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Analytical method development and validation of acyclovir of different market brands by UV-Spectroscopy

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ABSTRACT

A study was conducted to develop a UV-spectrophotometric assay method for accurately determining the concentration of Acyclovir in both bulk and tablet formulations. Although previous methods existed for estimating Acyclovir in dosage forms, none of them were stability-indicating UV spectroscopic studies focused on a single dosage form.In this study, a simple, accurate, rapid, and cost-effective UV-spectrophotometric assay method was established. The stock solution was prepared using water as the solvent, and subsequent concentrations were prepared using the same solvent. Acyclovir was detected at a wavelength of 252nm, which was determined as its maximum absorption wavelength (λmax).The linearity of Acyclovir was observed in concentrations ranging from 1 to 7 µg/ml, with a high regression coefficient (r2) of 0.9987. The percentage of Acyclovir content in the API formulation was found to be 99.7016 ± 1.4996, indicating good accuracy. The method also demonstrated precision, as evidenced by the recovery studies, with recoveries close to 100% and a low relative standard deviation (RSD) of less than 2.The inter-day precision for Acivir, Aciv, and Aclovir was determined to be 0.3382, 2.1933, and 2.7090, respectively. The intra-day variations were found to be 1.5207, 2.3961, and 1.2254 for Acivir, Aciv, and Aclovir, respectively. Accuracy was assessed by determining the values at 80%, 100%, and 120% of the expected concentrations. For Acivir, the accuracy values were 0.69627, 0.08166, and 0.41124 at 80%, 100%, and 120%, respectively. For Aciv, the values were 0.18316, 0.12809, and 0.19022, and for Aclovir, the values were 0.18149753, 0.16605322, and 0.19693346 at 80%, 100%, and 120%, respectively.The robustness of the method was evaluated by measuring the absorbance at different wavelengths. The robustness values for Acivir at wavelengths 252nm, 253nm, and 254nm were 2.98217216, 2.264530905, and 4.430021, respectively. For Aciv, the values were 3.5995176, 3.177528527, and 13.34564475, and for Aclovir, the values were 10.5995176, 5.634288012, and 8.09250084 at wavelengths 252nm, 253nm, and 254nm, respectively. These results indicate the sensitivity of the method to different wavelengths and highlight the importance of using the designated wavelength (252nm) for accurate measurements.

Keywords: Aclovir, Acivir, Aciv, 252nm.

INTRODUCTION

Qualitative and quantitative analyses are two main categories in analytical studies. Qualitative analysis involves determining the presence or absence of specific components in a sample, while quantitative analysis provides the precise concentration of the component. UV-visible spectroscopy can be used for both qualitative and quantitative analyses. Qualitative analysis helps identify unknown samples by

comparing their spectral characteristics with known standards, while quantitative analysis enables the determination of accurate concentrations.[1-4]

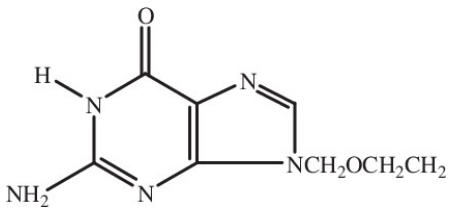
Method validation is of paramount importance in UV-visible spectroscopy due to the following reasons:

1. Ensuring accuracy and precision: Method validation establishes the accuracy and precision of the analytical method. It identifies and corrects potential errors stemming from instrument or analytical sources. By validating the method, one can ensure that the results obtained are reliable and trustworthy.
2. Ensuring reliability: Method validation ensures that the analytical method produces consistent and reliable results over time and across different operators or instruments. Even slight variations in instrument settings or analytical conditions can impact the accuracy and precision of the results in UV-visible spectroscopy. Validation helps identify and address these variations, ensuring reliable data.
3. Meeting regulatory requirements: Regulatory bodies such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) often require method validation for analytical methods used in quality control or product release. Validation ensures that the methods meet specific standards of accuracy, precision, and reliability, ensuring compliance with regulatory guidelines.
4. Ensuring method suitability: Method validation also helps determine the suitability of the UV-visible spectroscopy method for its intended use. Whether it is quantitative analysis, impurity profiling, or stability testing, validation ensures that the analytical method is fit for its intended purpose and provides meaningful and accurate results.[5-9]

Drug profile

Name: Acyclovir

Structure:



Weight: 225.2046

Chemical Formula: C8H11N5O3

IUPAC: 2-Amino-1,9-dihydro-9-((2-hydroxyethoxy)methyl)-3*H*-purin-6-one

METHODOLOGY

A quantitative analysis of Acyclovir in a marketed formulation was conducted using the following method. Ten tablets of Acyclovir 200mg were accurately weighed and crushed to a fine powder. From this powder, an equivalent weight of 100mg of the drug was transferred into a 100ml volumetric flask. The volume was made up to the mark with water, and the mixture was sonicated for 15 minutes. After sonication, the solution was filtered through Whatman filter paper no. 41. The resulting solution was further diluted with water to obtain a final concentration of 1000µg/ml of the drug. The solution was scanned in the UV range (200-400nm), and the λmax was found to be 252nm.

