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UTILISATION OF CHARGE TRANSFER COMPLEXATION REACTIONS FOR THE QUANTIFICATION OF ANTIFUNGAL DRUG: VORICONAZOL IN PURE AND DOSAGE FORMS

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ABSTRACT. Voriconazole, a pivotal antifungal agent, has been analysed using two uncomplicated, sensitive, rapid, and validated spectrophotometric methods. These procedures rely on the formation of charge transfer complexes in methanol, employing alizarin red S and quinalizarin as chromogenic reagents, each exhibiting absorption maxima at 568 and 513 nm, respectively. The optimisation of reaction conditions was explored, encompassing the choice of solvent, reagent concentration, and reaction duration. Both alizarin red S and quinalizarin demonstrated excellent adherence to Beer's law over concentration ranges of 1.0-18 and 1.0-24 μg mL^{-1}, respectively, with robust correlation coefficients ($r^2 \geq 0.9993$) and minimal relative standard deviations (RSD% ≤ 1.04). Additionally, calculations were conducted for molar absorptivity (1.1256×104 and 1.7624 ×104 L mol^{-1} cm^{-1}), Sandell sensitivity (31.0 and 19.82 ng cm^{-2}), detection and quantification limits (0.3 and 1.0 μg mL^{-1}) for alizarin red S and quinalizarin, respectively. Both methods were effectively applied for the determination of voriconazole in dosage forms, and their validity was confirmed using the standard addition technique. The results obtained from these proposed procedures for pure and dosage forms closely matched those from previously reported methods.

KEY WORDS: Voriconazole, Spectrophotometry, Charge transfer complex, Dosage forms, Method validation.

INTRODUCTION

A triazole derivative, voriconazole (VOR), is the most critical antifungal. Chemically, it is (2R, 3S)-2-(2,4-difluorophenyl)-3-(5-fluoro pyrimidin-4-yl)-1-(1,2,4-triazol-1-yl)-2-butanol (Figure 1) [1]. Similar to other azole antifungals, its mode of action involves inhibiting the 14-sterol demethylase enzyme, which is required for the formation of ergosterol in the fungal cell membrane. In contrast to human enzyme systems, this inhibition favours fungal enzyme systems. The approval of VOR by the FDA in May 2002 was granted for the treatment of refractory infections caused by Fusarium species, *Scedosporium apiospermum*, and invasive aspergillosis [2].

The literature review uncovered several analytical techniques that have been documented for quantifying VOR in various pharmacological dose forms, including pure drugs, pharmaceutical formulations, and biological samples. These methods primarily involve chromatography [3-12] and electrochemistry [13-15] has also been employed for this purpose. These approaches typically involve extensive sample pre-treatment, cleanup processes before analysis, and the use of costly instruments that are beyond the reach of most quality control labs.

Visible spectrophotometry is a commonly employed analytical technique in quality control and clinical laboratories, hospitals, and pharmaceutical industries. This method is favoured due to its simplicity, affordability, speed, sensitivity, selectivity, accuracy, precision, widespread accessibility, and suitability for pharmaceutical analysis. It finds extensive application in the

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determination of diverse classes of drugs in their pure form, pharmaceutical formulations, and biological samples. Based on current understanding, there have been only a limited number of spectrophotometric methods reported for the quantitative determination of VOR in pharmaceutical formulations [16-22].

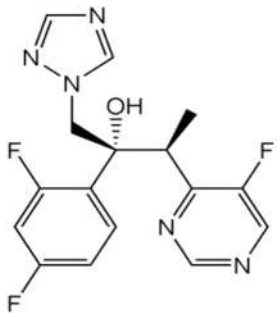


Figure 1. The chemical structure of voriconazole (VOR).

Several methods in the literature necessitate precise pH regulation and involve a laborious liquid-liquid extraction process. Additionally, measurements are conducted at shorter wavelengths. Certain alternative methods exhibit a relatively limited dynamic linear range, require a heating step, and/or employ costly reagents. Nevertheless, it is important to note that all of these limitations are inherent in the previously documented approaches. Consequently, the development of a novel, uncomplicated, cost-effective, and specific spectrophotometric method was deemed essential for the determination of VOR in pharmaceutical formulations.

