Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 2, 2015

Original Article

UTILITY OF CERTAIN σ AND π-ACCEPTORS FOR THE SPECTROPHOTOMETRIC DETERMINATION OF VORICONAZOL ANTIFUGAL DRUG IN PHARMACEUTICAL FORMULATION

AYMAN A. GOUDA[1,3*], RAGAA EL SHEIKH[1], ALAA S. AMIN[1], SARA H. IBRAHIM[1]

[1]Chemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt, [2]Chemistry Department, Faculty of Science, Benha University, Benha, Egypt. Faculty of public health and [3], health informatics Umm al-Qura, university, Makkah, Saudi Arabia. Email: aymangouda77@gmail.com

Received: 11 Sep 2014 Revised and Accepted: 05 Oct 2014

ABSTRACT

Objective: Studies were carried out, for the first time, to investigate the charge-transfer reactions of voriconazole antifungal drug (VOR) as n-electron donor with the σ-acceptor iodine (I_2) and various π-acceptors: 7,7,8,8-tetracyanoquinodimethane (TCNQ); 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and *p*-chloranilic acid (*p*-CLA).

Methods: The formation of the colored charge-transfer complexes were utilized in the development of simple, rapid and accurate spectrophotometric methods for the analysis of voriconazole in pure form as well as in its pharmaceutical formulation (tablets). Different variables affecting the reactions were studied and optimized.

Results: Under the optimum reaction conditions, linear relationships with good correlation coefficients (0.9994–0.9999) were found between the absorbance and the concentration of voriconazole in the range of 2.0–120 µg mL^{-1}. For more accurate analysis, Ringbom optimum concentration range was found to be between 4.0-110 µg mL^{-1}. The limits of detection ranged from 0.36 to 1.23 µg mL^{-1}andthe limits of quantification ranged from 1.20 to 4.10 µg mL^{-1}. A Job's plot of the absorbance versus the molar ratio of voriconazole to each of acceptors under consideration indicated (1:1) ratio.

Conclusion: The proposed methods were applied successfully for simultaneous determination of voriconazole in tablets with good accuracy and precision and without interferences from common additives. The results were compared favourably with the reported method.

Keywords: Voriconazole, Charge-transfer complexes, Spectrophotometry; Pharmaceutical formulations.

INTRODUCTION

Voriconazole (VOR) is a triazole antifungal that is a derivative of fluconazole. It is chemically (2R, 3S)-2-(2,4-difluorophenyl)-3-(5-fluoro pyrimidin-4-yl) -1- (1,2,4-triazol-1-yl) butan- 2-ol (Figure. 1) [1]. Like all azole antifungals, its mechanism of action is the inhibition of a cytochrome P-450-dependent enzyme, 14-á-sterol demethylase that is essential to the synthesis of ergosterol for the fungal cell membrane. This inhibition is more selective for fungal than for mammalian enzyme systems. The accumulation of 14-alphamethyl sterols results in a decrease in ergosterol, which is an essential component of fungal cell wall formation. The resulting cell wall abnormalities are thought to be responsible for VOR's antifungal activity. VOR was approved by the Food and Drug Administration in May, 2002 for the treatment of invasive aspergillosis and refractory infections of scedo sporiuma piospermum and fusarium. Studies have also shown it to be a promising agent for empiric treatment in febrile neutropenia.

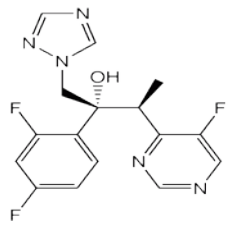


Fig. 1: The chemical structure of VOR

Literature survey revealed that a few analytical methods have been reported for the determination of VOR in pure drug, pharmaceutical dosage forms and in biological samples using liquid chromatography either in single or in combined forms [2-13], Electrochemical methods [14, 15] and spectrophotometric methods [16-19].

Visible spectrophotometry, because of simplicity and cost effectiveness, sensitivity and selectivity, fair accuracy, precision and available in most quality control laboratories, has remained competitive in an area of chromatographic techniques for pharmaceutical analysis. Furthermore, they do not need costly instrumentation required for published HPLC methods.

The molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge transfer complexes, which absorb radiation in the visible region [20]. A variety of electron donating compounds have been reported to yield charge-transfer complexes with various acceptors. The rapid formation of these complexes leads to their utility in the development of simple and convenient spectrophotometric methods for these compounds [21-27].

VOR is a good n-electron donor and will form charge transfer complexes with σ-acceptor like iodine (I_2) and π-acceptors such as 7,7,8,8-tetracyanoquinodimethane (TCNQ); 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and *p*-chloranilic acid (*p*-CLA); are known to yield charge transfer complexes and radical anions. The charge-transfer reaction has not yet been reported for VOR; therefore the aim of the present study was directed to investigate simple, direct, sensitive and precise spectrophotometric methods for simultaneous determination of VOR via complexation with σ- and π -acceptors in pure form and its pharmaceutical formulations (tablets).

MATERIALS AND METHODS

Apparatus

All absorption spectra were made using the double beam Unikon 930 spectrophotometer (Kontron Instruments, Munchen, Germany)

with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm, equipped with 10 mm matched quartz cells.

Materials and reagents

Materials

Pure grade voriconazole (VOR) was kindly supplied by Sigma Co. its potency was (99.38 ± 0.92%).

Pharmaceutical formulations

Vfend® tablets labeled to contain (200 mg VOR / tablet), purchased from Pfizer Pharmaceuticals Co. Ltd., USA.

