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Original Article

VALIDATED SPECTROPHOTOMETRIC METHODS BASED ON CHARGE TRANSFER COMPLEXATION REACTION FOR THE DETERMINATION OF VALACYCLOVIR HYDROCHLORIDE AS ANTIVIRAL DRUG IN PHARMACEUTICAL FORMULATIONS

ALI H. AMIN[1,2], RAGAA EL SHEIKH[3], AHMED O. YOUSSEF[4], GHADA M. ABDEL FATTAH[3], AYMAN A. GOUDA[3*]

[1]Deanship of Scientific Research, Umm Al-Qura University, Makkah, Saudi Arabia, [2]Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt, [3]Chemistry Department, Faculty of Science, Zagazig University, Zagazig, 44519, Egypt, [4]Chemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt

*Email: aymangouda77@gmail.com

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ABSTRACT

Objective: The present study aims to develop and validate two simple, sensitive, accurate, precise and economical spectrophotometric methods for the determination of valacyclovir HCl (VAL) in pure form and pharmaceutical formulations.

Methods: 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 565 and 540 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.

Results: Under the optimum conditions, Beer's law is obeyed in the concentration ranges 0.5–16 and 1.0–20 µg/ml using Quinz and ARS, respectively, with good correlation coefficient ($r^2 \geq 0.9994$) and with a relative standard deviation (RSD% ≤ 0.70). The limits of detection and quantification were found to be 0.15 and 0.30 µg/ml for Quinz and 0.50 and 1.0 µg/ml for ARS.

Conclusion: 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: Valacyclovir HCl, Spectrophotometry, Quinalizarin, Alizarin red S, Charge transfer reaction, Pharmaceutical formulations

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INTRODUCTION

Valacyclovir hydrochloride (VAL) is chemically designated as [(S)-2-[(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)methoxy]ethyl-2-amino-3-methylbutanoate] (fig. 1). VAL is an antiviral drug and a prodrug, being converted *in vivo* to acyclovir. The mechanism of action of VAL is inhibition of the viral DNA polymerase and also involved in the viral DNA chain termination. VAL used for the treatment of herpes simplex virus types, 1 (HSV-1) and 2 (HSV-2) varicella-zoster virus infections, herpes zoster (shingles), and herpes B [1-3].

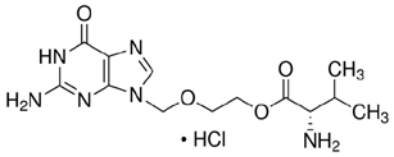


Fig. 1: The chemical structure of valacyclovir HCl (VAL)

The literature survey revealed that several analytical methods have been reported for the determination of VAL in pharmaceutical formulations and biological fluids, including high-performance liquid chromatography [5-10], electrochemical method [11-15], and spectrofluorimetry method [16-18]. These methods are complex and require long and tedious pre-treatment of the samples and laborious clean-up procedures prior to analysis.

A through literature search has revealed that some spectrophotometric methods have been developed for the determination of VAL [19-32] in pure and dosage forms and biological samples. Spectrophotometry is considered as the most convenient analytical technique in quality control laboratories, hospitals and pharmaceutical industries because of its inherent simplicity and low cost, sensitivity and selectivity, significant accuracy and precision. Alizarin derivatives have been used for the spectrophotometric determination of some drugs [33-37].

In the present work, we developed a simple, sensitive, rapid, accurate and validated spectrophotometric method for the determination of VAL in pure form and pharmaceutical formulations. The proposed method involves the formation of a charge transfer complex between VAL 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 [38].

MATERIALS AND METHODS

Instrumentation

All absorption spectra were made using Varian UV–Vis spectrophotometer (Cary 100 Conc., Australia) equipped with 5.0 and 10 mm quartz cell was 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.

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. Bidistilled water was used throughout the investigation.

Pure sample of VAL was kindly supplied by EVA Pharma S. A. E., Cairo, Egypt, with a purity of 99.30±1.20% by applying the offical

method [1]. Valysernex tablets, labeled to contain 1000 mg VAL per tablet, the product of EVA Pharma, Cairo, Egypt. Valtrovir tablets, labeled to contain 1000 mg VAL per tablet, the product of Hikma Pharma, Amman, Jordon.