The objective of this research is to develop spectrophotometric techniques for the determination of VOR in pure and dosage forms. These methods aim to be user-friendly, highly sensitive, accurate, precise, cost-efficient, and validated. The approaches under consideration involve the utilisation of chromogenic reagents, namely quinalizarin (Quinz) and alizarin red S (ARS), which have the capability to establish enduring charge transfer complexes with VOR. The VOR experiments conducted with typical excipients at concentrations commonly found in dosage forms demonstrated no evidence of interference. The accuracy of these processes is substantiated by statistical evidence.

EXPERIMENTAL

Apparatus

All the absorption spectral measurements were made using a Shimadzu UV-1601 UV/Visible double beam spectrophotometer (Sweden) with a fixed slit width of 2 nm and equipped with 10 mm-matched quartz cells.

Chemicals and reagents

All employed chemicals and solvents (dimethyl sulfoxide, methanol, acetonitrile, acetone, and ethanol) were of analytical-reagent grade and used throughout the study. Pure-grade VOR was kindly supplied by Mash Pharmaceutical Company, Egypt; its potency was 99.38 ±0 .92%. The commercial pharmaceutical formulation is Vfend® tablets labeled to contain 200 mg VOR/tablet, purchased from Pfizer Egypt S.A.E. Pharmaceutical Company, Cairo, A.R.E. under the authority of Pfizer Inc., USA.

Standard stock solutions of VOR with concentrations of 100 μg mL^{-1} and 1.0×10^{-3} mol L^{-1} were created by dissolving 10 and 35.0 mg of pure medicine, respectively, in the minimum amount of methanol. The solutions were then diluted to a final volume of 100 mL with methanol to achieve the desired working concentration. The stability of the standard solution was demonstrated for a minimum duration of one week when it was stored in a cool environment with a temperature below 25 ºC and kept away from light exposure.

The compounds alizarin red S, 3,4-dihydroxy-9, 10-dioxo-2-anthracene sulfonic acid (ARS), and quinalizarin 1,2,5,8-tetrahydroxy-anthraquinone (Quinz) were acquired from Sigma-Aldrich and used without any modifications. In order to prepare a stock solution with a concentration of 1.0×10^{-3} mol L^{-1}, the necessary quantity of the reagent was dissolved in approximately 25 mL of methanol. Subsequently, the remaining volume of the 100-mL volumetric flask was filled with additional solvent until it reached the mark. After a duration of one week, the aforementioned response remained consistent.

General procedures

A set of volumetric flasks with a capacity of 10 mL were utilised to contain measured portions of the standard working solution of VOR. The aliquots were prepared to achieve concentration ranges of 1.0-18 and 1.0-24 μg mL^{-1} using Quinz and ARS, respectively. Two millilitres of either a Quinz or ARS solution with a concentration of 1.0×10^{-3} mol L^{-1} were added to each flask. Following vigorous agitation of the mixture to enhance the reaction rate, methanol was further introduced until the desired volume was attained. The absorbance of the resulting solutions at 568 nm and 513 nm was tested using Quinz and ARS, respectively. These measurements were compared against a reagent blank that was prepared simultaneously. The calibration graph was generated by graphing the absorbance values against the concentration of VOR. The regression equation was developed accordingly.

Assay procedure for dosage forms

A total of twenty Vfend tablets were meticulously pulverized, and an amount of the tablet's contents equivalent to 100 mg was subsequently transferred into a calibrated flask with a volume of 100 mL and subsequently dissolved in 10 mL of methanol. Following a sonication and agitation process lasting approximately 10 min to ensure comprehensive mixing, the contents of the flask were subsequently filtered using Whatman No. 42 filter paper. A stock solution with a concentration of 100 μg mL^{-1} was generated by removing the initial portion of the filtrate and adjusting the amount of the solution using methanol. The necessary concentration ranges were acquired through further dilution of this solution with the identical solvent. The indicated processes were implemented on aliquots covering the working concentration ranges for each technique using a set of 10 mL volumetric flasks. The nominal content of the tablets was determined by utilising regression equations or calibration graphs.