Preparation of stock standard solution

Standard stock solution of VOR (1.0 mg mL^{-1}) was prepared by accurately weighting and dissolving 100 mg of VOR in 2.0 mL methanol in a 100 mL calibrated flask and completed to volume with the same solvent (for DDQ), with 1,2-dichloroethane (for I_2), and with acetonitrile (for the other acceptors, TCNQ and *p*-CLA). These stock solutions were diluted with the respective solvents to obtain suitable concentrations that lie in the linear range of each particular assay method, In the same manner, stock solution of 1.0 x 10^{-3} M of the drug was also prepared. The solutions were stable for at least one week at 4 °C.

Reagents

All reagents and solvents used were of analytical-reagent grade.

- Iodine resublimed, (Riedel De-Haen, Germany) (1.0 x 10^{-3} M) in 1,2-dichloroethane was prepared. the solution was stable for at least one week at 4 °C.

- 7,7,8,8-tetracyanoquinodimethane (TCNQ), (Aldrich Chem. Co., Milwaukee, USA) and *p*-Chloranilic acid (*p*-CLA), (Fluka, Switzerland), (1.0 x 10^{-3} M) were prepared in acetonitrile, the solutions were stable for at least one week at 4 °C.

- 2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ), (Merck-Schuchardt, Munich, Germany) (1.0 x 10^{-3} M) was prepared in methanol, the solutions were prepared fresh daily and were stable for at least one week at 4°C.

General analytical procedure

Into 10-mL calibrated flasks were placed aliquot volumes (0.2-3.0 mL) of 100 µg mL^{-1} VOR in 1,2-dichloroethane for I_2, (0.1-1.8 mL) of 500 µg mL^{-1} VOR in methanol for DDQ, (0.1-2.5 mL) of 500 µg mL^{-1} VOR in acetonitrile for *p*-CLA and (0.1-1.0 mL) of 200 µg mL^{-1} VOR 200 µg mL^{-1} VOR in acetonitrile for TCNQ. 1.0 and 2.0 mL of (1.0 x 10^{-3} M) TCNQ and (I_2, DDQ and *p*-CLA), respectively was added. The reaction mixture was allowed to stand for 5.0 min at (25 ± 2 °C) in case of DDQ or *p*-CLA; the reaction mixture was heated in a water-bath at 60±2ºC for 10 min in case of TCNQ and the reaction in case of I_2 was achieved instantaneously. The volume was made up 10 mL with methanol for DDQ, 1,2-dichloroethane for I_2 and with acetonitrile for DDQ and *p*-CLA. The absorbance of the resulting solutions was measured at the wavelengths of maximum absorption 364, 840, 461 and 538 nm for I_2, TCNQ, DDQ and *p*-CLA, respectively against the reagent blank prepared in the same manner.

Procedure for determination of VOR in tablets

Twenty tablets of the drug were weighed and a quantity of the content tablets equivalent to 100 mg was transferred into a 50-mL calibrated flask, dissolved in 2.0 mL methanol, swirled and sonicated for 5.0 min, completed to volume with the corresponding solvent (as in stock solutions), shaken well for 10 min, and filtered. The first portion of the filtrate was rejected, and a measured volume of the filtrate was diluted quantitatively with a suitable solvent to yield suitable concentrations lie in the linear range of each particular assay method; then the procedure was followed as above.

Determination of molar ratio of reactants in complex

Job's method of continuous variation [28] was employed, a 1.0 x 10^{-3} M standard solution of drug and reagents were used. A series of solutions were prepared in which the total volume of VOR and reagent was constant (2.0 mL). The drug and reagents were mixed in various complementary proportions (0:10, 1:9,..., 9:1, 10:0, inclusive) and then diluted in a 10-mL calibrated flask with the optimum solvent. The reactions were allowed to proceed for the optimum reaction time (Table 1) and the absorbance of the resulting solutions was measured at the corresponding wavelengths of maximum absorbance (λ_{max}) against a reagent blank under the same conditions.

Association constant

Serial volumes of 1.0-5.0 mL of 1.0 x 10^{-3} M VOR solution (in 1.0 mL steps) in the optimum solvent were transferred in 10-mL calibrated flasks. To each flask, 1.0 mL of acceptor solution (1.0 x 10^{-3} M) was added and continued as directed under the general procedure.

RESULTS

Spectral characteristics of the reaction

Reaction with σ-acceptor (iodine)

The color of iodine in 1,2-dichloroethane is violet showing absorption maximum (λ_{max}) at 520 nm. This color was immediately changed into lemon yellow, and the absorption spectrum of VOR-I_2 reaction product showed absorption peaks at 292 and 364 nm. This change in color, and the appearance of these two peaks were attributed to the formation of charge-transfer complex between the VOR and I_2, having an ionized structure $DI^+ \ldots I_3^-$, taking into account that the absorption spectrum of I_3^- in 1,2-dichloroethane showed the two absorption maxima at 292 and 364 nm. This complex should originate from an early intermediate outer complex $D \ldots I_2$, according to the following scheme

$$D + I_2 \rightleftharpoons D\text{-}I^+ I^- \rightleftharpoons [D\text{-}I^+] + I^- \xrightleftharpoons{I_2} I_3^-$$

Outer complex — inner complex — tri-iodide ion pair

Further confirmation for the charge-transfer nature of the reaction, VOR was extracted from the complex by shaking with aqueous mineral acids. The color of iodine in 1,2-dichloroethane layer was restored to violet, confirming the charge-transfer nature of the reaction. Measurements were carried out at 364 nm due to the interference from the native UV absorption of VOR at 256 nm.

