

Analytical study of charge transfer complexation of rabeprazole with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

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ABSTRACT: A simple, sensitive spectrophotometric method was developed for the determination of rabeprazole sodium (RA) in pure form and for pharmaceutical formulations. The charge transfer interaction between RA and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) as an acceptor was investigated spectrophotometrically. Variables affecting the reaction were studied and optimized. The proposed method was applied successfully to the determination of RA in its pure form and pharmaceutical dosage forms with good accuracy and precision. The formation of charge transfer complex and the site of interaction was confirmed by using UV-Visible spectrophotometry, FT-IR spectrometry, and ¹H NMR techniques. Based on Job's method of continuous variation plots, the obtained results indicate the formation of 1:1 charge transfer complex with a general formula [(RA) (DDQ)]. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant differences in accuracy or precision.

KEYWORDS: spectrophotometry, rabeprazole sodium, NMR spectroscopy, FT-IR spectroscopy, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

INTRODUCTION

Rabeprazole (RA), or 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl]sulphonyl]-1H-benzimidazole, is a prodrug metabolized by P450 or CYP450. It acts as a selective and irreversible proton pump inhibitor suppressing gastric acid secretion by specific inhibition of the gastric hydrogen-potassium adenosine triphosphatase (H⁺/K⁺ ATPase) enzyme system at the secretory surface of the gastric parietal cells. It inhibits the final transport of hydrogen ions (via exchange with potassium ions) into the gastric lumen¹⁻⁴. RA is not officially listed in any pharmacopoeia. The analytical techniques that have been reported so far for the determination of this drug in biological samples as well as in pharmaceutical formulations are electrophoresis⁵, high performance liquid chromatography⁶⁻⁸, UV-Visible spectrophotometry^{9,10}, and liquid chromatography-mass spectrometry¹¹. The literature reveals no conventional UV-Visible spectrophotometric method so far for the determination of RA in pharmaceutical formulations. The conventional visible spectrophotometric methods are the instrumental methods of choice and commonly

used in industrial laboratories because of their simplicity, selectivity, and sensitivity. Therefore, the need for a fast, simple, sensitive, low-cost, and selective method is obvious, especially for a routine quality control analysis of RA in drug formulations.

This work describes a simple visible spectrophotometric method for the determination of RA by exploiting its basic nature and electron donating property. This method is based on the charge transfer complexation reaction of RA with 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) in acetonitrile medium. The formation of a charge transfer complex is supported by the chemical shift in ¹H NMR spectrometry as well as changes in the Fourier transform infrared spectrometry (FT-IR) spectra. The proposed method was validated statistically.

MATERIALS AND METHODS

Apparatus

Spectral runs and absorbance were recorded on a UV-2450 spectrophotometer (Shimadzu) using quartz cells. A water 2695 separation module (Waters, Milford) with 2996 PDA detector coupled with Micromass Q-TOF micro (Waters, Milford) was used

for LC-MS experiments. ^1H NMR was recorded on an Oxford NMR AS 400 system (Varian Company) and FT-IR analysis was done on a Spectrum 100 FT-IR spectrometer (Perkin Elmer) to support the charge transfer complex formation. LABOROTA 4003-control $>260^\circ\text{C}$ flash point oils (Heidolph Instruments GmbH & Co. KG) were used to evaporate the solvent after drug extraction from pharmaceutical formulations.

Chemicals and reagents

RA was kindly provided by Inogent Laboratories, Hyderabad. Commercial dosage forms of RA were purchased from a local market. DDQ was purchased from Sigma Aldrich. All other reagents used were of analytical grade. The standard solutions of RA (0.1%) and DDQ (0.1%) were prepared in gradient grade acetonitrile and stored in a refrigerator at 4°C .

