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Spectrophotometric Determination of Rabeprazole Sodium Using Two Charge Transfer Complexation Reactions

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ABSTRACT

This work presents two simple and direct spectrophotometric methods for determination of rabeprazole sodium (RB) through charge transfer complexation reactions. The first method is based on the reaction of the drug with p-chloranilic acid (p-CA) in acetonitrile to give a red colored product with maximum absorbance at 518 nm. The second method is based upon the interaction of RB and 7,7,8,8-tetracyanoquinodimethane (TCNQ) in acetone resulting in the formation of a bluish-green complex measured at 845 nm. Factors affecting the color development were studied and optimized. The proposed colorimetric procedures were effectively validated with respect to linearity, ranges, precision, accuracy, robustness, detection and quantification limits. Regression analysis for the calibration curves of the formed color products with p-CA and TCNQ showed good linear relationships over the concentration ranges of 20–200 and 2–16 µg/mL respectively. The method was successfully applied to the assay of rabeprazole enteric coated tablets with good accuracy and precision. Assay results were statistically compared to a reference HPLC method where no significant differences were observed between the proposed methods and reference method.

INTRODUCTION

Rabeprazole sodium (RB) (Figure 1) chemically known as 2-[[[4-(3-Methoxypropoxy)-3-methyl-2-pyridinyl] methyl] sulfinyl]-1*H*-benzoimidazole sodium, is a proton pump inhibitor. It suppresses secretion of gastric acid by inhibiting the enzyme system of hydrogen/potassium adenosine triphosphatase (H^+/K^+ ATPase), the proton pump of the gastric parietal cell. It is used in conditions where inhibition of gastric acid secretion is required, including aspiration syndromes, dyspepsia, gastro-oesophageal reflux, peptic ulcer and the Zollinger-Ellison syndrome (Sweetman, 2009). Numerous methods have been described for the determination of RB in its single, multi-component dosage forms or in other complex matrices. These methods include HPTLC (Gandhi *et al.*,2009), capillary electrophoresis (Garcia *et al.*,2005), HPLC-tandem mass spectrometry (Yu *et al.*,2010), HPLC with UV detection (Garcia *et al.*,2004) and UPLC coupled with photodiode array detector (Seshadri *et al.*,2013). Additionally, several spectrophotometric methods have been reported depending on the formation of extractable colored complexes of the drug with orange G dye (Pillai, 2006), crystal violet (Pillai and Singhvi, 2006) and several sulphonphthalein acid dyes (Mohamed *et al.*,2010). Other colorimetric procedures include the reaction of RB with 3-methyl-2-benzothiazolinone hydrazone hydrochloride in the presence of cerium(IV) (Rahman *et al.*,2008) and reaction with 1-chloro-2,4-dinitrobenzene to form a Meisenheimer complex (Rahman *et al.*,2008).

Some spectrophotometric methods exploited oxidation of the drug using potassium iodate (Mohamed *et al.*,2010), alkaline potassium permanganate (Bhandare *et al.*,2008) and bromide/ bromate mixture followed by reaction of the excess reagent with either methyl orange or indigo carmine (Satyanarayana and Rao, 2010). Moreover, spectrophotometric measurement of RB in the UV region has been applied for assay of its single and multi-component pharmaceutical preparations (Garcia *et al.*,2006; Prasad and Sharma, 2010). The interest to establish simple, fast, and adequately sensitive spectrophotometric methods for routine analysis in control laboratories has been one of the main targets for analytical chemists. The formation of a charge transfer complex involves transfer of electronic charge from an "electron rich" molecule to an "electron deficient" molecule.

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The molecular interactions between electron donors and electron acceptors are generally associated with the formation of intensely colored products. Typically, the electron donor and electron acceptor combine in a 1:1 molar ratio to form the charge transfer complex. A variety of electron donating basic compounds have been reported to yield charge-transfer complexes with various acceptors. The rapid formation of charge transfer complexes leads to their utility in the development of simple and convenient spectrophotometric methods for numerous pharmaceutical compounds (Al-Ghannam and Belal, 2002; Abdel-Hay *et al.*,2004; Darwish, 2005).

