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Quantitative analysis of Acyclovir in pure form and pharmaceutical preparations

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ABSTRACT

Three spectrophotometric methods are described for quantitative analysis of acyclovir in pure and dosage forms. The first method was depended on insist generated bromine as oxidizing agent and either methyl orange or methyl blue as a chromogenic agents acyclovir was treated with known excess amounts of bromine and residual unreacted bromine is determined by treating with fixed amount of either methyl orange and measure absorbance at 550 mm or methyl blue and measure absorbance at 650 nm. The amount of bromine reacts equal to acyclovir. Calibration curves were cover over ranges (1-30) µg/ml, for methyl orange and (2.5-40) µg/ml. The second method was depended on diazo coupling of acyclovir with resorcinol to form yellow- orange color with maximum absorption at 462 µm, Beer's law was obeyed in the range of (1-40) µg/ml. The third method was depended on the formation of yellow Schiff base complex as a result of condensation reaction between cited drug and 2.4-dihydroxy benzaldehyde in ethanol solution which absorbed maximally at 435nm with abeyance with Beers law in the concentration range of (5-100) µg/ml, the methods were satisfactory applied for determination of acyclovir in pure and dosage forms and results were compared statistically with reference method (HPLC).

Keywords: A acyclovir, spectrophotometry, methyl blue methyl orange, potassium bromate/ bromide solution, resorcinol, 2,4-dihydroxy benzaldehyde.

1. INTRODUCTION

Acyclovir (ACL), has the molecular structure as below, (Figure 1).

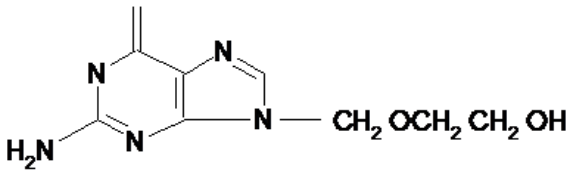


Figure - 1: Structure of Acyclovir.

Acyclovir (ACL), 9-[(2-hydroxyethoxy) methyl] guanine (Figure 1), is an antiviral drug used extensively in the treatment of skin infections caused by herpes simplex virus [1]. It is official in European pharmacopeia [2], British pharmacopeia [3] and United States pharmacopoeia [4], the therapeutic importance of the drug has prompted the development of analytical methods for its assay. The most extensively used technique for the quantification of ACL in body fluids is high performance liquid chromatography (HPLC) [5-19]. The techniques such as media immunoassay [20,21], high performance capillary electrophorsis [22], liquid chromatography [23], and micellar liquid chromatography [24], are also confined to biological fluids including plasma, urine and serum. Chromatographic methods including HPGC [25-27], HPLC [28,29] have been applied for the determination of ACL in pharmaceutical formulations. Besides being tedious and difficult to perform these procedures lack sensitivity, methods based on derivative [30], and differential [31] UV, spectrophotometry have also been reported for the assay of ACL in dosage forms. Recently [32-36], a visible spectrophotometric methods using Foliiv-cioalten reagent for the determination of ACL in pharmaceutical formulations has been reported. The present paper describes there simple spectrophotometric methods, these methods were based on measuring absorbance of the formed colored complex as a result of bromination reaction. Diazo-coupling reaction and formation of Schiff base complex, these proposed spectrophotometric methods were more sensitive than HPLC [27-28], and

derivative UV-spectrophotometric methods reported previously.

2. MATERIALS AND METHODS

2.1. Apparatus

All of spectrophotometric measurements were carried out using a Shimadzu 1160-uv/Vis with matched 1cm quartz cells. A digital pH-meter was used for pH-adjustment.

