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Utilization of charge transfer complexation reaction for the spectrophotometric determination of acyclovir as antiviral drug in pharmaceutical formulations

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ABSTRACT : ***The present work aims to develop and validate two simple, sensitive, accurate, precise and economical spectrophotometric methods for the determination of acyclovir (ACV) in pure form and pharmaceutical formulations. The developed methods are based on the formation of charge transfer complex between VAL as n-electron donor and quinalizarin (Quinz) or alizarin red S (ARS) as π-acceptor in methanol to form highly colored chromogens which showed an absorption maximum at 560 and 538 nm using Quinz and ARS, respectively. The optimization of the reaction conditions such as the type of solvent, reagent concentration and reaction time were investigated. Under the optimum conditions, Beer's law is obeyed in the concentration ranges 1.0–20 and 1.0–24 µg/ml using Quinz and ARS, respectively with good correlation coefficient (r^2 ≥ 0.9994) and with a relative standard deviation (RSD% ≤ 0.80). The limits of detection and quantification were found to be 0.30 and 1.0 µg/ml for Quinz and 0.29 and 0.97 µg/mL for ARS. The methods were successfully applied to the determination of VAL in its pharmaceutical formulations and the validity assesses by applying the standard addition technique. Results obtained by the proposed methods for the pure VAL and commercial tablets agreed well with those obtained by the reported method.***

Keywords: Acyclovir , Spectrophotometry, Quinalizarin, Alizarin red S, Charge transfer reaction, Pharmaceutical formulations.

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I. INTRODUCTION

Acyclovir (ACV); chemically named as 9-[(2-hydroxyethoxy)-methyl]-guanine) (Fig. 1). It is antiviral drug used for the treatment of viral infections due to herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and varicella-zoster virus (herpes zoster and chicken pox) [1,2].

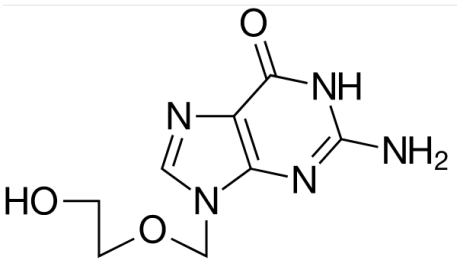

Figure (1). The chemical structure of acyclovir (ACV)

ACV has been determined in pure form, dosage forms, and biological fluids using a variety of analytical methods, including chromatography [3-6], electrochemistry [7-10], spectrofluorimetry [11-13], flow injection [14], FT-IR [15], and spectrophotometry [13, 16-29]. The depicted analytical techniques that have been reported for the determination of ACV appear to depend on the use of a practical instrument for the majority of these techniques. Additionally, a number of spectrophotometric techniques required cooling, buffer preparation, and/or incubation reaction times to finish the reaction. Most of the reported procedures for the determination of ACV suffer from the use of complex instruments, the need high expertise in their use, in addition the unavailability of these instruments in several quality control laboratories. Accordingly, there is a need for simpler, less costly as well as easier to be applied methods for the routine analysis of ACV as a drug of widespread use.

Table (1): Comparison between the reported methods for spectrophotometric determination of ACV.

Methods	Wavelength (nm)	Beer's law (µg mL^{-1})	Molar absorptivity ($L.mol^{-1}.cm^{-1}$) × 10^4	LOD (µg mL^{-1})	Samples	Reference
Ce(IV) in acidic mdium	320	2.0-8	2.56	25.39	Pharmaceutical preparations	13
UV	252.8	1.0-20	1.5899	NA	Bulk and its pharmaceutical preparations	16
Ninhydrin-Ascorbic acid at pH 5	540	10-30	4.1071	0.3	Dosage forms	17
UV	252	1.0-30	1.5899	NA	Bulk drug and pharmaceutical preparations	18
Perchloric acid-crystal violet	570	2.0-20	1.78	1.696	Pure form and pharmaceutical preparations	19
2, 4-dinitrophenyl hydrazine (2, 4 DNP)	414	20-60	NA	NA	Tablet preparations	20
UV	253	2-20	1.3733	NA	Bulk and pharmaceutical dosage forms	21
Cerium (IV) ammonium sulfate/ 3-methylbenzothiazolin 2-one hydrazone	630	5.0-50	0.41	0.18	Pharmaceutical formulations	22
Potassium persulfate/ 3-methylbenzothiazolin 2-one hydrazone	630	5.0-45	0.503	1.40		
N-bromosuccinimide (NBS)/ methyl orange	508	1.0-5.0	NA	0.2		23
Copper (II) in borax/sodium hydroxide buffer pH 9	290	112-1620	NA	NA	Pure and dosage forms	24
Cobalt (II) in 1 % pyridine in methanol	287	112-1620	NA	NA		
3-methyl benzothiazoline-2-one hydrazone (MBTH) /FeCl3	616	20-200	0.0941	1.06	Pharmaceutical formulations	25
Folin–Ciocalteu (F–C) in alkaline medium	760	50-450	0.0165	5.86	Bulk drug and formulations	26
Vanillin	470	2.0-10	NA	NA	Dosage form	27
p-dimethylaminobenzaldehyde	404	1.81-9.06	1.10	0.024	Bulk and dosage forms	28
UA-DLLME	495	0.1-3.0	2.5324	0.03	Pure and dosage forms	This work