Reaction with π-acceptors

The interaction of VOR with selective polyhaloquinone and polycyanoquinone π-acceptors in non-polar solvents such as 1,2-dichloroethane was found to produce colored charge-transfer complexes with low molar absorptivity values. In polar solvents such as methanol or acetonitrile, complete electron transfer from the VOR (D), as an electron donor, to the acceptor moiety (A) takes place with the formation of intensely colored radical ions with high molar absorptivity values, according to the following Scheme:

$$D^{\cdot\cdot} + A \rightleftharpoons [D^{\bullet\bullet} \rightarrow A] \xrightleftharpoons{\text{Polar solvent}} D^{\bullet+} + A^{\bullet-}$$

Donor — Acceptor — DA complex — radical anion

The dissociation of the (D–A) complex was promoted by the high ionizing power of the polar solvent and the resulting peaks in the absorption spectra of VOR-acceptor reaction mixtures were similar to the maxima of the radical anions of the acceptors obtained by the iodide reduction method [29].

The interaction of VOR with π-acceptors at optimum temperature gave colored chromogens showing different absorption maxima at 538, 461 and 840 nm for DDQ, *p*-CLA and TCNQ, respectively (fig. 2, 3 and 4).

The resulting maxima of the investigated drug with DDQ and *p*-CLA are similar to that of the radical anion of these acceptors obtained by the reduction method and coincide with the values reported in previous work [30].

The predominent chromogen with TCNQ in acetonitrile is the bluish-green colored radical anion, which exhibits strong absorption

maxima at 840, 825, 762, and 742 nm. These bands may be attributed to the formation of the radical anion $TCNQ^{\bullet-}$, which was probably formed by the dissociation of an original donor–acceptor (D–A) complex with VOR. The dissociation of the complex was promoted by the high ionizing power of acetonitrile.

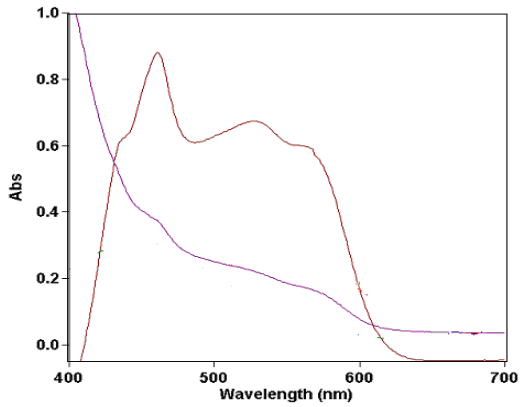


Fig. 2: Absorption spectra of DDQ and the reaction product of 90 µg mL^{-1} VOR in methanol

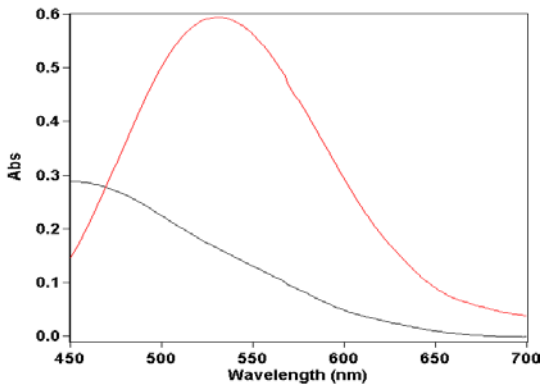


Fig. 3: Absorption spectra of *P*-CLA and the reaction product of 120 µg mL^{-1} VOR in acetonitrile

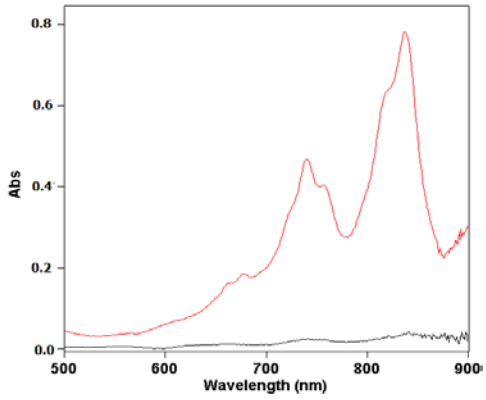


Fig. 4: Absorption spectra of TCNQ and the reaction product of 20 µg mL^{-1} VOR in acetonitrile

Further support of this assignment was provided by the absorption maxima with those of TCNQ radical anion produced by the iodide reduction method [31].

Chloranilic acid (*p*-CLA) exists in three ionic forms, the neutral yellow-orange H_2A at very low pH, the dark purple HA^- which is stable at pH 3.0 and a colorless A^{2-}, which is stable at high pH; these transformations are illustrated in the following scheme:

$H_2A \rightleftharpoons H^+ + HA^-$ (violet),

$HA^- \rightleftharpoons H^+ + A^{2-}$ (colorless).

Since the interaction of VOR with *p*-CLA in acetonitrile gave a violet product, it might be concluded that HA^- was the form of *p*-CLA involved in the reaction described herein.

The relative higher molar absorptivities and sensitivity of the four acceptors employed in the present analytical work may be attributed to their difference in electron affinities, as well as the conditions employed in the reaction (reagent concentration, reaction time, and solvent).

Optimization of reaction conditions

Effect of reagent concentration

The results for variation of reagent concentration, indicated that 1.0 and 2.0 mL of (1.0 x 10^{-3} M) (I_2, *p*-CLA or TCNQ) and DDQ, respectively are suitable. The higher concentrations of the reagents may, on the other hand, be useful for rapidly reaching equilibrium and complete color development, the results are given in (fig. 5). This minimizes the time required to attain the maximum absorbance at the corresponding wavelength of the charge transfer complexes.