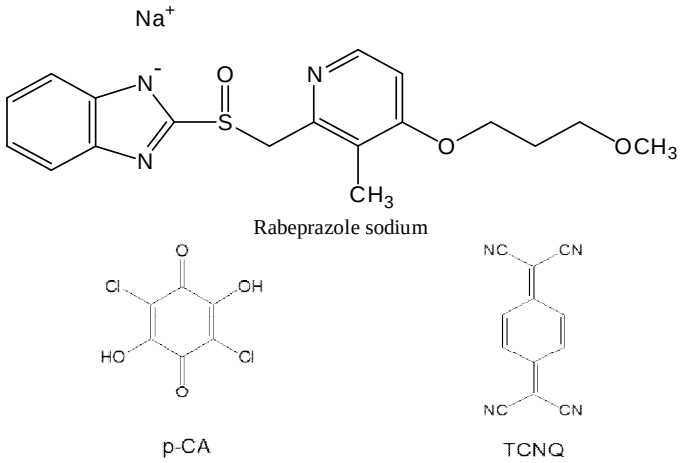

Fig. 1: Chemical structures of rabeprazole sodium, p-chloranilic acid (pCA) and 7,7,8,8-tetracyanoquinodimethane (TCNQ).

Being a salt of weak acid and a negative charge carrier, RB is a good electron donor and can form charge transfer complexes with various acceptors. Only a single report can be found in the scientific literature describing the charge transfer interaction between RB and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ), and the formed color product was measured spectrophotometrically at λ_{max} 444 nm (Khan *et al.*,2009). This work reports two simple and direct spectrophotometric methods for assay of RB in pure form as well as in tablets using p-chloranilic acid (p-CA) and 7,7,8,8- tetracyanoquinodimethane (TCNQ) as chromogenic reagents. The structures of the chosen chromogenic reagents are shown in Figure 1.

MATERIALS AND METHODS

Instrumentation

Spectrophotometric measurements were performed using Shimadzu 1700 UV-VIS spectrophotometer with matched 1-cm quartz cells.

Materials and reagents

Authentic sample of Rabeprazole sodium (RB) was kindly provided by SIGMA Pharmaceutical Industries, Quwasna, Monofeeia, Egypt. Analytical grade of 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (p-chloranilic acid, pCA) (BDH Chemicals, Poole, UK) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) (Aldrich Chemical Co, Milwaukee, USA) were used. HPLC grade acetonitrile and methanol (LAB-SCAN Analytical Sciences, Poland) and analytical grade acetone (Tedia Company Inc., Fairfield, OH, USA) were used.

Preparation of standard, sample and reagents' solutions

Preparation of stock RB and reagents' solutions

Stock standard solutions of RB, 500 µg/mL and 1000 µg/mL, were separately prepared in acetonitrile. pCA solution, 4000 µg/mL (4 mg/mL), and TCNQ solution, 1000 µg/mL (1 mg/mL), were also prepared in acetonitrile. All solutions were freshly prepared.

Preparation of RB tablets sample solutions

Pharmaceutical preparations assayed in this study are Pariet® tablets (Eisai Co. Ltd, Tokyo, Japan for Janssen-Cilag, Beerse, Belgium) labeled to contain 20 mg RB per tablet and Rabicid® tablets (SIGMA Pharmaceutical Industries, Quwasna, Monofeeia, Egypt) labeled to contain 10 mg RB per tablet. For each preparation, ten tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 25 mg RB is extracted into 10 mL acetonitrile with the aid of sonication for 15 min then filtered into a 25 mL-volumetric flask. The residue was washed with 2 × 5 mL portions of acetonitrile and washings were added to the filtrate, then the solution was diluted to volume with acetonitrile to reach a final concentration 1000 µg/mL for RB (stock sample solution for reaction with p-CA). Similarly, an accurately weighed portion of the powder equivalent to 25 mg RB is extracted into 25 mL acetonitrile with the aid of sonication for 10 min then filtered into a 50 mL-volumetric flask. The residue was washed with 2 × 10 mL portions of acetonitrile and washings were added to the filtrate. Finally, the solution was diluted to volume with the same solvent to reach a concentration of 500 µg/mL RB (stock sample solution for reaction with TCNQ).