Stoichiometric relationship

The stoichiometric ratios of the charge transfer complexes formed by VOR and reagents were calculated using the continuous variation technique, which was initially proposed by Job [23] and later refined by Vosburgh and Coober [24], at the optimal wavelengths. The VOR standard solution and the reagent solution with a concentration of 1.0×10^{-3} mol L^{-1} were both made via Job's method of continuous variation. Every individual solution within the series was prepared by combining a combined volume of 2.0 mL of medicine and reagents. In accordance with the

aforementioned procedure, a calibrated flask with a volume of 10 mL was utilised to contain methanol, which was then combined with the drug in differing quantities.

RESULTS AND DISCUSSION

Absorption spectra

In optimal conditions, the radical anion, which functions as the species responsible for absorption, was rapidly formed in the medium subsequent to the combination of the reagents. The highest amount of absorption was seen at wavelengths of 568 nm and 513 nm while utilising Quinz and ARS, respectively, within a methanol medium (Figure 2). It is noteworthy to mention that in a methanol solution, Quinz and ARS exhibit maximal absorption wavelengths at 492 nm and 421 nm, respectively. The significant difference in the maximum absorption wavelengths between the reagent and the product, notably 76 nm for Quinz and 92 nm for ARS, enabled a precise evaluation of the products while reducing the impact of any surplus reagents in the solution.

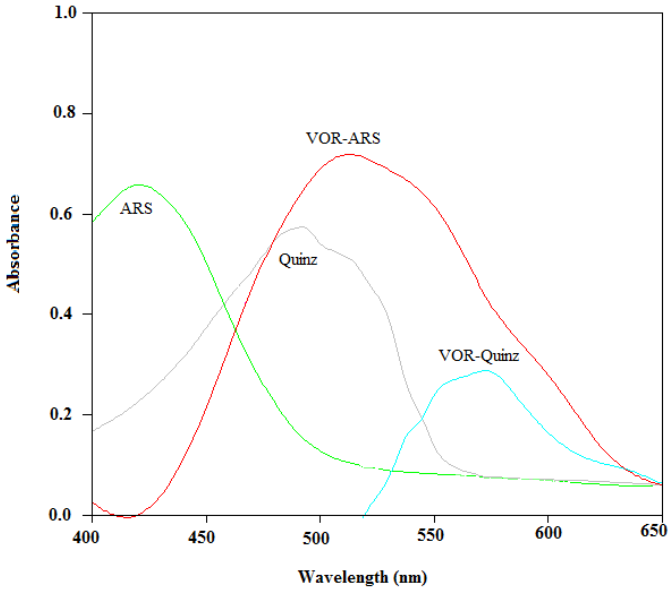


Figure 2. Absorption spectra of charge transfer complexes of 18 µg mL^{-1} VOR with (1.0×10^{-3} mol L^{-1}) Quinz and ARS in methanol solvent obtained against reagent blank solution prepared in the same solvent.

The optimization of the experimental conditions

Impact of the solvent

The investigation focused on the charge transfer reaction in different solvents, specifically dimethyl sulfoxide (DMSO), methanol, acetonitrile, acetone, and ethanol. Although DMSO and acetonitrile possess the highest dielectric constants, it is worth noting that methanol demonstrated the most favorable sensitivity. The observed result can plausibly be attributed to the capacity of

methanol to form enduring hydrogen bonds with the radical anion. Following this, methanol was chosen as the ingredient for the following experimental inquiries (Figure 3).

