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Research Article

Utility of p-Chloranilic Acid and 2,3-Dichloro-5,6-dicyano-p-benzoquinone for the Spectrophotometric Determination of Rizatriptan Benzoate

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Rizatriptan is a new selective 5-$HT_{1B/1D}$ agonist which is used in the treatment of migraine headaches. Two simple, rapid, accurate, and economical spectrophotometric methods are described for the determination of rizatriptan benzoate (RTB) in its pure form and pharmaceutical preparations. These methods are based on the charge-transfer complexation reaction between rizatriptan benzoate as *n*-electron donor and p-chloranilic acid (p-CA) or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as π-acceptor to form highly colored chromogens. The chromogens formed by the reaction between RTB and p-CA peaked at 530 nm (method A) and that formed by the reaction between RTB and DDQ peaked at 590 nm (method B). Under the optimum conditions Beer's law is obeyed in the concentration range of 14–245 μg mL^{-1} for method A and 4–70 μg mL^{-1} for method B. The coefficient of correlation was found to be 0.9999 for both methods. The molar absorptivity, Sandell sensitivity, limits of detection, and quantification are also reported. The stoichiometric relationship determined by Job's continuous method was found to be 1 : 1 (drug : reagent) for both methods. Both methods were applied to determination of RTB in the pharmaceutical formulations. Results of the analysis were validated statistically.

1. Introduction

Drug quality control is a branch of analytical chemistry that has a wide impact on the health of human being, so the development of a reliable, quick, and accurate method for the active ingredient determination is always welcomed. Of the chemical neurotransmitter substances, serotonin (5-hydroxytriptamine or 5-HT) is perhaps the most implicated in the treatment of migraine, one among them is rizatriptan benzoate (RTB). RTB is a selective 5-hydroxytryptamine1B/1D (5-$HT_{1B/1D}$) receptor agonist which is chemically described as *N*, *N*-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indole-3-ethanamine monobenzoate. It has a weak affinity for other 5-HT receptor subtypes and was launched in 1998 for the acute treatment of migraine in adults [1]. Migraine headache is recognized as a chronic disease with episodic occurrences and is frequently accompanied by gastrointestinal disturbance including nausea and vomiting. The headache may be preceded or accompanied by aura which is characterized by visual disturbances. The effectiveness of triptans, which are serotonin 5-$HT_{1B/1D}$ receptor agonist drugs, in these conditions is due to their ability to block the stimulated secretion of neuropeptides from trigeminal nerves to break the nociceptive cycle of migraine. These actions also include constriction of meningeal and cerebral blood vessels [2–4].

Rizatriptan (RTB) is not official in any pharmacopoeia. The literature survey revealed several reported analytical approaches for the determination of RTB in dosage forms and biological materials including liquid chromatography-electrospray tandem mass spectrometry, LC-MS/MS [9, 10] for human plasma and high performance liquid chromatography with fluorescence detection [11, 12] and in human serum by LC-MS/MS [13]. Development of a rapid, sensitive, and selective method for the determination of RTB is essential for the analysis of drug in bulk, in drug delivery

system and for release dissolution studies. A few methods are found in the literature for the determination of RTB in pharmaceuticals and include UV-spectrophotometry [14–18], spectrofluorimetry [17], HPLC when present alone [19, 20] or in combination with other antimigraine drugs [21]. A microemulsion electrokinetic chromatography (MEEKC) has also been reported for the determination of RTB and its degradation products [5].

In the literature, only a few visible spectrophotometric methods have been described for the determination of RTB. Shanmukha Kumar et al. [6] have described two methods, one based on the chloroform-soluble ion-pair with methyl orange, and the other, redox-complexation reaction involving iron (III) and 2,2′-bipyridyl. The reaction of RTB with 2,6-dichloroquinone-4-chlorimide, 1,2-naphthaquinone-4-sulphonic acid or brucine and metaperiodate has resulted in colored products serving as basis for the assay of drug [7]. Three more methods have been reported by Shanmukha et al. [8]. The first method is similar to one described earlier involving iron (III) except that 1, 10-phenantroline is used in place of 2,2′-bipyridyl. In the second method, Folin-Ciocalteu (F-C) reagent is reduced by RTB in alkaline medium and the resultant blue chromogen measured. The last method uses alizarin red as the ion-pair reagent to form a chloroform-soluble ion-pair complex with RTB. A method based on the reaction of RTB as *n*-electron donor with 7,7,8,8-tetracyanoquinodimethane (TCNQ) as a π-acceptor to give highly colored complex species peaking at 570 nm is also found in the literature [22]. Present authors have also been reported a few visible spectrophotometric methods for the quantification of RTB [23, 24].

However, the reported methods, particularly those based on chromatography are complex, require expensive experimental setup, and skilled personnel and inaccessible to many laboratories in developing and underdeveloped nations. In contrast, visible spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories. Spectrophotometric methods have several advantages, such as low interference level, good analytical selectivity, and they are easier, less expensive, and less time consuming compared with most of the other methods [25]. The reported spectrophotometric methods [6–8, 22] suffer from one or the other disadvantage such as poor sensitivity, rigid pH control, heating or extraction step, complicated experimental setup, and meticulous control of experimental variables as can be seen from Table 1.