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.

Preparation of standard solutions

A standard stock solutions of VAL equivalent to 100 µg/ml and 1.0 × 10^{-3} mol/l were prepared by dissolving an appropriate weight of pure VAL 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 x 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.

General procedures

Aliquots of the standard VAL working solution in the concentration ranges (0.5-16 µg/ml) and (1.0-20 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 565 and 540 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.

Applications to pharmaceutical formulations

The contents of twenty tablets were were crushed, finely powdered, weight out and the average weight of one tablet was determined. An accurate weight of the powdered tablets equivalent to 10 mg of VAL 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 stock solution 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 graphs.

Stoichiometric relationship

The stoichiometric ratios of the ion-associates formed between VAL and the reagents were determined by applying the continuous variation method [39] at the wavelengths of maximum absorbance. In the continuous variation method, equimolar solutions were employed: a 1.0 x 10^{-3} mol/l standard solution of VAL and 1.0 x 10^{-3} mol/l solution of reagent was used. A series of solutions was prepared in which the total volume of VAL and the reagent 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 following the above-mentioned procedure. The absorbance of the prepared solutions was measured at the optimum wavelength for each complex.

Validation procedure

The proposed method was validated according to ICH guidelines [38] concerning linearity, accuracy, precision, the limit of detection (LOD), the limit of quantitation (LOQ), robustness and ruggedness.

Linearity

The linearity of the detector response with the concentrations of VAL was evaluated. Absorbance were plotted versus the corresponding concentration to obtain the calibration graph. Regression data analysis was performed using least-squares linear regression statistical analysis.

LOD and LOQ

ICH guideline [38] describes several approaches to determine the detection (LOD) and quantitation limits (LOQ). These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD and LOQ for the proposed methods were calculated using the following equation [38, 40]:

$$LOD = 3s/k \text{ and}$$

$$LOQ = 10\ s/k$$

Where s is the standard deviation of ten replicate determinations values of the reagent blank or the standard deviation of intercepts of regression lines and k is the sensitivity, namely the slope of the calibration graph.

Accuracy

The accuracy of the proposed method was evaluated by analyzing three different concentrations (5, 10 and 15 µg/ml) of VAL within the linearity range. The concentrations were obtained from the corresponding regression equation. Accuracy was expressed as the recovery % for VAL.

Precision

The intraday precision (repeatability) for the determination of VAL was carried out by analyzing VAL sample solutions at three concentrations (5, 10 and 15 µg/ml), within the same laboratory, using the same analyst, with the same equipment, in the same day. The inter-day precision (intermediate precision**)** for the determination of VAL was carried out by analyzing sample solutions at three concentrations using the proposed methods, within the same laboratory, using the same analyst, with the same equipment but on three consecutive days. Precision was expressed as the percentage relative standard deviation (RSD %) for VAL.

Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation of method variables, including the concentration of reagents and reaction time on the performance of the proposed methods. In these experiments, one parameter was changed, whereas the others were kept unchanged, and the recovery percentage was calculated each time.

Methods ruggedness was expressed as the RSD% and was also tested by applying the proposed methods to the assay of drug using the same operational conditions but using three different instruments as well as three different anaysts.

RESULTS AND DISCUSSION

Absorption spectra

The proposed method is based on the charge transfer reaction between VAL 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 constants were derived. Best conditions for the analytical determination of VAL were observed in a 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 565 and 540 nm using Quinz and ARS, respectively in the methanol medium (fig. 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 ≥ 74 and 118 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.