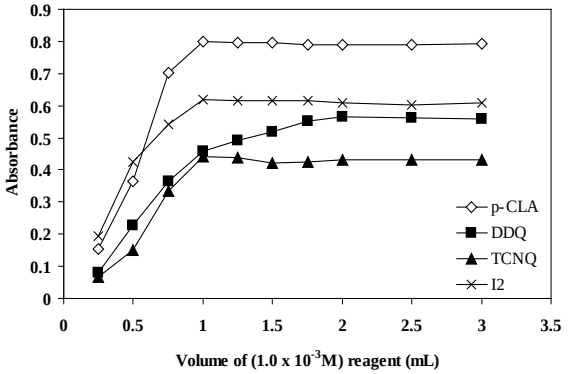


Fig. 5: Effect of reagent concentration on the absorbance of Charge transfer complex

Effect of solvent

1,2-Dichloroethane was found to be an ideal solvent in case of iodine, because it is favourable for the formation of tri-iodide ion pair (inner complex). Dichloromethane and chloroform produced lower absorbance readings. Polar solvents such as acetonitrile and alcohols were found to be unsuitable as their blanks with iodine gave high absorbance. It is obvious that, the rate of transformation of outer complex to inner complex is in the order of 1,2-dichloroethane>dichloromethane>chloroform [31]. There is actually a considerable decrease in the energy of activation along with an increased dielectric constant ε_r of the medium; in 1,2-dichloroehane (ε_r = 10.2) the transformation of inner complex proceeds much faster than that in dichloromethane (ε_r = 9.1) and chloroform (ε_r = 4.8). This is in support of the proposed three-steps mechanism. In fact, the resulting charged transition states in going from the outer complexes to the inner ones (as the rate determining step of the mechanism) are expected to be more stabilized in 1,2-dichloroethane because of higher solvating ability and relative permittivity than dichloromethane and chloroform [32].

For *p*-CLA and TCNQ complex formation, acetonitrile afforded maximum sensitivity, due to its high dielectric constant (37.5) [33] when compared with all other solvents (benzene, chloroform, ethylene chloride and methanol), a property which is known to promote the dissociation of the original charge transfer complexes to the radical anions in addition to the high solvating power of the reagent and drug. On the other hand, methanol affords maximum sensitivity and full color development with DDQ. In addition, it is a good solvent for the reagent.

Effect of reaction time

Complete color development, was attained instantaneously using iodine, whereas for DDQ and *p*-CLA, complete color development was attained after 5.0 min at (25 ± 2 °C). For TCNQ complete color development was attained after 90 min. To consume the time required for complete color development heating in a water bath at 60 ± 2 °C for 10 min. The color remained stable for 3.0, 2.0 and 4.0 h using TCNQ, DDQ and *p*-CLA, respectively. In the case of iodine, the yellow color remained stable at least a further 1.0 h in the dark.

Molar ratio of the reaction

Job's continuous variation graph [26] for the reaction between VOR and different reagents (fig. 6) shows that the interaction occurs on an equimolar basis via the formation of a charge transfer complex (1:1). The colored reaction product can be represented, taking TCNQ as an example, by Scheme 1. A more detailed examination was made for VOR complexes with the studied acceptors. The absorbance of the complex was used to calculate the association constant using the Benesi-Hildebrand equation [34].

$$\frac{[A_\circ]}{A_\lambda^{AD}} = \frac{1}{\varepsilon_\lambda^{AD}} + \frac{1}{K_c^{AD}\,\varepsilon_\lambda^{AD}} \times \frac{1}{[D_\circ]}$$

Where $[A_o]$ and $[D_o]$ are the total concentrations of the interacting species, A_λ^{AD} and $\varepsilon_\lambda^{AD}$are the absorbance and molar absorptivity of the complex at their λ_{max}, and K_c^{AD} is the association constant of the complex. On plotting the values of $[A_o]/A_\lambda^{AD}$ versus $1/[D_o]$, a line was obtained with slope equals $(\varepsilon_\lambda^{AD}\ K_c^{AD})^{-1}$ and intercept of this line with the ordinate is $(\varepsilon_\lambda^{AD})^{-1}$.

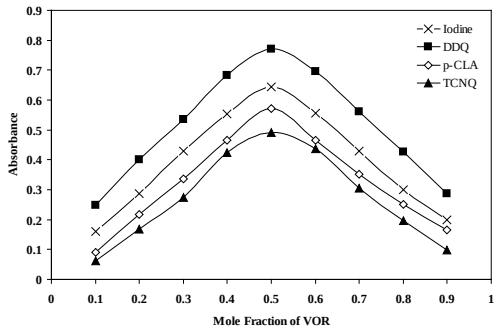
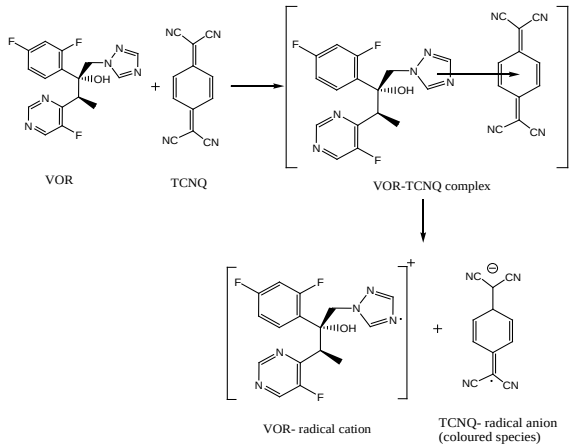


Fig. 6: Continuous variation plots for the reaction of VOR with Iodine, *p*-CLA, DDQ and TCNQ. λ= 364, 538, 461 and 840 nm, respectively. Total molar concentration = 1.0 x 10^{-4} mol L^{-1}



Scheme 1: Proposed reaction pathway for the formation of charge transfer complex between VOR and TCNQ

The calculated association constants are recorded in table 3, whereas the molar absorptivities were comparable with those obtained from the regression line equation of Beer's law. The low values obtained for the association constants are common in these complexes due to the dissociation of the original donor–acceptor complex to the radical anion.

