Development and Validation of Liquid Chromatographic and UV Derivative Spectrophotometric Methods for the Determination of Famciclovir in Pharmaceutical Dosage Forms

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A high-performance liquid chromatographic method and a UV derivative spectrophotometric method for the determination of famciclovir, a highly active antiviral agent, in tablets were developed in the present work. The various parameters, such as linearity, precision, accuracy, specificity, robustness, limit of detection and limit of quantitation were studied according to International Conference on Harmonization guidelines. HPLC was carried out by using the reversed-phase technique on an RP-18 column with a mobile phase composed of 50 mM monobasic phosphate buffer and methanol (50 : 50; v/v), adjusted to pH 3.05 with orthophosphoric acid. The mobile phase was pumped at a flow rate of 1 ml/min and detection was made at 242 nm with UV dual absorbance detector. The first derivative UV spectrophotometric method was performed at 226.5 nm. Statistical analysis was done by Student's *t*-test and *F*-test, which showed no significant difference between the results obtained by the two methods. The proposed methods are highly sensitive, precise and accurate and therefore can be used for its Intended purpose.

Key words famcivclovir; HPLC; UV derivative spectrophotometry; validation; pharmaceutical dosage form

Chemically, famciclovir (FCV), known as 2-[2-(2-amino-9*H*-purin-9-yl)ethyl]-1,3-propanediol diacetate (Fig. 1) is a novel antiviral drug, which is highly efficient in treatment of acute uncomplicated herpes zoster. It was reported that FCV dosed at 250 mg three times daily for 7 d was effective as 800 mg acyclovir dosed five times daily for 7 d, in the treatment of the acute signs and symptoms of herpes zoster.[1] This drug is also used for the treatment of the ophthalmic zoster.[2] FCV is a synthetic guanine derivative, which is metabolized to penciclovir having the potent antiviral activity as another 9-substituted guanine derivative like acyclovir. Penciclovir is active against herpes simplex virus type 2, vericella zoster virus I, Epstein-Barr virus and hepatitis B,[3] as another 9-substituted guanine derivative like acyclovir, penciclovir is selectively phosphorylated in virus-infected cells to a monophosphate ester by thymidine kinase, followed by further phosphorylation to triphosphate ester which inhibits virus DNA polymerases.[4] Compared with acyclovir, penciclovir administration leads to higher triphosphate ester concentrations in virus-infected cells and consequently its antiviral activity persists for a longer time after removal of the compound.[4,5] FCV is absorbed rapidly and extensively after oral administration, and total systemic availability of penciclovir is 77%,[6] which is about four times higher than that of acyclovir.[7] Metabolism of FCV involves sequential hydrolysis of both acetyl groups to give 6-deoxypenciclovir, which is subsequently oxidized to penciclovir,[8,9] which was synthesized by Briony Brand *et al.*[10] FCV stability in different buffer solutions was studied by Zhang *et al.*[11] Since penciclovir is widely used in the antiviral therapy, it is important to develop and validate analytical methods for its determination in pharmaceutical dosage form. The HPLC method has been highly used in the quality control of drugs because of its sensitivity, reproducibility and specificity. On the other hand, the derivative UV spectrophotometric (UVDS) method is very simple, rapid, economical and allows the determination of drugs with sufficient reliability. The present work reports the development and validation of a HPLC method and a UVDS method for the estimation of FCV in tablets.

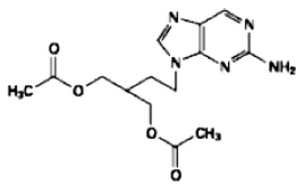

Fig. 1. Structure of Famciclovir

Experimental

Chemicals FCV was kindly supplied by Cipla Laboratories Ltd. (India). Pharmaceutical dosage forms (famtrex®, varovir®) containing FCV was obtained commercially. Methanol, potassium dihydrogenphosphate (A.R. grade), and orthophosphoric acid (A.R. grade) were obtained from Quiligens (Mumbai, India). Ultra pure water was obtained from a Milli-Q® UF-Plus apparatus (Millipore) and was used to prepare all solutions for the HPLC method and distilled water was used to prepare all solutions for the UVDS method. All solutions were prepared daily.

Instrumentation and Analytical Conditions The HPLC method was performed on a Waters HPLC system, equipped with a model binary pump, UV dual absorbance detector (2487), Rheodyne injector (033381) fitted with a 20-μl loop and breeze software used to monitoring the results. The method was conducted using a reversed-phase technique. FCV was eluted isocratically with a flow rate of 1.0 ml/min using a mobile phase consisting of 50 mM monobasic phosphate buffer and methanol (50 : 50; v/v), adjusted to pH 3.05 with orthophosphoric acid. The wavelength of the UV detector was set to 242 nm. The mobile phase was prepared daily, filtered through a 0.45-μm membrane filter (Millipore) and sonicated before use. A symmetry C18 analytical RP-18 column (250 mm×4.0 mm i.d., 5-μm particle size) (Waters) was used. The HPLC system was operated at 23±1 °C. UVDS method was performed on a UV-visible Spectrophotometer model 164 (Elico, India) at 226.5 nm using 1.0 cm quartz cells and spectra treats software was used for all absorbance measurements.

