four individual 10ml volumetric flasks. The volume was made upto the mark by adding individual buffer solutions (method A, B, C, D) to each volumetric flasks. Then they were analyzed in UV-visible spectrophotometer individually taking buffer as reference solution at wavelength 200nm-400nm.

Table-1: Assay of Famciclovir from tablet dosage form

Methods	Labeled claim(mg)	Drug recovered(mg)	%Drug recovered ±SD	%RSD
A	250	249.72	99.88±0.39	0.52
B	250	248.92	99.56±0.41	0.94
C	250	248.56	99.42±0.82	0.83
D	250	249.43	99.77±0.49	0.59

Accuracy

Accuracy of the method was expressed as recovery study, ascertained by standard addition method at 3 levels. Standard quantity equivalent to 50%, 100% and 150% is to be added in samples.

Intra and Inter day Precision

It is determined by analyzing the drug at three different known concentrations and each concentration is analyzed for three times, on a same day and calculated the values of mean, SD, and %RSD. The inter day study determined likewise, but the analysis was carried out for three consecutive days and Mean, SD, and %RSD values were calculated.

RESULTS AND DISCUSSION

The solutions of famciclovir in methanol (10µg/mL) were scanned individually at the series of wavelengths of 200nm-400nm at zero order derivative and the first order derivative spectra was taken at smoothening factor of the instrument using Shimadzu 1800 Spectronic UV Visible spectrophotometer.

Overlain spectra were depicted in fig 2-5 and summary of validation parameters were represented in table-1

Table-1: Summary of validation parameters

Methods	**Parameters**						
	Beer's Law Limit	**Correlation coefficient**	**%Recovery± SD**	**LOD (µg/mL)**	**LOQ (µg/mL)**	**Sandell's sensitivity**	**Molar absorptivity**
Method A *i)zero order*	10-80 µg/mL	0.9997	99.5±0.3	0.068	0.182	0.05406	6105.308
ii)first-order derivative		0.9996	99.7±0.8	0.066	0.184	-	-
Method B *i)zero order*	10-80 µg/mL	0.9995	99.7±0.3	0.082	0.198	0.05556	5944.642
ii)first-order derivative		0.9992	98.52±1.1	0.085	0.189	-	-
Method C *i)zero order*	10-90 µg/mL	0.9997	99.51±0.13	0.094	0.177	0.06579	4884.2464
ii)first-order derivative		0.9996	100.4±0.13	0.098	0.184	-	-
Method D *i)zero-order*	10-90 µg/mL	0.9990	100.63±1.3	0.076	0.192	0.05263	5783.976
ii)first-order derivative		0.9991	99.3±1.43	0.081	0.197	-	-

Accuracy of the methods was ascertained by standard addition method at 3 levels. Standard quantity equivalent to 50%, 100% and 150% is to be added in sample. The result shown that best recoveries in between 98-102% indicating that the method was accurate and the results were given in table no.2-5.

Table-2: Accuracy data of Method A (Borate buffer pH-9)

Method A	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	Mean ± SD
Zero order	10	30	29.9	99.7±0.3
	10	40	40.47	101.2±0.3
	10	50	49.63	99.3±0.3
First order	10	30	29.68	99.0 ±0.6
	10	40	39.82	99.0 ±1.0
	10	50	50.07	100.2 ±0.5

Table -3: Accuracy data of Method B (0.1N NaOH)

Method B	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	Mean ± SD
Zero order	10	30	29.9	99.7±0.3
	10	40	40.47	101.2±0.3
	10	50	49.63	99.3±0.6
First-order	10	30	29.68	99.0±0.6
	10	40	39.61	99.0±0.6
	10	50	50.07	100.2±0.5

Table-4: Accuracy data of method D (Phosphate buffer pH 4)

Method C	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	Mean ± SD
Zero order	10	30	29.68	99±0.74
	10	40	39.44	98.93±0.41
	10	50	50.02	100.18±0.45
First order	10	30	29.84	99.48±0.92
	10	40	39.72	99.31±0.46
	10	50	50.6	101.2±0.38

Table-5: Accuracy data of Method C (Phosphate buffer pH 7)

Method D	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	Mean ± SD
Zero order	10	30	30.28	100.93±1.1
	10	40	40.26	100.66±0.69
	10	50	50.3	100.7±0.63
First order	10	30	29.08	99.35±0.8
	10	40	40.54	101.36±0.6
	10	50	50.54	101.09±0.6

Precision of the method was considered by repetitive measurements of drug solution and the result showed lower %RSD values. The %RSD for intra-day precision and inter-day precision for famciclovir were given in table 6-13.