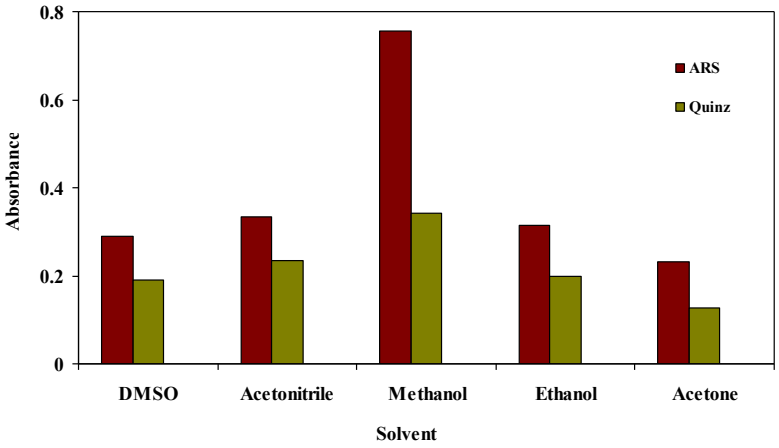


Figure 3. Impact of various solvents on the charge transfer complexes of 18 µg mL^{-1} VOR with (1.0×10^{-3} mol L^{-1}) Quinz and ARS in methanol solvent obtained against reagent blank solution prepared in the same solvent.

Impact of the reagent concentration

To achieve the stated objective, an experiment was carried out in which various volumes of reagent solutions, each with a concentration of 1.0×10^{-3} mol L^{-1}, were added to a fixed concentration of VOR (18 µg mL^{-1}); see Figure 4 for further information. The results of the experiment show that a volume of 2.0 mL of a reagent solution of Quinz or ARS with a concentration of 1.0×10^{-3} mol L^{-1} was adequate to obtain the maximum colour intensity and consistently provided the highest absorbance values.

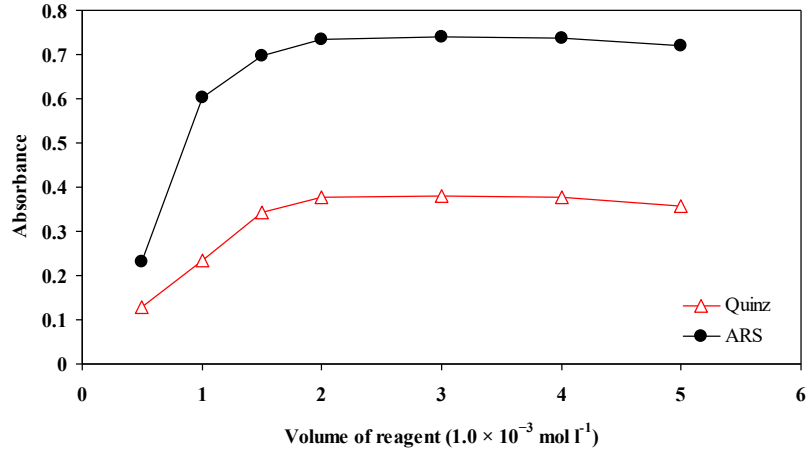


Figure 4. Impact of reagent concentration on the absorbance of the formed complexes. VOR concentration = 18 µg mL^{-1}.

Impacts of reaction time and temperature

Monitoring the development of colour under laboratory ambient conditions allowed researchers to determine the ideal reaction time. When both reagents were applied, full colour development for VOR took place after 2.0 min. The absorbance of the charge transfer complex decreased as the temperature rose and underwent a hypochromic shift before dying at a temperature of 50 °C.

Order of the additions

"VOR-reagent-solvent" is the best order of addition for attaining full colour development, maximum absorbance, and stability at the designated wavelength. A longer length was required for other sequences, which also showed poorer stability. The compounds displaying this specific sequence exhibit stability for at least 10 h.

Stoichiometric ratio

The molar ratio between the charge transfer complex's VOR and reagent (Quinz or ARS) was determined using Job's approach [23]. This method entails making successive adjustments while keeping the total molar concentrations of the VOR and reagent constant. Based on the findings depicted in Figure 5, it was ascertained that the molar ratio yielding the maximum absorbance was (1:1) (VOR: reagent).

The literature review suggests that in non-polar solvents, there is a tendency for the formation of molecular charge-transfer complexes, whereas in polar solvents, there is a preference for the predominance of radical anion species [25-30]. Furthermore, there is a commonly held consensus that the incorporation of fundamental compounds that include an unshared pair of electrons, such as VOR, results in the formation of charge-transfer complexes that exhibit n-π characteristics. The aforementioned complexes can be seen as an intermediary molecular-association compound that leads to the generation of a corresponding radical anion in polar solvents. In the current context, radical anions are generated as a result of a full transfer of charge, as illustrated in Scheme 1.