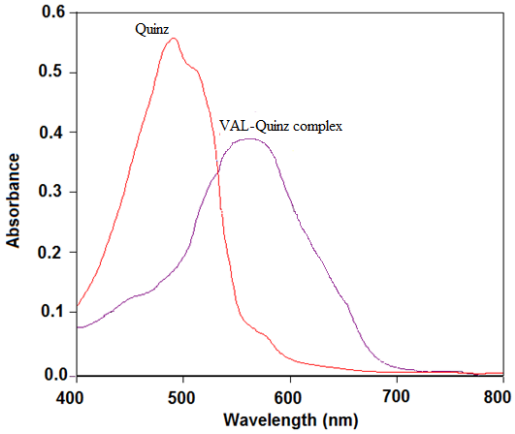


Fig. 2: Absorption spectra for the reaction product of (16 µg/ml) VAL against (0.2%, w/v) Quinz reagent blank solution

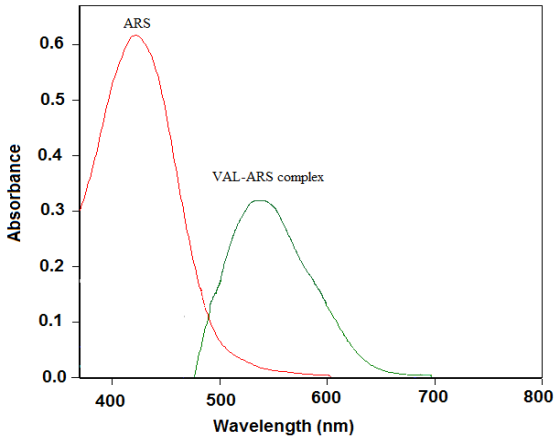


Fig. 3: Absorption spectra for the reaction product of (20 µg/ml) VAL against (0.2%, w/v) ARS reagent blank solution

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).

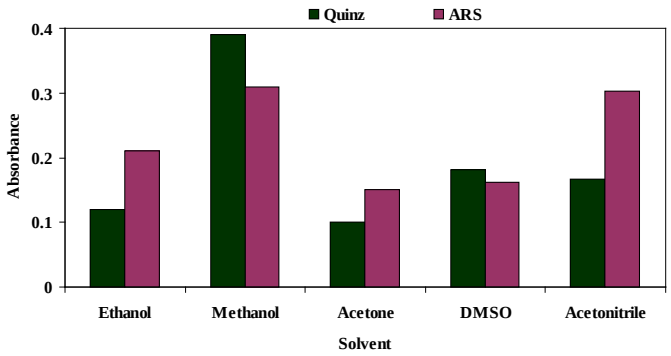


Fig. 4: Effect of different solvents on the charge transfer complex formation obtained against (0.2%, w/v) Quinz or ARS solutions also prepared in each solvent. VAL drug concentration; (16 and 20 µg/ml) for Quinz and ARS, respectively

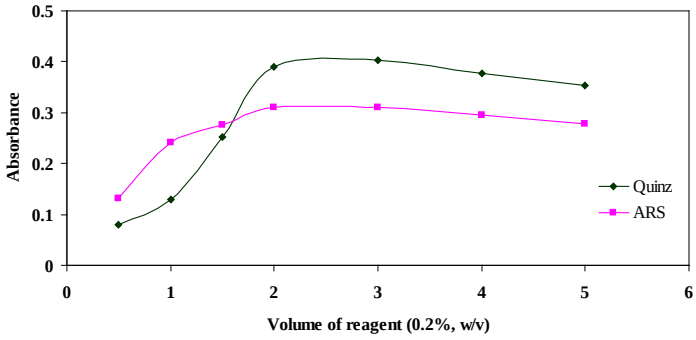


Fig. 5: Effect of (0.2%, w/v) reagent concentration on the absorbance of VAL-reagent complex. VAL concentration; (16 and 20 µg/ml) for Quinz and ARS, respectively

Effect of reagents concentration

In order to achieve this objective, an experiment was performed by varying the concentration of the reagent in the range of 0.5-5.0 ml of (0.2%, w/v) Quinz and ARS solutions, while the VAL 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, respectively, 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.

Effect of the reaction time

The optimum reaction time was evaluated by monitoring of the absorbance at optimum wavelengths of a VAL solution containing 16 and 20 µg/ml in case of Quinz and ARS reagents, respectively at ambient laboratory temperature (25±2 °C). Complete colour development and measurements were carried out after 5.0 min of mixing of VAL 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.

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 h. After this time, absorbance suffered a slight decrease.

Stoichiometric ratio

Job's method of the continuous variation [39] 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) (VAL: 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 VAL to the charge-deficient center of Quinz or ARS molecule from the total transfer of charge.