Table-6: Intraday precision data of method A (Borate buffer pH-9)

Method A	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	20	19.75	98.8±0.8	0.8
	40	39.73	99.3±0.2	0.2
	80	79.14	98.9±0.2	0.2
First order	20	19.83	99.2±0.8	0.8
	40	39.71	99.3±0.3	0.3
	80	79.08	98.8±0.1	0.1

Table-7: Inter day precision data of method A (Borate buffer pH-9)

Method A	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	20	19.79	99.0±0.5	0.5
	40	39.87	99.7±0.6	0.6
	80	79.23	99.0±0.9	0.9
First order	20	19.75	98.8±0.5	0.5
	40	39.66	99.1±0.4	0.4
	80	78.9	98.6±0.2	0.2

Table-8: Intraday precision data of method B (0.1 N NaOH)

Method B	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	20	19.63	98.52±1.1	1.2
	40	40.8	101.93±0.1	0.1
	80	79.86	99.83±0.1	0.1
First order	20	19.92	99.63±1.0	1.0
	40	40.09	100.22±0.3	0.3
	80	80.21	100.26±0.1	0.1

Table-9: Inter day precision data of method B (0.1 N NaOH)

Method B	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	20	19.61	98.1± 0.4	0.4
	40	40.72	101.80±0.3	0.3
	80	79.83	99.79±0.1	0.1
First order	20	19.82	99.28±0.5	0.5
	40	40.23	100.59±0.5	0.5
	80	79.85	99.82±0.4	0.4

Table-10: Intraday precision data of method D (Phosphate buffer pH 4)

Method C	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	20	19.74	98.74±0.51	0.51
	40	39.45	98.65±0.34	0.35
	80	79.61	99.51±0.13	0.13
First order	20	19.76	98.84±0.66	0.66
	40	39.41	98.54±0.42	0.42
	80	80.30	100.4±0.7	0.7

Table-11: Inter day precision data of method D (Phosphate buffer pH 4)

Method C	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	20	19.96	101.3±1.37	1.36
	40	39.81	98.86±0.67	0.67
	80	80.12	98.1±0.4	0.4
First order	20	20.15	101.8±1.03	1.01
	40	39.84	99.62±0.47	0.47
	80	79.84	99.84±0.18	0.18

Table-12: Intraday precision data of method C (Phosphate buffer pH 7)

Method D	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	20	19.61	98.1±0.6	0.6
	40	40.40	101.0±0.2	0.2
	80	80.79	101.0±0.1	0.1
First order	20	19.8	99.5±1.6	1.6
	40	40.38	101.0±0.4	0.4
	80	79.78	99.74±0.15	0.15

Table-13: Inter day precision data of method C (Phosphate buffer pH 7)

Method D	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	20	19.7	98.3±0.55	0.6
	40	40.0	100.0±1.44	1.4
	80	80.8	101.03±0.1	0.1
First order	20	19.65	98.3±1.8	1.8
	40	40.33	100.9±0.5	0.5
	80	79.83	99.8±0.23	0.23

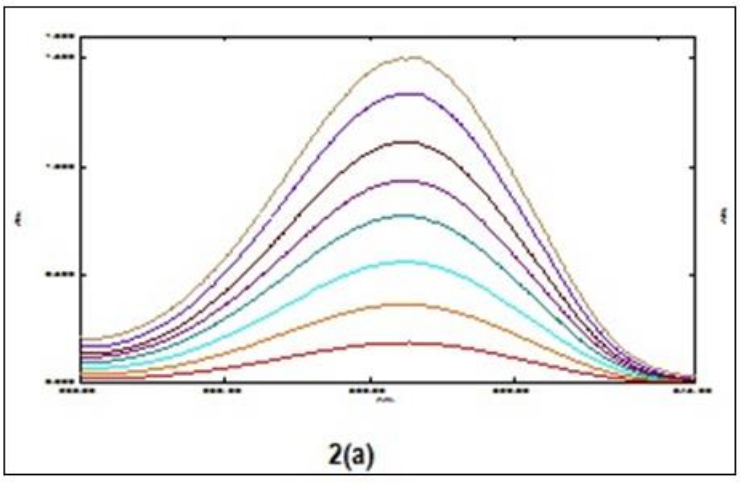


Fig-2(a): Overlain spectra (zero order derivative).