2.2. Materials and reagents

All solvents and reagents were of analytical grade and double distilled water was used throughout the work,

a. Acyclovir pure sample was obtained from (sigma, pharmaceuticals, Egypt). Stock solution was prepared by dissolving 100 mg in least amounts of DMF and complete to 100.0 ml with water. Working solution (5µg/ml) was prepared by diluting 1.0 ml of stock solution to 100.0 ml with water.

b. Methyl orange: was prepared by dissolving 0.3g in distilled water.

c. Methyl blue was prepared by dissolving 0.3g in distilled water.

d. Bromide and bromate stock solution was prepared by dissolving 0.1g of potassium bromate and 1.0g of potassium bromide in 100 ml of water, working solution was prepared by diluting 2.5 ml of stock solution to 100.0 ml with distilled water.

e. 0.1% sodium nitrite was prepared by dissolving 100 mg of sodium nitrite in water.

f. 1.0% sodium carbonate was prepared by dissolving 1g of sodium carbonate in water.

g. 0.2% resorcinol solution was prepared by dissolving 0.2g of resorcinol in water.

h. Drug working solution was prepared by dissolving 100 mg of drug in 100 ml ethanol, then dilute 0.5 ml of this solution to 100 ml with the same solvent to obtain solution of (5µg/ml).

i. 1M hydrochloric acid dissolved in either water or ethanol.

j. 0.2% 2,4-dihydroxy benzaldehyde in ethanol.

k. Acyclovir (25 mg / tablet) was obtained from local markets.

2.3: General procedures

2.3.1. Method I

Into a series of 10-ml calibrated flasks, added 0.5 ml of bromate working solution to (0.1-3.0) ml of working drug solution (5 µg/ml), and 1 ml of 1M HCL and leave reaction mixture for 5-minutes, then added 1ml of dye solution (methyl orange or Methyl blue) and leave reaction mixture for another 5-minutes, then complete to the mark with distilled water and measure absorbance of reaction mixture against a blank prepared similarly omitting drug, the reaction mixture showed absorption maximum at 550 nm and 650 nm for methyl orange and methyl blue, respectively.

2.3.2. Method II

Into a series of 10-ml calibrated flasks, transfer aliquots, of acyclovir ranging from (0.2-4.0) ml portion of working drug solution, to each flask added 0.5 ml of 1M HCL, followed by 1.0 ml of 1% sodium nitrite, the contents of flasks were set aside for 5-minutes for diazotization, then added 1.5 ml of 1.0% urea and the flasks were kept aside for 5-minutes for complete neutralization of excess nitrous acid formed in the reaction, then finally added 2.0 ml of sodium carbonate solution followed by 1.5 ml of resorcinol solution and mixed well and volume of flasks were completed to the mark with distilled water and measure absorbance of the formed yellow-orange chromogenic, which absorbed maximally at 462 nm, against reagent blank, prepared similarly omitting drug.

2.3.3. Method III

Into a series of 10-ml test tubes transfer aliquots of drug solution (mg/ml) equal to (0.1-1.0) ml, which corresponding to (10-100) µg/ml then added 2.0 ml of 2,4-dihydroxy benzaldehyde in ethanol, followed by adding 3.0 ml of 1M of hydrochloric acid in ethanol, then the contents of all tubes were transferred to water bath and heated at 55°C for 20 minutes, then the contents of test tubes were cooled to room temperature and carefully transferred to 10-ml calibrated flasks and complete to the mark with ethanol and measure the absorbance of the formed yellow chromogenic in the range of (300-500) nm, it is clear that the yellow Schiff base absorbed maximally at 435 nm, against the measurements obtained against reagent blank, prepared similarly omitting drug.

2.3.4. Procedures for pharmaceutical preparations

10 tablets were crushed and a weight equaling to two tablets was taken, extracted three successive times of DMF (3x20ml), then filtered into 100ml measuring flask and complete to volume with distilled water or ethanol and

proceed as described as above for method I, II and III respectively for pure samples.

3. RESULTS AND DISCUSSION

The proposed spectrophotometric methods were simple, rapid and easy for determination of acyclovir in pure and dosage forms.