Preparation of the Standard Solutions HPLC: Accurately weighed 100 mg of FCV reference standard was transferred to 100 ml volumetric flask and dissolved in ultra pure water (final concentration of 1 mg/ml). From this solution, working standard solution 100 μg/ml was prepared. The concentrations of 5, 10, 20, 30, and 40 μg/ml were made in 10 ml volumetric

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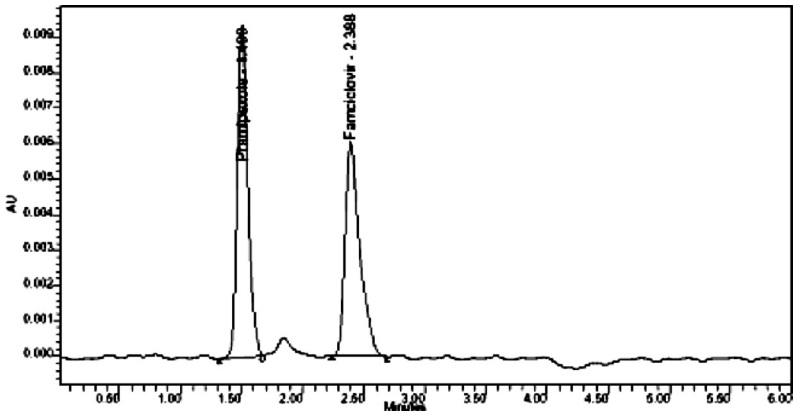

Fig. 2. Chromatogram of Famciclovir with Internal Standard Pramipexole

flasks and the volume was adjusted with mobile phase.

UVDS: Accurately weighed 100 mg of reference standard was transferred to 100 ml volumetric flask and dissolved in distilled water (final concentration of 1 mg/ml). From this solution, the concentrations of 10, 25, 50, 75, and 100 μg/ml were made in 10 ml volumetric flasks and volume was adjusted with distilled water.

Preparation of Famciclovir Samples from Accolate Tablets: About 20 tablets of famtrex, varovir (each tablet contains 250, 500 mg of famciclovir as API) were weighed and thoroughly powdered. The amount of powder equivalent to about 100 mg was placed in a 100 ml volumetric flask. To it around 90 ml of solvent (water) was added and the flask was placed in an ultrasonic bath for 15 min. The solution was then cooled and diluted to volume with the same solvent. The solution was filtered though a 0.45-μm filter and then the filtrate were used to prepare sample solutions of different concentrations.

Conditions HPLC: HPLC separation was carried out by a Symmetry Shield RP18, 5-μm, 250×4.6 mm column (Waters make). The mobile phase flow rate was 1.0 ml min^{-1}. The analysis was carried out at ambient temperature (23 °C). The sample injection volume was 20 μl. The UV detection was carried out at 242 nm for the determination of famciclovir.

UVDS: For FCV solutions, the first derivative spectra were recorded in the wavelength range 200—400 nm using water as reference. The instrument settings were optimized to produce a spectrum with about 80% full-scale deflection and acceptable noise level. Each spectrum was recorded in triplicate. For each replicate measurement the cell was refilled with fresh solution.

Method Validation The methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures.[12] Student's *t*-test and *F*-test were used to verify the validity of the methods. Pramipexole was used as an internal standard.

Linearity: The calibration curve was obtained with five concentrations of the standard solution, as 5—40 μg/ml for HPLC method and 10—100 μg/ml for UVDS method, respectively. The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision: The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples, at same concentration and during the same day. The intermediate precision was studied by comparing the assays on different days (3 d). Five sample solutions were prepared and assayed.

Robustness: The robustness of the HPLC method was determined by analysis of samples under a variety of conditions such as small changes in the pH (3.0—3.6) and in the percentage of methanol (40—50%) in mobile phase and changing the column (Metachem® LC RP-18, with 250 mm×4.6 mm i.d. and 5 μm particle size). The effect on retention time and peak parameters were studied.

Limit of Detection and Limit of Quantitation: The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation.