Validation of the proposed method

Linearity, detection, and quantitation limits

Following the proposed experimental conditions, the relationship between the absorbance and concentration was quite linear in the concentration ranges 2.0-120 µg mL^{-1} as showed in table 1. The regression equations were derived using the least-squares method [35]. The intercept (a), slope (b), correlation coefficient (r), molar absorptivities (ε), and sandell sensitivity values are summarized in table 2. The percentage recoveries of the pure drugs using the proposed methods compared with that given by the reported methods are illustrated in table 1. The validity of the proposed methods was evaluated by statistical analysis [36], between the results achieved from the proposed methods and that of the reported methods. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Table 1), there is no significant difference between the proposed and reported methods regarding accuracy and precision.

Table 1: Statistical Analysis for determination of VOR by the proposed methods

Parameters	***Iodine***	***DDQ***	***p-CLA***	***TCNQ***
Wavelengths λ_{max} (nm)	364	461	538	840
Solvent	1,2- dichloroethane	Methanol	Acetonitrile	Acetonitrile
Color stability	1.0	2.0	4.0	3.0
Beer's law limits (µg mL^{-1})	2.0-24	5.0-90	5.0-120	2.0-20
Ringbom optimum concentration range (µg mL^{-1})	5.0-20	10-80	10-110	4.0-16
Molar absorptivity ε, (L/mol^{-1} cm^{-1}) x 10^4	0.8535	0.2839	0.1915	1.0872
Sandell·s sensitivity (ng cm^{-2})	40.92	12.30	18.24	32.13
Regression equation [a]				
Slope (b)	0.0242	0.0088	0.0049	0.0313
Intercept (a)	-0.0003	- 0.0084	0.0075	-0.0004
Correlation coefficient (*r*)	0.9997	0.9996	0.9994	0.9999
Mean ± SD	99.50 ± 0.74	99.70± 0.86	99.80±0.98	99.60±1.14
Relative standard deviation, RSD%	0.74	0.86	0.98	1.14
Relative error, RE%	0.78	0.90	1.03	1.20
LOD, (µg mL^{-1})[b]	0.36	0.86	1.23	0.43
LOQ, (µg mL^{-1})[b]	1.20	2.87	4.1	1.43
Calculated *t*-value[c]	0.23	0.57	0.70	0.34
Calculated *F*-value [c]	1.55	1.14	1.13	1.54

[a] $A = a + bC$, where C is the concentration in µg mL^{-1}, A is the absorbance units, a is the intercept, b is the slope, [b] LOD, limit of detection; LOQ, limit of quantification; ε, molar absorptivity, [c] The theoretical values of t and F are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

The intra-day and inter-day precision and accuracy results show that the proposed methods have good repeatability and reproducibility (Table 2).

Ruggedness and robustness

The robustness of the procedures was assessed by evaluating the influence of small variation of experimental variables, i. e., concentration of reagents and reaction time and the effect of the changes was studied on the absorbance of the complex systems. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the changes had negligible influence on the results as revealed by small intermediate precision values expressed as RSD (≤ 3.0%).

Method ruggedness was demonstrated having the analysis done by three analysts, and also by a single analyst performing analysis on three different spectrophotometer instruments in the same laboratory. The robustness and the ruggedness were checked at three different drug levels. The intermediate precision, expressed as percent RSD was in the range 1.25–2.80%, which is a measure of robustness and ruggedness was within the acceptable limits as shown in the table 3. This provided an indication of the reliability of the proposed methods during routine work.

Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure VOR at different levels and the total was determined by the proposed methods using the standard addition technique. The percent recovery of pure VOR added was in the range 99.27–99.90% with relative standard deviation of 0.41–0.73% (Table 5) indicating that the recoveries were good, and that the co- formulated substance and common excipients did not interfere in the determination.

The limit of detection (LOD) is defined as the minimum level at which the analyte can be reliably detected was calculated using the following equation [36, 37], and listed in table 1:

$$LOD = 3s / k$$

Where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits were found to be 0.36, 0.86, 1.23 and 0.43 μg mL^{-1} using I_2, DDQ, *p*-CLA and TCNQ, respectively. The limits of quantization (LOQ) is defined as the lowest concentration that can be measured with acceptable accuracy and precision [36, 37],

$$LOQ = 10 s / k$$

According to this equation, the limit of quantization was found to be 1.20, 2.87, 4.10 and 1.43 μg mL^{-1} using I_2, DDQ, *p*-CLA and TCNQ, respectively, respectively.

Accuracy and precision

The accuracy and precision of the methods were evaluated by performing six replicate analyses on pure drug solution at three different concentration levels (within the working range). Percentage relative standard deviation (RSD%) as precision and percentage relative error (RE %) as accuracy of the proposed spectrophotometric methods were calculated. The relative standard deviation (RSD) values were less than 2.0% in all cases, indicating good repeatability of the suggested methods. This level of precision of the proposed methods was adequate for the quality control analysis of VOR. The percentage relative error calculated using the following equation:

RE % = [(founded – added) / added] x 100

Table 2: Evaluation of intra-day and inter-day precision and accuracy obtained by the proposed methods.

