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Spectrophotometric Determination, Analysis And Validation Of Acyclovir 0n Solid Dosage Form

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Abstract: *A new, economical, sensitive, simple, rapid UV spectrophotometric method has been developed for the estimation of Acyclovir in pure form and pharmaceutical formulation. This UV method was developed using NaOH (0.1N) as a solvent. In the present method, the wavelength selected for analysis was 253nm. Using 1cm match quartz cells, all of the spectrum and absorbance measurements were performed using an UV-VIS spectrophotometer (Shimadzu 1800) was used to carry out the spectral analysis. The ICH guidelines were used to validate the method. Throughout the study, we compared the purity of the drugs, with standard drugs (HPLC grade purity >99%) purchased from SIGMA ALDRICH Ltd. The test drugs from pharmaceutical companies (CIPLA) in its dosage form taken from the market.The method was validated for linearity, range, accuracy, precision, drug content , LOD and LOQ. Linearity was found in the range of 2.5-40µg/ml. Accuracy was performed by using a recovery study. The percentage recovery for all the methods performed was in the range of 99-100% indicating of zero interference of the excipients (in the formulation). LOD and LOQ were found to be 1.5766434 and 4.7777075 respectively [SD: 0.474474135; SE: 0.212191284]. Relative error (<2) values showed that the proposed procedure exhibited excellent Interday and intraday precision.The proposed UV spectroscopic method was found to be accurate, precise, stable, linear, specific, and simple for quantitative estimation of acyclovir in bulk and pharmaceutical dosage form. Hence the present UV spectroscopic method is suitable for the routine assay of acyclovir in bulk and pharmaceutical formulations.*

Keywords: *Acyclovir, UV-Visible spectrophotometric method, Method validation*.

INTRODUCTION:

One of the most frequently employed techniques in the pharmaceutical analysis is UV-Visible spectrophotometry. The amount of ultraviolet or visible radiation absorbed by a substance in a solution is measured by UV spectrophotometer [1].

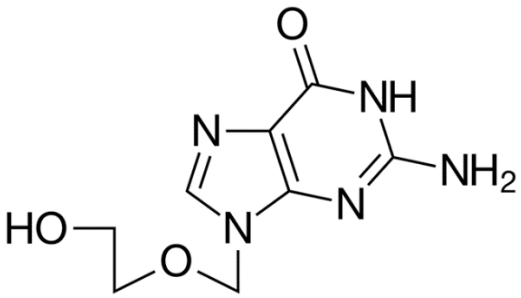

Fig. 1: Chemical structure of acyclovir [2]

Acyclovir is also known as Aciclovir (ACV). Its molecular formula is C8H11N5O3. IUPAC name of acyclovir is 2-amino-9-[(2-hydroxyethoxy) methyl]-6, 9-dihydro-3H-purin-6-one (fig. 1). It is a nucleotide analog antiviral primarily used for the treatment of herpes simplex virus infections2 [3]. Acyclovir is converted into acyclovir monophosphate due to the action of viral thymidine kinase then it is converted into acyclovir triphosphate (ACV-TP) by the action of host cell kinase [4]. ACV-TP competitively inhibits and inactivates the action of DNA

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polymerases by preventing further synthesis of viral DNA without affecting the cellular processes [5].

MATERIALS AND METHODS:

Instrumentation: Using 1cm match quartz cells, all of the spectrum and absorbance measurements were performed using an UV-VIS spectrophotometer (Shimadzu 1800).
Ultrasonicator (micro clean-103) was used to sonicate the formulation sample. Drug sample was weighed by using an electronic analytical balance (Shimadzu AY220).

Materials:

Glass pipettes, Micropipettes, Beakers, Conical flasks (1000ml, 500ml, 250ml), volumetric flask (100ml, 50ml).

Chemicals and reagents: Acyclovir tablets (800 mg) were obtained from Manufacturers (Care formulation labs Pvt Ltd, Mumbai). Standard acyclovir was obtained from Sigma Aldrich ltd and all the reagents and chemicals used in the study are procured from Sigma Aldrich and were of analytical grade.