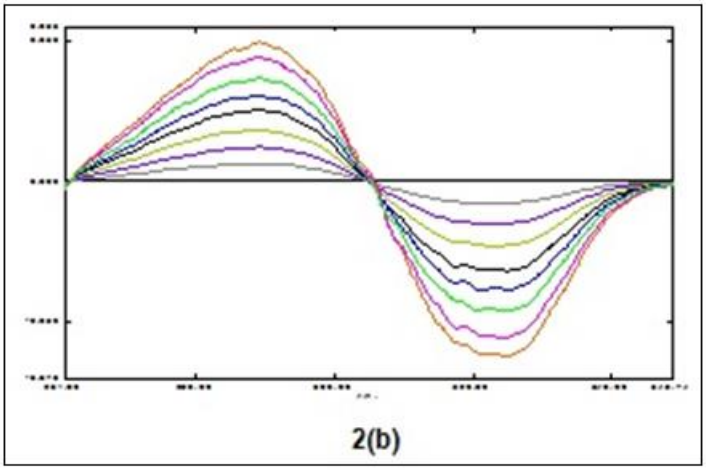


Fig-2 (b): Overlain spectra (first-order derivative: Method A (Borate buffer pH 9)

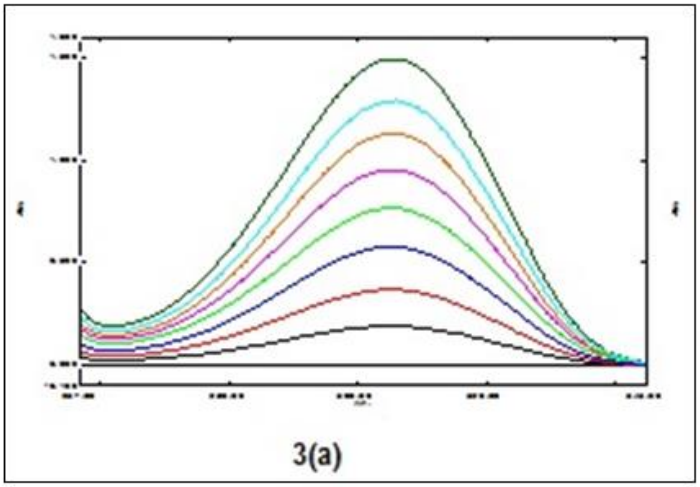


Fig-3 (a): Overlain spectra (zero order derivative)**.**

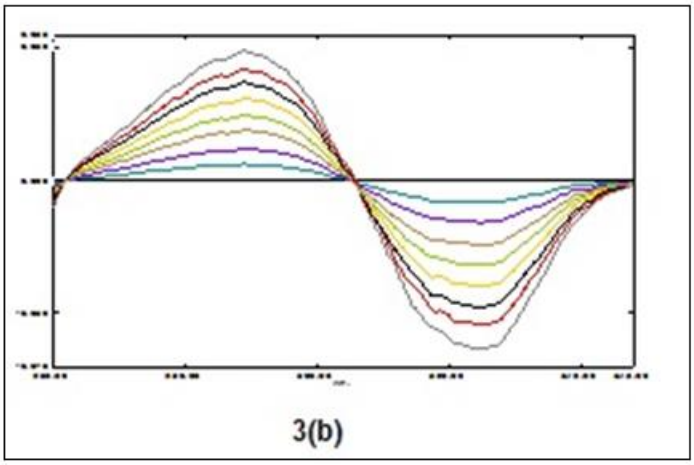


Fig-3 (b): Overlain spectra (first-order derivative): Method B (0.1N NaOH).