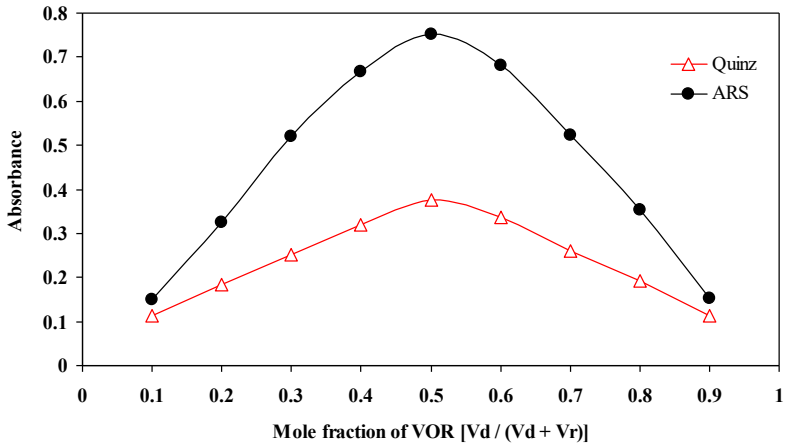
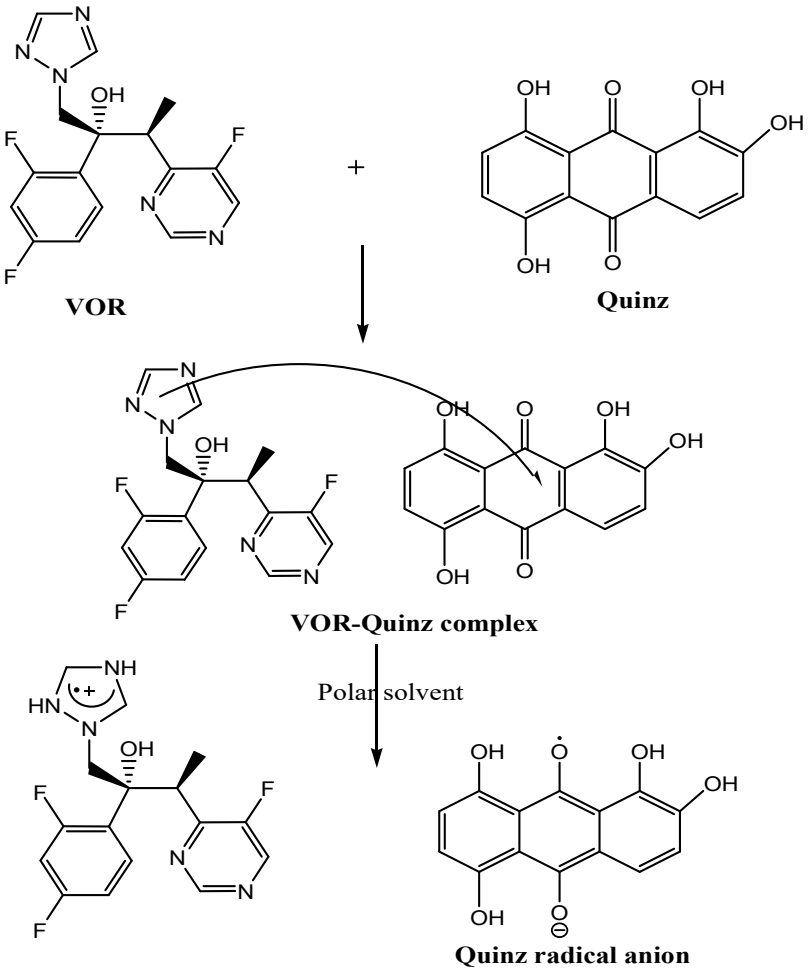


Figure 5. Application of Job's method to the reaction between VOR and reagents.



Scheme 1. Possible mechanism of radical anion formation from VOR and Quinz reaction.