Results and Discussion

HPLC A reversed-phase HPLC method was proposed as a suitable method for the estimation of FCV in pharmaceutical dosage forms. The chromatographic conditions were adjusted in order to provide a good performance of the assay. The HPLC procedure was optimized with a view to develop an accurate and reproducible method so as to resolve the internal standard from the drug. Various conditions such as mobile phase compositions, analyzing columns with different packing materials (C18, C8, phenyl), and configurations (10, 15, 25 cm columns) were tested so as to obtain a sharp peak and also to resolve the peak of internal standard. Mobile phase was selected from peak parameters (symmetry, tailing), run time, easy of preparation and cost. Figure 2 shows a typical chromatogram obtained from the standard and a FCV solution using the proposed method. As shown in this figure, FCV was eluted forming symmetrical peak, well separated from the internal standard. The retention time observed (2.38 min) allows a rapid determination of the drug, which is important for routine analysis. From the peak of drug, the mobile phase consisting of 50 mM phosphate buffer and methanol in the ratio 50 : 50 (v/v), pH adjusted to 3.05 was found to be an appropriate mobile phase on the column used at a flow rate of 1.0 ml/min. In the proposed system FCV peak was eluted with a capacity factor (k') 4.73, tailing factor (T) 1.2. The calibration curves for FCV were constructed by plotting concentration *versus* peak area ratio, and showed good linearity in the 5—40 μg/ml range. The representative linear equation was $y=0.26571x-0.0285$, with a correlation coefficient ($r=0.999$) indicating a high sensitivity of the method (Table 1). The LOD and LOQ were found to be 0.19 and 0.61 μg/ml, respectively. The precision of this method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as R.S.D. (%) of a series of measurement. The experimental values obtained for the determination of FCV in samples are presented in Table

Table 1. Results of the Analysis of the Data for the Quantitative Determination of Famcilovir by the Proposed Methods

Statical parameter	HPLC	UVDS
Concentration range (μg/ml)	5—40	10—100
Regression equation	$y=0.2657x-0.0285$	$y=0.00469x-0.01820$
Correlation coefficient (r)	0.9999	0.99679
Stand error on estimations (S_c)	−0.02271	0.01571
Standard deviation on slope (S_b)	0.00079	0.00022
Standard deviation on intercept (S_a)	0.01689	0.01321
Limit of detection (LOD) (μg/ml)	0.19068	8.53
Limit quantification (LOQ) (μg/ml)	0.61	28

2. The result obtained shows R.S.D. of 0.26%, indicating good intra-day precision. Inter-day variability was also calculated from assays on 3 d a mean R.S.D was 0.24%. The mean recovery was found to be 99.33 for famtrex 250 mg, 99.39 for famtrex 500 mg, 99.58 for varovir 250 mg, and 98.85 for varovir 500 mg (Table 3), indicating an agreement between the true value and the value found.

Table 2. Results of the Determination in Bulk by the Proposed Methods

Method		Experimental amount	% purity	R.S.D.
HPLC	1 d	502.42	100.48	0.2666
		501.43	100.28	
		499.42	99.88	
		499.81	99.86	
		499.53	99.90	
	2 d	501.31	100.26	0.2704
		499.45	99.89	
		502.01	100.40	
		498.95	99.79	
		499.31	99.86	
	3 d	496.37	99.27	0.1803
		498.45	99.69	
		498.36	99.67	
		497.61	99.52	
		498.41	99.68	
UVDS	1 d	501.15	100.23	0.4621
		502.32	100.46	
		497.48	99.49	
		502.32	100.46	
		503.45	100.69	
	2d	502.25	100.45	0.7903
		503.45	100.69	
		496.32	99.26	
		497.32	99.45	
		505.43	101.08	
	3 d	500.67	100.02	0.7797
		503.53	100.76	
		496.43	99.28	
		497.45	99.49	
		493.56	98.71	

UVDS Method The spectrum of a 40-μg/ml FCV solution in water (against a blank of the same) is shown in Figs. 3a and b. Two intense absorbance peaks with λ_{max} at 221 and 304 nm are apparent. Several assays were carried out using the first, second, third and fourth derivative of the spectra, and the best results were obtained when using the amplitude from the valley at a wavelength of 226.5 nm to the zero base line. With first derivative spectra good linearity was obtained on standard solutions of FCV over the 10—100 μg/ml concentration range. The linearity equation was $y=-0.00174x-0.01820$ ($r=0.9968$), where x is the FCV concentration (expressed as μg/ml) and y is the amplitude from the valley at a wavelength of 226.5 nm to the zero base line was chosen. Precision assessed on the standard solutions was satisfactory; R.S.D.% values of 0.46% (repeatability) and 0.67% (intermediate precision) were found for five replicates at a concentration of 500 μg/ml. The first derivative spectra of formulation sample solutions (Fig. 3b) are morphologically identical to those of the standard solutions. The results obtained shows R.S.D of 0.46 indicating good intra-day precision. Inter-day variability was calculated from assays on 3 d and a mean R.S.D. was found to be 0.68 (Table 2). Accuracy was calculated adding known amounts of FCV pure substance to powdered formulations, obtaining additions of 25, 50, 75, 100, 125 μg/ml (total concentrations: 25, 50, 75, 100, 125 μg/ml). As seen from Tables 2 and 3, all assays gave satisfactory results: the mean amount found of declared was always between 98.8 and 99.8% for all formulations, while precision R.S.D.% values were always under 1.7% and accuracy above 98.6%. The LOQ was 28 μg/ml and the LOD 8.5 μg/ml, according to ICH guidelines.[12)]

Comparison between HPLC Method and UVDS Method The Student's *t*-test was applied and does not reveal significant difference between the experimental values obtained in the sample analysis by the two methods. The calculated *t*-value and *F*-value was found to be less than the tabular values at 95% confidence limits (Table 4).