Methods	*Added (μg mL^{-1})*	*Intra-day*				*Inter-day*			
		Recovery %	*Precision RSD % [a]*	*Accuracy Er %*	*Confidence Limit [b]*	*Recovery %*	*Precision RSD % [a]*	*Accuracy Er %*	*Confidence Limit [b]*
Iodine	5.0	99.55	0.53	-0.45	4.98±0.03	99.75	0.80	-0.25	4.99±0.04
	15	100.30	0.90	0.30	15.05±0.13	99.40	1.15	-0.60	14.91±0.16
	25	99.80	1.05	-0.20	24.95±0.25	99.60	1.40	-0.40	24.95±0.33
DDQ	20	100.10	0.74	0.10	20.02±0.14	100.40	0.79	0.40	20.08±0.15
	40	99.30	0.97	-0.70	39.72±0.37	99.10	0.81	-0.90	39.64±0.31
	80	99.70	1.30	-0.30	79.76±0.99	99.80	0.63	-0.20	79.84±0.48
***p*-CLA**	30	99.50	0.85	-0.50	29.85 ± 0.24	99.90	0.59	-0.10	29.97 ± 0.17
	60	100.50	0.98	0.50	60.30 ± 0.56	99.60	0.69	-0.40	59.76 ± 0.39
	90	99.60	1.20	-0.40	89.64 ± 1.02	99.30	0.92	-0.70	89.37 ± 0.78
TCNQ	5.0	99.80	0.56	-0.20	4.99 ± 0.03	100.20	0.47	0.20	5.01 ± 0.02
	10	100.30	0.90	0.30	10.03 ± 0.09	100.50	0.70	0.50	10.05 ± 0.07
	15	99.50	1.50	-0.50	14.93 ± 0.21	99.40	1.50	-0.60	14.91 ± 0.21

[a] Mean of six determination, RSD%, percentage relative standard deviation; Er%, percentage relative error, [b] Mean ± standard error.

Table 3: Method robustness and ruggedness expressed as intermediate precision (RSD, %).

Method	*VOR taken μg mL^{-1}*	*Robustness*		*Ruggedness*	
		Parameters altered		*Inter-analysts (RSD, %)*	*Inter-instruments (RSD, %)*
		Volume of reagent[a]	**Reaction time [b]**	**(n = 3)**	**(n = 3)**
Iodine	5.0	1.25	2.16	2.08	1.85
	15	1.18	2.24	2.28	1.73
	25	1.46	1.88	2.50	1.90
DDQ	20	1.52	1.90	1.82	2.14
	40	1.73	2.42	1.66	2.40
	80	1.86	2.10	1.48	2.60
p-CLA	30	1.90	1.65	2.02	2.10
	60	1.50	1.80	2.60	1.87
	90	1.26	1.55	2.74	1.79
TCNQ	5.0	1.30	1.72	1.94	2.20
	10	1.95	1.84	1.84	2.64
	15	2.04	2.26	1.98	2.80

[A]The volumes of I_2, p-CLA or TCNQ added were 1.0±0.1 mL and the volume of DDQ added was 2.0±0.2 mL, [b] the reaction times were 5.0±1.0 min for I_2, *p*-CLA or DDQ and 10±1.0 min for TCNQ.

Table 4: Application of the standard addition technique for the determination of VOR in dosage forms using the proposed methods

Sample	*Iodine*			*DDQ*			*p-CLA*			*TCNQ*			*Reference method (Ahmed et al., 2010)[12]*
	Taken (μg mL⁻¹)	*Added (μg mL⁻¹)*	*Recovery[a] (%)*	*Taken (μg mL⁻¹)*	*Added (μg mL⁻¹)*	*Recovery[a] (%)*	*Taken (μg mL⁻¹)*	*Added (μg mL⁻¹)*	*Recovery[a] (%)*	*Taken (μg mL⁻¹)*	*Added (μg mL⁻¹)*	*Recovery[a] (%)*	
Vfend tablets	2.0	-	99.20	10	-	99.40	10	-	99.00	2.0	-	98.90	
		4.0	99.50		10	99.90		20	100.70		2.0	99.30	
		8.0	98.80		20	99.30		40	100.50		4.0	99.80	
		16	98.60		40	99.50		60	99.60		8.0	99.90	
		24	99.80		60	100.20		80	100.40		12	100.20	
		28	99.70		80	99.10		100	99.20		16	100.40	
Mean ± SD			99.27±0.49			99.57±0.41			99.90 ±0.73			99.75 ±0.56	99.56
V			0.24			0.168			0.533			0.31	0.15
R. S. D%			0.49			0.41			0.73			0.56	0.39
S. E			0.20			0.167			0.30			0.23	0.16
***t*-value[b]**			1.04			0.04			0.92			0.91	
***F*-value[b]**			1.58			1.11			3.5			2.06	

[a]Average of six determinations, [b] The theoretical values of *t* and *F* are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (*p*= 0.05).

Specificity and interference

The proposed spectrophotometric methods have the advantages that the measurements in all of these methods are performed in the visible region, away from the UV-absorbing interfering substances that might be co-extracted from VOR containing dosage forms. Regarding the interference of the excipients and additives usually presented in pharmaceutical formulation (lactose monohydrate, starch, croscarmellose sodium, povidone, magnesium stearate and a coating containing hypromellose, titanium dioxide, lactose monohydrate and triacetin), the energy of the charge transfer (ECT) depends on the ionization potential (IP) of the donor and the electron affinity of the acceptor (EA), hence the λ_{max} values of the other π-donors mostly differ from that of the investigated compounds if they are able to form CT complexes. Preliminary experiments showed that all additives and excipients did not form CT complexes with the studied acceptors indicating the high selectivity of the proposed methods and applicability to use for routine determination in pure and in dosage forms.