NO.	Chemical name	Purity	Molecular weight	Company
1	Sodium hydroxide	99.99%	40	Qualigens

Experimental work

Method Development: Acyclovir (Pure and Tablet Powder), which is 100mg in exact weight, was dissolved in 100ml of 0.1N NaOH and further dilutions were made with 0.1 N NaOH. A series of standard solutions containing 2.5-40µg/ml of acyclovir were prepared in 0.1N NaOH and absorbance was measured at 253nm against reagent blank. The same process was used for recovery trials, which involved adding a known quantity of pure medication to the formulation that had already been examined. The amount of drug discovered was used to compute the percentage recovery.

Standard drug solution (10µg/ml): About 1mg of standard drug was weighed and made upto 100ml following sonication in a volumetric flask. Each ml contains 10µg. Drug was dissolved in 0.1N NaOH.

Sample preparation: Test samples of varying drug concentrations (8-40µg/ml) were studied for spectrophotometric estimation. Respective weight of powder was weighed and made upto 100ml following sonication in a volumetric flask. Sample tablet was dissolved in 0.1N NaOH.

Selection of wavelength: To determine the wavelength for measurement, Acyclovir (50µg/ml) solution was scanned in the range of 200-400 nm against distilled water as blank. Wavelength

of maximum absorption was determined for the drug. Acyclovir showed maximum absorption at 253 nm.

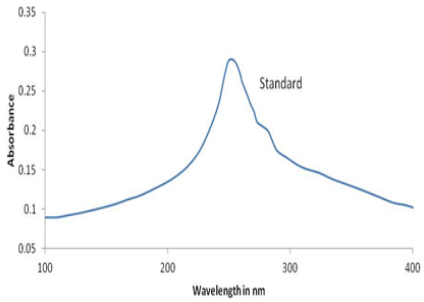

Figure 2: UV spectra showing the peaks of the standard (20µg/ml). Peak absorbance was seen at 253nm. Standard Peak absorbance was seen at 253nm. Standard shows maximum peak than the sample dissolved in 0.1N NaOH.

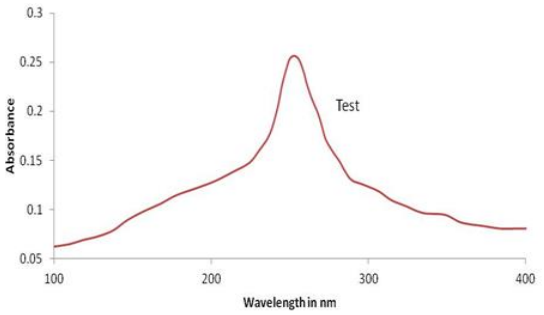

Figure 3: UV spectra showing the peaks of the test sample (20µg/ml). shows maximum peak than the sample dissolved in 0.1N NaOH.

Method Validation: The process of creating concrete proof to give a high degree of assurance that a certain action will consistently deliver the anticipated outcome or a product that satisfies its set standards and quality attributes is known as method validation. The accuracy, intraday precision, linearity, and percent recovery of the analytical technique development (ICH-Q2 R1, 2005) for ACV in pharmaceutical dosage forms and bulk samples were validated (USP, 2000).

Linearity: Stock solutions of varying concentrations (8-40µg/ml) of that were appropriate for the suggested procedures were measured. It was discovered that the Beer-Lambert concentration ranges were 8-40µg/ml. Following working standards preparation, absorbance was recorded at 253nm.

Accuracy: By conducting recovery studies on commercially available formulations (tablets) and prepared solutions containing known amounts of drug using the standard addition method, accuracy was assessed. Standard drug was added to pre-analyzed samples at three different concentration levels (80%, 100%, and 120%) in accordance with ICH guidelines. At each level, the recovery trials were conducted in triplicate.

Precision (intraday and inter day): Studies of intraday and interday variance provided proof of precision. By obtaining various solutions with the same concentration (5µg/ml and 20µg/ml), analyzing them three times daily, and recording the results, the intraday precision was ascertained. Solutions of the same concentration (5µg/ml and 20µg/ml) were made for the

interday investigation, analyzed, and the results were provided as % RSD. We studied precision using two concentrations.

