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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ACYCLOVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM UNDER STRESS DEGRADATION CONDITIONS

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ABSTRACT:

A simple, sensitive, specific, and accurate RP-HPLC approach is used to estimate the of acyclovir in tablet and bulk dosage forms. Mobile phase contained 0.1% OPA, and the chromatogram was performed using the Kromosil C18 (4.1 x 250mm, 5µm). With the flow rate of 1 ml/min, acetonitrile that had been diluted with water in a proportion, 60:40 was injected through the column at 30°C. The chosen optimized wavelength was 254.0 nm. Acyclovir's retention time was discovered to be 2.346 minutes. Acyclovir's %RSD was determined to be 0.9%. %RSD of Acyclovir's Repeatability Precision was discovered as 0.5%. Acyclovir had a 99.99% recovery rate. The result of the % assay for acyclovir was 100.08%. Acyclovir's LOD and LOQ values were 0.32 and 0.98, respectively. Acyclovir's regression equation is y = 32813x + 4220. As a result of shorter retention times and shorter run times, the method was created to be simple, cost-effective, and it may be used for routine Quality Control Tests in Industries.

Key Words: Acyclovir, RP-HPLC, Method Development, Validation, Forced Degradation.

INTRODUCTION:

Being a nucleotide analog, antiviral used for the treatment of herpes simples, varicella zoster, herpes labialis, herpes zoster and acute herpetic keratitis. It is a nucleoside analogue that prevents the replication of DNA by certain herpesviruses[1,5,6,7, and 8], as well as the activity of viral DNA polymerase. The viral thymidine kinase converts acyclovir to acyclovir monophosphate. Guanylate kinase changes acyclovir monophosphate into acyclovir diphosphate. Nucleoside diphosphate kinase, pyruvate kinase, creatine kinase, phosphoglycerate kinase, succinyl-CoA synthetase, phosphoenolpyruvate carboxykinase, and adenyl succinate synthetase[1,2,4] convert acyclovir diphosphate to acyclovir triphosphate. Acyclovir (Fig.1) is also known chemically as 2-amino-9-[(2-hydroxyethoxy)methyl]. -6,9-dihydro-3H-purin-6-one. On March 29, 1982[7], the FDA had approved acyclovir.

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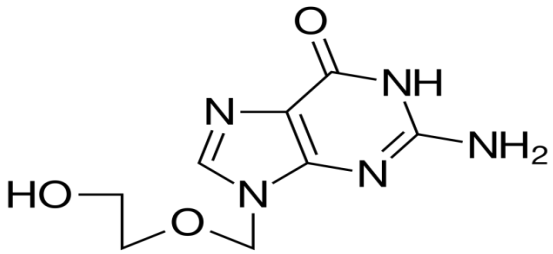

Fig 1: Chemical Structure of Acyclovir

UPon the identification of Acyclovir in bodily fluids from various species, numerous researches and articles have been published. There are several HPLC methods that use UV or fluorescence detection[18-19] to find aciclovir[12–17] in human serum. Regarding the forced deterioration investigations, there are no specific approaches, however, ann effort has been made to create a new stability-indicating RP-HPLC method that is quick, easy, accurate, and economical, as well as the best method with the simplest and most accessible mobile phase for the quantitative determination of acyclovir in pharmaceutical dosage form, taking into account the benefits of earlier proposed methods in the literature. The International Conference on Harmonization (ICHQ2 (R1) principles were followed in the validation of this approach.

MATERIALS AND PROCEDURES

Chemicals and reagents

Chemicals and reagents were acquired from the Spectrum Labs: Acyclovir standard (Purity≥ 99.7 as is basis), Acetonitrile (HPLC grade), HPLC grade water (Millipore), 0.1% Orthophosphoric acid, Sodium hydroxide, Hydrochloric acid, and Hydrogen peroxide.

Instrumentation: Electronic balance (Mettler Toledo, Model: XP56), sonicator (Elma, Model: S300H), hot air oven (Serve well Instruments, Model: H02436), digital pH metre (Mettler Toledo), and UV-Visible chamber were the instruments utilized in the investigation (Mack Equipment, Model: MK-2). Empower 2 software was used to monitor and integrate the HPLC (Waters 2695 with PDA detector 2996).