Method validation is a crucial step in establishing the reliability and accuracy of an analytical method. The validation process ensures that the performance characteristics of the method meet the requirements for the intended application. Key aspects of method validation include:

Linearity

The linearity of the method was determined by preparing standard solutions of Acyclovir at concentrations ranging from 1 to 7 µg/ml. A calibration graph of concentration versus absorbance was plotted to assess the linearity of the method.

Precision

The precision of the method was evaluated through intraday and interday variation studies. In the intraday study, the working solutions of standard and sample were analyzed three times in a day, and the percentage of relative standard deviation (RSD) was calculated. For the interday study, the working solutions of standard and sample were analyzed on three consecutive days, and the percentage of RSD was determined. Repeatability of the method was also assessed by analyzing six samples of the tablet formulation.

Accuracy

Recovery studies were conducted at three different levels (80%, 100%, and 120%) to evaluate the accuracy of the method. For each level, tablet powder weighing about 106mg, equivalent to 100mg of Acyclovir, was taken. Standard amounts of Acyclovir were added to these samples, and the resulting mixtures were triturated well. From each mixture, a portion was taken and added to a 100ml volumetric flask to prepare Acyclovir solution. The accuracy of the method was assessed based on the recovery percentages obtained from these studies.

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value. It is calculated using the formula LOD = 3.3 * σ/a, where σ is the standard deviation of the response and a is the slope of the calibration curve.

The limit of quantitation (LOQ) is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. It can be expressed as LOQ = 10 * σ/a, where σ is the standard deviation of the response and a is the slope of the calibration curve.
Robustness refers to the ability of an analytical procedure to remain unaffected by small, deliberate changes in method parameters, ensuring reliable and consistent results under varying conditions.[10-19]

RESULTS AND DISCUSSION

Wavelength Selection: A standard solution of Acyclovir was scanned in a UV spectrophotometer between 200 nm to 400 nm using the diluent as a blank. The maximum absorption wavelength (λmax) for Acyclovir was found to be 252 nm. This selected wavelength is suitable for the proposed analytical method.

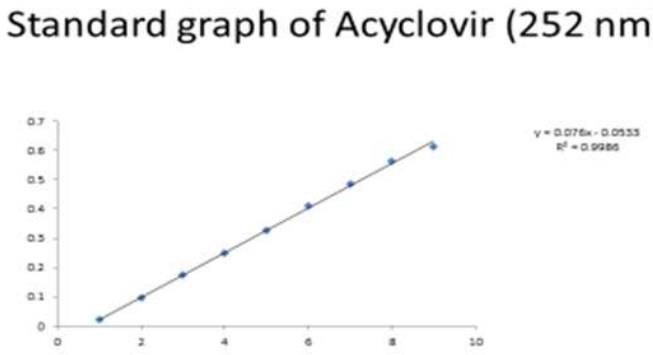
Method Validation

Linearity: A calibration curve was constructed using seven points in the concentration range of 1-9 ppm for Acyclovir. The response of the drug exhibited linearity within the investigated concentration range. The linear regression equation was determined as y = 0.076x - 0.0533, with a correlation coefficient of 0.9986.

Linearity, LOD & LOQ of acyclovir

CONC	ABS	FOUND CONC(µg/ml)	%RECOVERY
2	0.1320667	2.1815267	109.076305
4	0.2727667	3.87670683	96.9176707
6	0.4352333	5.83413655	97.2356091
8	0.6370667	8.26586345	103.323293
12	0.9544667	12.0899598	100.749665
14	1.1222333	14.111245	100.794607
MEAN			101.349525
SD			4.49070809
	SE OF INTERCEPT		0.01303035
SD OF INTERCEPT	SD OF INTERCEPT*n		0.03191785
LOD	3*3(SD OF INTERCEPT/SLOPE)		1.26902284
LOQ	10*(SD OF INTERCEPT/SLOPE)		3.84552375

Standard graph of acyclovir



Limit of detection

The detection limit (LOD) of an analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value. LOD is commonly expressed as the concentration of the analyte (in parts per million) in the sample. It can be determined using three methods: visual evaluation, signal-to-noise ratio, and calculation based on the standard deviation of the response and slope of the calibration curve. The formula for LOD calculation is LOD = 3.3 * (Standard error of the intercept / Slope).
The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. LOQ is also expressed as the concentration of the analyte (in parts per million) in the sample. Like LOD, LOQ can be determined using visual evaluation, signal-to-noise ratio, or calculation based on the standard deviation of the response and slope of the calibration curve. The formula for LOQ calculation is LOQ = 10 * (Standard error of the intercept / Slope).
Precision is assessed to determine the reproducibility and consistency of an analytical method. It is evaluated by conducting the analysis as per the prescribed procedure and normal weight taken for analysis. The analysis is repeated a certain number of times, and the % assay, mean assay, % deviation, % relative standard deviation (%RSD) are calculated. In the present study, the developed method demonstrated good precision, with %RSD values of 0.307%

for repeatability and 0.591% for intermediate precision studies.