General analytical procedure

Stock solutions of 0.1% RA was prepared by transferring 50 mg of RA to a 50 ml standard flask and diluting it up to the mark with acetonitrile. Aliquots of 0.1% RA solution (0.1–1.0 ml) were placed in 10 ml standard volumetric flasks. To each flask, 1.5 ml of 0.1% DDQ was added and diluted up to the mark with acetonitrile. The absorbance was measured at 444 nm within the stability period of 35 min against the reagent blank (DDQ + acetonitrile) prepared similarly.

Molar ratio of the reaction

Job's method of continuous variation¹² was employed to establish the stoichiometric ratio of RA to DDQ (Fig. 1) and was found to be 1:1. This indicates the presence of one n-donating centre in the RA for the charge transfer complexation reaction. Our proposed reaction mechanism is given in Fig. 2.

Preparation of RA from RA sodium

Five 20 mg tablets were powdered, mixed thoroughly and weighed accurately to an amount equivalent to 100 mg of RA. The mixture was stirred well with 80 ml water and filtered through a piece of Whatmann No. 42 filter paper. The residue was washed with 20 ml water to make the solution up to the mark in a 100 ml standard volumetric flask. The solution was then transferred to a separation flask followed by neutralization to pH 7.0 by adding 0.1 N HCl. The drug was then extracted by using different volumes of ethyl acetate for complete recovery. Ethyl acetate was evaporated by using a rotavapor and the filtrate was diluted to 100 ml with acetonitrile to make a 0.1% solution. The LC/MS purity of the extracted drug was

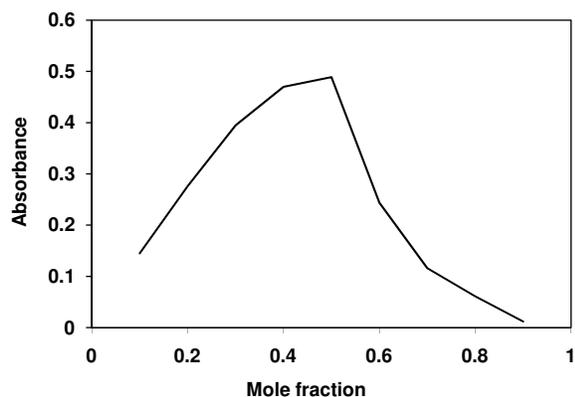


Fig. 1 Continuous variation plot for the reaction of RA with DDQ.

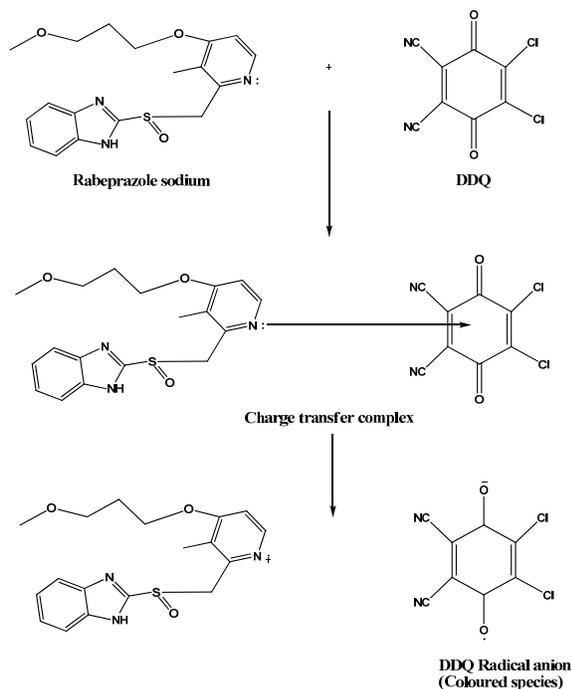


Fig. 2 Proposed mechanism for the reaction of RA with DDQ.

found to be 96.60% with m/z ions at 360 $[\text{M}+\text{H}]^+$ and 381.98 $[\text{M}+\text{Na}]^+$ and the spectrum was recorded at 254 nm.