The aim of this study was to establish simple, sensitive, precise, and inexpensive procedures for the quantification of RTB in pharmaceutical preparations. The basis of the proposed methods is molecular interactions between electron donors and electron acceptors. These interactions are generally associated with the formation of intensely colored charge transfer (CT) complexes which absorb radiation in the visible region [26, 27]. Substituted quinones such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and p-chloranilic acid (p-CA) have been used as π-acceptors with various donors to form CT complexes and radicals, thus enabling the determination of a number of pharmaceutical substances [28–35]. This work describes the application of the above reagents (p-CA and DDQ) for the spectrophotometric assay of RTB. The proposed methods have been validated statistically for their accuracy, precision, sensitivity, selectivity, robustness, and ruggedness as per ICH guidelines.

2. Experimental

2.1. Instrument. All absorption measurements were made using a Systronics model 106 digital spectrophotometer (Systronics Ltd, Ahmedabad, India) with 1 cm path length matched quartz cells.

2.2. Materials. Pharmaceutical grade RTB certified to be 99.65% pure was obtained as a gift sample from Jubilant life Sciences, Nanjangud, Mysore, India, and used as received. Rizora-10 and Rizora-5 from Intas pharmaceuticals Ltd., Ahmedabad, India, both tablets were purchased from local commercial sources. 1,4-Dioxane and acetonitrile (spectroscopic grade) were purchased from Merck Specialities Pvt Ltd., Mumbai, India.

2.3. Reagents. p-Chloranilic acid (p-CA) and 2,3-dichloro-5,6-dicyanoquinone (DDQ) both 0.1% solutions (both from S.D. Fine Chem Ltd, Mumbai), were prepared freshly in 1,4-dioxane.

2.4. Standard RTB Stock Solution. For p-CA method, a 350 μg mL^{-1} RTB stock standard solution was prepared by dissolving 35 mg of pure drug in acetonitrile in a 100 mL volumetric flask and the solution was diluted to the mark with the same solvent; and the above 350 μg mL^{-1} RTB stock solution was diluted with acetonitrile to get 100 μg mL^{-1} and used for the assay in DDQ method.

2.5. Construction of Calibration Curves

2.5.1. p-CA Method (Method A). Varying aliquots of standard RTB solution equivalent to 14.0–245.0 μg mL^{-1} (0.2–3.5 mL of 350 μg mL^{-1}) were accurately measured and transferred into a series of 5 mL calibrated flasks and the total volume in each flask was brought to 3.5 mL by adding acetonitrile. After the addition of 1 mL of 0.1% p-CA solution, the volume was adjusted to the mark with acetonitrile and mixed well. The absorbance was measured at 530 nm against a reagent blank similarly prepared without adding RTB.

2.5.2. DDQ Method (Method B). Into a series of 5 mL calibration flasks, aliquots (0.2–3.5 mL) of standard 100 μg mL^{-1} RTB solution equivalent to 4.0–70.0 μg mL^{-1} RTB were accurately transferred to 5 mL calibrated flasks, and to each flask, 1 mL of 0.1% DDQ solution was added. The volume was made up to the mark with acetonitrile, mixed well and the absorbance of the red coloured C-T complex was measured at 600 nm against the reference blank similarly prepared.

Standard graphs were prepared by plotting the absorbance versus RTB concentrations, and the concentration of the unknown was read from the calibration graph or computed

Table 1: Comparison of the proposed and the existing visible spectrophotometric methods.

Sl. No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range, μg mL^{-1} and ε, l mol^{-1} cm^{-1}	Reaction time, min	Remarks	Ref.
(1)	(a) Methyl orange.	Extracted ion-pair complex was measured.	420	10–50 ($\varepsilon = 1.02 \times 10^4$)	2	Involves strict pH control and extraction step.	[5]
	(b) Ferric chloride, 2,2′-bipyridyl	Complex formed between reduced Fe(II) and 2,2′-bipyridyl measured.	490	4–20 ($\varepsilon = 1.0 \times 10^4$)	5	Heating required, multistep reaction.	
(2)	(a) 2,6-Dichloro quinone-4-chlorimide (DCQC)	Oxidative coupling product measured.	610	5–25 NA	NA	Narrow linear range.	[6]
	(b)1,2-Napthoquinone-4-sulphonic acid (NQS).	Color produced by replacement of imino group of RTB by sulfonate group of NQS measured.	480	15–75 NA	NA	Narrow linear range.	
	(c) Brucine, sodium metaperiodate.	Oxidative coupling product measured.	530	8–40 NA	NA	Narrow linear range.	
(3)	(a) Ferric chloride,1,10-phenanthroline.	Complex formed with 1,10-phenanthroline, ferric chloride measured.	510	2–10 ($\varepsilon = 3.85 \times 10^4$)	10	Heating required, multi step reaction, narrow linear range.	[7]
	(b) Folin-Ciocalteu reagent, sodium hydroxide.	Reduced FC-reagent was measured.	610	2–10 ($\varepsilon = 3.5 \times 10^3$)	5	Narrow linear range.	
	(c) Alizarin red	Extracted ion-pair complex was measured.	425	4–20 ($\varepsilon = 5.4 \times 10^3$)	2	Involves strict pH control and extraction step.	
(4)	7,7,8,8-Tetracyanoquin-odimethane (TCNQ)	Charge transfer complex measured	744	10–100 NR	15	Heating required, reagent is expensive.	[8]

Table 1: Continued.