NA: not available.

In contrast, visible spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories, hospitals and pharmaceutical industries for the assay of different classes of drugs in pure, pharmaceutical formulations and biological samples due to its simplicity, reproducibility, speed, less analysis time and reasonable sensitivity with significant economic advantages.

Alizarin derivatives have been used for the spectrophotometric determination of some drugs [30-34].

In the present work, we developed simple, sensitive, rapid, accurate and validated spectrophotometric method for the determination of ACV in pure form and pharmaceutical formulations. The proposed method involves the formation of charge transfer complex between ACV and two alizarin derivatives; quinalizarin (Quinz) and alizarin red S (ARS) as chromogenic reagents. The proposed methods have been validated statistically for their accuracy, precision, sensitivity, selectivity, robustness, and ruggedness as per ICH guidelines [35].

II. Materials and Methods

2.1. Apparatus:

All absorption spectra were made using Varian UV–Vis spectrophotometer (Cary 100 Conc., Australia) equipped with 10 mm quartz cell used for absorbance measurements. This spectrophotometer has a wavelength accuracy of ±0.2 nm with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm in the wavelength range of 200–900 nm.

2.2. Materials and reagents:

All chemicals, solvents and reagents used in this work were of analytical reagent or pharmaceutical grade and all solutions were prepared fresh daily. Double distilled water was used throughout the investigation.

2.2.1. Materials:

Pure sample of ACV was kindly supplied by Misr Pharmaceutical Co., Cairo, Egypt, with a purity of 100.16 ± 0.47% by applying the official method [1]. Pharmaceutical preparations containing ACV Purchased from different commercial sources in the local market; Zovirax 400 tablets Labeled to contain 400 mg ACV/tablet (Glaxo Wellcome, London, UK) and acyclovir 200 tablets Labeled to contain 200 mg ACV/tablet (mempheis, cairo, Egypt).

Quinalizarin 1,2,5,8-tetrahydroxy-anthraquinone (Quinz) and alizarin red S, 3,4-dihydroxy-9, 10-dioxo-2-anthracene sulfonic acid (ARS) (Sigma-Aldrich) were used without further purification.

2.2.2. Stock standard solutions:

A stock standard solutions of ACV equivalent to 100 µg/ml and 1.0×10^{-3} mol/l were prepared by dissolving an appropriate weight of pure ACV in methanol in a 100 ml measuring flask. The standard solutions were found stable for at least one week without alteration when kept in an amber coloured bottle and stored in a refrigerator when not in use. Serial dilution with the same solvent was performed to obtain the appropriate concentration ranges.

A stock solutions (0.2%, w/v) and (1.0×10^{-3} mol/l) of Quinz and ARS reagents were prepared by dissolving the appropriate weight of the reagent in approximately 25 mL of methanol, then completed to the mark with methanol in 100 ml volumetric flask. These solutions were stable for at least one week if kept in the refrigerator.

2.3. General procedures:

Aliquots of the standard ACV working solution in the concentration ranges (1.0-20 µg/ml) and (1.0-24 µg/ml) for Quinz and ARS, respectively were transferred into a set of 10 ml volumetric flasks. To each flask 2.0 ml of (0.2%, w/v) Quinz or ARS solution were added. Then the mixture was shaken in order to promote the reaction and the volume was completed to the mark with methanol. The absorbance of the resulting solutions were measured at 560 and 538 nm for Quinz and ARS, respectively against a reagent blanks prepared simultaneously. The calibration graph was constructed by plotting the absorbance *versus* the final concentration of VAL. The corresponding regression equation was derived.