General procedures

For the reaction with p-CA: aliquots of RB stock standard solution (1000 µg/mL) were transferred into a series of 25-mL volumetric flasks to obtain final concentrations 20-200 µg/mL, treated with 2 mL of pCA solution, the volume was completed with acetonitrile and absorbance was measured at 518 nm against reagent blank. For the reaction with TCNQ: aliquots of RB stock standard solution (500 µg/mL) were transferred into a series of 25-mL volumetric flasks to obtain final concentrations 2-16 µg/mL, treated with 3 mL of TCNQ solution, and the volume was completed with acetone. The reaction mixtures were kept at room temperature for 30 min, and then absorbance was measured at 845 nm against reagent blank.

Procedure for the assay of tablets

Aliquots of the RB stock sample solutions were transferred into a series of 25-mL volumetric flasks and the general procedures were then followed. Recovery values were calculated from similarly treated standard solutions. For standard

addition assay, sample solutions were spiked with aliquots of stock standard solutions of RB to obtain final concentrations within the previously specified ranges then treated as under general procedures. Recovered concentrations were calculated by comparing the analyte response with the increment response attained after addition of the standard.

RESULTS AND DISCUSSION

Being an ionized negatively charged species, RB reacts instantaneously with p-CA giving a characteristic deep purple colored product which exhibits an absorption maximum at 518 nm (Figure 2).

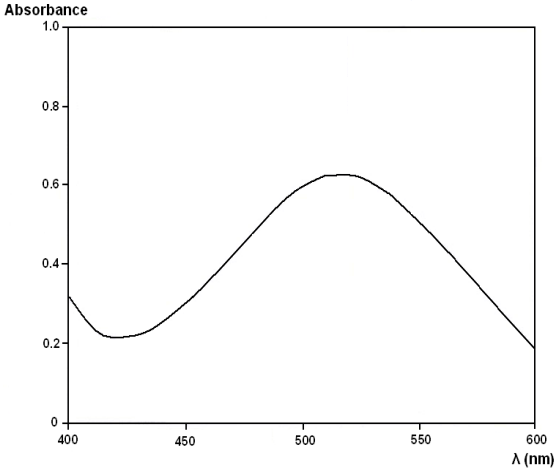

Fig. 2: Absorption spectrum of the reaction product of 120 µg/mL RB with p-CA in acetonitrile.

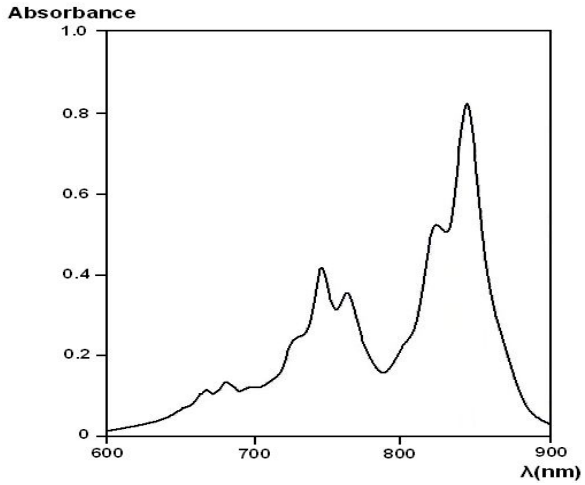

Fig. 3: Absorption spectrum of the reaction product of 12 µg/mL RB with TCNQ in acetone.

Similarly, RB reacts with TCNQ to yield a bluish-green product which exhibits a broad absorption spectrum with several maxima at 681, 745, 763, 823 and 845 nm where the maximum at 845 nm is the most prominent (Figure 3). These colored products can be attributed to the formation of charge-transfer complexes between RB, as electron donor, and p-CA or TCNQ, as π-acceptors, followed by subsequent dissociation of the donor-acceptor complexes to form the purple and the bluish-green radical anions of p-CA and TCNQ respectively. The dissociation of the complexes was promoted by the high dielectric constants of the polar solvents (acetonitrile and acetone). These reactions can be demonstrated by the following scheme:

$$D + A \underset{}{\overset{}{\rightleftharpoons}} D{-}A \overset{\text{Polar solvent}}{\rightleftharpoons} D^{\bullet+} + A^{\bullet-}$$

Donor π-acceptor Complex Radical ions

Optimization of experimental conditions

Different experimental parameters affecting the color development and its stability were carefully studied and optimized. Such factors were changed individually while keeping the others constant. These factors include reagent concentration, diluting solvent and time. The influence of reagent concentration (in terms of reagent volume) on the absorbance values was tested. It was found that increasing the volume of p-CA solution increased the color intensity up to 2.0 mL, after which no more increase in absorbance was recorded. Similarly, the effect of the volume of TCNQ solution was studied and it was found that 3.0 mL was sufficient to get the highest color intensity (Figure 4).