Sl. No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range, μg mL^{-1} and ε, l mol^{-1} cm^{-1}	Reaction time, min	Remarks	Ref.
(5)	(a) Bromophenol blue (BPB)	Extraction free ion-pair complexes measured.	425	0.8–16 ($\varepsilon = 1.76 \times 10^4$)	5	Simple, rapid, sensitive, selective, and no heating step. Use of single reagent and no extraction step involved.	
	(b) Bromocresol purple (BCP)		425	1–20 ($\varepsilon = 1.96 \times 10^4$)	5		
	(c) Bromothymol blue (BTB)		420	1.2–24 ($\varepsilon = 1.63 \times 10^4$)	5		
(6)	NBS (a) JG	Resulting color measured	620	0.5–8 ($\varepsilon = 3.03 \times 10^4$)	15	Highly sensitive, no heating or extraction step, inexpensive instrumental setup, use of ecofriendly chemicals, and aqueous system	
	(b) CMG		540	1.5–30 ($\varepsilon = 1.15 \times 10^4$)	15		
(7)	(a) p-Chloranilic acid (p-CA)	Radical anion was measured.	530	14–245 ($\varepsilon = 1.30 \times 10^3$)	Instantaneous	Single-step instantaneous reaction, no heating or extraction step, no pH control, wide linear dynamic ranges.	Present methods
	(b) 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)	Radical anion was measured.	590	4–70 ($\varepsilon = 5.22 \times 10^3$)	Instantaneous		

NR: not reported; NA: not available.

from the respective regression equation derived using the absorbance-concentration data.

2.5.3. Assay Procedure for Pharmaceutical Preparations. Twenty tablets were weighed and pulverized. An amount of tablet powder equivalent to 35 mg RTB was transferred into a 100 mL volumetric flask and about 70 mL of acetonitrile was added to the flask. The content was shaken well for 20 min and diluted to the mark with the same solvent. The resulting solution was filtered through Whatman No. 42 filter paper and used for the assay by following the general procedure described for p-CA method. The resulting tablet extract ($350\,\mu g\,mL^{-1}$) was diluted to $100\,\mu g\,mL^{-1}$ with acetonitrile and suitable aliquot was used for the assay using the general procedure described for DDQ method.

2.5.4. Procedure for the Analysis of Placebo Blank and Synthetic Mixture. A placebo blank containing starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg), and sodium alginate (10 mg) was prepared and its solution prepared as described under tablets and then subjected to analysis.

A synthetic mixture was separately prepared by adding pure RTB (50 mg) to the above mentioned placebo blank and the mixture was homogenized. The mixture containing 35 mg of RTB was weighed and its extract was prepared as described for tablets. The extracts containing three different concentrations of RTB were subjected to assay according to the general procedures described earlier and the percentage recovery of RTB was computed.

2.5.5. Stoichiometric Relationship. Job's method of continuous variations of equimolar solutions was employed to establish the stoichiometry of the colored products. The solutions equivalent to 7.66×10^{-4} and 2.55×10^{-4} M RTB were prepared. Further, 7.66×10^{-4} M p-CA (method A) and 2.55×10^{-4} M DDQ (method B) solutions were prepared in 1,4-dioxane. A series of solutions was prepared in which the total volume of RTB and reagent was kept at 5 mL. The drug and reagent solutions were mixed in various complementary proportions (0 : 5, 1 : 4, 2 : 3,. . .,5 : 0, inclusive) and made up to mark with acetonitrile. The absorbance of the resulting colored species was measured at 530 nm in method A and 590 nm in method B.

3. Results and Discussion

3.1. Absorption Spectra. The reaction of p-CA as a π-acceptor with rizatriptan benzoate as *n*-electron donor results in the formation of an intense orange-red product which exhibits absorption maxima at 530 nm (Figure 1) due to the formation of the corresponding p-CA radical anion. DDQ also acts as a π-acceptor and the RTB-DDQ charge transfer complex resulted in the formation of an intense reddish violet color which exhibit three maxima at 600, 545, and 455 nm (Figure 2). These bands can be attributed to the formation of DDQ radical anions arising from the complete transfer of *n*-electrons from donor to acceptor moieties in acetonitrile. The absorption band at 590 nm was selected as analytical wavelength keeping in view the sensitivity of the reaction product and low blank absorbance.