The first one was an indirect method, depended on determination of residual bromine after allowing the reaction between cited drug and a measured amount of bromine to be complete, the residual bromine was determined by reacting it with fixed amount of methyl orange or methyl blue the method depended on the bleaching action of bromine on the dye, The discoloration being caused by the oxidative destruction of the dyes. Acyclovir when added in increasing amounts to fixed amount insitu generated bromine consumes the latter proportionately and therefore occurs a concomitant fall in the concentration of bromine when a fixed amount of dye is added to the decreasing amounts, of hormone, A concomitant increase in the concentration of dye results. Consequently a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentration of drug. The insider generation of bromine is carried out using a mixture of potassium bromide and potassium bromate in the presence of 1M hydrochloric acid (1M HCL), according to the following equation:

$$5Br^{+} + (BrO_3)^{-} + 6H^{+} \longrightarrow 3\,Br_2 + 3H_2O$$

The second method was depended on the diazotization coupling of acyclovir with resorcinol in sodium carbonate, forming yellow orange complex, has absorption maximum at 462 nm, the third method was depended on the condensation reaction between acyclovir and 2,4-dihydroxy benzaldehyde in ethanolic hydrochloric acid forming yellow Schiff base complex has absorption maximum at 435 nm.

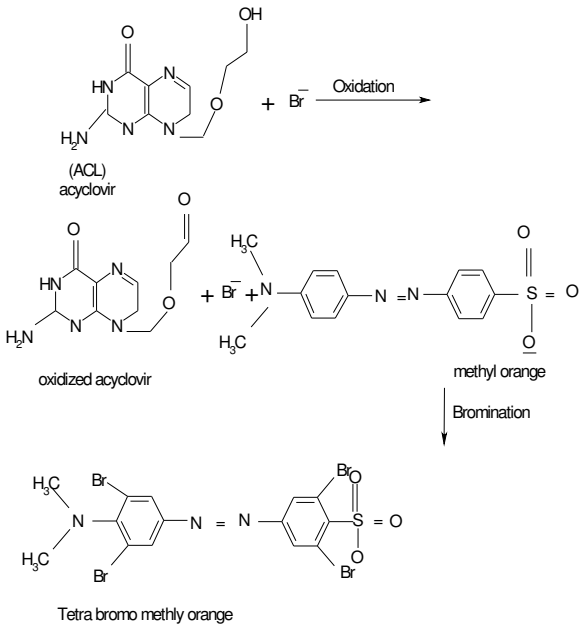
3.1. Absorption spectra

3.1.1. Method I

The resulting absorption spectrum was due to the red color of the oxidized methyl orange, which absorbed maximally at 550 nm or blue color of the residual oxidized methyl blue which absorbed maximally at λ_{max} = 650 nm. As shown in scheme 1.

3.1.1.1. Effect of dye concentration:-

The effect of dyes concentration were performed to found appropriate dyes concentrations by stabilizing other experimental conditions and using different volumes of dyes (methyl orange or methyl blue), the results showed that increasing volume of dyes more than one does not change value of absorbance indicating that the best volume was found to be 1.0 ml for both methyl orange or methyl blue (Figure 2).



Scheme - 1: Mechanism of reaction of acyclovir with Potassium bromate / Bromide mixture and methyl orange in acid medium.

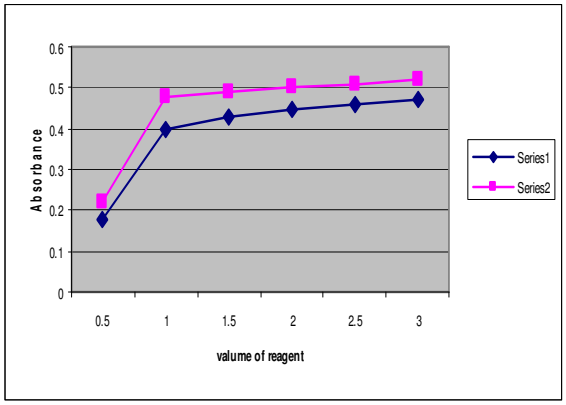


Figure - 2: Effect of volume of reagent of reaction of acyclovir with methyl orange (1) and methyle blue (2).

3.1.1.2. Effect of acids of medium

Effect of acidity of the medium of generating bromine was carried out by using different acids like sulphuric acid, by hydrochloric acid, nitric acid, acetic acid, and phosphoric acid, the results showed that hydrochloric acid was the best acid which gives the most precise and accurate results. The concentration of hydrochloric acid and its volumes was studied to show the most appropriate conditions for reaction, the results showed that 1.0 ml of 1M HCL were the most appropriate ones.