Drug Content Estimation in Formulations: By using this procedure, the amount of ACL in the commercial formulations was estimated. The average weight of 5 tablets was calculated, and it was then finely pulverized. We studied 200 and 400mg of tablet form. The specified amount of ACL (200 and 400mg) from a precisely weighed tablet powder was placed into a 100ml volumetric flask containing 0.1N NaOH and sonicated for 10min. The alkali solution was used to bring the volume up to the required level once the medicines had completely dissolved. The final product was filtered via a membrane filter with a 0.45µm pore size.

To obtain a concentration of 20µg/ml ACL (200mg form), 1ml of the filtrate solution was transferred into a 100ml volumetric flask and the volume was made up with alkali solution. To obtain a concentration of 20µg/ml ACL (400mg form), 0.5ml of the filtrate solution was transferred into a 100ml volumetric flask and the volume was made up with alkali solution. The samples were then subjected to the suggested methods, and the amount of ACV was calculated using calibration curves using the two developed methods.

Recovery Studies: Analytical recovery studies were carried out by adding known quantities of pure drug to pre-analyzed samples of the tablet formulations with concentration ranges of 2, 10, and 20µg/ml in order to further validate the accuracy of the method developed. By comparing concentrations from spiked samples against actual added amounts (2, 10 and 20 µg /ml) covering the desired range, percent analytical recovery values were determined.

Table 1: Results of linearity

Concentration in µg/ml	Absorbance
8	0.126
16	0.243
24	0.342
32	0.512
40	0.611

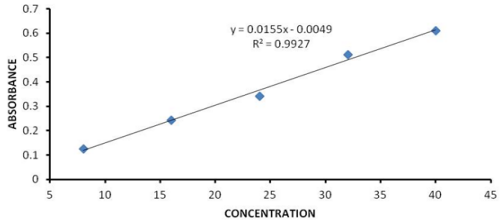

Fig. 3: Calibration curve for acyclovir

Table 2: The accuracy data along with SE, COV and relative error

Sample in µg/ml	Calculated amount	SD	COV	SE	% recovery	Relative error
5	4.98	0.02081	0.4183	0.0120	99.5206	0.4794
10	9.78	0.02318	0.2369	0.0134	97.8349	2.1651
20	19.72	0.06	0.3043	0.0346	98.5919	1.4081
25	24.85	0.03055	0.1230	0.0176	99.3843	0.6157

Table 3: Table predicting the linearity of ACL as obtained using UV-Vis method. (n=3).

Parameters	
Correlation coefficient (r)	0.999676773
Regression equation (Y*)	
Slope (a)	0.9931
Intercept (b)	0.105804657
SE of intercept	0.212191284
SD of intercept	0.474474135
LOD	1.576643486
LOQ	4.777707532

Table 4: The absorbance and recovery values for intraday validation study done at 5 and 20 µg/ml.

Sample (5µg /ml)	Mean Abs	Calculated amount	SD	COV	SE	% recovery	Relative error
Day 1	0.070	4.9763	0.0020	0.0402	0.0012	99.5256	0.4744
Day 2	0.070	4.9549	0.0015	0.0308	0.0009	99.0983	0.9017
Day 3	0.070	4.9763	0.0050	0.1005	0.0029	99.5256	0.4744
Day 4	0.069	4.9335	0.0012	0.0234	0.0007	98.6710	1.3290
Day 5	0.072	4.9763	0.0020	0.0402	0.0012	99.5256	0.4744

Sample (20µg/ml)	Mean Abs	Calculated amount	SD	COV	SE	% recovery	Relative error
Day 1	0.300	19.7183	0.02000	0.10143	0.01155	98.59190	1.40810
Day 2	0.3030	19.9320	0.00577	0.02897	0.00333	99.66017	0.33983
Day 3	0.300	19.7183	0.02000	0.10143	0.01155	98.59190	1.40810
Day 4	0.300	19.7183	0.02000	0.10143	0.01155	98.59190	1.40810
Day 5	0.297	19.5047	0.01155	0.05920	0.00667	97.52364	2.47636

Table 5: Table showing the absorbance and recovery values for intraday validation study done at 5 and 20µg/ml.

