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High-Pressure Liquid Chromatographic Method Development and Validation for Estimation of Acyclovir in Raw and Tablet Formulation

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Original Research Article

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Abstract: A simple, precise and gradient RP- HPLC method has been developed and validated for Aciclovir raw and in tablet formulation. The proposed method was validated to obtain official requirements including accuracy, linearity, precision, selectivity and stability. The estimation was developed on $C_{(18)}$ column reversed-phase using the mobile phase composition as Phosphate buffer: methanol in the ratio (60:40 %. v/v). 1ml /minute was the flow rate and the maximum absorption were observed at 290 nm using Shimadzu SPD-20A Prominence UV-Vis detector. Aciclovir showed a precise and good linearity, the concentration range was 5-25 µg/mL. The RP-HPLC, assay showed the highest purity ranging 99.59 to 100.97% for Acyclovir tablet formulation. 100.19 % was the mean percentage purity. The Aciclovir retention time was found to be 3.01 minutes. The method accuracy was showed by statistical analysis.

Keywords: Aciclovir, methanol, buffer, HPLC and UV

INTRODUCTION

Acyclovir, 9-[(2-hydroxyethoxy)-methyl]-guanosine, is an acyclic guanosine derivative, which exhibits a selective inhibition of herpesviruses replication with potent clinical antiviral activity against the herpes simplex and varicella-zoster viruses [1, 2]. It is available by mouth and intravenously. Acyclovir is generally considered safe for use in pregnancy with no harm having been observed [3]. It appears to be safe during breastfeeding. Aciclovir is a nucleic acid analogue made from guanosine. It works by decreasing the production of the virus's DNA [4].

There are many works published for the determination of Acyclovir in biological fluids of different species. For a laboratory, to develop a method is sometimes a compromise between cost, time consumption, and purpose of study. Several HPLC methods have been reported for determination of Acyclovir in human serum using UV or fluorescence detection [5–6]. We present herein for the first time, a sensitive and selective HPLC method for the Acyclovir [7-9]. The HPLC method development for the estimation for Aciclovir is a new method, which will fulfil all ICH guidelines requirements of validation. Increasing the importance of speed, time and reliability of analysis in pharmaceutical analytical laboratories, a new method for Aciclovir determination in tablet formulation with a very short analysis time is described in this method. The proposed method is very fast, quick and accurate in terms of chromatographic retention time and run time compared with other reported methods. The proposed aim of this study was to develop simple, accurate, specific and precise RP-HPLC method for the Aciclovir estimation in the bulk and pharmaceutical tablet formulation.

MATERIALS AND METHODS

Chemicals: The Aciclovir reference standard (RS) was purchased from Sigma, Germany. The Acivir tablet (Aciclovir 400 mg) manufactured and marketed by Cipla Ltd, purchased from, local Pharmacy from Vellore, India. The HPLC grade acetonitrile, water and methnol were purchased from Sigma, German.

RP-HPLC instrumentation: Shimadzu LC-20 AT HPLC system, using SPD-10 detector (SPD-M20A, Japan). A Zodiac C(18) column (50mm x 4.6mm, 5µm) with Pore size 95Å. The temperature of the column was maintained at 27ºC and 1ml/min was the flow rate. The volume of injection was 20µl, 290nm was set as a wavelength and the HPLC run time was set for 7 minutes.

Preparation of mobile phase: Buffer preparation: 5 ml of Triethylamine transferred into 1000

ml of analytical gade water and Orthophosphoric acid is used to adjust the pH to 3, the resulting solution is then filtered by 0.45µm nylon membrane filter and then degassed. Mobile phase: Buffer and Methanol were mixed in 60:40 ratio which was then sonicated to degas. Approximately 400 ml of methanol was transferred into 1 liter volumaetric flask and 600 ml of Phosphate buffer was added and mixed thoroughly by shaking and the pH was adjusted to 6 by gradual adding of 0.5N phosphoric acid, the resulting solution was filtered with 0.45µ membrane filter. The final mobile phase was prepared by adding the ratio of 60:40 %. V/V Phosphate buffer: methanol.

Preparation of Aciclovir stock solution

Standard Aciclovir solution: 10 mg of raw Aciclovir raw drug weighed accurately and transferred to clean 100 ml volumetric flask and 25ml volume of mobile phase was added to the flask and volume was made upto 100 ml with mobile phase. Aciclovir raw drug results 100 µg/mL of drug concentration. The concentration of 5-25 µg/mL was achived by diluting the Aciclovir standard stock solution with mobile phase.

Pharmaceutical Preparations: 10 tablets of Aciclovir marketed tablet were weighed accurately and powdered. The powder amount equivalent to 10 mg of Aciclovir was taken in 100 ml volumateric flask and 50 ml of mobile phase was added to dissolved the drug substance and sonicate for 5-10 min, then 50 ml of mobile phase was added to make final volume of 100ml. The resulting mixture was filtered through a Whatman Filter paper No. 41 and transferred to a new 100 ml volumetric flask. The concentration of 5-25 µg/mL was achived by diluting the standard stock solution with mobile phase. Aciclovir powder were very freely soluble in water.