Mechanism verification

Molecular charge-transfer complexes are formed in non-polar solvents while radical anion species are predominant in polar solvents [33-37]. Also, it is believed that the addition of basic compounds that contains a lone pair of electrons, such as VAL results in the formation of charge-transfer complexes of n-π type (Scheme 1). This kind of complexes can be considered an intermediate molecular-association compound that forms a corresponding radical anion in polar solvents. In this case, radical anions results.

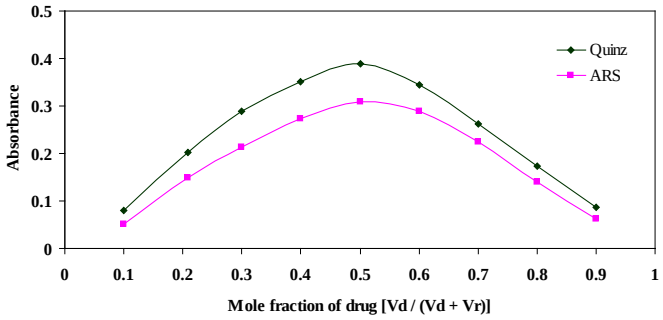
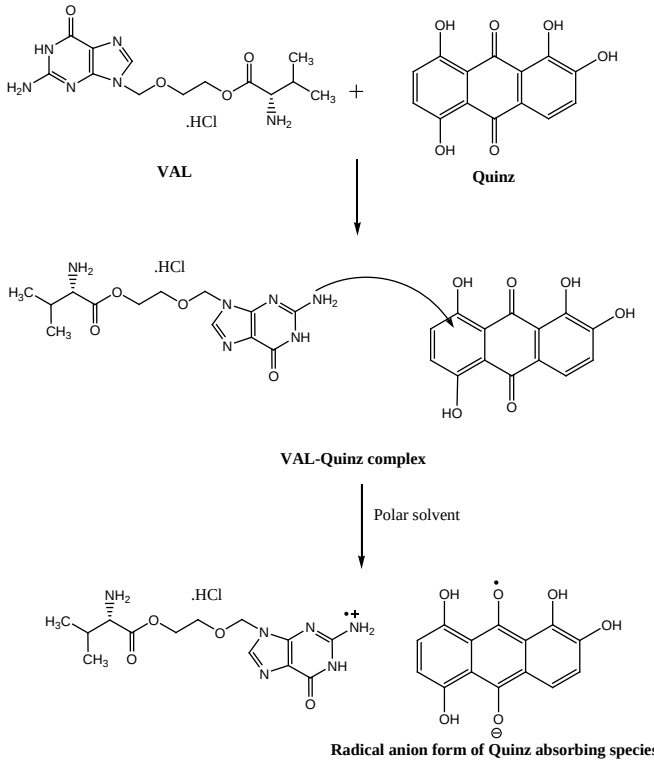


Fig. 6: Application of Job's method to the reaction between VAL and Quinz and ARS reagents



Scheme 1: Possible mechanism of radical anion formation from VAL and Quinz reaction

Method of validation

Linearity

Following the proposed experimental conditions, the relationship between the absorbance and concentration for VAL drug was quite linear in the concentration ranges 0.5–16 and 1.0–20 µg/mlusing Quinz and ARS, respectively. The calibration graph is described by the equation:

A = a+b C …. 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 1. The apparent molar absorptivity of the resulting colored charge transfer complexes and relative standard deviation were also calculated and recorded in table 1.

Sensitivity

In accordance with the formula, LODs were found to be 0.15 and 0.30 µg/ml and LOQ were found to be 0.50 and 1.0 µg/ml using Quinz and ARS, respectively.

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 VAL 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:

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

The analytical results obtained from this investigation are summarized in table 2. The low values of the relative standard deviation (RSD%) and percentage relative error (RE%) indicates good precision and accuracy of the proposed methods.

Robustness and ruggedness

The analysis was performed with altered conditions by taking three different concentrations of VAL 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 0.50-2.10 %. This provided an indication for the reliability of the proposed methods during its routine application for the analysis of VAL and so the proposed spectrophotometric methods are considered robust.