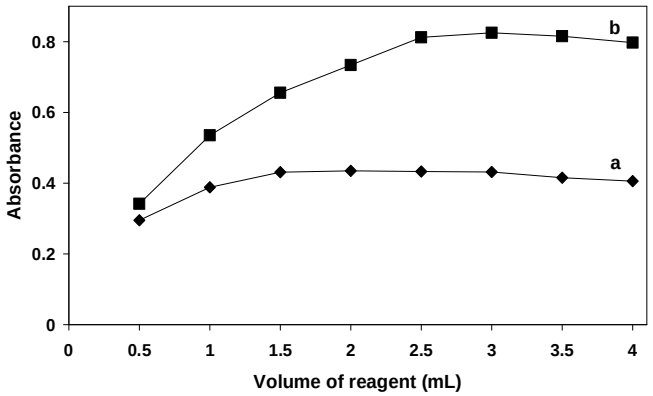

Fig. 4: Effect of volume of p-CA (a) and TCNQ (b) on the absorbance of the charge transfer complexes with RB.

In order to select the most appropriate diluting solvent, the reactions were carried out in different organic solvents such as acetone, acetonitrile, ethanol, isopropanol and methanol. Small shifts in the position of the maximum absorption peak were observed, whereas the absorption intensities were extensively influenced. Acetonitrile was considered as an ideal solvent for the reaction with p-CA, while acetone was found the best solvent regarding intensity of the color formed with TCNQ. The optimum reaction time was determined by monitoring the color development at room temperature (20±2 °C). Complete color development was attained instantaneously with pCA, and increasing the time of the reaction did not show any increase in the absorbance. Therefore, the absorbance readings were taken at zero time. In case of TCNQ, maximum color intensity was reached after 30 min; accordingly, the reaction was allowed to take place for 30 min before recording the absorbance (Figure 5).

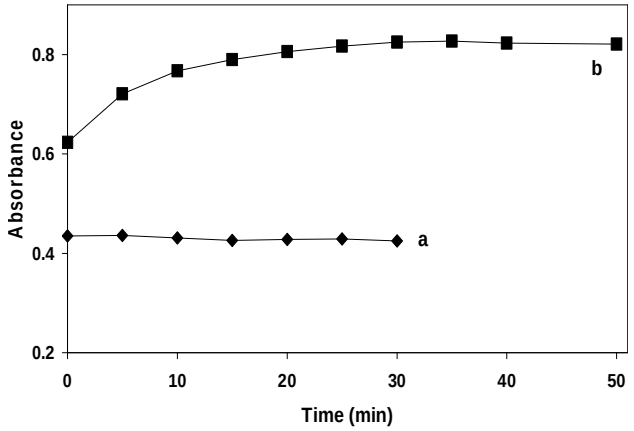

Fig. 5: Effect of reaction time on the absorbance of the charge transfer complexes of RB with p-CA (a) and TCNQ (b).

Validation of the proposed methods

Linearity and concentration ranges

Under the optimal experimental spectrophotometric conditions, a linear relationship exists between the absorbance readings of each reaction product and the corresponding concentrations of RB. Table 1 presents the linearity data and statistical parameters for the studied methods including linear regression equations, concentration ranges, correlation coefficients, molar absorptivity values, standard deviations of the intercept (S_a), the slope (S_b) and standard deviation of residuals ($S_{y/x}$). Regression analysis shows good linearity as revealed from the correlation coefficient values (r > 0.9996) and RSD% of the slope values which were found less than 1.4 %.

Table. 1: Analytical parameters for the determination of RB using the proposed charge transfer spectrophotometric methods.