Preparation of Standard stock solution:

Acyclovir, accurately weighed at 25 mg, was put separately to 50 ml volumetric flasks. The flasks was added 10ml of diluent, which was then sonicated for 10 minutes. Standard stock solutions 1, 2, and 3 were written on the flasks after they had been diluted to the appropriate level. (Acyclovir 500 µg/ml).

Preparation of Standard working solution: 1ml of each each stock solution was pipetted into a 10 ml volumetric flask, and made up with the diluent (50 µg /ml of acyclovir).

Preparation of Sample stock solution:

The weight of 10 Aciherpin tablets, which each contain 400 mg of Acyclovir, was calculated. Weight equivalent to one tablet was then put into a 100 ml volumetric flask, 25 ml of diluent was added, and the mixture was sonicated for 50 minutes. Finally, the volume was adjusted using diluent and filtered. (Acyclovir, 4000 µg/ml).

Preparation of Sample working solution:

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0.125 ml of the filtered solution was pipetted into a 10 ml volumetric flask and diluted to a final volume of 10 ml. (Acyclovir 50 µg/ml).

Chromatographic conditions:

Flow rate	**:**	1ml/min
Column	**:**	Kromosil C18 150x 4.6mm, 5µm.
Wavelength	**:**	254.0 nm
Column temperature	**:**	30°C
Injection volume	**:**	10.0µL
Run time	**:**	5.0minutes
Diluent	**:**	0.1% OPA:Acetonitrile (50:50)

Observation: Retention time for acyclovir was 2.346 minutes with good resolution (fig.2). The procedure conditions were optimized, the plate count and tailing factor were very good, and the same conditions were employed for the validation.

Degradation: For the forced degradation experiment, standard, stock solutions of acyclovir were subjected to a range of stress conditions, including oxidative degradation using 20% H_2O_2, acidic degradation using 2N HCL, and alkalline degradation using 2N NaOH. The generated solutions were refluxed at 60^{o}C for 30 minutes the standard solutions were also heated and exposed to UV light in order to assess the degradation towards thermal and photolytic stress conditions. In order to get 50 µg/ml of Acyclovir for degradation investigations, the resultant solutions were diluted. 10 µl of samples were taken in the apparatus, and chromatograms were recorded to test the sample stability.

Method Validation: The method was approved in line with ICH Q2R1 guidelines. Among the validation parameters are system suitability, specificity, linearity, accuracy, precision, LOD& LOQ, and robustness are thoroughly checked.

RESULTS AND DISCUSSION

System suitability parameters: Six standard injections of Acyclovir (50µg/ml) solutions were used to evaluate the system suitability parameters. The USP plate count, resolution, and peak tailing were all identified. The USP Plate count for three drug samples taken together was greater than 2000, and the tailing factor was under 2. The system's proper parameters were all passed and stayed within the limit. Table 1 displays the outcome.

Specificity: Interference is examined using the optimized method. Retention time for acyclovir was 2.346 minutes. In our method, we did not detect any interfering peaks in the chromatograms of blank and placebo samples during the drug's retention time. As a result, this procedure was described as particular. The chromatograms for specificity are displayed in Figures 3, 4, and 5.

Linearity: Six linear Acyclovir concentrations (12.5-75 µg/ml) were administered in triplicate manner. The obtained correlation coefficients for all three samples were 0.999. Table 2 and Fig. 6 presented the findings.

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Precision:

Repeatability: Six working sample solutions of the same strengths (50 µg / ml Acyclovir) were made after multiple samples of a sample stock solution were taken. Every working sample solution was used for each injection, and the outcomes are displayed in table 3. Acyclovir's average area, standard deviation, and percent RSD were calculated and found to be 0.5%. Since the precision restriction for this procedure was less than "2%," the system precision was successful. The data results are displayed in Table 3.

Intermediate Precision: From a sample stock solution, many samples were obtained, and six working sample solutions (containing 50 µg/ml of Acyclovir) were prepared and used for recording chromatograms. The areas obtained are listed in table 4 after each injection from each working sample solution was administered the day after sample preparation. Acyclovir's average area, standard deviation, and percent RSD were calculated and found to be 0.7%. The intermediate precision was used for this procedure since the precision limit was less than "2%". The data results are displayed in Table 4.