The inter-analysts RSD% were in the range 0.45-2.20%, whereas the inter-instruments RSD% ranged from 0.50-1.80% suggesting that the developed methods were rugged. The results are shown in table 3.

Table 1: Statistical analysis and analytical data in the determination of VAL using the proposed methods

Parameters	Quinz	ARS
Wavelengths (nm)	565	540
Linearity (µg/ml)	0.5-16	1.0–20
Molar absorptivity ε, (L/mol. cm) x 10^4	1.9365	1.4126
Sandal's sensitivity (ng cm^{-2})	18.63	25.54
Regression Equation[a]		
Intercept (*a*)	0.0148	0.0015
Slope (b)	0.0185	0.0193
Correlation coefficient (r)	0.9994	0.9995
mean±SD[b]	99.80±1.14	99.60±0.74
RSD%[b]	1.14	0.74
RE%[b]	1.20	0.77
LOD (µg/ml)[c]	0.15	0.30
LOQ (µg/ml)[c]	0.50	1.0
t-test [d]	0.23	0.50
F-test[d]	3.49	1.47

[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).

Table 2: Intra-day and Inter-day accuracy and precision data for VAL obtained by the proposed methods

Method	Taken concentration (µg/ml)	Recovery %[a]	Precision RSD % [a]	Accuracy RE %[a]	Confidence limit [b]
		Intra-day			
Quinz	4.0	99.50	0.68	-0.50	3.98±0.028
	8.0	99.80	1.04	-0.20	7.984±0.087
	12	99.20	0.89	-0.80	11.904±0.11
ARS	5.0	99.30	0.53	-0.80	4.996±0.026
	10	99.50	0.65	-0.50	9.950±0.065
	15	99.10	0.82	-0.90	14.865±0.122
		Inter-day			
Quinz	4.0	99.90	0.90	-0.10	3.996±0.038
	8.0	99.30	0.55	-0.70	7.944±0.046
	12	99.10	0.71	-0.90	11.892±0.089
ARS	5.0	99.60	0.65	-0.40	4.98±0.032
	10	100.10	0.88	0.10	10.01±0.088
	15	100.40	0.93	0.40	15.06±0.140

[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).

Table 3: 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	4.0	0.55	0.68	0.45	0.50
	8.0	1.20	1.15	0.90	0.97
	12	1.50	1.65	1.70	1.80
ARS	5.0	1.0	0.50	0.80	0.70
	10	1.70	1.30	1.40	1.0
	15	2.0	2.10	2.20	1.55

[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.

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 VAL (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:

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

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 4 revealed that the accuracy of the proposed methods was unaffected by the various excipients present in tablets which did not interfere in the assay.

Analysis of pharmaceutical formulations

The proposed methods were applied to the determination VAL 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 5). 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 [40]. Hence, no significant difference between the proposed methods and the reported method at the 95 % confidence level with respect to accuracy and precision.

Table 4: Results of recovery experiments by standard addition method for the determination of VAL in tablets using the proposed methods

Samples	Taken drug in tablet (µg/ml)	Pure drug added (µg/ml)	Valysernex tablets		Valtrovir tablets	
			Total found (µg/ml)	Recovery[a] (%)±SD	Total found (µg/ml)	Recovery[a] (%)±SD
Quinz	6.0	3.0	8.937	99.30±0.36	8.964	99.60±0.30
	6.0	6.0	12.024	100.20±0.50	12.084	100.70±0.34
	6.0	9.0	15.15	101.0±0.85	14.85	99.00±0.68
Mean Recovery %				100.17		99.77
SD				0.85		0.86
RSD%				0.85		0.86
ARS	6.0	3.0	8.937	99.30±0.44	9.063	100.70±0.38
	6.0	6.0	12.036	100.30±0.60	12.012	100.10±0.78
	6.0	9.0	15.12	100.80±1.10	14.91	99.40±0.50
Mean Recovery %				100.13		100.07
SD				0.76		0.65
RSD%				0.76		0.65

[a](n=6).