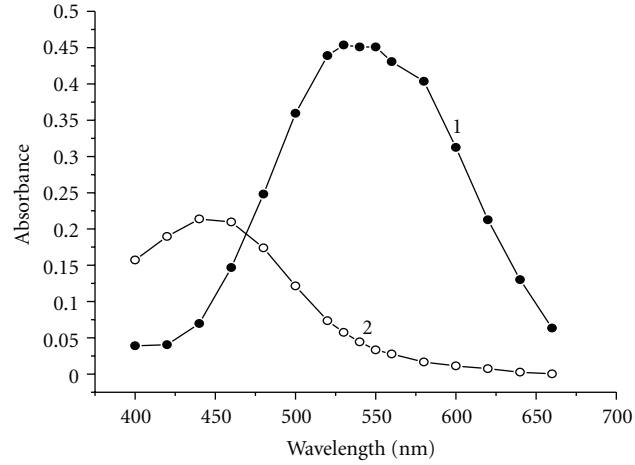


Figure 1: Absorption spectra of charge transfer complex of RTB-p-CA ($140\,\mu g\,mL^{-1}$ RTB): (1) p-CA radical anion (2) p-CA in acetonitrile.

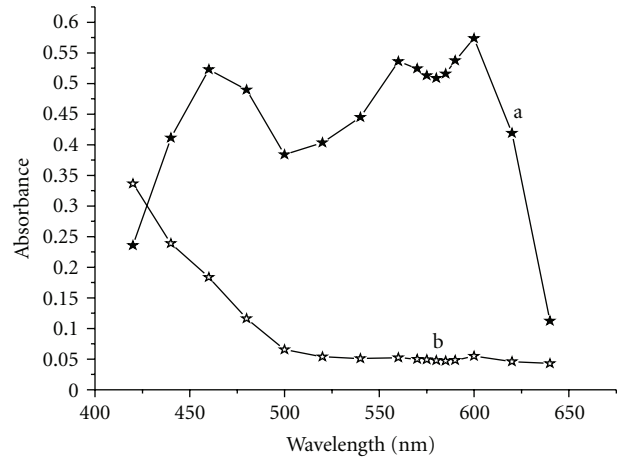
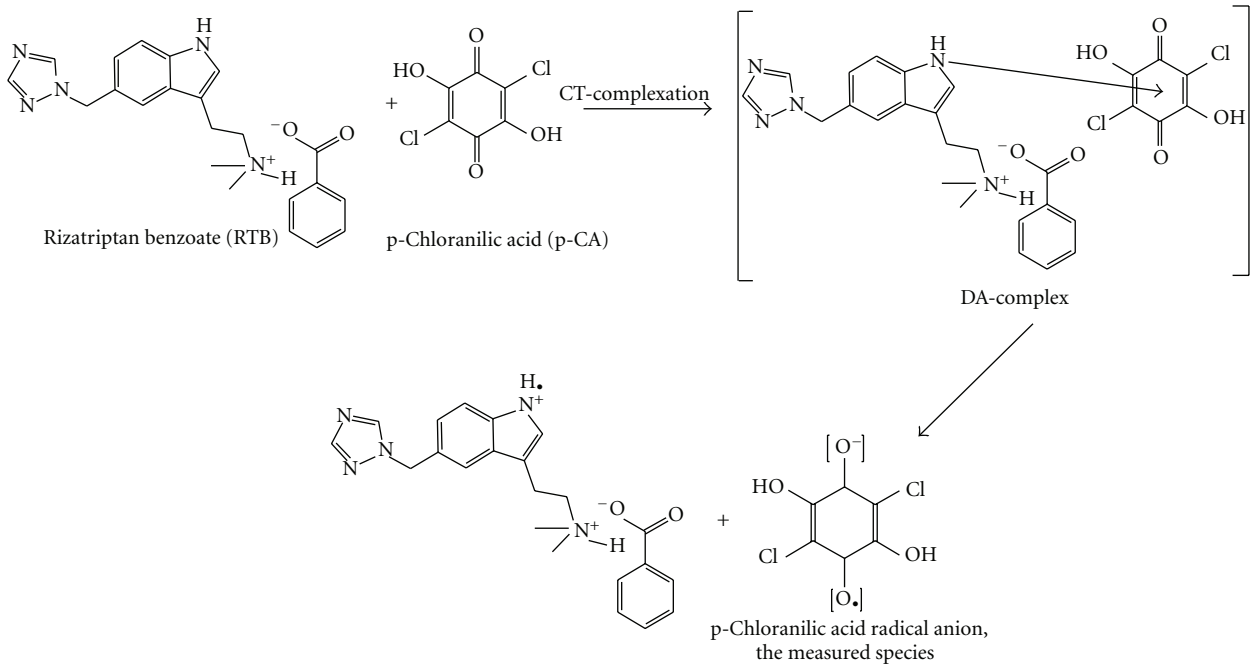