Accuracy: Using the conventional addition technique, samples of three levels of accuracy were created. Triplicate injections were administered at each level of accuracy, and it was found that the mean percent recovery for acyclovir was 99.99%.. The results are shown in Table 5. Since acceptable recover values were obtained, this approach's accuracy was approved.

Robustness: Robustness conditions including flow minus (0.9 ml/min), flow plus (1.1 ml/min), mobile phase minus (65:35 v/v), mobile phase plus (55:45 v/v), temperature minus (25°C), and temperature plus (35°C), were maintained for the Acyclovir samples (50 µg/ml) were injected in duplicate. The percentage RSD was calculated and found to be acceptable. Data are shown in Table 6.

Assay: Aciherpin tablets were labelled as containing 400 mg of acyclovir per unit. The experiment was conducted using the aforementioned formulation. Acyclovir's average assay success rate was 100.08%.

Degradation Studies: The stock standard solution was used for the degradation investigations, and the proposed method was used to analyse the degraded samples. All of the injected samples passed the limits of degradation when the assay % of acyclovir in each sample was computed. The outcomes were displayed in table 7. In figs. 7,8,9,10, and 11, the purity plots derived from degradation studies are displayed.

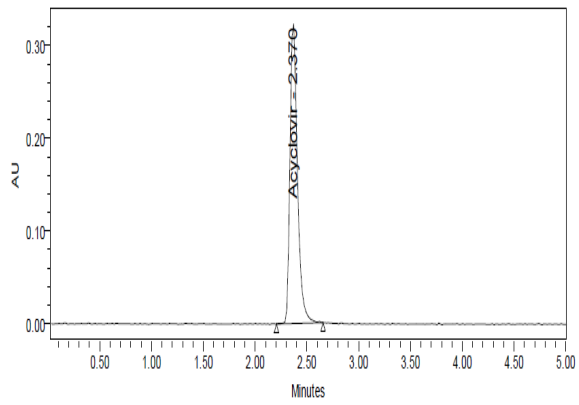

Fig No.2: Optimised Chromatogram

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Table No.1: System suitability parameters

S no	Acyclovir		
Injection	**RT(min)**	**USP Plates Count**	**Tailing factor**
1	2.370	5477	1.36
2	2.386	5465	1.34
3	2.391	5542	1.35
4	2.391	5364	**1.37**
5	2.394	5247	1.36
6	2.395	5684	1.36