2.4. Application to pharmaceutical formulations:

The contents of twenty tablets of each drug were crushed, finely powdered, weight out and the average weight of one tablet was determined for each drug. An accurate weight of the powdered tablets equivalent to 10 mg of each drug was dissolved in 10 mL methanol with shaking for 5.0 min and filtered using a Whatman No. 42 filter paper. The filtrate was diluted to the mark with methanol for in a 100 mL measuring flask to give and 100 µg mL^{-1} stock solution of ACV for analysis by the proposed spectrophotometric methods. A convenient aliquot was then subjected to analysis by the spectrophotometric procedures described above. Determine the nominal content of the tablets using the corresponding regression equation of the appropriate calibration graph.

2.5. Stoichiometric relationship:

The stoichiometric ratios of the ion-associates formed between VAL and the reagents were determined by applying the continuous variation method [36]at the wavelengths of maximum absorbance. In continuous variation method, equimolar solutions were employed: a 1.0×10^{-3} mol/l standard solution of VAL or ACV and 1.0×10^{-3} mol/l solution of Quinz or ARS reagent were used. A series of solutions was prepared in which the total volume of VAL or ACV and the reagents was kept at 2.0 mL. The drug and reagent were mixed in various complementary proportions (0:2, 0.2:1.8, 0.4:1.6,……..2:0, inclusive) and completed to volume in a 10 mL calibrated flask with the appropriate solvent methanol following the above mentioned procedure. The absorbance of the prepared solutions was measured at the optimum wavelength for each complex.

III.RESLUTS AND DISCUSSIONS

3.1. Absorption Spectra

The proposed method is based on the charge transfer reaction between ACV and quinalizarin (Quinz) or alizarine red S (ARS) in methanolic medium through two steps: (*i*) optimization of the experimental conditions in order to achieve both maximum sensitivity and selectivity. This step comprised the evaluation of the effect of the solvent nature, investigation of the influence of the reagent concentration and evaluation of the time required to complete the reaction and; (*ii*) study and characterization of the reaction, which was carried out by the evaluation of the reaction stoichiometry (Job's continuous variation method), calculation of the association constant and molar absorptivity in methanol medium and the verification of the proposed reaction

mechanism. In order to achieve maximum sensitivity the effect of some chemical variables such as the type of solvent, reagent concentration and reaction time were evaluated. The reaction was characterized in terms of stability of the product formed and its stoichiometry, and the apparent molar absorptivity and association constant were derived. Best conditions for the analytical determination of ACV were observed in methanol medium with Quinz and ARS.

At optimum conditions, the radical anion (absorbing species) was formed in the medium immediately after mixing of the reagents and showed maximum absorption at 560 and 538 nm using Quinz and ARS, respectively in methanol medium (Figs. 2 and 3). Thus, these wavelengths was chosen for all further measurements in order to obtain highest sensitivity for the proposed methods. It is important to point out that the Quinz and ARS alone, in methanol medium, exhibits maximum absorption at 491 and 422 nm, respectively. The high difference between maxima of the reagent and the product absorption bands ≥ 69 and 116 nm for Quinz and ARS, respectively allowed the measurement of the products with only a small contribution of the reagents that was added in excess in the medium.