3.1.1.3. Effect of time

The time required for bromination and oxidized acyclovir before adding dyes was studied, also time required for irreversibly oxidized dye after its addition was also studied. The results showed that the bromination reaction was completed at 5-minutes and after oxidation time, contact times up to 60-minutes had been examined and non-further bromination was detected. A contact time of 5-minutes was necessary for the bleaching of the dyes color by the residual bromine and the color of the residual oxidized dye was stable for at least two hours after mixing of the reaction mixture.

3.2. Method II

The optimum conditions for color development in the diazotization of drug with resorcinol in alkaline medium were established by varying the parameters, one at a time and keeping the other parameter fixed and show the effect of the absorbance of the formed yellow-orange chromogenic, which absorbed maximally at λ_{max} = 462 nm. As shown in figure 2. The parameters affecting the formation of yellow colored chromogen complex are included.

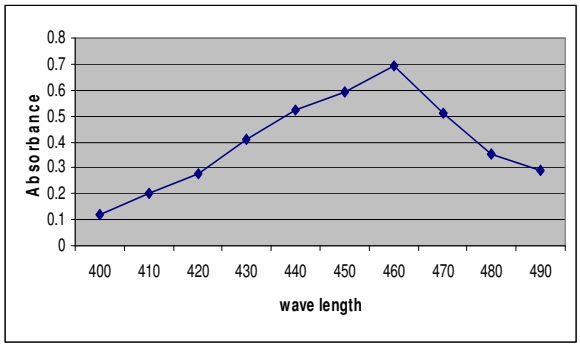


Figure - 2: Absorption spectrum of reaction of acyclovir and resorcinol.

3.2.1. Effect of type of acid

By taking different acids like hydrochloric acid, sulphuric acid, nitric acid and acetic acid results show that hydrochloric acid gives the highest absorbance.

3.2.2. Effect of molarity of acids

By taking different molarities of acid results show that 1M of hydrochloric acid gives the highest absorbance.

3.2.3. Effect of volumes of acids

By taking different volumes of 1M HCL ranging from (0.5, 1.0, 1.5, 2.0) results exhibit 0.5 of 1M hydrochloric acid was given the highest absorbance

3.2.4. Effect of sodium nitrite solution

By taking different volumes of 1.0% sodium nitrite (0.5, 1.0, 1.5 and 2.0) ml, experiments show that 1.0 ml of 1.0% sodium nitrite solution gives the highest results.

3.2.5. Effect of type of alkali solution

This effect was done by taking different types of alkali like sodium hydroxide, potassium hydroxide and sodium carbonate, experimental results shows that sodium carbonate was given the best results as it was given the highest color intensity and highest maximum absorption value.

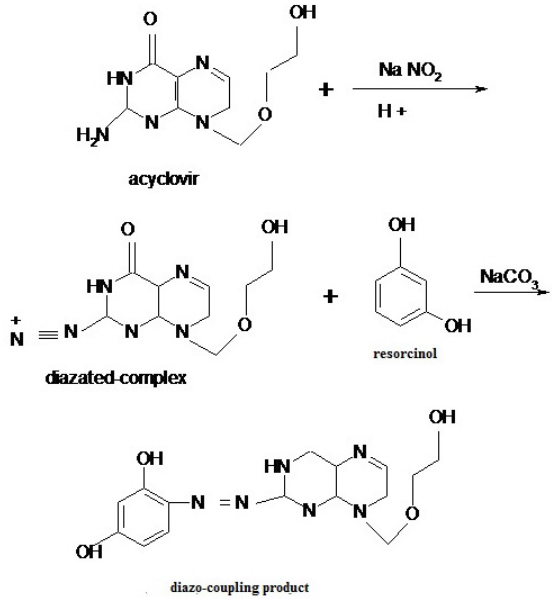
3.2.6. Effect of sodium carbonate solution

By taking different volumes of 1.0% sodium carbonate solution (0.5-3.0) ml, results exhibits 2.0 ml of 1.0% sodium carbonate gives higher absorbance values.