Methods validation

The validity of the methods was evaluated based on several criteria, including linearity, specificity, accuracy, repeatability, and precision. These criteria were assessed in accordance with the principles outlined by the International Conference on Harmonisation (ICH) [31].

Linearity

The linear regression equations were obtained using the aforementioned approaches. The regression plots exhibited a distinct linear correlation between the absorbance (A) and the VOR concentration (C) (μg mL^{-1}) within the designated ranges as outlined in Table 1.

In accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines [31], the equations used to calculate the Limit of Quantification (LOQ) and Limit of Detection (LOD) were as follows:

$$LOQ = 10s/b$$

$$LOD = 3.3s/b$$

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. b: is the slope of the calibration curve. The findings pertaining to the LOQ and LOD are also presented in a concise manner in Table 1.

Table 1. Analytical parameters utilised in the determination of VOR by the described methods.

Parameters	Quinz	ARS
Beer's law limits, μg mL^{-1}	1.0-18	1.0-24
Ringboom limits, μg mL^{-1}	3.0-16	3.0-22
Molar absorptivity, $\times 10^4$ L mol^{-1} cm^{-1}	1.1256	1.7624
Sandell sensitivity, ng cm^{-2}	31.0	19.82
Regression equation[a]		
Intercept (a)	0.0051	-0.0043
Standard deviation of intercept (S_a)	0.09	0.03
Slope (b)	0.0185	0.0293
Standard deviation of slope (S_b)	0.08	0.06
Correlation coefficient, (r)	0.9993	0.9996
Mean ± SD	99.20±1.04	99.60±0.85
RSD%	1.04	0.85
RE%	1.10	0.89
Limit of detection, μg mL^{-1}	0.30	0.30
Limit of quantification, μg mL^{-1}	1.0	1.0
Calculated *t*-value[b]	0.29	0.39
Calculated *F*-value[b]	1.28	1.17

[a]A = a + bC, where C is the concentration in (μg mL^{-1}), A is the absorbance, a is the intercept and b is the slope. [b]Mean of six determinations. [c]Theoretical values of t (2.57) and F (5.05) for five degrees of freedom and 95% confidence level at p = 0.05.

Accuracy and precision

In order to assess the soundness and consistency of the suggested procedures, the measurement of VOR was carried out both inside a single day and across many days, encompassing three different concentrations for each method. The intraday investigations were carried out within a single day, whilst the inter-day examinations were undertaken over a span of five days, with each level (n = 6) being evaluated. The figures documented in Table 2 encompass the percent relative error (RE%) and relative standard deviation (RSD%) as indicators of accuracy and precision, correspondingly. Furthermore, the results of both intraday and inter-day assessments were incorporated. The results exhibited a notable degree of accuracy and precision in relation to the procedures that were developed.

Robustness and ruggedness

The evaluation of the suggested approach's robustness involved an examination of the effects of minor adjustments in experimental variables, namely the concentrations of the reagent and the reaction time, on the analytical performance of the method. During the experiments, a single experimental parameter was modified while keeping all other variables constant. Following that, the percentage of recovery was calculated for each occurrence. The negligible deviations observed in any of the variables did not have a significant effect on the results, as evidenced by the relative standard deviation (RSD) values falling within the range of 0.7-2.50%. This observation offers

insight into the dependability of the suggested techniques when routinely employed for VOR analysis, thus establishing the robustness of the proposed spectrophotometric methodologies. The measure of ruggedness was quantified as the relative standard deviation (RSD) with the utilisation of three distinct instruments and three different analysts. The relative standard deviations (RSDs) of the inter-analysts and inter-instruments fell within the ranges of 0.60-2.30% and 0.85-2.50%, respectively. These findings indicate that the methodologies established exhibited ruggedness.

Table 2. Intra-day and inter-day precision and accuracy for VOR obtained by the suggested methods.