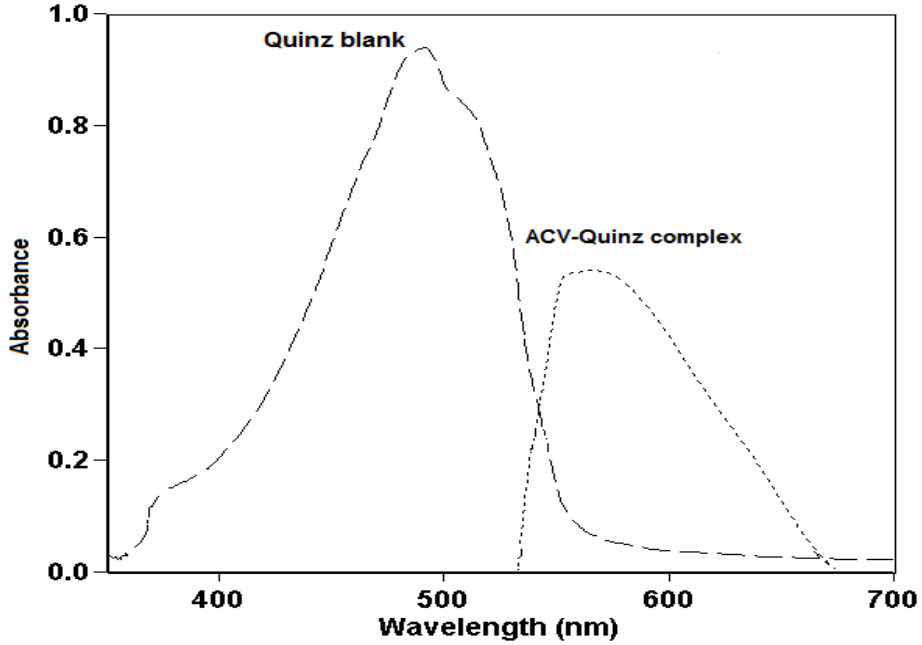

Figure (2). Absorption spectra for the reaction product of (20 µg/ml) ACV against (0.2%, w/v) Quinz reagent blank solution.

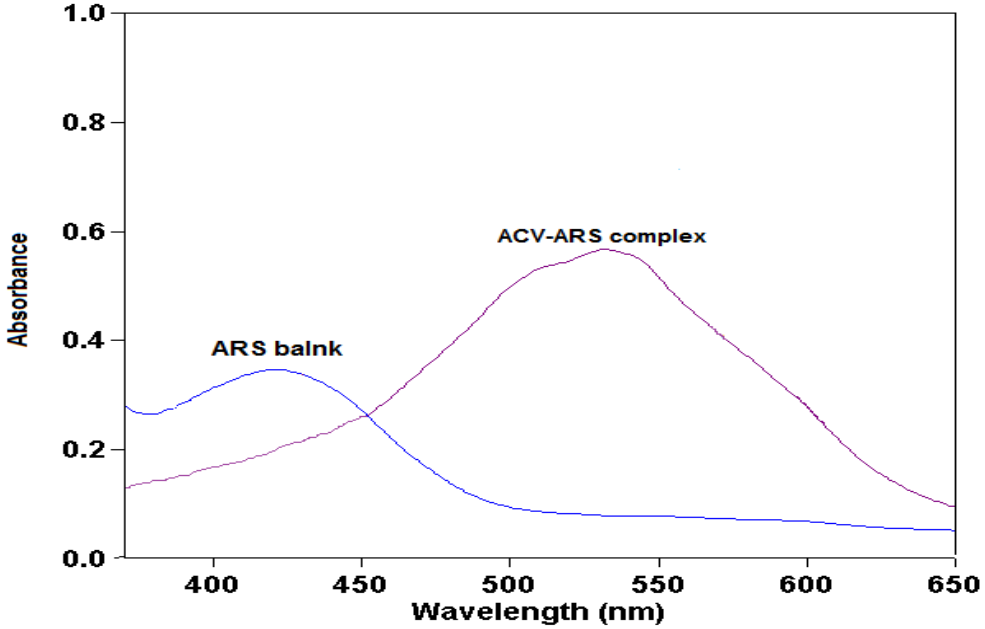

Figure (3). Absorption spectra for the reaction product of (24 µg/ml) ACV against (0.2%, w/v) ARS reagent blank solution.

3.2. Evaluation of the effect of the solvent nature

The solvent plays an important role in some charge transfer reactions, since it must be able to facilitate the total charge transfer and then allow the complex dissociation and stabilization of the radical anion formed, which is the absorbing species. According to the literature, solvents with high dielectric constant are more effective to execute this task. Taking this fact into account, water would be an excellent solvent for the procedure. However, the poor solubility of the Quinz and ARS in water did not allow its use in the present case. So, the reaction was tested in ethanol, methanol, acetone, DMSO and acetonitrile media. Although the highest dielectric constant of DMSO and acetonitrile, best sensitivity was achieved with methanol, probably because of the capacity of this solvent to form stable hydrogen bonds with the radical anion. Then, methanol was chosen for further experiments (Fig. 4)