Table 3: Precision Of Acyclovir Intraday

ACIVIR DT tab (CIPLA)							
TIME	**CONC µg/ml**	**A1**	**A2**	**A3**	**AVG**	**FOUND CONC**	**%RECOVERY**
10:30AM	5	0.3159	0.3156	0.3056	0.3167	4.83689887	96.73684231
12:30PM	5	0.3198	0.3143	0.2870	0.3189	4.72104236	94.42105345
3:30PM	5	0.3176	0.3045	0.2919	0.3076	4.70394897	94.67894887
MEAN							95.07894897
SD							1.445934768
%RSD							1.52078904

ACIVI tab (ZEE LABS)							
TIME	**CONC µg/ml**	**A1**	**A2**	**A3**	**AVG**	**FOUND CONC**	**%RECOVERY**
10:30AM	5	0.3256	0.3124	0.3134	0.311456	4.79298678	95.85964934
12:30PM	5	0.3145	0.2456	0.2889	0.295679	4.53903589	90.78070185
3:30PM	5	0.3076	0.3267	0.3124	0.295342	4.58114036	91.62280678
MEAN							92.75436789
SD							2.22254567
%RSD							2.396116478

ACLOVIR DT tab (MAXCARE)							
TIME	**CONC µg/ml**	**A1**	**A2**	**A3**	**AVG**	**FOUNDCONC**	**%RECOVERY**
10:30AM	5	0.3056	0.3132	0.3155	0.3112665	4.792982458	95.85964913
12:30PM	5	0.2985	0.2878	0.2857	0.2919668	4.539035077	90.78070185
3:30PM	5	0.2846	0.295	0.3055	0.2951668	4.581140341	91.62280705
MEAN							100.9176333
SD							1.236676243
%RSD							1.225431277

Interday precision

ACIVIR DT tab (CIPLA)							
DATE	**CONC µg/ml**	**A1**	**A2**	**A3**	**AVG**	**FOUNDCONC**	**%RECOVERY**
5-04-2022	5	0.3099	0.3110	0.307	0.3093	4.76710526	95.34210526
6-04-2022	5	0.3076	0.3086	0.3085	0.3085	4.75657895	95.13157895
7-04-2022	5	0.3502	0.3104	0.3069	0.3069	4.73552632	94.71052632
MEAN							95.06140351
SD							0.3215584259
%RSD							0.338291091

ACIVI (ZEE LABS)							
DATE	**CONC µg/mL**	**A1**	**A2**	**A3**	**AVG**	**Found Conc**	**% recovery**
5-04-2022	5	0.3249	0.3188	0.3221	0.3219	4.93289474	98.6578947
6-04-2022	5	0.3165	0.3155	0.3155	0.3156	4.85247504	97.5632114

7-04-2022	5	0.3045	0.3031	0.3101	0.3059	4.72236842	94.4473684
				MEAN			96.7017544
				SD			2.12104828
				% RSD			2.19339173

ACLOVIR DT tab (MAXCARE)							
DATE	**CONC µg/ml**	**A1**	**A2**	**A3**	**AVG**	**FOUNDCONC**	**%RECOVERY**
23-03-2023	5	0.3198	0.3156	0.312	0.3158	4.852631573	97.05263158
24-03-2023	5	0.3216	0.2806	0.2974	0.2974	4.610526316	92.21052632
25-03-2023	5	0.3406	0.3242	0.2986	0.2986	4.626315789	92.52631579
				MEAN			93.92982456
				SD			2.709035529
				%RSD			2.884105821

Accuracy

Accuracy of the method is as curtained by standard addition method at 3 levels. Standard quantity equivalent to 80%, 100% and 120% is to be added in sample .The result shown that best recoveries (99.92-100.94%) of the spiked drug were obtained at eachadded concentration, indicating that the method was accurate.