3.2.7. Effect of resorcinol reagent

By taking different values of 2.0% resorcinol reagent, experimental results show that 1.5ml of 2.0% resorcinol solution gives the highest absorbance value.

The diazotization and coupling of acyclovir and resorcinol was shown in scheme 2 as shown below.



Scheme - 2: Mechanism of reaction of diazocoupling of acyclovir with resorcinol.

3.3. Method III

This method was depended on condensation reaction between acyclovir and 2,4-dlydroxy benzaldehyde in acid medium, producing yellow color Schiff base complex absorbed maximally at 435 nm. As shown in figure 3. The optimum conditions for reaction were obtained by changing one parameter and keeping the others were constant, the parameters affecting formation of yellow colored Schiff base are:

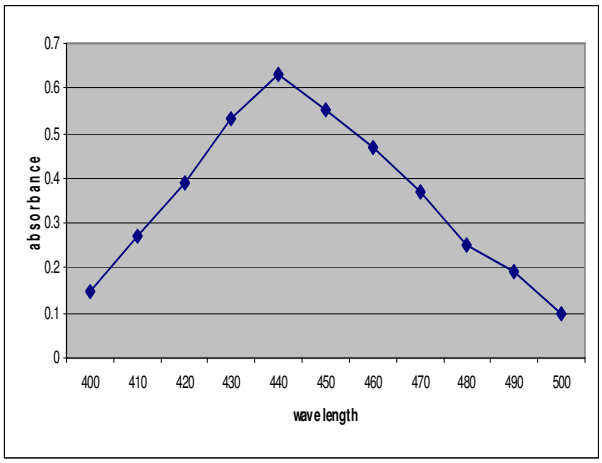
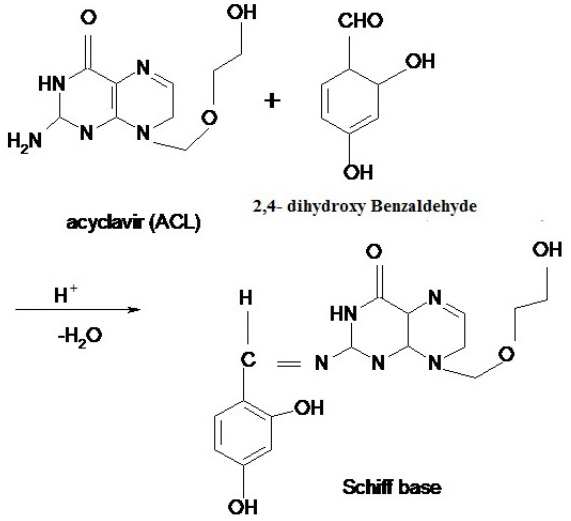


Figure - 3: Absorption spectrum of reaction of acyclovir with 2,4-dilydroxybenzaldelyde.



Scheme - 3: Mechanism of reaction of acyclovir with 2,4-dihydroxy benzaldehyde in acid medium.

3.3.1. Effect of type of acids

By using different types of acids like hydrochloric acid, sulphuric acid, nitric acid and acetic acid results show that 3.0ml of HCL in ethanolic solution give the highest absorbance value.

3.3.2. Effect of volume of 2,4-dihydroxy benzaldehyde

By taking different volumes of 2,4-dihydroxy benzaldehyde from (0.5-3.0) ml, results exhibit that 2.0ml of 2,4-dihydroxy benzaldehyde were given the highest absorbance value.

3.3.3. Effect of temperature

By taking reaction mixture at different temperatures from room temperature, 30, 35, 40, 45, 50, 55 and 60 C° experiments show that heating reaction mixture at temperature equals to 55C° gives the highest absorbance value.

3.3.4. Effect of heating time

By heating reaction mixture at different time intervals starting from 5, 10, 15, 20, 25, 30, 40, 50 and 60 minutes at constant temperature equals to 55C°, the data showed that heating reaction mixture at 55C° for 20.0 minute were the best which gives the highest absorbance value. As shown below in scheme 3.