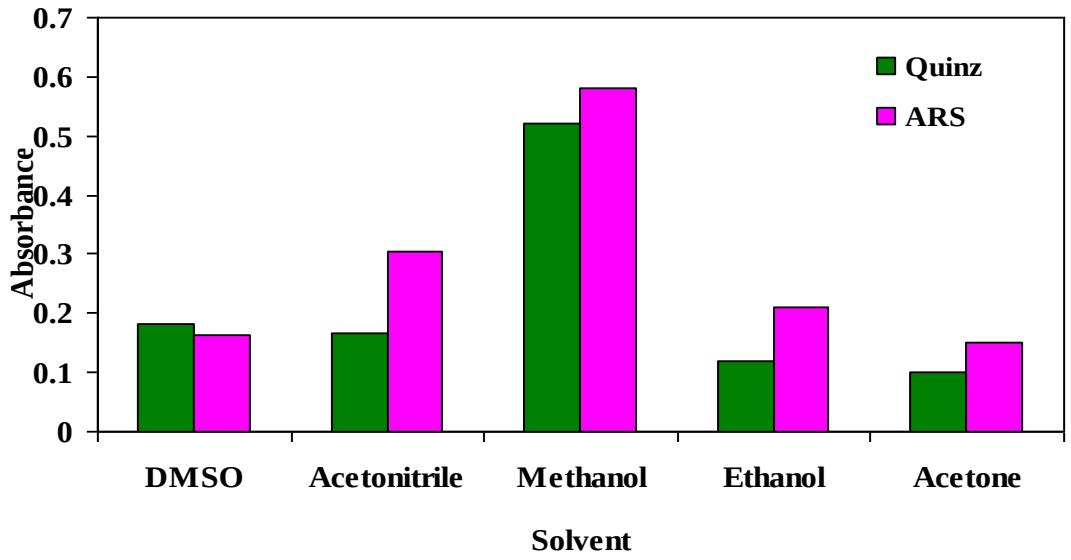

Figure (4). Effect of different solvents on the charge transfer complex formation obtained against (0.2%, w/v) Quinz or ARS solutions also prepared in each solvent. ACV drug concentration; (20 and 24 µg/ml) for Quinz and ARS, respectively.

3.3. Effect of reagents concentration

In order to achieve this objective, an experiment was performed by varying the reagents concentration in the range of 0.5-5.0 ml of (0.2%, w/v) Quinz and ARS solutions, while the ACV concentration was maintained constant. As it can be seen, remarkable increase of the absorbance was verified up to 2.0 ml of (0.2% w/v) Quinz and ARS reagents, respectivelly, after this point, the absorbance remain constant (Fig. 5). Therefore, 2.0 ml of (0.2%, w/v) Quinz and ARS reagents, is a sufficient and optimum reagent volume.

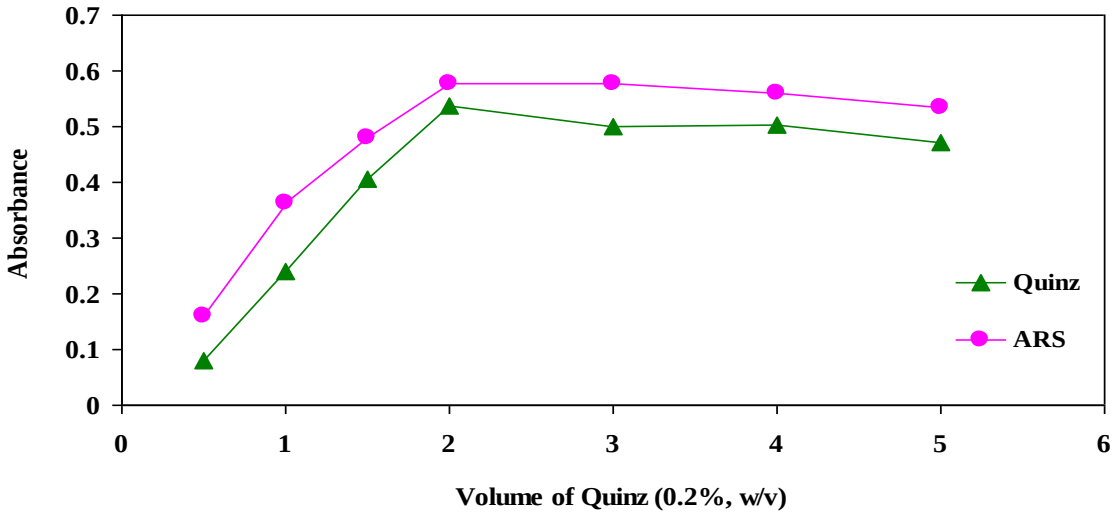

Figure (5). Effect of (0.2%, w/v) reagent concentration on the absorbance of ACV-reagent complex. ACV concentration; (20 and 24 µg/ml) for Quinz and ARS, respectively.