Parameter	p-CA	TCNQ
Wavelength (nm)	518	845
Linearity range (µg/mL)	20 – 200	2 – 16
Molar absorptivity (ε) (L mol^{-1} cm^{-1})	2021	25630
Intercept (a)	-0.0076	0.01876
Slope (b)	0.0053	0.0672
Correlation coefficient (r)	0.99988	0.99964
S_a (1)	0.0051	0.0084
S_b (2)	4.17×10^{-5}	9.01×10^{-4}
RSD% of slope	0.79	1.34
$S_{y/x}$ (3)	0.0065	0.0105
LOD (4) (µg/mL)	3.18	0.41
LOQ (5) (µg/mL)	9.62	1.25

(1) S_a: Standard deviation of intercept
(2) S_b: Standard deviation of slope
(3) $S_{y/x}$: Standard deviation of residuals (standard error of estimate)
(4) Limit of detection
(5) Limit of quantification

Limits of detection and quantification

In accordance with the recommendations of ICH guidelines on Validation of Analytical Procedures (ICH, 2005), the limit of detection, LOD = 3.3 σ/s, where σ is the standard deviation of the intercept of the regression line and s is the sensitivity, namely the slope of the calibration curve. On the other hand, the limit of quantification (LOQ) is defined as 10 σ/s. The LOD and LOQ values were calculated and presented in Table 1.

Accuracy and precision

The accuracy and within-day (intra-day) precision for the proposed procedures were studied at three concentration levels for RB using three replicate determinations for each concentration within one day. Similarly, the accuracy and between-day (inter-day) precision were tested by analyzing the same three concentrations using three replicate determinations repeated on three days.

The recovered concentrations were calculated using the corresponding regression equations and were found to be satisfactory. The percentage relative standard deviation (RSD %) values were less than 1.4 % and the percentage relative error (E_r %) values were less than 1.7 % proving the high repeatability and accuracy of the developed methods for the estimation of RB in bulk form (Table 2).

Table. 2: Accuracy and precision for the analysis of RB in bulk form using the proposed charge transfer spectrophotometric methods.

Reagent	Parameter	Nominal value (µg/mL)	Found ± SD[a] (µg/mL)	RSD (%)[b]	E_r (%)[c]
p-CA	Within-day	40	40.61 ± 0.26	0.64	1.53
		80	79.13 ± 0.33	0.42	-1.09
		160	161.92 ± 1.40	0.87	1.20
	Between-day	40	40.53 ± 0.37	0.91	1.33
		80	80.60 ± 0.93	1.15	0.75
		160	161.51 ± 1.99	1.23	0.94
TCNQ	Within-day	4	4.03 ± 0.033	0.82	0.75
		8	8.05 ± 0.059	0.73	0.63
		12	11.97 ± 0.078	0.65	-0.25
	Between-day	4	4.05 ± 0.043	1.06	1.25
		8	8.10 ± 0.112	1.38	1.25
		12	12.20 ± 0.128	1.05	1.67

[a] Mean ± standard deviation for three determinations.
[b] % Relative standard deviation.
[c] % Relative error.

Table. 3: Study of robustness of the proposed spectrophotometric methods.

Reagent	Parameter	Absorbance
p-CA	**Reagent volume (mL)**	
	1.8	0.608
	2.0	0.622
	2.2	0.621
	RSD%	**1.27**
	Working wavelength (nm)	
	515	0.617
	518	0.622
	521	0.620
	RSD%	**0.41**
TCNQ	**Reagent volume (mL)**	
	2.7	0.805
	3.0	0.823
	3.3	0.825
	RSD%	**1.35**
	Working wavelength (nm)	
	842	0.802
	845	0.823
	848	0.795
	RSD%	**1.81**

Robustness

The robustness of an analytical procedure is a measure of its capability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its

reliability during normal usage (ICH, 2005). Robustness was examined by making small variations in the working wavelengths (± 3 nm) and volume of reagents (± 10 %) then examining the results. These variations did not have any significant effect on the measured absorbance values of the reaction products. RSD% of the measured absorbance for the studied variations did not exceed 2%. Table 3 shows the effect of the studied variations on the absorbance readings of the reaction products.

Stability of solutions

The stability of the colored products at room temperature was examined. No spectrophotometric changes were observed within 30 min after measurement. Also, the stock standard solutions of RB prepared in acetonitrile were stable for at least 3 days when stored refrigerated at 4 ºC.