Procedure for the reference method

Various volumes containing 10 $\mu\text{g}/\text{ml}$ of the drug in acetonitrile (0.1%; 1 mg/ml) were diluted to volume with acetonitrile in 10 ml standard volumetric flasks. The absorbance was measured against a solvent blank at 262 nm, where DDQ was added to RA solution

for the charge transfer complexation reaction and was monitored at 444 nm. The amount of drug in a given sample was computed from the calibration equation.

Preparation of the complex for infrared measurements

To 0.5 ml of 0.1% RA in acetonitrile, 0.5 ml of 0.1% DDQ solution in acetonitrile was added in a round bottom flask containing 9 ml of acetonitrile and stirred for 15 min. Solvent was evaporated under reduced pressure, and the resulting residue dried over calcium chloride. The dried residue was used for FT-IR measurements.

Solutions for ^1H NMR measurements

Preparation of the complex for ^1H NMR experiments was done in the same way as for the IR measurements; 50 mg of complex was placed in d_6 -DMSO and stirred for 5 min and then used directly for the ^1H NMR experiment.

RESULTS AND DISCUSSION

Reaction of RA with DDQ

DDQ is a π -acceptor which reacts instantaneously with basic nitrogenous compounds to form a charge-transfer complex of n - π type¹³⁻¹⁸. The spectrum of DDQ in the acetonitrile mixture exhibits an absorption band at 372 nm. The addition of RA to this solution caused an immediate shift with a new characteristic band at 444 nm (Fig. 3). The band may be attributed to the formation of a DDQ radical anion which probably resulted from the dissociation of the donor-acceptor complex in the highly polar solvent (acetonitrile). The acetonitrile was selected as an ideal solvent because it gives maximum sensitivity due to its high dielectric constant, which gives the maximum yield of radical anions in addition to its high solvating power of the reagents.

The addition of DDQ to RA (n -donor) results in the formation of a charge transfer complex of the n - π type. This compound was believed to be an intermediate molecular association complex which dissociates in acetonitrile producing a DDQ radical anion. In Job's method of continuous variation, absorbance was measured by varying the mole fraction of RA and DDQ keeping in mind that the maximum change will occur when each reactant is a limiting reactant. Hence the graph of absorbance versus mole fraction will show a region starting when the mole fraction of this reactant is zero and increasing as the mole fraction of this reactant increases until the stoichiometric mole ratio of reactants is reached. In this region, the

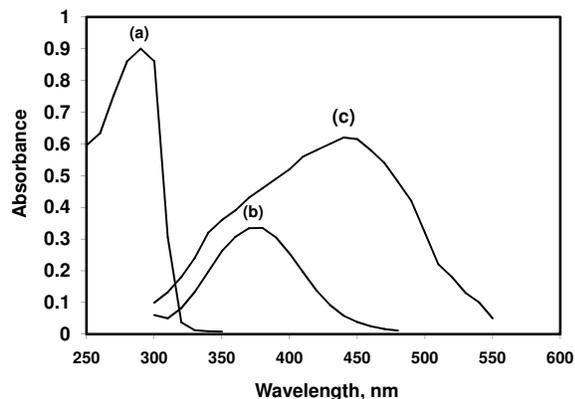


Fig. 3 Absorption spectra of (a) RA (b) DDQ and (c) reaction product of RA with DDQ in acetonitrile.

slope of the change will be positive and the limiting reactant will be the reactant being graphed. When the maximum change is reached, the other reactant becomes limiting, and the magnitude of the change drops, resulting in a negative slope.

When the change is biphasic, there will be a region with a positive slope for the mole fraction range in which the reactant graphed is the limiting reactant, and a region with a negative slope for the range in which the reactant graphed is in excess. The point at which these lines intersect is the experimental value for the mole fraction of the reactant that produces maximum change when both reactants are limiting reactants and was found to be 1:1 for the RA-DDQ complex. This indicated the presence of one n -donating centre in the RA for the charge transfer complexation reaction (Fig. 2).