Application of the proposed method to the analysis of tablets

The obtained satisfactory validation results made the proposed procedures suitable for the routine quality control analysis of VOR and its pharmaceutical formulations (V fend® tablet). The results obtained by the proposed methods were statistically compared with those obtained by the reported method (Ahmed *et al.,* 2010). The obtained mean values of the labeled amounts ranged from 99.27±0.49% to 99.90 ±0.73% (Table 4). In the *t*- and F-tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level [38]. This indicated similar precision and accuracy in the analysis of VOR in its formulations. It is evident from these results that all the proposed methods are applicable to the analysis of VOR in its tablets with comparable analytical performance. However, the critical recommendations of some of these methods might be based on the experimental conditions (e. g. reaction time), and the ultimate sensitivity that determines the amount of specimen required for analysis. For example, the methods involving DDQ and *p*-CLA are recommended whenever rapid analysis is required; this because they have very short reaction time. The method involving TCNQ is recommended, as high sensitivity is required on the expense of the analysis time.

DISCUSSION

The color of iodine in 1,2-dichloroethane is violet showing absorption maximum (λ_{max}) at 520 nm. This color was immediately changed into lemon yellow, and the absorption spectrum of VOR-I_2 reaction product showed absorption peaks at 292 and 364 nm. This change in color, and the appearance of these two peaks were attributed to the formation of charge-transfer complex between the VOR and I_2, having an ionized structure $DI^+ \ldots I_3^-$, taking into account that the absorption spectrum of I_3^- in 1,2-dichloroethane showed the two absorption maxima at 292 and 364 nm. This complex should originate from an early intermediated outer complex $D \ldots I_2$, according to the following scheme:

$$D + I_2 \rightleftharpoons \underset{\text{Outer complex}}{D\text{-}I^+ I^-} \rightleftharpoons \underset{\text{inner complex}}{[D\text{-}I^+]} + I^- \overset{I_2}{\rightleftharpoons} \underset{\text{tri-iodide ion pair}}{I_3^-}$$

The interaction of VOR with selective polyhaloquinone and polycyanoquinone π-acceptors in non-polar solvents such as 1,2-dichloroethane was found to produce colored charge-transfer complexes with low molar absorptivity values. In polar solvents such as methanol or acetonitrile, complete electron transfer from the VOR (D), as an electron donor, to the acceptor moiety (A) takes place with the formation of intensely colored radical ions with high molar absorptivity values, according to the following Scheme:

$$\underset{\text{Donor}}{D^{\bullet\bullet}} + \underset{\text{Acceptor}}{A} \rightleftharpoons \underset{\text{DA complex}}{[D^{\bullet\bullet} \rightarrow A]} \overset{\text{Polar solvent}}{\rightleftharpoons} \underset{\text{radical anion}}{D^{\bullet +}} + \underset{\text{radical anion}}{A^{\bullet -}}$$

The dissociation of the (D–A) complex was promoted by the high ionizing power of the polar solvent and the resulting peaks in the absorption spectra of VOR-acceptor reaction mixtures were similar to the maxima of the radical anions of the acceptors obtained by the iodide reduction method [29].

Chloranilic acid (*p*-CLA) exists in three ionic forms, the neutral yellow-orange H_2A at very low pH, the dark purple HA^- which is stable at pH 3.0 and a colorless A^{2-}, which is stable at high pH; these transformations are illustrated in the following scheme

$$H_2A \rightleftharpoons H^+ + HA^- \text{(violet)},$$

$$HA^- \rightleftharpoons H^+ + A^{2-} \text{(colorless)}.$$

Since the interaction of VOR with *p*-CLA in acetonitrile gave a violet product, it might be concluded that HA^- was the form of *p*-CLA involved in the reaction described herein.

CONCLUSION

The charge-transfer complexation reaction of VOR as electron donor and some electron acceptors has been investigated. The obtained colored complexes were utilized in the development of four simple, accurate and sensitive with good precision and accuracy spectrophotometric methods for the analysis of VOR in pure form as well as in dosage forms. With these methods, one can do the analysis at low cost without losing accuracy. The proposed methods can be used as alternative methods to the reported ones for the routine determination of Vfend® tablet. This encourages their successful use in routine analysis of these drugs in quality control laboratories and they involve very simple procedures.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests with the company name used in the paper.