3.4. Effect of the reaction time

The optimum reaction time was evaluated by monitoring of the absorbance at optimum wavelengths of a ACV solution containing 20 and 24 µg/ml in case of Quinz and ARS reagents, respectively at laboratory ambient temperature (25±2°C). Complete colour development and measurements were carried out after 5.0 min of mixing of ACV with the reagents. By increasing the temperature, the absorbance of the charge transfer complex was decrease with a hypochromic shift, until decayed at 50 °C.

3.5. Sequence of additions

The most favorable sequence of addition is "VAL-reagent-solvent" for complete colour development, highest absorbance and stability at the recommended wavelength. Other sequences needed longer time in addition to lower stability. The complexes with this sequence remain stable for at least 6.0 hrs. After this time, absorbance suffered a slight decrease.

3.6. Stoichiometric ratio

Job's method of the continuous variation [36] of equimolar solutions was employed to determine the stoichiometry of the charge transfer reaction in methanol medium. As shown in (Fig. 6), the molar ratio which gave maximum absorbance was found to be (1:1) (ACV: reagent). In view of this result a reaction mechanism was proposed considering the transfer of free electron of the nitrogen atom present in one molecule of ACV to the charge-deficient center of Quinz or ARS molecule from the total transfer of charge.

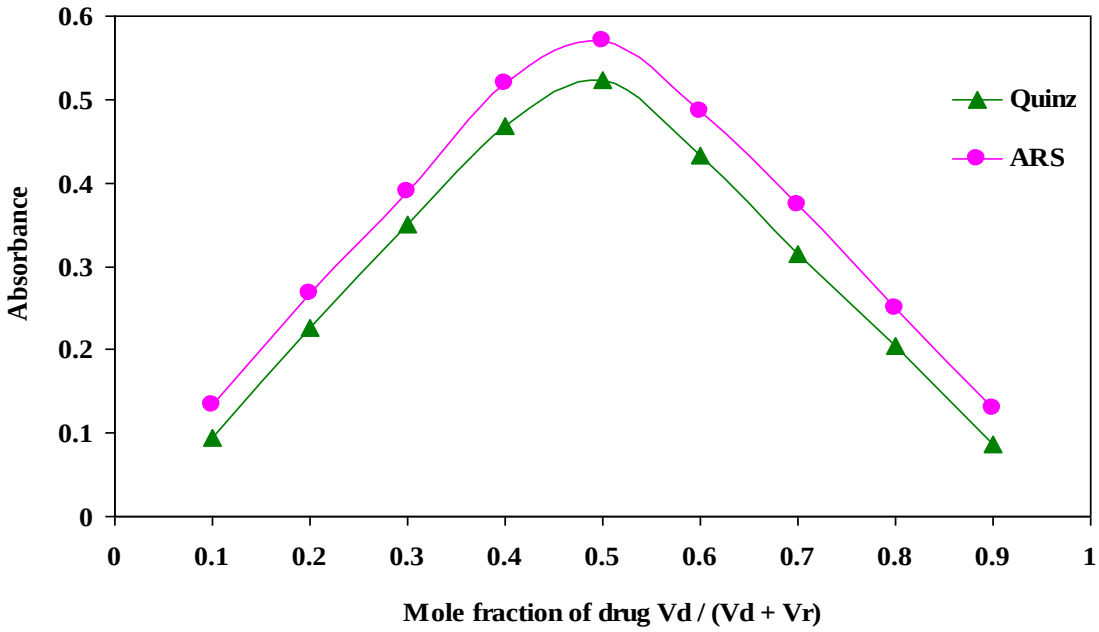

Figure (6). Application of Job's method to the reaction between ACV and Quinz and ARS reagents. from VAL and Quinz reaction.

3.7. Method of validation

3.7.1. Linearity

Following the proposed experimental conditions, the relationship between the absorbance and concentration for ACV drug was quite linear in the concentration ranges 1.0–20 and 1.0–24 µg/ml using Quinz and ARS, respectively. The calibration graph is described by the equation:

$$A = a + b\,C \qquad \text{Eqn. 1.}$$

(where A = absorbance, a = intercept, b = slope and C = concentration in µg/ml) obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 2. The apparent molar absorptivity of the resulting colored charge transfer complexes and relative standard deviation were also calculated and recorded in Table 2.