The molar absorptivities and association constants for RA-DDQ reaction products were calculated using the Ross and Labes equation¹⁹:

$$\frac{1}{A_{\lambda}^{AD}} \frac{[A][D]}{[A] + [D]} = \frac{1}{K\xi_{\lambda}} \left(\frac{1}{[A]} + \frac{1}{[D]} \right) + \frac{1}{\xi_{\lambda}},$$

where $[A]$ and $[D]$ are the molar concentrations of the acceptor and donor, respectively, A_{λ}^{AD} and ξ_{λ} are the absorbance and the molar absorptivity of the complex at the specified λ_{max} , and K is the association constant of the complex. The values of K and ξ_{λ} were obtained from the plot in Fig. 4 and were found to be $2500 \text{ mol}^{-1} \text{ cm}^{-1}$ and 308 mol^{-1} , respectively.

Optimization of reaction conditions

The spectrophotometric properties of the coloured species formed with DDQ and the different parameters affecting the colour development were extensively

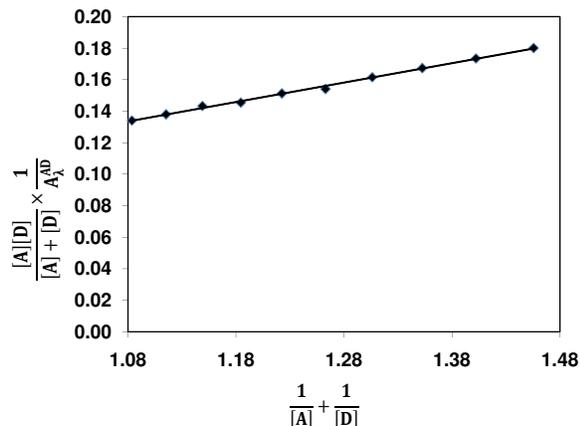


Fig. 4 Plot of $(1/[A] + 1/[D])$ versus $[A][D]/([A]+[D]) \times 1/A_x^{A,D}$ for 1:1 complex of RA-DDQ.

studied. The optimum conditions for the assay method were established by studying the reaction as a function of the concentration of reagent, the nature of the solvent, and the stability of the coloured species. For the proposed method, the effect of the volume of 0.1% DDQ was studied over the range of 0.1–2.0 ml in a solution containing 50 $\mu\text{g/ml}$ RA. The results revealed that 1.2 ml of DDQ was required to achieve the maximum intensity of colour. Therefore, 1.5 ml was used as an optimum value and maintained throughout the experiment. The reaction is stabilized within 2.0 min of mixing at room temperature and the absorbance remains constant for a further 35 min.

Spectroscopic investigations for the structure of charge transfer complex

The structure of the donor-acceptor complex was investigated by both FT-IR and ^1H NMR spectroscopy. The majority of the FT-IR measurements of the complex were concerned with shifts in vibrational frequencies in the donor, acceptor, or both. The FT-IR spectra of the complex showed some differences compared with the sum of the individual spectra of the two components. These differences were used to distinguish between the weak charge transfer complex and the products of the charge transfer reaction. In the IR spectra of free DDQ and its complex between RA and DDQ, decrease in the vibrational frequency of the cyano band and carbonyl band were observed from 2232.52 cm^{-1} (in DDQ) to 2219.01 cm^{-1} (in the complex) and 1674.29–1621.06 cm^{-1} , respectively. Along with this, some other changes were also observed in the fingerprint region of the FT-IR spectra as shown in Fig. 5. These changes could be due to the expected symmetry and electronic structure changes