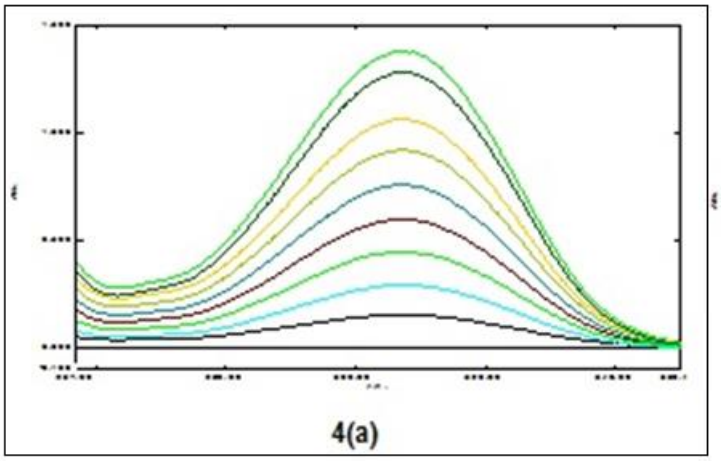


Fig-4 (a): Overlain spectra (zero order derivative)**.**

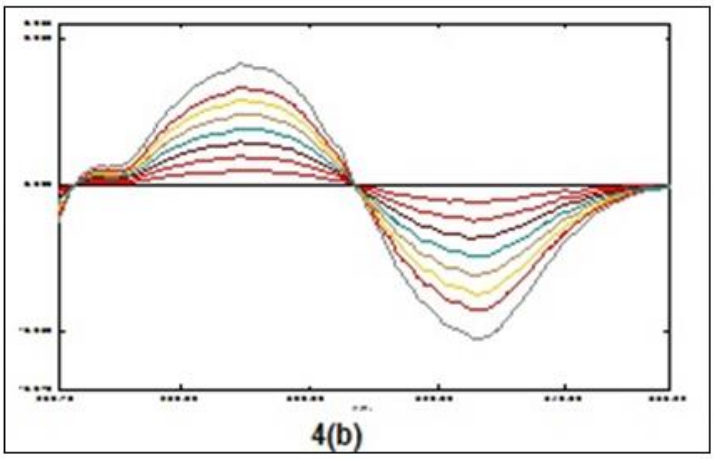


Fig-4(b): Overlain spectra (first-order derivative). Method C (Phosphate buffer pH-4).

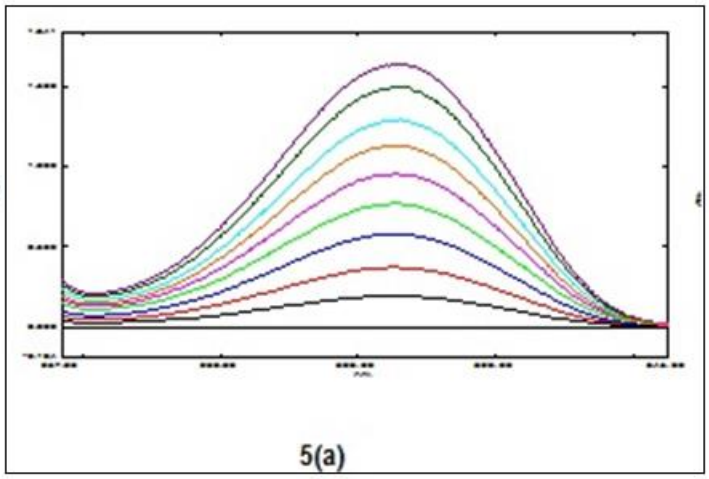


Fig-5(a): Overlain spectra (zero order derivative**).**

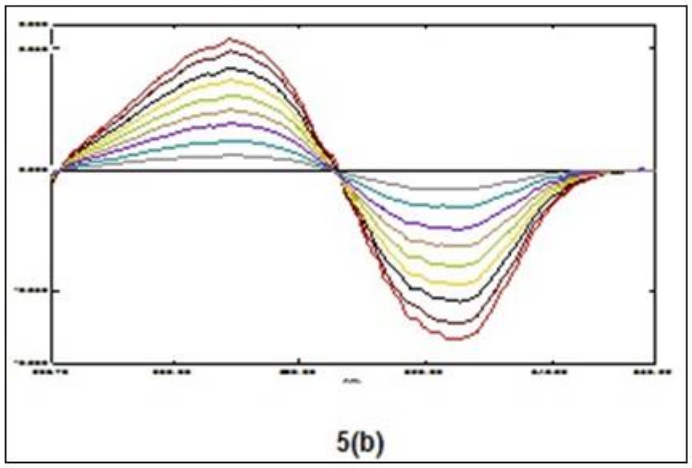


Fig-5(b): Overlain spectra (first-order derivative): Method D (Phosphate buffer pH-7)

Beer Lambert's law was obeyed in the concentration range of 0-80µg/mL and 0-90 µg/mL for famciclovir. The response of the drug was found to be linear in the investigation. Calibration curves for all the methods (A, B, C and D) were shown in fig 6-9.