Assay of tablets

The proposed colorimetric methods were applied to the determination of RB in the enteric coated tablet formulations: Pariet® and Rabicid®, and recoveries were calculated from similarly treated external standards. The assay results revealed satisfactory accuracy and precision as indicated from % recovery, SD and RSD% values (Table 4). A reference HPLC method was applied for the estimation of RB in its commercial products (Garcia *et al.*,2004). For each pharmaceutical preparation, the results of the proposed methods were statistically compared with those of the reference method using the one-way analysis of variance test (Single factor ANOVA) (Miller and Miller, 2000). The ANOVA test is a useful statistical tool for comparing recovery data obtained from more than two methods of analysis. The calculated F-values did not exceed the critical value, indicating that there were no significant differences between the proposed methods together with the reference method (Table 4). Moreover, the proposed methods were employed for the assay of RB preparations using the standard addition technique. Again, the proposed methods proved to be successful, accurate and precise for the determination of RB in its tablets dosage forms (Table 4).

CONCLUSIONS

The charge-transfer complexation reactions of rabeprazole sodium (RB) as electron donor and some electron acceptors have been investigated. The obtained colored complexes were utilized for the development of two simple, rapid and accurate spectrophotometric methods for the analysis of RB in tablets. Better sensitivity was achieved with TCNQ; on the other hand, the reaction with p-CA was instantaneous and time saving. The proposed methods are advantageous to the previously reported UV-based spectrophotometric methods (Garcia *et al.*,2006), as the measurements are performed in the visible region, away from any possible interfering UV-absorbing excipients that might be co-extracted from RB dosage forms. In addition, the proposed methods are superior or comparable to the already available spectrophotometric methods working in the visible region (Bhandare *et al.*,2008; Mohamed *et al.*,2010; Pillai, 2006; Pillai and Singhvi, 2006; Rahman *et al.*,2008; Satyanarayana and Rao, 2010) regarding simplicity (only one reagent and no extraction with harmful organic solvents such as chloroform) and/or sensitivity (in terms of concentration ranges and LOD values). Only a single report can be found in the scientific literature for the charge transfer spectrophotometric determination of RB based on its reaction with DDQ (Khan *et al.*,2009). This previous study described a procedure for the preparation of rabeprazole acid from its sodium salt by acidification, extraction with ethyl acetate, evaporation of the solvent and finally dissolution in acetonitrile.

Table. 4: Analysis of RB in its pharmaceutical preparations using the proposed spectrophotometric methods and the reference method.

Using external standard analysis						
Pharmaceutical preparation	**Parameters**		**p-CA**	**TCNQ**		**Reference method**
Pariet® tablets	**%Recovery ± SD[a]**		100.81 ± 0.97	100.61 ± 1.24		100.31 ± 0.82
	RSD%[b]		0.96	1.23		0.82
ANOVA (single factor)						
Source of Variation	SS	df	MS	**F**	P-value	**F critical**
Between Groups	0.643985	2	0.321992	**0.306313**	0.741744	**3.885294**
Within Groups	12.61423	12	1.051186			
Total	13.25822	14				
Rabicid® tablets	**%Recovery ± SD[a]**		98.29 ± 1.34	99.18 ± 0.93		98.84 ± 0.89
	RSD%[b]		1.36	0.94		0.90
ANOVA (single factor)						
Source of Variation	SS	df	MS	**F**	P-value	**F critical**
Between Groups	2.016341	2	1.008171	**0.873781**	0.442317	**3.885294**
Within Groups	13.84563	12	1.153802			
Total	15.86197	14				
Using standard addition analysis						
Pharmaceutical preparation	**Parameters**		**p-CA**	**TCNQ**		
Pariet® tablets	**%Recovery ± SD[a]**		99.54 ± 0.95	100.43 ± 1.13		
	RSD%[b]		0.95	1.13		
Rabicid® tablets	**%Recovery ± SD[a]**		98.73 ± 1.26	99.30 ± 1.04		
	RSD%[b]		1.28	1.05		

a Mean ± standard deviation for five determinations.
b % Relative standard deviation.

The rabeprazole acid then reacts with DDQ producing a charge transfer complex which is measured at 444 nm with linearity range 10–90 μg/mL. Obviously, the proposed methods are much more simple, rapid and reliable. The proposed TCNQ method provides better sensitivity in terms of linearity range than the already published DDQ method.

Moreover, the proposed methods depend on the already available electron donor nature of RB. Furthermore, the developed methods do not require elaborate treatment or sophisticated experimental setup usually associated with HPLC methods of analysis. The developed methods used only a spectrophotometer, which is available in all quality control laboratories. The applicability of the developed methods was evaluated through the determination of RB in pharmaceutical formulations with good accuracy and precision, therefore they can be considered useful and convenient for the routine and quality control assay of the drug.

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