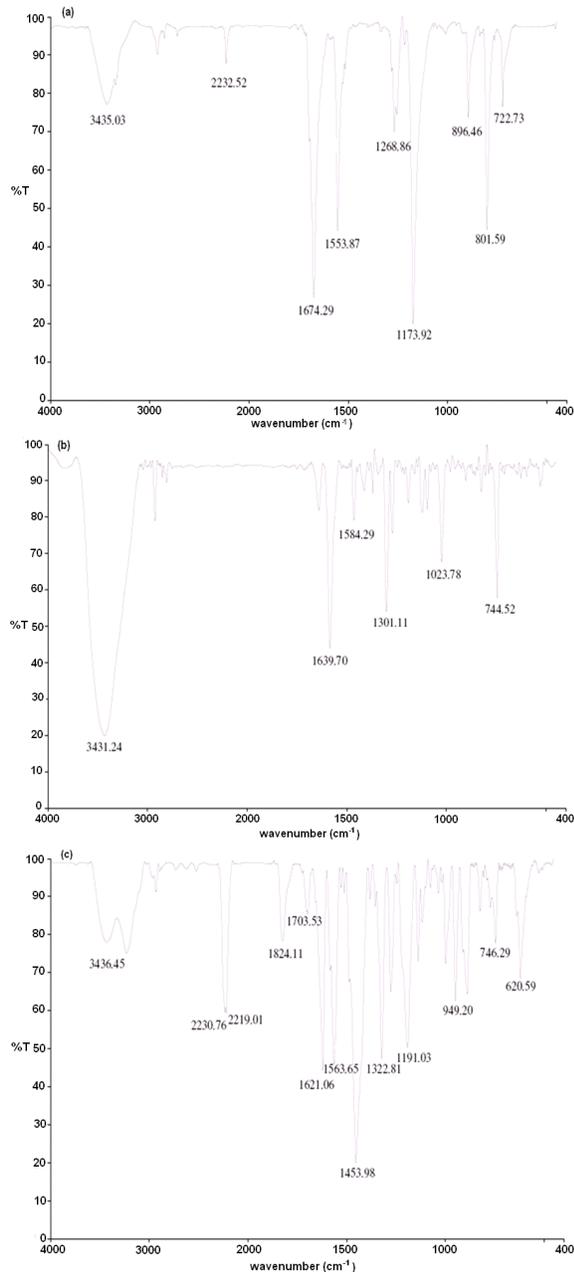


Fig. 5 Infrared spectra of (a) DDQ (b) RA (c) RA-DDQ complex.

upon the formation of the charge transfer complex¹³. The two new bands at 1824.11 and 1703.53 cm^{-1} occurred as a result of the red shift of the CN frequency in comparison to the free DDQ.

In the ^1H NMR spectra of the complex, generally the protons of the donor are shifted to downfield (paramagnetic shift)^{14–18}. The ^1H NMR spectra of the charge transfer complex of RA with DDQ as an accep-

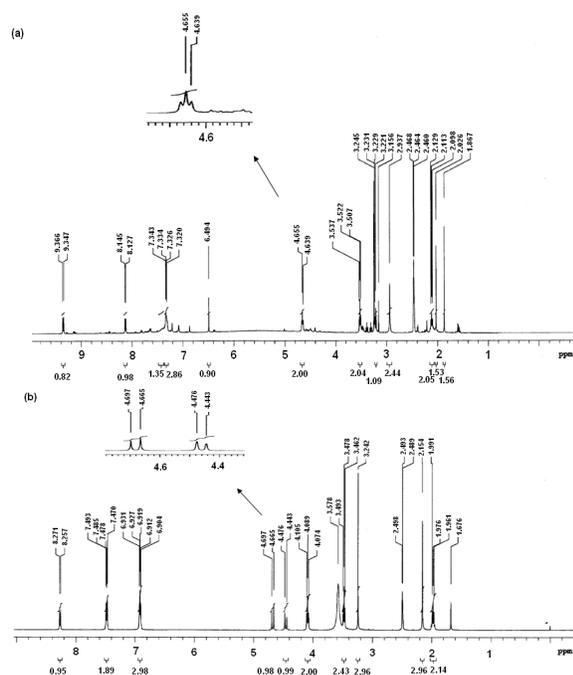


Fig. 6 ^1H NMR spectra of (a) RA-DDQ complex (b) RA.