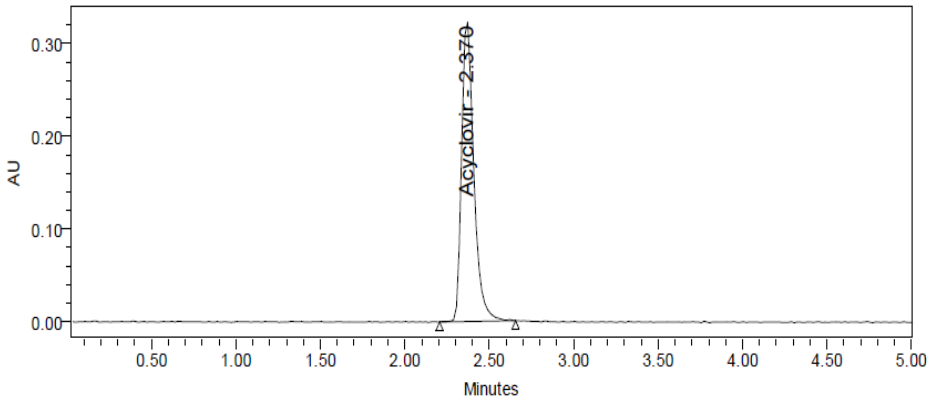

Fig No.3: Standard solution chromatogram

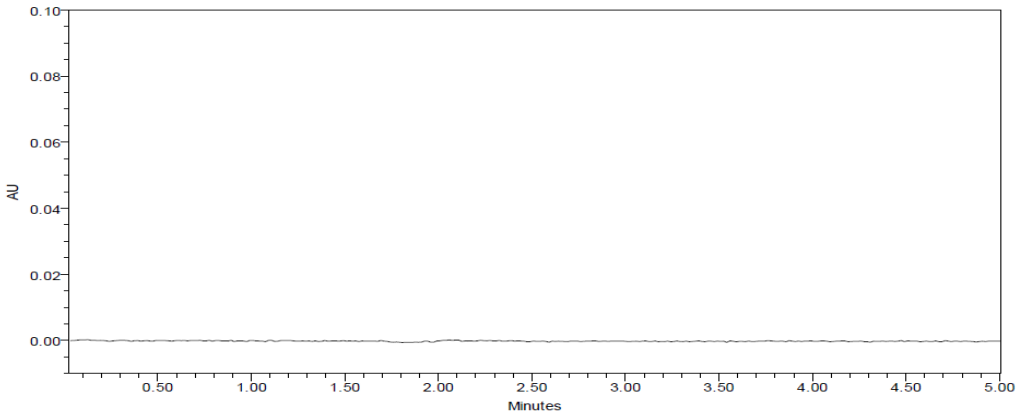

Figure No.4: Blank chromatogram

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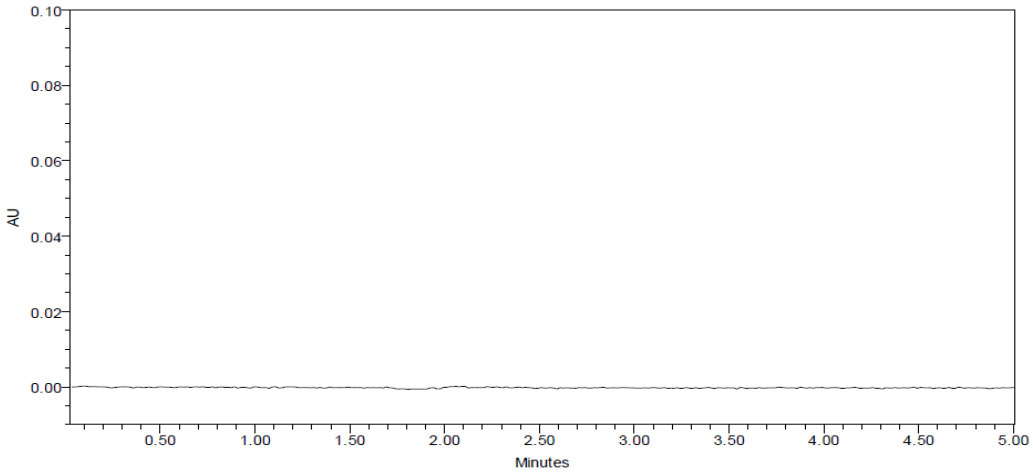

Fig No.5: Placebo chromatogram

Table No.2: Linearity table for Acyclovir,

Acyclovir	
Conc.in µg/mL	**Area of peak**
12.5	426439
25	817904
37.5	1238515
50	1664158
62.5	2031878
75	2483189

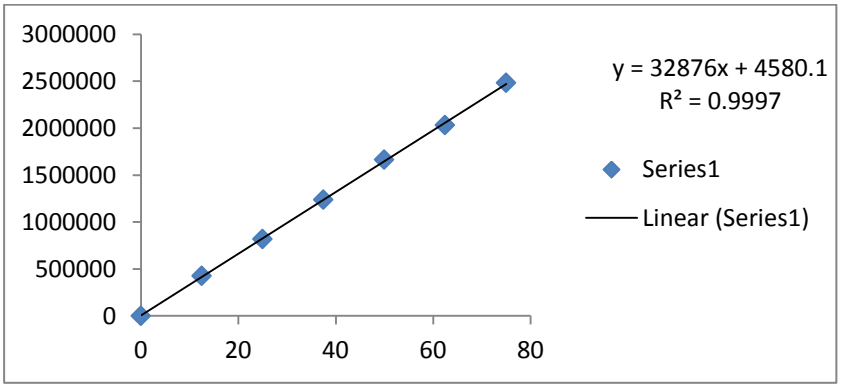

Fig No 6: Calibration curve of Acyclovir

Table No.3: Repeatability for Acyclovir

S.no.	Acyclovir
1	1627623
2	1622075

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3	1634510
4	1622820
5	1611921
6	1619530
Mean	1623080
S.D	7609.09
%RSD	0.5