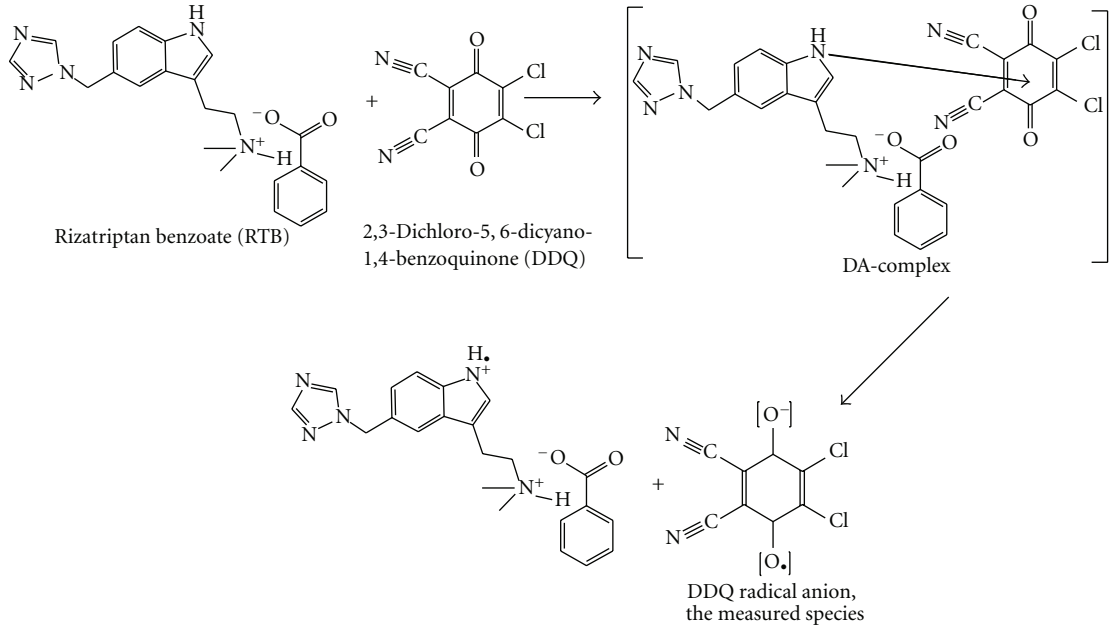
Figure 2: Absorption spectra of charge transfer complex of RTB-DDQ ($40\,\mu g\,mL^{-1}$ RTB): (a) DDQ radical anion (b) DDQ in acetonitrile.

3.2. Reaction Mechanism. The chemistry involved in the proposed methods is based on the reaction of the basic nitrogen (The more basic secondary amine) of RTB as *n*-donor with the π-acceptors, namely, p-CA and DDQ to form charge transfer complexes which subsequently dissociate into radical anions depending on the polarity of the solvent used. In polar solvents, such as acetonitrile, complete electron transfer from the donor to the acceptor moiety takes place with the formation of intensely colored radical anions [36], according to the following equation:

$$\overset{\bullet\bullet}{D} + A \longrightarrow \underbrace{\left[\overset{\bullet\bullet}{D} \longrightarrow A\right]}_{\text{charge transfer complex}} \xrightarrow{\text{Polar solvent}} \overset{\bullet+}{D} + \underbrace{A^{\bullet-}}_{\text{radical anion}} \quad (1)$$



Scheme 1: The tentative reaction mechanism for RTB-p-CA complex.



Scheme 2: The tentative reaction mechanism for RTB-DDQ complex.

The dissociation of the (D → A) complex is promoted by the high ionizing power of the acetonitrile. The tentative reaction mechanisms for RTB-p-CA and RTB-DDQ complexes are proposed and illustrated in Schemes 1 and 2, respectively.

3.3. The Effect of Different Experimental Variables

3.3.1. Effect of Reagent Concentration. The effect of the reagent concentration on the intensity of the color developed at the selected wavelengths was ascertained by adding different amounts of the reagents p-CA and DDQ to fixed concentrations of 150 and 40 μg mL^{-1} RTB in method A and method B, respectively. It was found that 1.0 mL of 0.1% p-CA and 1.0 mL of 0.1% DDQ solutions were sufficient for the production of maximum and reproducible color intensity and the sensitivity decreases by further addition of these reagents (Figure 3). Higher volumes are undesirable since they resulted in increasing blank absorbances.