Method	Taken ($\mu g\ mL^{-1}$)	Recovery %	Precision RSD %[a]	Accuracy RE %	Confidence limit[b]
Intra-day					
Quinz	5.0	99.35	0.64	-0.65	4.968±0.012
	10	100.30	0.86	0.30	10.03±0.082
	15	99.90	1.07	-0.10	14.99±0.15
ARS	6.0	100.30	0.90	0.30	6.018 ± 0.057
	12	99.50	1.40	-0.50	11.94 ± 0.18
	18	99.20	1.50	-0.80	17.86± 0.28
Inter-day					
Quinz	5.0	99.80	0.54	-0.20	4.99±0.026
	10	99.45	0.70	-0.55	9.945±0.066
	15	100.35	0.96	0.35	15.05±0.14
ARS	6.0	99.10	0.70	-0.90	5.946 ± 0.044
	12	99.30	1.10	-0.70	11.92± 0.14
	18	99.50	1.80	-0.50	17.91 ± 0.34

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

Specificity and effect of excipients

The study aimed to assess the impact of excipients usually found in tablets on the suggested strategy, specifically examining potential interference. The experimental methodology employed in this study entailed the application of the usual addition technique. This involves the deliberate addition of specified amounts of pure VOR to a previously analysed solution of tablets. The assessment of the recovery of the supplementary compound quantification method was conducted by comparing the concentrations of intentionally introduced mixtures with the specified reference value. Table 3 presents the results, indicating that the obtained findings were deemed satisfactory and exhibited a higher level of performance compared to the spectrophotometric techniques documented in existing scholarly works. The presented methodologies exhibited favourable recovery outcomes, indicating that the inclusion of excipients did not yield any significant interference. This observation provides more evidence to support the idea that the proposed methods provide a significant degree of selectivity.

Analysis of the pharmaceutical preparations

The methodology employed in this research was applied to measure the VOR in pharmaceutical formulations. The method was subjected to testing in order to assess its linearity, specificity, accuracy, repeatability, and precision in accordance with the criteria outlined by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

A statistical comparison was performed to assess the data acquired from the suggested procedures in relation to those obtained from the reference method [20]. The recovery values, along with their respective standard deviations (SD), were gathered. The data underwent statistical analysis using the Student's t-test and the variance ratio F-test, both performed at a confidence level of 95%. The findings of the study revealed that there was no statistically significant disparity observed in the accuracy and precision of the proposed approaches compared to the reference techniques. The relevant data can be located in Table 3 of the cited research paper [32]. The findings reported in this study illustrate the suitability of the suggested approaches for assessing VOR in its several dosage forms, exhibiting comparable analytical performance.

Table 3. Application of the standard addition technique for the determination of VOR in Vfend tablets using the proposed methods, (taken concentration 6.0 μg mL^{-1}).

Parameters	Added (μg mL^{-1})	Recovery (%)[a]		Reported method [20]
		Quinz	ARS	
	-	99.20	99.10	99.00
	3.0	99.00	100.30	99.7
	6.0	100.40	99.00	100.0
	9.0	99.50	99.70	99.60
Mean ± SD[b]		99.53 ± 0.62	99.90 ± 0.63	99.57 ± 0.41
RSD%[b]		0.62	0.63	0.41
V [b]		0.38	0.40	0.21
S.E.[b]		0.30	0.32	0.17
t-value[c]		0.12	0.98	
F-value[c]		2.29	2.36	

[a]The average six determinations. [b]V = variance; RSD% = percentage relative standard deviation; SE = standard error. [c]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).

CONCLUSION

The goal of the current work was to develop novel low-cost, easy, fast, sensitive, accurate, and precise spectrophotometric methods for the determination of VOR in dosage forms by utilising the coloured charge transfer complexes of VOR with Quinz and ARS. When applying them, there's no need for extraction, heating, or pH changes. The sensitivity of the suggested methods (limit of detection as low as 0.3 μg mL^{-1}) is lower than that of all reported spectrophotometric methods for determination of VOR, making them superior to the previously reported spectrophotometric methods in terms of sensitivity and improved selectivity. The compounds that are produced retain an impressive degree of stability. These characteristics make the recommended methodologies incredibly suitable for regular VOR analysis in quality control labs.

Conflict of interest

There was no conflict of interest declared by the authors.

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