tor was recorded in d_6 -DMSO along with the spectra of the free drug (Fig. 6). In free RA, the protons attached to the sulfinyl group are dissymmetric and the spectrum shows an AB type of quartet at $\delta = 4.44$, 4.47, 4.66, and 4.69 ppm with coupling constant, $^2J = 13$. However, in the spectra of the complex, the doublet of the proton at high field is shifting towards another proton doublet and giving an overlapped AB quartet with $^2J = 6.2$ to $\delta = 4.65$. A change in coupling constant for the multiplet of propoxy protons from 6.2 to 7.2 with a shift of 0.137 ppm was observed. The change in coupling constant is due to two-bond geminal coupling as described by Karplus²⁰, and depends on the H-C-H bond angle. The formation of charge transfer complex seems to be reducing the coupling constant value. The decrease in 2J for protons attached with sulphanyl group from 13 to 6.2 reveals the involvement of a lone pair of nitrogen in the benzo-imidazole ring of RA for the formation of n - π complex with DDQ. Similar shifts were also observed for other protons (Fig. 6).

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD

Calibration curves, linearity, and sensitivity

The linear calibration curves were obtained over the concentration range of 10–90 $\mu\text{g}/\text{ml}$ of RA. The least square treatment of calibration data yielded the

regression equation $A = 0.0019 + 1.3979C$ with a correlation coefficient of 0.9999, indicating the excellent linearity of the calibration curve. The 95% confidence limits²¹ for slopes and intercepts of the regression lines pointed towards a high reproducibility of the proposed method. In order to verify that the developed method is free from procedural errors, the experimental intercept, a , of the regression line was tested for significance of the deviation from the expected value zero. For this justification, a simplified method was used to calculate the quantity from the relation $t = a/S_a$ ²² and its comparison with the tabulated data from the t -distribution. The t -value for the proposed method was found to be 0.217, which did not exceed the 95% criterion ($t = 2.365$, when $\nu = 7$). It confirmed that the intercept for the proposed method is not significantly different from zero. Thus the present method is free from constant errors independent of the concentration of RA.

The detection limit²³ for the proposed method was calculated and found to be 0.298 $\mu\text{g}/\text{ml}$. The small value of the variance (2×10^{-7} $\mu\text{g}/\text{ml}$) confirmed the small degree of scatter of experimental data points around the line of regression. Both the detection limit and the slope of the calibration graphs indicated the good sensitivity.

The error (S_c) in the determination of the given concentration of RA was calculated by using²⁴

$$S_c = \frac{S_0}{b} \left[1 + \frac{1}{n} + \frac{(A - \bar{A})^2}{b^2 \sum_i (C_i - \bar{C})^2} \right]^{1/2}$$

The value of S_c may be used to establish the confidence limit for the determination of unknown concentrations by using $C_i \pm tS_c$. The results are shown in Fig. 7 by plotting percentage uncertainty versus the concentration of RA²⁵ at 95% confidence level. Hence, the confidence limits established for the above method can be used to evaluate the relative uncertainty

Table 1 Test of precision and accuracy of the proposed method.

Amount of RA ($\mu\text{g}/\text{ml}$) added	Recovery found \pm SD	RSD ^a (%)	SAE ^b	CL ^c	
30	30.01 \pm 0.39	100.05	1.32	0.162	0.416
50	49.88 \pm 0.47	99.76	0.94	0.191	0.493
80	80.05 \pm 0.29	100.06	0.36	0.117	0.302

^a relative standard deviation

^b standard analytical error

^c 95% confidence limit with 5 degrees of freedom ($t = 2.571$)