Solution stability: The prepared drug solution stability was analysed during the time of analysis and also repeated the same analysis method on same day with different time intervels. The same analysis was repeated after 24 hrs by keeping the drug solution under laboratory temperature (37 ± 1°C) and in refrigeration (5 ± 1°C).

Method validation: The proposed method was preceded to achieve a new, sensitive and easy method for estimation of Aciclovir by RP-HPLC from tablet formulation. The experimental analysis was validated according to the ICH guidelines, recommendations and USP-30.

System suitability: The resolution, retention time, tailing factor and column theoretical plates parameters was performed by six replicates of standards and three replicates of sample preparation.

RESULTS AND DISCUSSION

The calculated RSD values are well below 2%, which indicating the accuracy and the precision of the proposed method and also shows the low standard error values. The result shows the good index of accuracy and reproducibility of the method. Flow rate, detection wavelength was maintained constant. The typical RP-HPLC conditions are presented in Table -1.

The HPLC chromatogram of Aciclovir standard and Aciclovir tablet formulation is presented in figure 1 and 2.

Table-1: HPLC conditions for estimation of Aciclovir

Parameters	Description
Column	Zodiac C(18) column (50mm x 4.6mm, 5µm)
Column temperature	27 ± 1°C
Mobile phase	Phosphate buffer: methanol (60:40 %. v/v)
Detection	Photodiode array detection at 290 nm
Injection volume	20 µl
Flow rate	1 ml min^{-1}

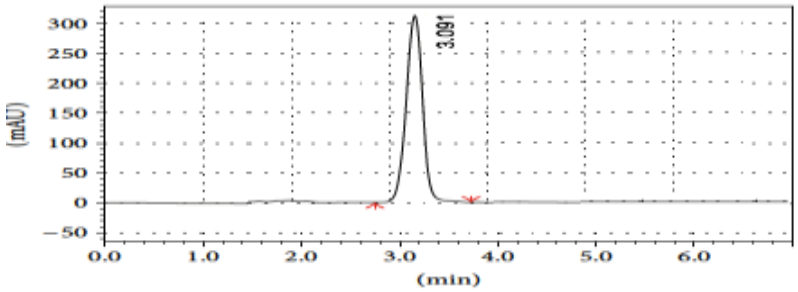

Fig-1: A Typical Chromatogram of Aciclovir Standard

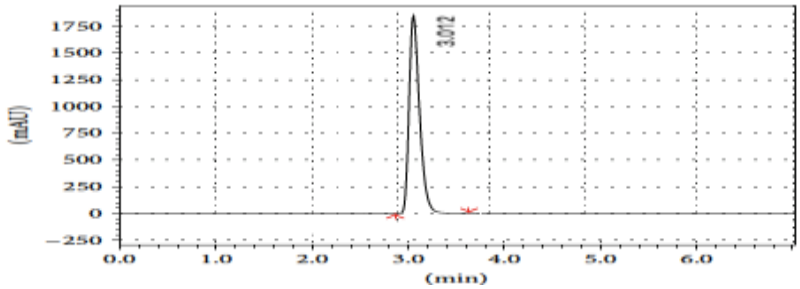

Fig-2: A Typical Chromatogram of Aciclovir tablet formulation

Linearity: The proposed method linearity was examined for five different concentrations. The concentration ranges from 5-25 µg/ml. The linearity of Aciclovir standard was determined by the plotting concentration vs peak area. By peak area as a functional of analyte concentration linearity was evaluated for Aciclovir. The linearity graphs presented in figure 3, and data presented in Table 2. The system suitability is demonstrated by the linearity analysis.

Table-2: RP- HPLC linearity for Aciclovir

Concentration (µg/ml)	Peak area
5	24567.67
10	50344.22
15	74352.35
20	99097.45
25	127649.79

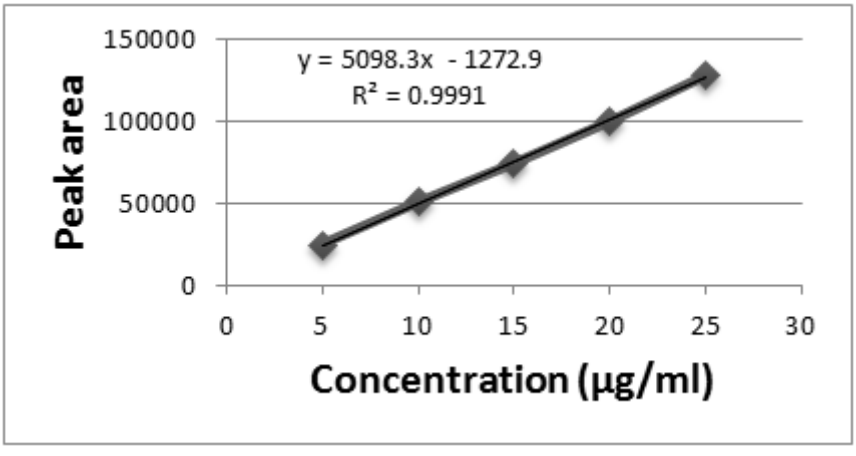

Fig-3: Calibration graph of Aciclovir 5-25 µg/ml precision

Accuracy: The experiment done for recovery study showed the method accuracy. The good recovery showed the method was accurate. The analysis for recovery was performed by known amount of Aciclovir working standard added to pre-analyzed solution of the formulation in the test concentration range of (40%,

