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Research Article

The Estimation of Acyclovir in Bulk and Tablet Dosage form by Using Specificity and Analytical Method Development

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Abstract

A selective, accurate, HPLC method was developed by this study for the determination of Acyclovir in bulk and tablet dosage form. This method was developed by Thermo Fisher Scientific Software Chromquest Version 4.1 using C18 column in solvents 700 ml of Acetonitrile and 300 ml of buffer pH 3.0 ± 0.10 70:30) HPLC grade were set, C8 (4.6 mm x 1.5 cm, 5 µm) column, flow rate 0.50 ml/min as mobile phase. the mobile phase was pumped, and the sample was detected at 254 nm. For standard Acyclovir the retention time was 5 min. The method was validated for analytical standards such as linearity, accuracy, precision, and robustness. In a wide range of 5-25 (µg/ml) the linearity was observed.

Keywords: Acyclovir; Specificity; Reagent

Introduction

Category Acyclovir drug belongs to Antiviral Dru (Nucleoside RT inhibitors) Acyclovir is an antiviral agent and act against herpes viruses. Chemical Name of drug 2-Amino-1,9-dihydro-9-((2-hydroxyethoxy)methyl)-3H-purin-6-one with Molecular formula $C_8H_{11}N_5O_3$. It is a purine nucleoside analogue, used as antiviral agent against herpes viruses. It is mainly used for the treatment of herpes simplex virus infections, chickenpox and shingles. For herpes virus infections the administered dose for immuno-suppressed patients is up to 10 mg/kg body weight every 8h. Structure of Acyclovir as shown in figure 1.

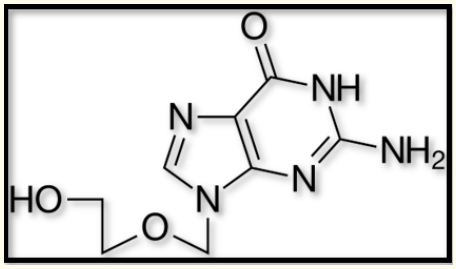

Figure 1: Structure of Acyclovir.

Material and Methods

- **Chemicals and reagents**: This method was developed by Thermo Fisher Scientific Software Chromquest Version 4.1 using C18 column in solvents 700 ml of Acetonitrile and 300 ml of buffer pH 3.0 ± 0.10 as mobile phase. The drug Acyclovir was obtained as gift sample of drug: Swapnroop Drugs and Pharmaceuticals, Aurangabad-431003 India. As the mobile phase. 700 ml of Acetonitrile and 300 ml of buffer pH 3.0 ± 0.10 (70:30) buffer used as mobile phase. Before injecting the drug, to acquire the saturation the column was equilibrated with mobile phase of stationary phase
- **Mobile Phase Preparation**: To make a buffer solution to dissolved 3.4g of potassium dihydrogen phosphate in 500 ml of HPLC grade water adjusted pH of this solution to 3.4 ± 0.10 with orthophosphoric acid sonicated for 5 minutes and filtered through 0.45 µ filter paper. So that mobile Phase taken 700 ml of Acetonitrile and 300 ml of buffer pH 3.0 ± 0.10 (70:30) sonicated for 5 min.
- **Selection of Analytical UV Wavelength (λmax)**: To fix wavelength for analysis the prepared stock solution was scanned in ultraviolet spectroscopy over the range of 200-800 nm from

resultant spectrum wavelength at 254 nm was chosen as in this range maximum absorption of drug occurs. So, this range is taken to analyze the sample.

- **Preparation of Standard Stock Solution of Acyclovir**: 100 mg of Acyclovir was weighed accurately and transferred into the 100 ml flask, diluted with mobile phase. The resulted concentration of solution is 1 mg/ml
- **Preparation of Sample**: 1 ml of stock solution is diluted with 100 ml of mobile phase to get concentration 10 µg/ml.

Results and Discussion

Method development

The method was developed by 700 ml of Acetonitrile and 300ml of buffer pH 3.0 ± 0.10 as a high concentration in mobile phase yield tailing in the peak due to the presence of water in the buffer. During method development, a number of variations have been done with mobile phase in different concentration and 0.50 ml/min flow rate to give asymmetric peak.

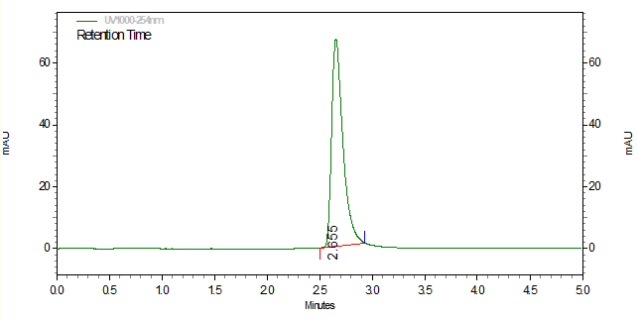

Figure 2: Chromatogram of Acyclovir.