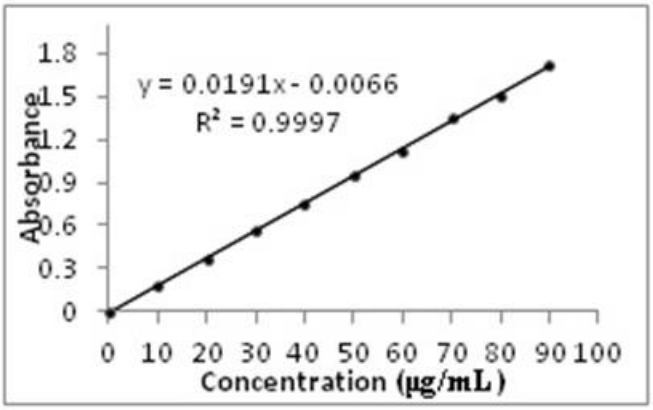


Fig-6a: Calibration curve of Borate buffer pH-9 (zero order derivative)**.**

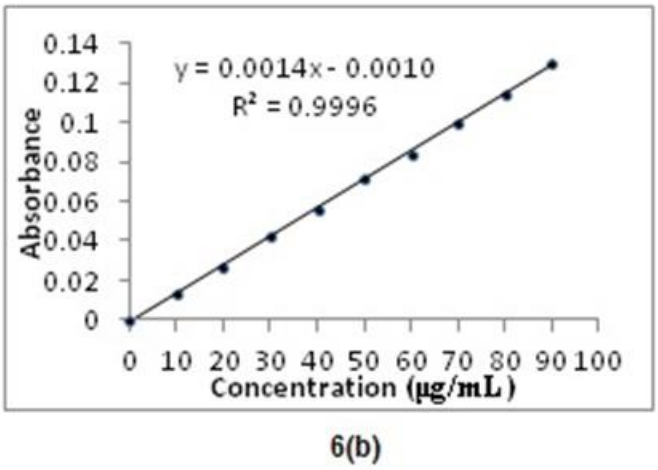
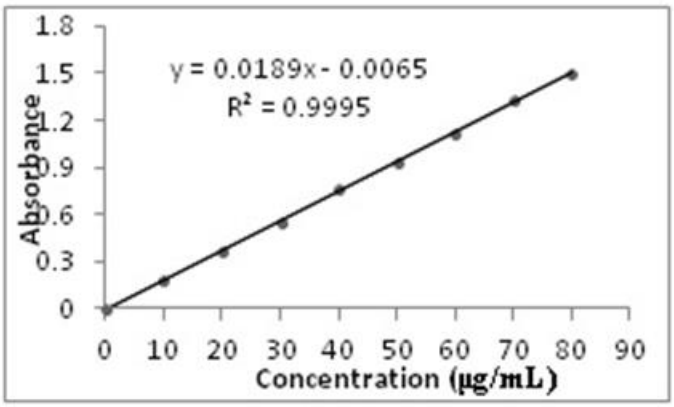
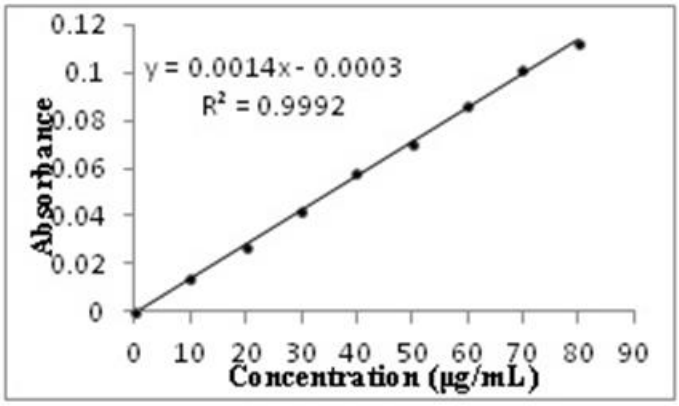


Fig-6b: Calibration curve of Borate buffer pH-9 (first-order derivative)



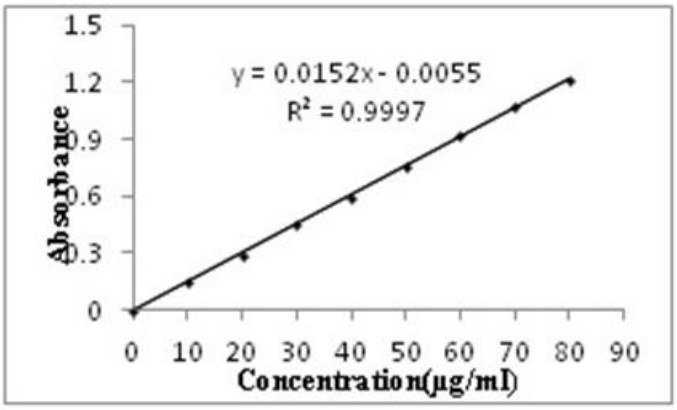
7(a)

Fig-7a: Calibration curve of 0.1N NaOH (zero order derivative).