Sensitivity

In accordance with the formula, LODs were found to be 0.3 and 0.29 µg/ml and LOQ were found to be 1.0 and 0.97 µg/mL using Quinz and ARS, respectively.

Table (2): Statistical analysis and analytical data in the determination of ACV using the proposed methods.

Parameters	Quinz	ARS
Wavelengths (nm)	560	539
Linearity (µg/ml)	1.0-20	1.0–24
Molar absorptivity ε, (L/mol.cm) x 10^4	6.377	4.657
Sandal's sensitivity (ng cm^{-2})	35.31	48.36
Regression Equation [a]		
Intercept (*a*)	0.0016	-0.0041
Slope (b)	0.0273	0.0217
Correlation coefficient (r)	0.9997	0.9994
Mean ± SD[b]	99.40±0.80	99.80±0.65
RSD%[b]	0.80	0.65
RE%[b]	0.84	0.68
LOD (µg/ml) [c]	0.3	0.29
LOQ (µg/ml) [c]	1.0	0.97
t-test [d]	1.73	0.93
F- test [d]	1.97	1.30

[a] $A = a + bC$, where C is the concentration in µg/mL, A is the absorbance units, a is the intercept, b is the slope.
[b] SD, standard deviation; RSD%, percentage relative standard deviation; RE%, percentage relative error.
[c] LOD, limit of detection; LOQ, limit of quantification; ε, molar absorptivity.
[d] The theoretical values of t and F at P= 0.05 are 2.571and 5.05, respectively, at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

3.7.2. Accuracy and precision

To evaluate the accuracy as percent relative error (RE%) and precision as relative standard deviation (RSD%) of the proposed methods, solutions containing three different concentrations of ACV were prepared and analyzed in six replicates.

The intra-day precision were performed in the same day and inter-day precision i over five different days (for each level n=6). The percentage relative error (RE%) was calculated using the following equation:

$$\%\ R.E. = \left[\frac{found - taken}{taken} \right] \times 100 \qquad \text{Eqn. 3.}$$

The analytical results obtained from this investigation are summarized in Table 3. The low values of the relative standard deviation (RSD%) and percentage relative error (RE%) indicates good precision and accuracy of the proposed methods.

Table (3): Intra-day and Inter-day accuracy and precision data for ACV obtained by the proposed methods.

Method	Taken concentration (µg/ml)	Recovery % [a]	Precision RSD % [a]	Accuracy RE % [a]	Confidence limit [b]
		Intra-day			
Quinz	5.0	99.10	0.50	-0.90	4.955 ± 0.026
	10	100.30	0.75	0.30	10.03± 0.079
	15	99.60	0.90	-0.40	14.94 ± 0.141
ARS	5.0	99.40	0.60	-0.60	4.97 ± 0.031
	10	99.20	0.90	-0.80	9.92 ± 0.094
	15	100.10	1.30	0.10	15.02 ± 0.205
		Inter-day			
Quinz	5.0	99.70	0.75	-0.30	4.98 ± 0.039
	10	98.70	0.80	-1.30	9.87 ± 0.083
	15	99.00	1.70	-1.0	14.85 ± 0.265
ARS	5.0	99.40	0.40	-0.60	4.97 ± 0.021
	10	100.20	0.80	0.50	10.02 ± 0.084
	15	100.50	1.50	0.50	15.08 ± 0.237

[a] Mean ± SE (n=6), RSD%, percentage relative standard deviation; RE%, percentage relative error.
[b] Confidence limit at 95% confidence level and five degrees of freedom (t = 2.571).

3.7.3. Robustness and ruggedness

The analysis was performed with altered conditions by taking three different concentrations of ACV and it was found that small variation of method variables did not significantly affect the procedures as shown by the RSD% values in the range of 0.40-2.40 %. This provided an indication for the reliability of the proposed methods during its routine application for the analysis of ACV and so the proposed spectrophotometric methods are considered robust. The inter-analysts RSD% were in the range 0.65-2.10%, whereas the inter-instruments RSD% ranged from 0.70-2.50 % suggesting that the developed methods were rugged. The results are shown in Table 4.

Table (4): Results of methods robustness and ruggedness (all values in RSD%) studies.