Validation

Linearity

The different concentration varies from 5-30 µg/ml were prepared Chromatograms were recorded by injecting 20 µl from each concentration of the solution. All estimation were carried out at triplicate for each concentration. As shows in table 1 calibration: linearity and range of Acyclovir.

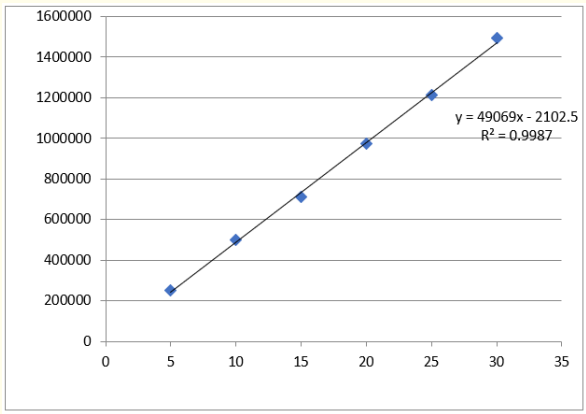

Figure 3: Linearity Curve and range for Acyclovir.

Sr No	Concentration µg/ml.	Area
1	5	252394
2	10	500095
3	15	712399
4	20	972894
5	25	1210151
6	30	1491666

Table 1: Linearity and range of Acyclovir.

Precision

Intraday precision

The intraday precision was determined by analyzing standard solution of Acyclovir at three different concentrations in the concentration range 17,22, and 27 µl for three times on the same day Each concentration was applied in triplicate and % RSD was calculated.

Interday precision

The intraday precision was determined by analyzing standard solution of Acyclovir at three different concentrations in the concentration range 17,22, and 27 µl for three times on three different days. Each concentration was applied in triplicate and % RSD was calculated.

Concentration (µg/ml.)	Area	Average Area	SD	%RSD
17	874071	878604	3801.60	0.431
	883375			
	878368			
22	1145932	1145399	388.90	0.033
	1145015			
	1145250			
27	1327744	1335462	8594.79	0.643
	1347453			
	1331189			

Table 2: Intraday precision of Acyclovir.

Concentration (µg/ml.)	Area	Average Area	S D	%RSD
17	874071	879742	4332.99	0.492
	880567			
	884588			
22	1145932	1145648	384.54	0.033
	1145105			
	1145909			
27	1327744	1366761	16716,59	1.223
	1391827			
	1380714			

Table 3: Inter day precision of Acyclovir.

Wavelength	Area	Average area	S D	% RSD
252 nm	650052	650754	498.60	0.076
	651055			
	651157			
256 nm	651307	651272	208.40	0.031
	651509			
	651001			

Table 4: Change in Wavelength ± 2 nm.

Flow rate	Area	Average area	S D	% RSD
0.4 min/ml	657068	656666	432.81	0.062
	656866			
	656065			
0.6 min/ml	651792	651157	496.49	0.076
	651099			
	650580			

Table 5

Sr No	Name of Drug	Label claim	Peak Area	Concentration of drug in (µg/ml.)	% drug contetns	% RSD
1	Acyclovir	400 mg	627561	10 µg/ml.	98.5%	0.21

Table 6: Assay of Acyclovir.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variation in the analytical procedure parameters. To evaluate HPLC method robustness a few parameters were deliberately varied. The parameters included variation in flow rate and change in the wavelength.

Assay of acyclovir

Avg. wt. of 10 tablets = 0.6970 g Brand name- Zoster 400 DTMfd. By- Leeford Heathcare Ltd.LC: 400 mg. Weighed 10 tablets, crushed and powder equivalent to 100mg of Acyclovir was weighed, added 50 ml of mobile phase, sonicated and diluted to 100 ml with it. Solution was filtered to get clear solution. 1 ml of above solution was diluted to 100 ml with mobile phase. Avg wt of 10 tablet = 0.6970 g Label claim = 400 mg.

Accuracy (recovery studies)

10 tablets were powdered and mixed. This powder was then spiked with a quantity Acyclovir corresponding to 50%, 100% and 150% of the labelled claim. Each of these powder mixtures was analyzed in triplicate and the quantity of Acyclovir was determined using calibration equation. Accuracy was reported as % Acyclovir recovered.

Conclusion

The proposed RP-HPLC method is simple, sensitive, precise and accurate. Since the analysis is completed within 5 --minutes, it clearly indicates that the method is rapid and thus it could be for routine analysis of Acyclovir from bulk drug and its tablet dosage forms [1-17].

Spike concentration	Spike solution area	Std Solution area	Recovery in (µg/ml)	% Recovery
50%	867763	580095	149.59	99.7%
100%	1169853	580095	201.66	100.8%
120%	1282591	580095	221.10	100.5%

Table 7: Recovery of Acyclovir.

Conflicts of Interest

Nil.

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