Table No4: Intermediate Precision ofAcyclovir

S.no.	**Acyclovir**
1	1614987
2	1623761
3	1590778
4	1601062
5	1614771
6	1614266
Mean	1609938
S.D	11870.5
%RSD	0.7

Table No. 5: Acyclovir Accuracy level

% level	**amount spiked in µg/mL**	**Amount recovered In µg/mL**	**% recovery**	**mean % recovery**
50%	25	25.18	100.74	99.99%
	25	24.91	99.63	
	25	25.05	100.21	
100%	50	50.07	100.15	
	50	49.64	99.27	
	50	49.86	99.73	
150%	75	75.38	100.50	

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	75	75.02	100.03	
	75	74.74	99.65	

Table No.6: Robustness Data

S.no	Condition	%RSD of Lamivudine
1	Flow rate (-) 0.9ml/min	0.9
2	Flow rate (+) 1.1ml/min	0.4
3	Mobile phase (-) 65B:35A	0.8
4	Mobile phase (+) 55B:45A	0.7
5	Temperature (-) 25°celsius	0.8
6	Temperature (+) 35°celsius	0.7

Table No.7: Degradation studies Data

S. No.	Condition	Acyclovir %degraded	Acyclovir %obtained	purity angle	purity threshold
1	**Acidic**	5.56	94.44	0.697	0.854
2	**Basic**	4.76	95.24	0.646	0.812
3	**Oxidation**	4.72	95.28	0.527	0.737
4	**Dry heat**	3.27	96.73	0.567	0.764
5	**UV Light**	1.67	98.33	0.831	0.793

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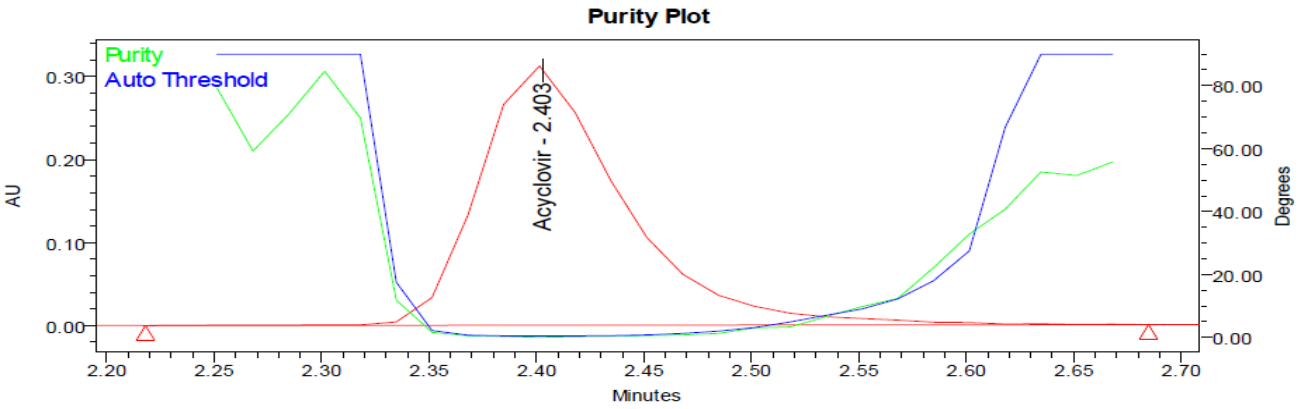