Table 5: Results of analysis of tablets by the proposed methods for the determination of VAL and statistical comparison with the official method [1]

Samples	Recovery[a] (%)±SD		
	Proposed methods		Official method [1]
	Quinz	ARS	
Valysernex SR tablets	100.17±0.85	100.13±0.76	99.60±0.86
t-value[b]	1.05	1.03	
F-value[b]	1.02	1.28	
Valtrovir tablets	99.77±0.86	100.07±0.65	99.40±0.90
t-value[b]	0.66	1.35	
F-value[b]	1.10	1.92	

[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).

Comparison between the proposed methods and reported methods

Table 6 shows comparison between the proposed spectrophotometric methods and other reported methods in the literature for the quantification of VAL in pharmaceutical formulations [19-30]. The proposed methods are new, simple, cost effective and selective spectrophotometric methods for the determination of VAL in pharmaceutical dosage forms. The reported methods are less selective, poor sensitivity, depending on critical experimental variables, few methods require a rigid pH control and tedious and time-consuming liquid–liquid extraction step; some other methods have a relatively narrow dynamic linear range, and/or use of expensive reagent or large amounts of organic solvents.

Table 6: Comparison between the reported spectrophotometric methods for determination of VAL

Methods	Wavelength (nm)	Beer's law (µg/ml)	Molar absorptivity (L/mol. cm) × 10^4	Samples	Reference
Zero order UV method	254	5-25	NA	Bulk and tablet	19
Brucine–$NaIO_4$	543	5-110	0.4642	Pharmaceutical formulations and human body fluids	20
MBTH	644	3-45	0.9761		
Sodium acetate	251	1-80	NA	Pharmaceutical dosage forms	21
Phosphate buffer pH 5.0	251	1-80	NA		
Phosphate buffer pH 7	252	1-80	NA		
borate buffer pH 9.0	253	1-80	NA		
0.1N NaOH	265	1-80	NA		
Paradimethylamino cinnamaldehyde	524	10-100	NA	Bulk and its pharmaceutical formulations	22
Phenyl hydrazine hydrochloride/hexacyano ferrate (III) in acidic medium	520	2-10	2.66	Pure and pharmaceutical dosage forms	23
Fe^{3+}/1,10-phenanthroline		5-25	0.506		
MBTH/Fe^{3+}	630	5-25	0.817	Bulk and tablet dosage form	24
MBTH/$NaIO_4$	624	2-10	2.83		
UV/0.1 M sulphuric acid	255	4-12	NA	Pharmaceutical dosage forms	25
DDQ	450	20-100	0.22	Pharmaceutical formulations	26
UV/0.1 M HCl	255	5-25	1.0910	Bulk and tablet dosage form	27
p-dimethyl aminobenzaldehyde	388	100-500	NA	Bulk and pharmaceutical dosage forms	28
Vanillin	428	20-100	NA		
UV	274	5-50	2.69167	Bulk and tablet dosage forms	29
UV	252	4-24	NA	Pharmaceutical dosage form	30
Quinz	565	0.5-16	1.9365	Pure form and pharmaceutical formulations	The proposed work
ARS	540	1.0-20	1.4126		

NA: not available. MBTH: 3-methyl-2-benzothiazolinonehydrazone; $NaIO_4$: sodium periodate; DDQ: 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone; Quinz: quinalizarin; ARS: Alizarin red S.

CONCLUSION

This work describes the application of charge transfer complexation reaction with two alizarin derivatives for the quantification of valacyclovir hydrochloride (VAL) 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 VAL 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 VAL in pure form and pharmaceutical formulations.

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AUTHORS CONTRIBUTIONS

Prof. Dr. Ragaa El Sheikh and Prof. Ayman A. Gouda has generated the research idea, interpreted the data and helped to draft the manuscript, helped in check spelling, reducing the plagiarism, interpreting the data, reviewed the manuscript and submit the manuscript for publication. Prof. Dr. Ahmed O. Youssef and Prof. Dr. Ali H. Amin has suggested the research idea and participated in the design of the study. Ms. Ghada M. Abdel Fattah was prepared the solutions, carried out the experiments, interpreted the data and helped to draft the manuscript.

CONFLICTS OF INTERESTS

The authors confirm that this article content has no conflict of interest.

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