3.4. Pharmaceutical preparation

Twenty tablets containing acyclovir were weighed and finely powdered, accurately weighted portions of the powered equivalent to 100 mg of acyclovir was dissolved in water and mixed for about 5-minutes and filter with whatmann filter paper no. 42, the volume was completed to 100.0 ml with distilled water for method I and II, but for method III, the filtrate was evaporated to residue and was dissolved in 100.0 ml of ethanol. And proceeds as described above for method I,II, III.

3.5. Validation of the methods

The proposed methods were validated, the linearity range of the absorbance as a function of drug concentration (Table 1) provides an accurate measure of the sensitivity of reagents used, calibration curves have correlation coefficients (r) higher than 0.999 indicating good linearity, the accuracy of the methods were determined by investigating the recovery of drug at concentration levels covering the specified range (Three replicates of each concentration). The results showed excellent recoveries (Table 2,3). Intra-day precision was evaluated by calculating standard deviation (SD) of five replicate determinations using the same solution containing pure drug pure drug at three different levels. The SD values measured the high precision of the methods. For interday precision, reproducibility on a day to –day basis, a series was run, in which the standard drug solution at three levels were analyzed each for five days. The results of inter-day (SD) revealed also a high precision, limit of detection (L.O.D) and limit of quantitation (L.O.Q) were also calculated. The obtained values of (L.O.D) and (L.O.Q) indicate high sensitivity of the proposed method, the robustness of the methods was evaluated by making small changes in volume of 2-parameters and kept other parameters were fixed and show its effects on the percentage recovery of the drug, for example in method I (change vol. of HCL and volume of methyl orange or methyl blue), in the results indicated that these changes had negligible influence on the values of recoveries as indicated by small values of SD.

Table - 1: Analytical parameters for determination of a acyclovir with the proposed methods.

Parameters	Method I		Method II	Method III
	Methyl orange	Methyl blue		
λ_{max} nm	550	560	462	433
Beer's law range	(5-25) µg/ml	(3-30) µg/ml	(5-35) µg/ml	(10-90) µg/ml
Ring bom range (µg/ml)	(1-30) µg/ml	(2.5-40) µg/ml	(1-40) µg/ml	(3-100) µg/ml
Molar absorptivity – $L.mol^{-1}.cm^{-2}$	$4.43x10^3$	$3.83x10^3$	$2.85x10^3$	$8.11x10^3$
Intercept	0.0231	0.0133	-0.0233	-0.0353
Slope	0.211	0.101	0.231	0.139
Correlation coefficient	0.9996	0.9999	0.9998	0.9998
Sandal sensitivity $\mu g/cm^2$	0.022	0.025	0.05	0.04
Relative standard deviation %	0.81	0.76	0.54	0.66
Limit of detection (LOD)	9.24	17.2	3.89	9.49
Limit of quantitation (LOQ)	30.8	57.42	12.98	31.6

A= a+b C where C = conc. of stated drug, b= slope, a= intercept.

Table - 2: Intra-day procedures for determination of acyclovir by the proposed methods

Method	Intra-day-precision				
Method I	**Taken (µg/ml)**	**Found ±SD**	**Recovery (%)**	**RSD (%)**	**Confidence limits ±SD at p = 0.05**
Methyl Orange	2	202±0.25	101%	0.5	2.02±0.35
	4	4.04±0.44	101%	0.66	4.04±0.47
	6	5.99±0.68	99.83%	0.82	3.99±0.75
	8	7.99±0.63	99.87%	0.79	7.99±0.58
	10	10.05±0.40	100.5%	0.63	10.03±0.42
Methyl blue	3	3.05±0.27	101.66%	0.51	5.05±0.40
	10	10.01±0.32	100.1%	0.56	10.01±0.68
	15	15.03±0.33	100%	0.57	15.03±0.53
	20	20.01±0.41	100.05%	0.64	20.01±0.76
	25	25.03±0.5	100.12%	0.70	25.03±0.38
Method II	3	2.99±0.21	99.66%	0.45	2.99±0.45
	9	8.95±0.33	99.44%	0.57	8.95v0.39
	12	11.96±0.28	99.66%	0.52	11.96±0.37
	15	14.98±0.45	99.86%	0.67	14.98±0.76
	20	19.97±0.33	98.95%	0.57	19.97±0.69
Method III	10	10.03±0.31	100.3%	0.55	10.05±0.39
	20	19.98±0.40	99.9%	0.63	19.98±0.48
	30	29.95±0.38	99.83%	0.61	29.95±0.75
	40	40.05±0.58	100.12%	0.76	40.03±0.60
	50	50.05±0.68	100.1%	0.82	50.05±0.29