Sample (5µg/ml)	Mean Abs	Calculated amount	SD	COV	SE	% recovery	Relative error
S1	0.0687	4.8908	0.0012	0.0236	0.0007	97.8163	2.1837
S2	0.0703	4.9976	0.0006	0.0116	0.0003	99.9529	0.0471
S3	0.0697	4.9549	0.0042	0.0840	0.0024	99.0983	0.9017
S4	0.0703	4.9976	0.0035	0.0703	0.0020	99.9529	0.0471
S5	0.0703	4.9976	0.0015	0.0306	0.0009	99.9529	0.0471

Sample (20µg /ml)	Mean Abs	Calculated amount	SD	COV	SE	% recovery	Relative error
S1	0.3033	19.9320	0.04041	0.20276	0.02333	99.6602	0.33983
S2	0.3033	19.9320	0.00577	0.02897	0.00333	99.6602	0.33983
S3	0.3000	19.7184	0.03464	0.17568	0.02000	98.5919	1.40810
S4	0.3000	19.7184	0.01000	0.05071	0.00577	98.5919	1.40810
S5	0.3033	19.9320	0.04509	0.22623	0.02603	99.6602	0.33983

RESULTS AND DISCUSSION:

Absorbance and recovery:

From our study, we found potent recovery from the samples by all the methods. Results of analysis were shown in the table. The percentage recovery for all the methods performed was in the range of 99-100% indicating of zero interference of the excipients (in the formulation). All of the proposed methods are found to be free from any interference from the excipients in the formulation.

Absorbance values obtained at 253nm are shown in the table. The proposed methods spectrophotometric analysis was linear and in the range of 1- 20µg/ml at 252.8nm with correlation coefficients (R^2) of 0.9967.

Accuracy: The accuracy was evaluated by analysing mixture of the pure standard drugs and test drugs at four different concentration levels within the linear range by the proposed procedure. Percent recoveries and the relative standard deviations were calculated and Student's t-test was applied to validate the accuracy and sensitivity of the proposed method.

Regression analysis was done for method validation to see the significant effects at varying concentrations. **LOD** and **LOQ** were found to be 1.5766434 and 4.7777075 respectively [SD: 0.474474135; SE: 0.212191284].

Interday validation: The Interday precision of the proposed method was also determined by analysing test drugs at two different concentration levels by the proposed procedure for 5 different days. The % recovery was found to be in the range of 98.67 to 99.66%. The low Relative error (<2) values showed that the proposed procedure exhibited excellent Interday precision. However, no significant observation was noted on the interday precision between the concentrations ($p<0.05$). F value were found to be too low than the F critical value ($p<0.05$).

Intraday validation: The Intraday precision of the proposed method was also determined by analysing test drugs at two different concentration levels by the proposed procedure at 5 different times. The % recovery was found to be in the range of 97.81 to 99.95%. The low Relative error (<2) values showed that the proposed procedure exhibited excellent Intraday precision. However, no significant observation was noted on the intraday precision between the concentrations ($p<0.05$). F value were found to be too low than the F critical value ($p<0.05$).

Drug Content Estimation in Formulations:

From the drug content analysis obtained, we could confirm the recovery to be 99.66% with a relative error less than or equal to 0.12 [SD: 0.0208; SE: 0.01202]. It is evident from these results that this method is applicable for the analysis of the drug in its bulk and tablet forms with comparable analytical performance.

It is evident from the aforementioned results that the proposed methods gave satisfactory results with ACL in bulk. Thus, its capsules were subjected to the analysis for their contents for ACL by the proposed methods and the official method.

The label claims, as percentages, ranged from 99.38 to 99.83% % (Table 3). These results were compared with those obtained from the official method by statistical analysis with respect to the accuracy (t-test) and precision (F-test). No significant differences were found

between the calculated and theoretical values of t- and F-tests at 95 % confidence level proving similar accuracy and precision in the analysis of ACL in its capsules. It is evident from these results that all the proposed methods are applicable to the analysis of the drug in its bulk and capsule forms with comparable analytical performance.

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