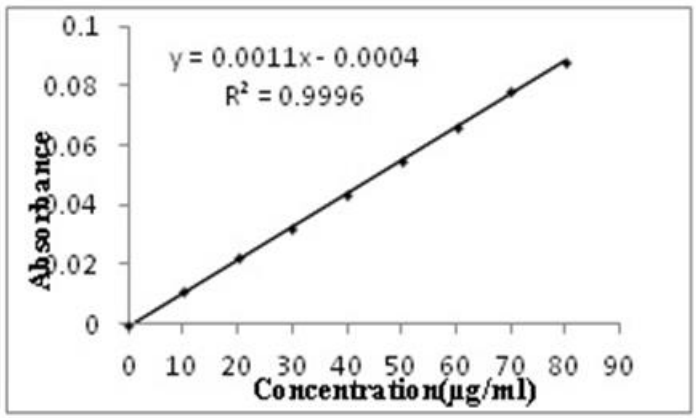
7(b)

Fig-7b: Calibration curve of 0.1N NaOH (first-order derivative).



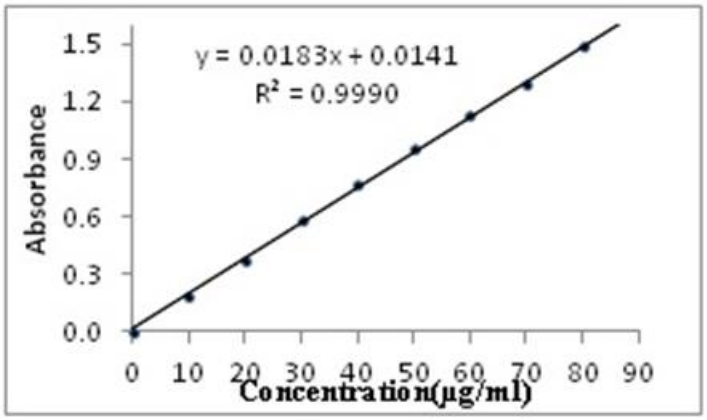
8(a)

Fig-8a: Calibration curve of Phosphate buffer pH 4 (zero order derivative).



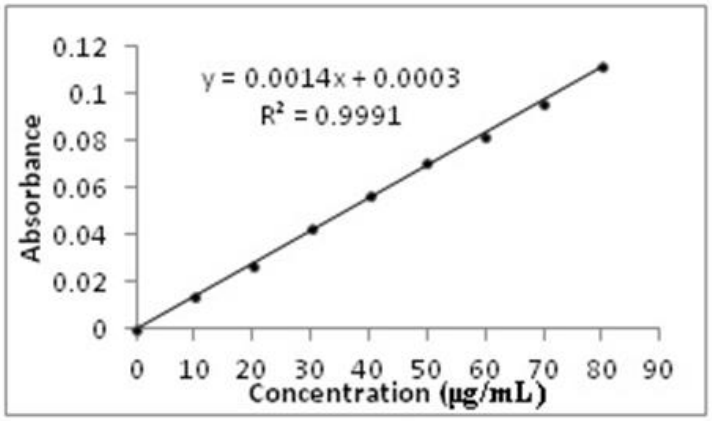
8(b)

Fig-8b: Calibration curve of Phosphate buffer pH 4 (first order derivative)



9(a)

Fig--9a: Calibration curve of Phosphate buffer pH 7 (zero order derivative).



9(b)

Fig-9b: Calibration curve of Phosphate buffer pH 7 (first order derivative**).**

CONCLUSION

The λ_{max} of famciclovir was found to be 305nm. The calibration curves were obtained for the series of concentrations 0-80 (µg/mL) and 0-90 (µg/mL) and they were found to be linear and obeys Beer lambert's law. The developed methods were validated in terms of linearity, accuracy and precision The percentage recoveries were found to be 98-102% and %RSD was <2%. Sandell's sensitivity, molar absorptivity, LOD&LOQ values are within the limits in accordance with the ICH guidelines. Correlation coefficient was found to be 0.999. The summary of

validation parameters were reported in Table-1. The proposed methods are simple for estimation of Famciclovir in bulk and dosage form. Hence it is concluded that the analytical methods were specific, precise, accurate and stability indicating characteristics. As the results are satisfactory these developed methods can be used for routine analysis of formulations without interference from excipients.

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