Methods	Nominal concentration (µg/ml)	RSD%			
		Robustness		Ruggedness	
		Variable alerted [a]			
		Reagent volume (n=3)	Reaction time (n=3)	Different analysts (n=3)	Different instruments (n=3)
Quinz	5.0	0.50	0.90	0.65	0.80
	10	0.85	1.30	1.20	1.50
	15	1.70	2.10	2.10	2.50
ARS	5.0	1.30	0.55	0.70	0.70
	10	1.70	0.85	1.80	1.60
	15	2.40	2.0	2.10	2.40

[a] Volume of (0.2%, w/v) reagent is (2.0±0.2 mL) and reaction time is (5.0±1.0 min) (after adding reagent) were used.

3.7.4. Recovery studies

To ascertain the accuracy, reliability and validity of the proposed methods, recovery experiment was performed through standard addition technique. This study was performed by spiking three different levels of pure ACV (50, 100 and 150% of the level present in the tablet) to a fixed amount of drug in tablet powder (pre-analysed) and the total concentration was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard was calculated from:

$$\% \text{ Recovery} = ([C_F - C_T]) / C_p \times 10 \qquad (4)$$

Where C_F is the total concentration of the analyte found, C_T is a concentration of the analyte present in the tablet preparation; C_P is a concentration of analyte (pure drug) added to tablets preparations. The results of this study presented in Table 5 revealed that the accuracy of the proposed methods was unaffected by the various excipients present in tablets which did not interfere in the assay.

Table (5): Results of recovery experiments by standard addition method for the determination of ACV in tablets using the proposed methods.

Samples	Taken drug in tablet (µg mL^{-1})	Pure drug Added (µg mL^{-1})	Zovirax tablets		Acyclovir tablets	
			Total found (µg mL^{-1})	Recovery [a] (%) ± SD	Total found (µg mL^{-1})	Recovery [a] (%) ± SD
Quinz	6.0	3.0	8.946	99.40 ± 0.21	8.91	99.00 ± 0.15
	6.0	6.0	12.084	100.70 ± 0.29	11.976	99.80 ± 0.40
	6.0	9.0	15.225	101.50 ± 0.45	14.91	99.40 ± 0.60
ARS	8.0	4.0	11.94	99.50 ± 0.27	11.856	98.80 ± 0.20
	8.0	8.0	15.792	98.70 ± 0.36	15.888	99.30 ± 0.48
	8.0	12	19.82	99.10 ± 0.58	20.12	100.60 ± 0.67

[a] (n=6).

3.8. Analysis of pharmaceutical formulations

The proposed methods were applied to the determination ACV in pharmaceutical formulations (tablets). A statistical comparison of the results obtained from the assay of VAL by the proposed methods and the official method [1] regarding accuracy and precision (Table 6). When the results were statistically compared with those of the official method by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value

and F-value at 95% confidence level did not exceed the tabulated values for five degrees of freedom [38]. Hence, no significant difference between the proposed methods and the reported method at the 95 % confidence level with respect to accuracy and precision.

Table (6): Results of analysis of tablets by the proposed methods for the determination of ACV and statistical comparison with the official method [1].

Samples	**Recovery [a] (%) ± SD**		
	Proposed methods		**Official method** [1]
	Quinz	**ARS**	
Zovirax tablets	100.30 ± 1.20	100.60 ± 0.90	100.80 ± 1.03
t-value [b]	0.71	0.33	
F-value [b]	1.36	1.31	
Acyclovir tablets	99.60 ± 0.70	99.90 ± 1.10	100.52 ± 0.63
t-value [b]	2.18	1.09	
F-value [b]	1.23	3.05	

[a] (n=6).

[b] The theoretical values of t and F are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).

IV. Conclusion

This study describes the application of charge transfer complexation reaction with two alizarin derivatives for the quantification of acyclovir (ACV) in pure form and pharmaceutical formulations. Compared with the existing spectrophotometric method, the proposed methods are relatively simple, rapid, cost-effective, sensitive, accurate, and robust for determination of ACV in pure form and pharmaceutical formulations. Moreover, the proposed methods are free from tedious experimental steps such as extraction step, heating and pH adjustment. The most attractive feature of these methods is the relative freedom from interference, by the usual diluents and excipients in amounts higher than their normal existence in pharmaceutical formulations. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. Therefore, the proposed validated methods could be useful for routine quality control assay of ACV in pure form and pharmaceutical formulations.

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