Accuracy of acyclovir

Acivir (CIPLA)

ACIVIR (CIPLA)					
S.NO	**CONC**	**ABS**	**MEAN**	**SD**	**%RSD**
1	80%	0.5623	0.56623333	0.003947	0.696217
2		0.5694			
3		0.5668			
4	100%	0.682	0.68203516	0.000559	0.081667
5		0.6824			
6		0.6814			
7	120%	0.7039	0.70323334	0.002894	0.411236
8		0.7003			
9		0.7059			

Aciv (ZEELABS)

ACIVI (ZEE LABS)					
S.NO	CONC	ABS	MEAN	SD	%RSD
1	80%	0.5689	0.54511568	0.001039	0.18316
2		0.5662			
3		0.5681			
4	100%	0.6646	0.66396667	0.000851	0.12809
5		0.6632			
6		0.6643			
7	120%	0.7131	0.71373334	0.001358	0.19022
8		0.7129			
9		0.7153			

Aclovir (MAXCARE)

ACLOVIR (MAX CARE)					
S.NO	**CONC**	**ABS**	**MEAN**	**SD**	**%RSD**
1	80%	0.5938	0.594267	0.00107858	0.1814975
2		0.5935			
3		0.5955			

4	100%	0.6718	0.670752	0.00111355	0.1660532
5		0.6696			
6		0.6704			
7	120%	0.7109	0.710867	0.23160015	0.1969335
8		0.7095			
9		0.7123			

Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variation in analytical conditions ,the analytical condition should be suitably controlled or a precautionary statement should be included in the procedure. The result of robustness study of the developed assay method was established. The results shown that during all variance conditions, assay value of the test preparation solution was not affected and it was accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust

Robustness of acyclovir

ACIVIR(CIPLA)				
S.NO	**CONC (µg/ml)**	**252nm**	**253nm**	**254nm**
1	5	0.2918	0.3154	0.2945
2		0.2978	0.3254	0.2746
3		0.2899	0.3146	0.2845
4		0.2789	0.3164	0.2689
5		0.2759	0.3325	0.2897
6		0.2812	0.3258	0.2456
MEAN		0.28591666	0.32168333	0.27635
SD		0.00852652	0.00728461	0.01776
%RSD		2.98217216	2.26453090	4.43002

ACIV(ZEE LAB)				
S.NO	**CON C (µg/ml)**	**252nm**	**253nm**	**254nm**
1	5	0.3015	0.3215	0.2987
2		0.3158	0.3263	0.2165
3		0.3256	0.3197	0.2756
4		0.3174	0.3249	0.2455
5		0.3345	0.3485	0.2685
6		0.3275	0.3282	0.2145
MEAN		0.3203833	0.3281833	0.2532166
SD		0.0115206	0.0104281	0.0337933
%RSD		3.5958994	3.1775285	4.3456447

ACLOVIR(MAXCARE)				
S.NO	**CON C (µg/ml)**	**252nm**	**253nm**	**254nm**
1	5	0.2458	0.3158	0.2368
2		0.2145	0.3257	0.2345
3		0.2547	0.3658	0.2685
4		0.2687	0.3457	0.2185
5		0.2156	0.3189	0.2574
6		0.2758	0.3358	0.2658
MEAN		0.2458512	0.3346166	0.246916
SD		0.0260589	0.0188532	0.019981
%RSD		3.5995176	0.6342880	4.092500

CONCLUSION

The Acyclovir tablet formulations (Acivir, Aciv, Aclovir) were validated according to ICH guidelines, covering various parameters including linearity, range, LOD, LOQ, accuracy, precision (intraday and interday), and robustness. Linearity was observed in the concentration range of 1-7 µg/ml, demonstrating adherence to Beer-Lambert's law. The regression analysis yielded a high correlation coefficient (R2) value of 0.9987, indicating a strong linear relationship between concentration and absorbance.

In terms of intraday precision, Acivir exhibited %RSD values below 2% (1.2254), meeting the acceptable criteria. However, Aciv and Aclovir showed slightly higher %RSD values, exceeding 2%.

For interday precision, Acivir demonstrated %RSD values below 2% (1.0143), while Aciv and Aclovir again exhibited slightly higher %RSD values, surpassing 2%.

Accuracy data indicated that all three formulations (Acivir, Aciv, Aclovir) achieved %RSD values below 2%, which is considered acceptable.

In the robustness analysis, all formulations displayed %RSD values within the acceptable limit, demonstrating the method's robustness under different conditions.

Based on the above data, it can be concluded that all three formulations (Acivir, Aciv, Aclovir) successfully complied with the tested parameters.

The validation study confirmed that the developed UV method for the determination of Acyclovir in bulk or pharmaceutical dosage forms is linear, accurate, rapid, precise, robust, and cost-effective. The method exhibited high correlation coefficients, low %RSD values, and acceptable standard deviations. Overall, the validated method is versatile and valuable for the reliable analysis of Acyclovir.

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