Table 3. Results of the Determination of Famciclovir in Tablets

	UVDS			HPLC		
	Taken (μg/ml)	Found (μg/ml)	% recovery[a)]	Taken (μg/ml)	Found (μg/ml)	% recovery
Famtrex 250	25	24.45	97.8	10	9.93	99.33
	50	49.15	98.34	25	24.55	98.2
	75	75.93	101.24	50	50.03	100.06
	100	99.13	99.13	75	74.79	99.72
	125	124.16	99.32	100	99.38	99.38
Famtrex 500	25	24.32	97.28	10	9.87	98.7
	50	49.10	98.20	25	24.61	98.24
	75	74.32	99.09	50	49.50	99.00
	100	98.65	98.65	75	75.54	100.74
	125	124.16	99.32	100	99.31	99.31
Varovir 250	25	25.15	100.6	10	9.84	98.4
	50	49.15	98.5	25	24.85	99.4
	75	73.93	98.57	50	50.01	100.02
	100	102.35	102.35	75	75.25	100.33
	125	123.45	98.76	100	99.75	99.75
Varovir 500	25	24.12	96.48	10	9.85	98.5
	50	48.95	97.79	25	24.81	98.56
	75	73.15	98.86	50	49.15	98.30
	100	98.93	98.93	75	75.51	100.58
	125	126.03	100.82	100	98.35	98.35

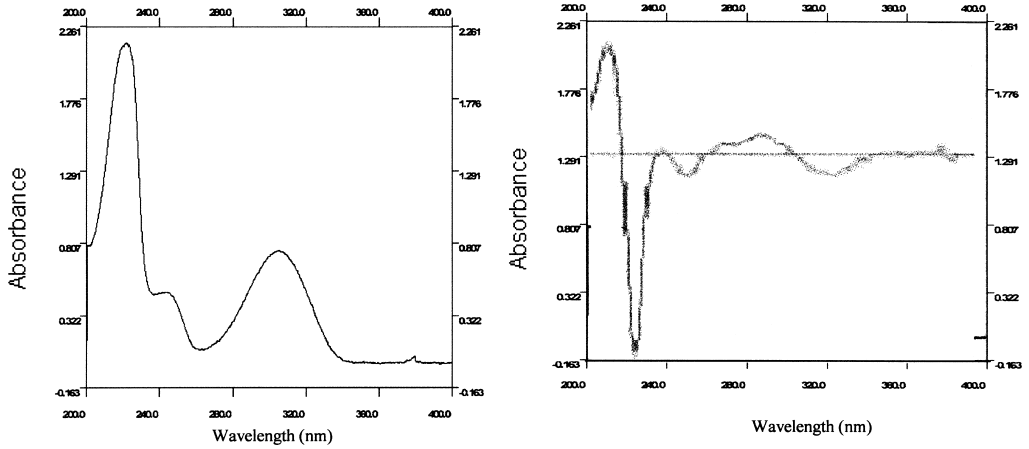

Fig. 3. (a) Ultraviolet Spectrum of a Famciclovir Standard Solution (10 μg/ml) and (b) First Derivative Spectrum of Famciclovir Standard Solution (10 μg/ml)

Table 4. Results Obtained in the Comparison of HPLC and UVDS Methods

Sample	HPLC (R.S.D.%)	UVDS (R.S.D.%)	*F*-test[a)]	*t*-test[b)]
Famtrex 250	0.7057	0.8956	0.2815	0.3461
Famtrex 500	0.8187	0.8217	1.3483	0.1950
Varovir 250	1.6936	0.7464	0.1958	0.4127
Varovir 500	0.9797	1.6241	0.3659	0.4356

a) Value at 95% confidence. *b*) Value at 95% confidence.

Conclusion

The two proposed methods based on the UVDS and HPLC, are suitable for determination of FCV in the commercial tablets. The methods are simple, reliable, fast and reproducible. The spectrophotometric method requires only wavelength scan and automatic calculation of the first derivative value, while the HPLC was less than three minutes. Furthermore, the proposed methods are inexpensive and low polluting, because small volumes are required for preparation of samples.

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