REFERENCES

1. Sweetman SC. *Martindale*, *The Complete Drug Reference*, 35th Edition Pharmaceutical Press: London; 2009. p. 550-1.
2. Claudia M, Jens T, Rainer P. Determination of voriconazole in human plasma and saliva using high performance liquid chromatography with fluorescence detection. J Chromatogr B 2008;865:74-80.
3. Penhourcq F, Jarry C, Bannwarth B. Direct injection HPLC micro method for the determination of voriconazole in plasma using an internal surface reversed phase column. Biomed Chromatogr 2004;18:719-22.
4. Srinubabu G, Raju Ch AI, Sarath N, Kumar PK, Rao JVLNS. Development and validation of a HPLC method for the determination of voriconazole in pharmaceutical formulation using an experimental design. Talanta 2007;71:1424-9.
5. Khoschsorur G, Fruehwirth F, Zelzer S. Isocratic high-performance liquid chromatographic method with ultraviolet detection for simultaneous determination of levels of voriconazole and itraconazole and its hydroxy metabolite in human serum. Antimicrob Agents Chemother 2005;49:3569-71.
6. Pennick G, Clark M, Sutton D, Rinaldi M. Development and validation of a high performance liquid chromatography assay for voriconazol. Antimicrob Agents Chemother 2003;47:2348-50.
7. Langman L, Boakye-Agyeman F. Measurerment of voriconazole in serum and plasma. Clin Biochem 2007;40:1378-85.
8. Adams AIH, Bergold AM. Development and validation of a high performance liquid chromatographic method for the determination of voriconazole content in tablets. Chromatogrphia 2005;62:429-34.
9. Adams AIH, Steppe M, Froehlich PE, Bergold AM. Comparison of microbiological and UV spectrophotometric assays for determination of voriconazole in tablets. J AOAC Int 2006;89:960-5.
10. Adams AIH, Gosmann G, Schneider PH, Bergold AM. LC stability studies of voriconazole and structural elucidation of its major degradation product. Chromatographia 2009;69:115-22.
11. Bharathi J, Sridhar B, Jitendra kumar P, Upendra rao U, Nagaraju P, Hanumantha rao K. Validated RP-HPLC method for the estimation of voriconazole in bulk and tablet dosage form. IJRPBS 2010;1:14-8.
12. Ahmed BE, Abdalla AS, Magda YE. Development and validation of a HPLC method for the determination of voriconazole and its degradation products in pharmaceutical formulation. Acta Pharm Sci 2010;52:229-38.
13. Gu P, Li Y. Development and validation of stability-indicating HPLC method for determination of voriconazole and its related substances. J Chromatogr Sci 2009;47:594-8.
14. Michael C, Teichert J, Preiss R. Determination of voriconazole in human plasma and saliva using high-performance liquid chromatography with fluorescence detection. J Chromatogr B 2008;865:74-80.
15. Theurillat R, Zimmerli S, Thormann W. Determination of voriconazole in human serum and plasma by micellarelectrokinetic chromatography. J Pharm Biomed Anal 2010;53:1313-8.
16. Alarfaj NA, El-Tohamy MF. Electrochemical sensors for direct potentiometric determination of Voriconazole in pharmaceutical dosage forms and biological fluids. IJPS 2012;7:1403-11.
17. Corbini G, Zanfini A, La Rosa C, D'Arpino A, Meucci R, Dreassi E. Polarographic determination of voriconazole in pharmaceutical formulations. Curr Anal Chem 2009;5:238-43.
18. Babu GS, Raju Ch AI. UV-spectrophotometric determination of voriconazole in bulk and its formulation. Asian J Chem 2007;19:1625-7.
19. Jain A, Maliwal D, Patidar V, Joshi A. UV spectrophotometric estimation of voriconazole in bulk and tablet dosage form. Asian J Chem 2009;21:1627-9.
20. Roy S, Ravi Kumar BVV, Tarafdar S. Development and validation of new analytical method for voriconazole by using UV-spectrophotometer. Int J Pharm Technol 2011;3:1904-12.
21. Tamilselvi N, Hassan B, Fadul FB, Kondapalli D, Anusha K, Kurian DS. UV Spectrophotometric estimation of voriconazole in tablet dosage form. Res J Pharm Technol 2011;4:1791-3.
22. Foster R. Organic Charge-Transfer Complexes. London, New York, Academic Press; 1969. p. 470.
23. Darwish IA, Refaat IH. Spectrophotometric analysis of selective serotonin reuptake inhibitors based on formation of charge-transfer complexes with tetracyanoquinodimethane and chloranilic acid. J AOAC Int 2006;89:326-33.
24. Gouda AA, EL-Sheikh R, Amin AS. Utility of some pi-acceptors for spectrophotometric determination of gatifloxacin in pure form and in pharmaceutical preparations. Chem Pharm Bull 2008;56:34-40.
25. Gouda AA, EL-Sheikh R, El Azzazy RM. Charge transfer spectrophotometric determination of zolmitriptan in pure and dosage forms. J Anal Bioanal Technol 2012;3:1-7.
26. Gouda AA. Utility of certain sigma-and pi-acceptors for the spectrophotometric determination of ganciclovir in pharmaceutical formulations. Talanta 2009;80:151–7.
27. Gouda AA, Amin AS, Youssef EH. Spectrophotometric methods based on charge transfer reactions for the determination of amisulpride in pure form andpharmaceutical formulation. Int J Pharm Pharm Sci 2014;6:154-60.
28. Job P. Advanced Physicochemical Experiments. 2nd edition. Oliner and Boyd, Edinburgh, 1964. p. 54.
29. Taha A, Rücker G. Utility of pi-acceptors in alkaloid assay. Arch Pharm (Weinheim) 1977;310:485-94.
30. Melby LR. In: Patai S, Editor, The Chemistry of the Cyano Group, Inter science Publisher/John Wiley & Sons, New York; 1970. p. 656.
31. Hasani M, Rezaei A. Spectrophotometric study of the charge transfer complexes of iodine with antipyrine in organic solvents" Spectrochim Acta A 2006;65:1093-7.
32. Gutmann V. The Donor-Acceptor Concepts of Molecular Interactions. Plenum, New York; 1978.
33. Cannors KAA. Textbook of Pharmaceutical Analysis. 3rd ed., John Wiley & Sons, New York; 1982. p. 50.

34. Benesi HA, Hildebrand JH. "A Spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons". J Amer Chem Soc 1949;71:2703-7.
35. Mendham J, Denney RC, Barnes JD, Thomas M. Vogel's Textbook of Quantitative Chemical Analysis, Pearson Education Ltd, England; 2000.
36. Miller JN, Miller JC. "Statistics and Chemometrics for Analytical Chemistry" 5th ed. Prentice Hall, England; 2005.
37. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (2005) ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2(R 1), Complementary Guideline on Methodology dated 06 November ICH, London; 1996.