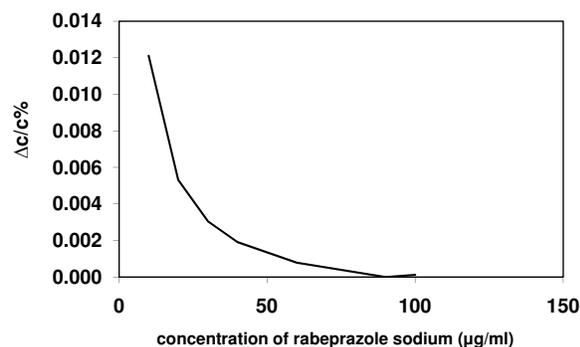


Fig. 7 Plot of the variation of the 95% confidence limits for the proposed method, presented as uncertainty percentage on the concentration of RA.

directly on the concentration over the full range of the concentration tested.

The reproducibility of the proposed method was checked by estimating three different concentration levels within the Beer's law limit (Table 1). The standard deviation, relative standard deviation, and standard analytical error are very satisfactory.

The validity of the proposed method for the determination of the drug in commercial dosage forms was tested by applying the standard addition technique. In this study, a known amount of pure RA was added to 30 mg of pre-analyse commercial dosage forms. The recoveries were obtained in the range 99.93–100.22%. The results are summarized in Table 2. The

common excipients present in formulations did not interfere. The applicability of the proposed method for the determination of RA in commercial dosage form was examined by analysing marketed products such as Rabez-20, Genix Pharma Ltd. (Hyderabad), Rabcip-20, Cipla Ltd. (Bombay) and Rabitop-20, Aristo Pharma Ltd. (Hyderabad).

The results of the proposed method were compared with those obtained by the reference method and are summarized in Table 3. It is evident from the table that the calculated t and F values are less than the theoretical ones at 95% confidence level, indicating no significant difference between the proposed method and reference method.

The proposed method is quite selective as the drug contains a basic centre, which preferentially interacts with DDQ. A substance having no nitrogen (no basic centre) in the organic moiety will not give any colour reaction with polyhalo/polycyanoquinones. The method shows no interference from the common excipients and additives. The statistical parameters and the recovery data reveal good accuracy and precision of the proposed method.

CONCLUSIONS

The proposed method is simple, sensitive, and rapid for the determination of RA in pure form and commercial dosage forms. Hence, the present approach encourages their successful use in routine analysis of the drug in quality control laboratories.

Table 2 Standard addition method for the determination of RA in dosage forms.

Formulation	Amount ($\mu\text{g/ml}$)		Recovery \pm RSD ^a (%)	SAE	CL
	Added	Found \pm SD			
Rabez 20 mg	30	60.08 \pm 0.28	100.13 \pm 0.46	0.113	0.291
	50	79.98 \pm 0.34	99.97 \pm 0.42	0.138	0.356
Rabcip 20 mg	30	60.01 \pm 0.21	100.01 \pm 0.34	0.085	0.220
	50	80.05 \pm 0.33	100.06 \pm 0.40	0.133	0.343
Rabitop 20 mg	30	59.96 \pm 0.28	99.93 \pm 0.45	0.111	0.286
	50	80.18 \pm 0.30	100.22 \pm 0.38	0.125	0.321

^a Mean \pm SD for six independent analyses.

Table 3 Comparison of proposed method with reference method.

Formulation	Proposed method						Reference method			
	Recovery	SD ^a ($\mu\text{g/ml}$)	SAE	CL	t -value ^b	F -value ^b	Recovery	SD ^a ($\mu\text{g/ml}$)	SAE	CL
Rabez 20 mg	100.18	0.228	0.56	0.239	0.381	2.30	99.56	0.346	0.141	0.363
Rabcip 20 mg	100.06	0.302	0.06	0.166	0.432	1.78	99.6	0.404	0.164	0.424
Rabitop 20 mg	100.26	0.187	0.08	0.196	0.338	2.21	99.56	0.278	0.113	0.291

^a Mean \pm SD for six independent analyses.

Theoretical t -value ($\nu = 10$) and F -value ($\nu = 5$) at 95% confidence level as 2.228 and 5.05.

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