Table - 3: Inter-day assays for determination of acyclovir by the proposed methods

Method	Taken (µg/ml)	Found ± SD (µg/ml)	Recovery (%)	RSD (%)	Confidence limits ± SD at p= 0.05
Method I	2	1.95±0.25	97.5%	0.5	1.95±0.28
methyl	4	3.96±0.20	99%	0.44	3.96±0.33
orange	6	5.97±0.38	99.5%	0.61	5.97±0.76
	8	7.99±0.40	99.87%	0.63	7.99±0.53
	10	9.98±0.52	99.8%	0.72	9.98±0.47
Methyl blue	5	4.96±0.19	99.2%	0.43	4.96±0.23
	10	9.96±0.34	99.6%	0.58	9.96±0.38
	15	15.01±0.48	100.1%	0.69	15.01v0.29
	20	19.95±0.67	99.57%	0.81	19.95±0.40
	25	25.05±0.33	100.2%	0.57	25.05±0.52
	3	3.05±0.13	101.6%	0.36	3.05±0.28
	9	9.01±0.20	100.11%	0.44	9.01±0.33
Method II	12	11.99±0.44	99.91%	0.66	11.99±0.22
	15	15.05±0.27	100.33%	0.51	15.05±0.53
	20	20.03±0.51	100.15%	0.71	20.03±0.44
	10	9.95±0.33	99.5%	0.57	9.95±0.25
	20	19.97±-0.41	99.85%	0.64	19.97±0.4
Method III	30	29.98±0.38	99.93%	0.61	29.98±0.20
	40	39.93±0.44	99.82%	0.66	39.95±0.33
	50	50.01±0.65	100.2%	0.8	50.01±0.55

Table - 4: Statistical analysis of results obtained from the proposed methods applied on tablets compared with official method.

Parameters	1st method		Method II	Method III	Official method (HPLC)
	Methyl orange	Methyl blue			
N	6	6	7	7	6
Mean recovery	100.05%	99.99%	99.98%	100.01%	99.98%
Variance	0.77	0.83	0.93	1.01	0.67
± SD	0.69	0.89	0.75	0.68	0.55
± RSD	0.87	0.91	0.86	0.82	0.74
±S.E	0.29	0.33	0.28	0.13	0.15
Student –t-test	0.5 (2.02)	0.413 (2.02)	0.527 (0.02)	0.231 (2.02)	-
F-test	1.57 (5.05)	2.61 (5.05)	1.85 (5.05)	1.52 (5.05)	-

N= number of different experiments; (2.02) tabulated value of T-test; (5.05) = tabulated value of F-test.

3.6. Applications

The proposed methods were successfully applied to the pharmaceutical preparation (tablets) of the stated drug. Excipients did not show interference. Results obtained were compared with official method (HPLC) and students t-test and F-test were calculated, results are shown in table 4.

4. CONCLUSION

The three new proposed spectrophotometric methods for determination of acyclovir in pure and dosage forms have been

developed, depending on insitu generation of bromine with using methyl orange and blue as chromogenic agents or diazo-coupling of acyclovir with resorcinol in alkaline medium or condensation reaction of acyclovir with 2,4-dihydroxy Benzaldehyde in acidic medium forming Schiff base complex, for method I,II and III respectively, they proved to be simple, accurate, rapid, sensitive and reproducible and can be successfully used in quality control and quality assurance laboratories.

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