60% and 100 %). For each recovery level three samples was prepared and repeated for 3 consecutive days. The statistical results for recovery study are well within the range (S.D. < 2.0). The Aciclovir tablet formulation recoveries results are presented in Table 3.

Table-3: Recovery studies of Aciclovir tablet formulation

Drug	% Level	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean recovery
Aciclovir	50	50	100	99.77	99.49
	50	100	149	99.45	
	50	150	199	99.55	

Precision: The proposed method precision (repeatability) experiment results of are shown in Table 4. The precision of intraday and interday was examined by analyzing the responeses of the sample on the same day for 4 repeatations and 3 alternate days for 2-10 µg/ml concentration range of Aciclovir. The obtained results are represented in % RSD. The % CV of the proposed method was precise as the values < 1.0 % for the repeatability study. The precision data are presented in Table 5.

Table-4: Method precision data of Aciclovir by RP-HPLC method

Aciclovir 20µg/ml (n=4)	Retention time	Area
1	3.01	99497.45
2	3.04	99897.75
3	3.07	99197.48
4	3.03	99297.40
Mean	3.01	99472.22
S.D [a]	0.0201	101.71
% CV[b]	0.68	2.07

n=4 observations

Table-5: Intermediate precision data of Aciclovir by RP-HPLC method

Aciclovir µg/ml	Inter-day measured mean area ± S.D.[a]	%CV[b] (n^c=4)	Intra-day measured mean area ± S.D.[a]	%CV[b] (n^c=4)
15	5057.25± 2.19	0.0872	4397.44± 4.65	0.0125
20	8919.75±2.22	0.0617	7772.75±2.15	0.0157
25	9613.12±2.11	0.0722	9976.12±4.26	0.1056

n^c = 4 observations

Limit of detection and quantitation

The limit of detection and quantification for Aciclovir is presented in table 6. Limit of detection (LOD) and limit of quantification (LOQ): LOD and LOQ were examined by minimum detectable peak area by injecting known concentration of drug solution. As per the International Conference on Harmonization guidelines the results are multiplied thrice to get LOD and 10 times to get LOQ. LOD and LOQ were found at concentrations of 0. 55µg/mL and 1.05 µg/mL respectively.

Table-6: Limit of detection and quantification

Parameters	**Aciclovir**
LOD (µg/ml)	0.47
LOQ (µg/ml)	1.45

Specificity: The Aciclovir standard reference and the drug formulation show the specificity of the method. The RP-HPLC chromatogram of Aciclovir both bulk and the tablet formulation are presented in figure 1, 2. The Aciclovir standard and tablet formulation shows the retention time was 2.10 min. For tablet formulation there was no excipient interference was detected, which shows the method specificity. The method showed the ability to determine the analyte in presence of excipients.

The system suitability

For the system suitability parameters five repeats of standards and two repeats of sample preparation are injected, the data is presented in Table 7. The Assay data of Aciclovir presented in Table 8. The statistical parameters results are presented in Table 9.

Table-7: Results of system suitability parameters

SNo	Parameters	Aciclovir
1.	Theoretical plates	7690
2.	Tailing factor	0.877
3.	Resolution factor	2.10
4.	Retention time	3.01± 0.1
5.	Calibration range or Linear dynamic range	5-25µg/ml

Table-8: Quantitative estimation (Assay) data of Aciclovir

Drug	Label claim (mg)	Amount found (mg)	Mean amount found (mg/ml)	Percentage purity (% w/w)	Mean purity (% w/w)	% Deviation
Aciclovir	400	400.01	400.01	100.97	100.19	+ 0.2
		399.77		99.59		+0.1
		400.21		100.21		+0.2
		399.93		99.97		+1.0
		400.14		100.24		+0.4

n= 4 observations

Table-9: Results of statistical parameters

SNo	**Parameters**	**Aciclovir**
1.	Standard deviation (SD)	1.07
2.	Relative standard deviation (RSD)	0.0776
3.	% RSD	0.776
4.	Standard error (SE)	0.02077
5.	Correlation Coefficient (r)	0.9990
6.	Slope (a)	5098.3
7.	Intercept (b)	1272.9

CONCLUSION

The developed RP-HPLC method was found to be precise, accurate, and sensitive. The proposed method is fast, reproducible and much economical and also no interference due to excipients.

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