3.3.2. Effect of Solvent. To dissolve RTB, acetonitrile was preferred to chloroform, dichloromethane, acetone, 2-propanol,

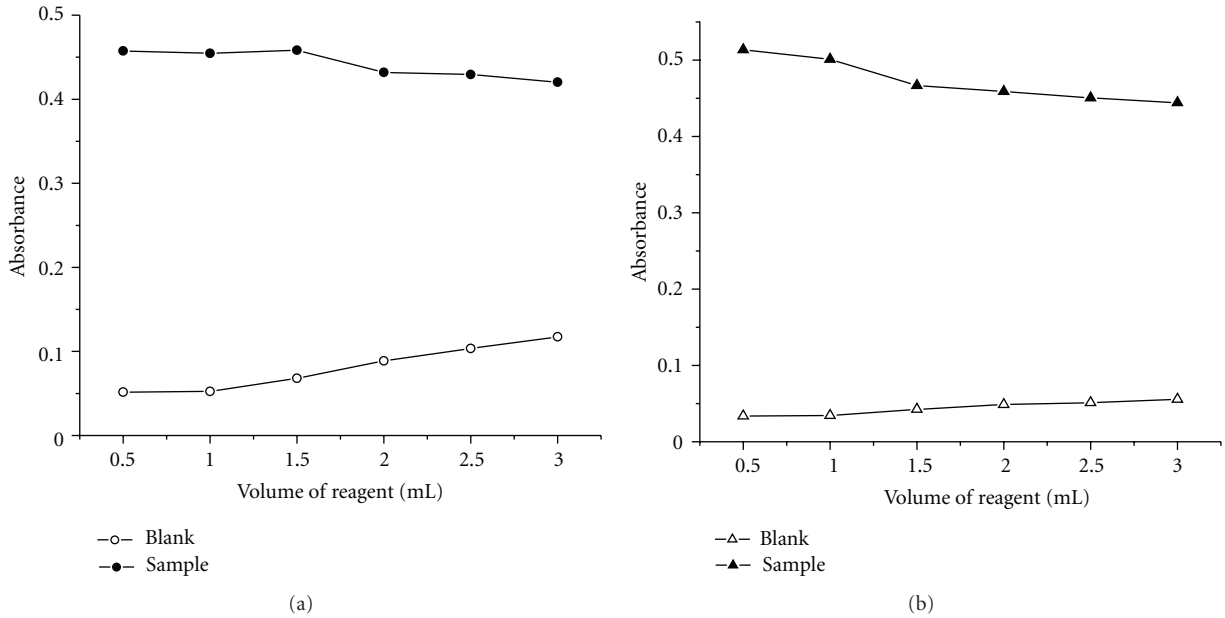


Figure 3: Effect of reagent concentration on color development: (a) RTB (150 μg mL^{-1}) and p-CA (0.1%); (b) RTB (40 μg mL^{-1}) and DDQ (0.1%).

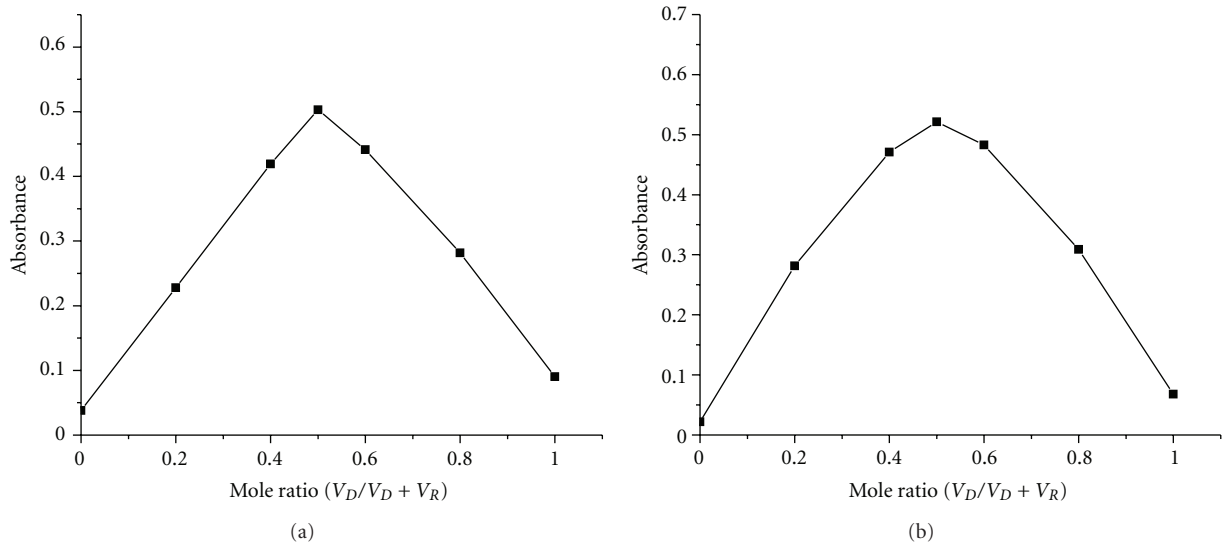


Figure 4: Job's Continuous-variation plot: (a) [RTB] and [p-CA] = 7.66 $\times 10^{-4}$ M (b) [RTB] and [DDQ] = 2.55 $\times$ 10^{-4} M.

dichloroethane, 1,4-dioxane, methanol or ethanol, because the complex formed in these solvents either had very low absorbance values or precipitated upon dilution. Whereas in the case of reagents, highly intense colored products were formed when 1,4-dioxane medium was used as a solvent to dissolve p-CA and DDQ. Therefore, acetonitrile and 1,4-dioxane were chosen as solvents to dissolve RTB and the reagents, respectively. Similarly, the effect of the diluting solvent was studied and the results showed that none of the solvents except acetonitrile favored sensitive and stable colored species in both methods. Thus, acetonitrile was used for dilution throughout the investigation.