Fig No 9: Acid Purityplot

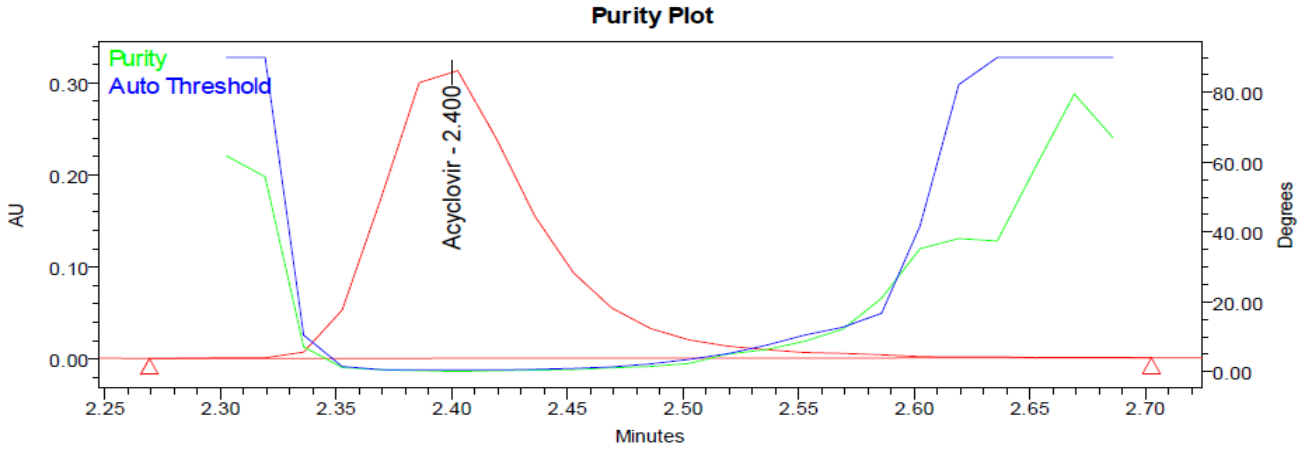

Fig No 10: Base Purity plot

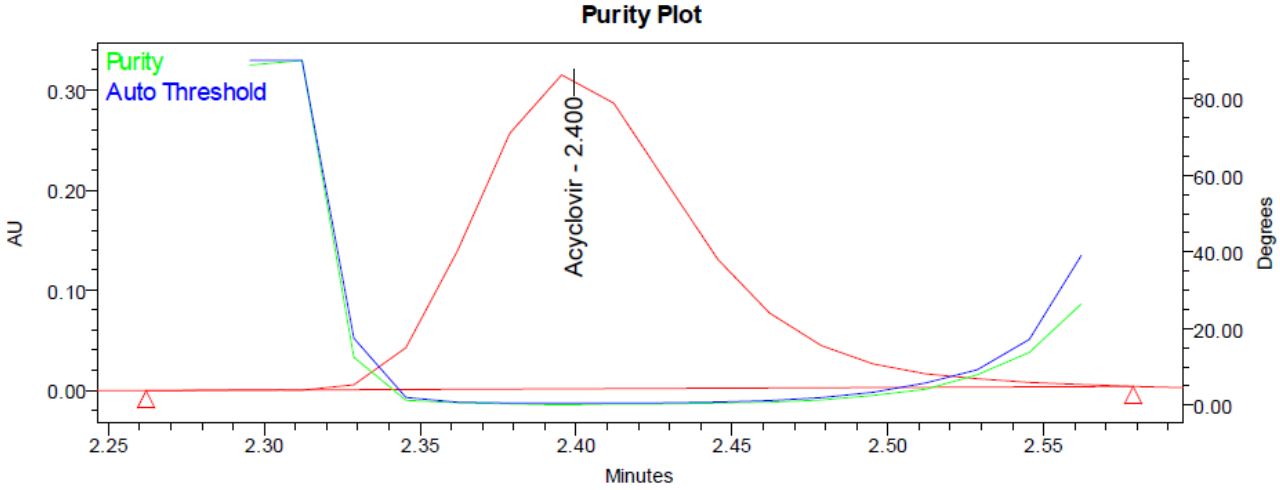

Fig No 11: Peroxide Purity plot

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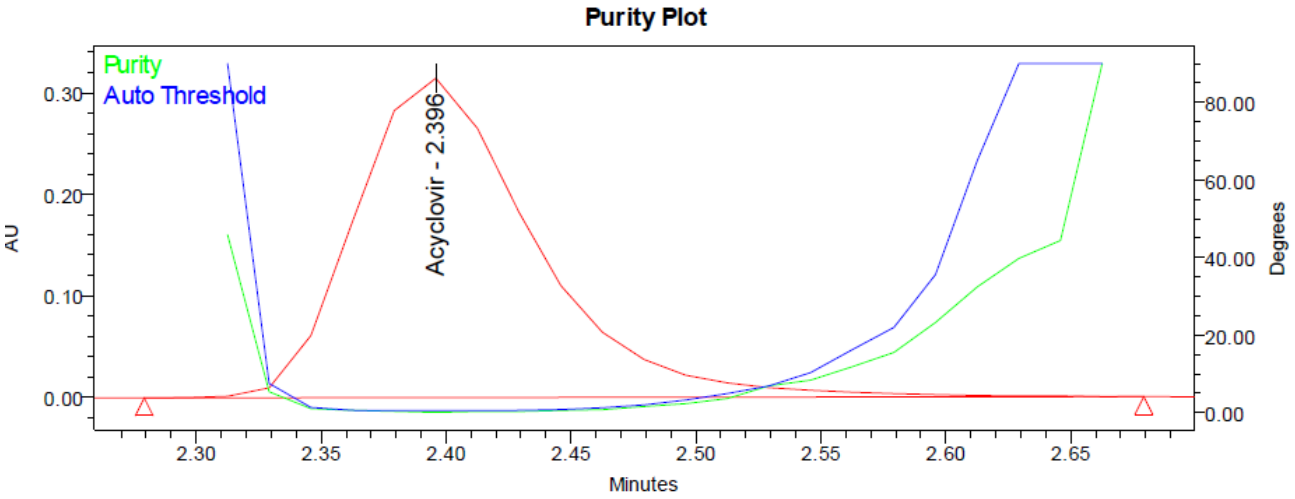

Fig No 12: Thermal Purity plot

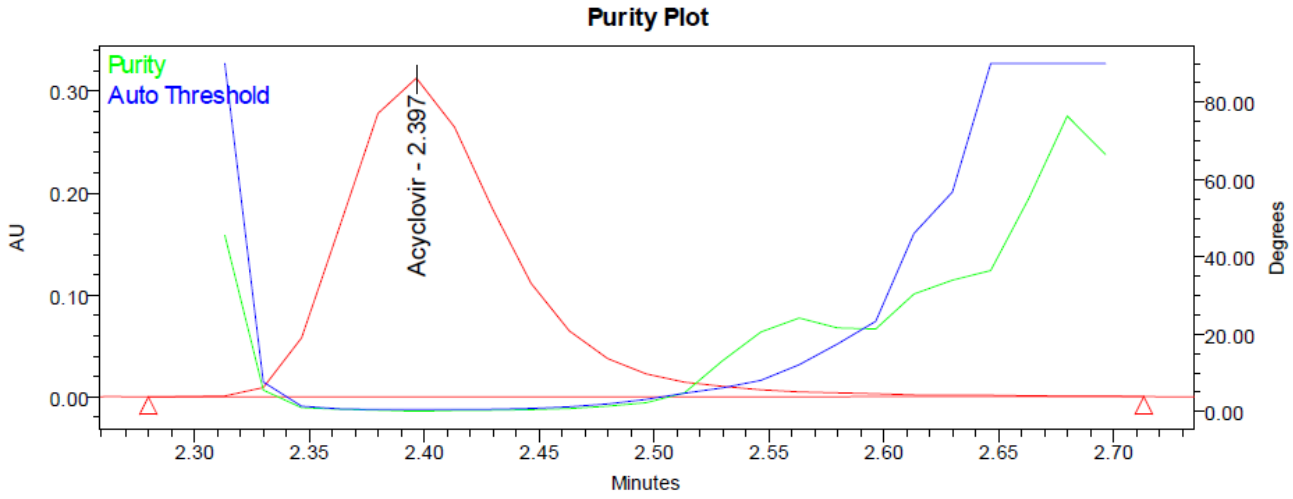

Fig No 13: UV Purity plot

Conclusion:

This work demonstrates the use of an established, simple stability-indicating RP-HPLC method to identify acyclovir in the presence of other compounds. The strategy was precise, particular, sensitive, efficient, and long-lasting. This method can distinguish active pharmaceutical ingredients from degradation byproducts produced during forced degradation tests. The recommended method can be used in the quality-control department for routine quantitative Acyclovir analysis.

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