3.3.3. Effect of Reaction Time. The optimum reaction time was determined by following the color development upon the addition of p-CA and DDQ solutions to the RTB solution at room temperature. Complete color development was attained instantaneously with both the reagents. The absorbance of these radical anions remained stable for at least 3 hrs and 2 hrs for method A and method B, respectively.

3.3.4. Molar Ratio of the Reaction. Job's continuous variations graph for the reaction between RTB and p-CA or DDQ (Figure 4) shows that the interaction occurs on an equimolar basis *via* the formation of a charge transfer complexes 1 : 1

(RTB: reagent). This finding was anticipated by the presence of more basic or electron donating centre (–NH) in the RTB.

3.4. Method Validation

3.4.1. Linearity. Under optimum experimental conditions for determination of the drug under study, the absorbance versus concentration plots were found to be linear over the concentration ranges stated in Table 2. The regression parameters calculated from the calibration graphs data, along with the standard deviations of the slope (S_b) and the intercept (S_a) are presented in Table 2. The linearity of the calibration graphs was demonstrated by the high values of the correlation coefficient (r) and the small values of the y-intercepts of the regression equations. The calculated molar absorptivity, Sandell sensitivity values of the methods A and B were also cited in Table 2.

3.4.2. Limit of Detection (LOD) and Limit of Quantification (LOQ). The LOD for the proposed methods were calculated using the equation [35]:

$$\text{LOD} = \frac{3.3 \times s}{b}, \tag{2}$$

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 "b" is the sensitivity, namely, the slope of the calibration graph.

The LOQ, defined as [36]:

$$\text{LOQ} = \frac{10 \times s}{b}. \tag{3}$$

Based on the above equations, the limits of detection and quantification were calculated and recorded in Table 2.

3.4.3. Assay Precision and Accuracy. In order to determine the precision of the proposed methods, solutions containing three different concentrations of RTB were prepared and analyzed in five replicates and the analytical results are summarized in Table 3. The low values of the percentage relative standard deviation (% R.S.D) and percentage relative error (% R.E) indicate the high precision and the good accuracy of the proposed methods. RSD (%) and RE (%) values were obtained within the same day to evaluate repeatability (intra day precision) and over five days to evaluate intermediate precision (inter day precision).

3.4.4. Robustness and Ruggedness. The robustness of the methods was evaluated by making small incremental changes in the volume of dye (1 ± 0.1 mL) and the effect of the change was studied on the absorbance of the C-T complex. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD ($\leq$1.28%). Method ruggedness was demonstrated having the analysis done by four analysts, and also by a single analyst performing analysis using four different cuvettes. Intermediate precision values (%RSD) in both instances were in the range 0.99–1.74 indicating acceptable ruggedness. These results are presented in Table 4.

3.4.5. Selectivity. In order to evaluate the selectivity of the proposed methods for the analysis of pharmaceutical formulations, the effect of the presence of common excipients, such as talc, starch, acacia, hydroxyl cellulose, sodium citrate, magnesium stearate, and sodium alginate was tested for possible interference in the assay by placebo blank and synthetic mixture analyses.

The analysis of synthetic mixture solution prepared as described earlier yielded percent recoveries which ranged between 99.02–102.1 and with standard deviation of 0.79–1.94 ($n = 5$). The results of this study showed that the inactive ingredients did not interfere in the assay indicating the high selectivity of the proposed method.

3.4.6. Applications to Analysis of Pharmaceutical Formulations. The proposed methods were successfully applied to the determination of RTB in two representative tablets Rizora-10 and Rizora-5. The results obtained are showed in Table 5 and were compared with those obtained by the reference method [17] by means of Student's t- and F-tests at 95% confidence level. The reference method consisted of the measurement of the absorbance of the tablet extract in water at 225 nm. In all cases, the average results obtained by the proposed methods and reference method were statistically identical, as the difference between the average values had no significance at 95% confidence level with respect to accuracy and precision, as demonstrated by the Student's t- and F-tests.

3.4.7. Recovery Study. To ascertain the accuracy and validity of the proposed methods, recovery experiment was performed *via* standard addition technique. To a fixed and known amount of RTB in tablet powder (preanalyzed), pure RTB was added at three concentration levels (50, 100, and 150% of the level present in the tablet) and the total was measured by the proposed methods. The determination with each concentration was repeated three times and the results of this study presented in Table 6 indicated that the various excipients present in the formulations did not interfere in the assay.

4. Conclusions

The methods are based on well-characterized charge-transfer complexation reaction, and have the advantages of simplicity, speed, accuracy and precision, and use of inexpensive equipment compared to the reported HPLC and LC-MS methods. Other advantage of these methods is wide linear range. The DDQ method is more sensitive than the p-CA method as seen from the higher molar absorptivity value. Moreover, the proposed methods can be performed at room temperature. Thus, the methods are useful for the quality control and routine analysis of RTB in pharmaceuticals since there is no interference from the common excipients that might be found in commercial formulations.

Table 2: Regression and analytical parameters.

Parameter	p-CA method	DDQ method
λ_{max}, nm	530	590
Beer's law limits (μg mL^{-1})	14–245	4–70
Molar absorptivity (l mol^{-1} cm^{-1})	1.30×10^3	5.22×10^3
Sandell sensitivity* (μg cm^{-2})	0.3002	0.0751
Limit of detection (μg mL^{-1})	1.36	0.60
Limit of quantification (μg mL^{-1})	4.11	1.83
Regression equation, Y**		
Intercept,(a)	0.0077	0.0078
Slope,(b)	0.0032	0.0127
Correlation coefficient (r)	0.9999	0.9999
Standard deviation of intercept (S_a)	0.00336	0.00402
Standard deviation of slope (S_b)	0.00002	0.00009

* Limit of determination as the weight in μg per mL of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of cross-sectional area 1 cm^2 and $l = 1$ cm. ** $Y = a + bX$, where Y is the absorbance, a is the intercept, b is the slope, and X is the concentration in μg mL^{-1}.

Table 3: Evaluation of intraday and interday precision and accuracy.

Method	RTB taken (μg mL^{-1})	Intraday ($n = 5$)			Interday ($n = 5$)		
		RTB found[a] (μg mL^{-1})	%RSD[b]	%RE[c]	RTB found[a] (μg mL^{-1})	%RSD[b]	%RE[c]
Method A	70.0	69.2	1.2	1.1	70.3	1.4	0.4
	140.0	142.2	1.0	1.6	142.3	1.1	1.7
	210.0	212.5	0.6	1.2	212.6	1.0	1.3
Method B	20.0	20.1	0.5	0.7	20.3	0. 9	1.6
	40.0	39.9	1.7	0.4	40.2	1.4	0.6
	60.0	60.8	1.1	1.4	61.0	1.5	1.6

[a] Mean value of five determinations; [b] Relative standard deviation (%); [c] Relative error (%).

Table 4: Robustness and ruggedness.

Method	RTB taken, μg mL^{-1}	Method robustness	Method robustness	
		Parameter altered		
		Reagent volume, mL[a]	Interanalysts	Intercuvettes
		RSD, % ($n = 3$)	RSD, % ($n = 4$)	RSD, % ($n = 4$)
Method A	70.0	0.9	1.7	1.5
	140.0	1.3	1.3	1.2
	210.0	1.0	1.1	1.3
Method B	20.0	1.0	1.0	1.6
	40.0	1.1	1.5	1.6
	60.0	1.4	1.6	1.4

[a] Volumes of reagent were 1 ± 0.1 mL.

Table 5: Results of analysis of tablets by the proposed methods.

Tablet brand name	Label claim mg/tablet	Found (percent of label claim ± SD)[a]		
		Reference method	Proposed methods	
			Method A	Method B
Rizora-10	10	100.17 ± 0.61	99.76 ± 0.78	101.12 ± 0.93
			$t = 0.93$	$t = 1.91$
			$F = 1.64$	$F = 2.32$
Rizora-5	5	99.89 ± 0.77	100.89 ± 1.13	101.01 ± 0.97
			$t = 1.60$	$t = 2.02$
			$F = 2.15$	$F = 1.59$

[a] Mean value of five determinations.
Tabulated t-value at the 95% confidence level is 2.78.
Tabulated F-value at the 95% confidence level is 6.39.

Table 6: Results of recovery study by standard addition method.

Tablets studied	Method A				Method B			
	RTB in tablets, $\mu g\,mL^{-1}$	Pure RTB added, $\mu g\,mL^{-1}$	Total found, $\mu g\,mL^{-1}$	Pure RTB recovered*, Percent ± SD	RTB in tablets, $\mu g\,mL^{-1}$	Pure RTB added, $\mu g\,mL^{-1}$	Total found, $\mu g\,mL^{-1}$	Pure RTB recovered*, Percent ± SD
Rizora 10	69.83	35.0	105.09	100.7 ± 1.98	20.22	10.0	30.32	101.0 ± 1.75
	69.83	70.0	140.55	101.0 ± 1.40	20.22	20.0	40.78	102.8 ± 0.57
	69.83	105.0	176.81	101.9 ± 1.00	20.22	30.0	50.62	101.3 ± 0.51
Rizora 5	70.62	35.0	106.38	102.2 ± 1.32	20.20	10.0	30.35	101.5 ± 0.49
	70.62	70.0	142.12	102.1 ± 0.85	20.20	20.0	40.83	103.2 ± 0.87
	70.62	105.0	176.75	101.1 ± 1.69	20.20	30.0	50.75	101.8 ± 0.51

* Mean value of three determinations.

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