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ORIGINAL ARTICLE

Sensitive and selective spectrophotometric assay of rizatriptan benzoate in pharmaceuticals using three sulphonphthalein dyes

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Abstract Three simple, rapid, selective and sensitive spectrophotometric methods are described for the determination of rizatriptan benzoate (RTB) in bulk drug and in tablets. The methods are based on the formation of intense yellow colored ion–pair complexes between RTB and sulphonphthalein acid dyes, namely, bromophenol blue (BPB), bromocresol purple (BCP), bromothymol blue (BTB) in chloroform medium. The colored products are measured at 425 nm (RTB–BPB complex, RTB–BCP complex) and 420 nm (RTB–BTB complex). The reactions were extremely rapid at room temperature and the absorbance values remained constant for 90 min (methods A and B) and over 12 h (method C). Beer's law was obeyed in the concentration ranges of 0.8–16.0, 1.0–20.0 and 1.2–24 μg ml^{-1} with molar absorptivity values of 1.76×10^4, 1.96×10^4 and 1.63×10^4 l mol^{-1} cm^{-1} for BPB, BCP and BTB methods, respectively. The limits of quantification (LOQ) were 0.39, 0.34 and 0.27 μg ml^{-1} for BPB, BCP and BTB methods, respectively. Other method validation parameters, such as precision, accuracy, robustness, ruggedness and selectivity, were satisfactory. The composition of the ion–pair was found to be 1:1 by Job's method. The proposed methods were successfully applied to the determination of RTB in commercial tablets. No interference was observed from common tablet adjuvants. Statistical comparison of the results with the reference method showed excellent agreement and indicated no significant difference in accuracy and precision.

1. Introduction

Rizatriptan benzoate (RTB) is chemically described as *N*,*N*-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indole-3-ethanamine monobenzoate (Fig. 1) and is a selective 5-hydroxytryptamine1B/1D (5-$HT_{1B/1D}$) receptor agonist. It has a weak affinity for other 5-HT receptor subtypes and was launched in 1998 for the acute treatment of migraine in adults (Goadsby, 1998). Migraine headache is recognized as a chronic disease with episodic occurrences, typically characterized by recurrent dis-

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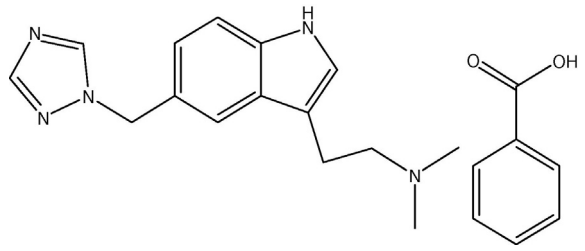

Figure 1 Structure of rizatriptan benzoate.

abling attacks of severe headaches, autonomic nervous system drug function and neurological aura symptoms. 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 (Gory et al., 2005; Oldman et al., 2002; Williamson et al., 1997).

Rizatriptan (RTB) is not official in any pharmacopoeia. A survey of literature reveals that RTB has been estimated in human plasma by liquid chromatography-electrospray tandem mass spectrometry, LC-MS/MS (Guo et al., 2006; Chen et al., 2006) and high performance liquid chromatography with fluorescence detection (Qin et al., 2006; Chen et al., 2004) and in human serum by LC-MS/MS (Vishwanathan et al., 2000). 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 (Amol et al., 2009; Kumari et al., 2010; Vivek et al., 2010; Altinoz et al., 2002), spectrofluorimetry (Altinoz et al., 2002), HPLC when present alone (Zecevic et al., 2008; Jocic et al., 2007) or in combination with other anti-migraine drugs (Sagar et al., 2010). A micro-emulsion electro-kinetic chromatography (MEEKC) has also been developed for the determination of RTB and its degradation products (Mahuzier et al., 2001).

To the best of our knowledge, there are four reports on the use of visible spectrophotometry for the assay of RTB in pharmaceuticals. The first report (Shanmukha Kumar et al., 2010) is concerned with two methods based on either the formation of ion–pair complex between RTB and methyl orange which is extracted into chloroform or redox-complexation reaction in which iron(III) is reduced by RTB and the resulting iron(II) complexed with 2,2′-bipyridyl. The second report (Dannana Gowry and Marothu Vamsi, 2007) describes three methods. First method was based on the oxidative coupling reaction between RTB with 2,6-dichloroquinone-4-chlorimide (DCQC) and the color developed was measured at 610 nm. Second method measures the color produced when sulfonate group of 1,2-napthoquinone-4-sulfonic acid (NQS) replaces imino group of indole moiety of RTB at 530 nm. In third method oxidative coupling product of RTB with brucine in presence of sodium metaperiodate was measured at 530 nm. Third report (Shanmukha Kumar et al., 2011) consists of three methods. First method is based on oxidation followed by complex formation reaction of RTB with 1,10-phenanthroline, ferric chloride and ortho-phosphoric acid to form an orange red colored chromogen measured at 510 nm. In the second method the blue colored chromogen formed by reduction of FC-reagent in alkaline medium by RTB was measured at 610 nm. Third method was based on the measurement of color developed by extracted ion association complex between alizarin red and RTB at 425 nm. Fourth report (Ramzia et al., 2011) renders one method based on the formation of charge transfer complex between RTB and 7,7,8,8 tetracyanoquinodimethane (TCNQ) measured at 744 nm.

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 under developed nations. In contrast, visible spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories. All the previously reported visible spectrophotometric methods are less sensitive and few methods require a rigid pH control and tedious liquid–liquid extraction steps, some methods have a relatively narrow linear range and involve heating step as cited in Table 1.

On the other hand, extraction-free spectrophotometry based on ion–pair reactions has, in recent years, received considerable attention for the quantitative determination of several pharmaceutical compounds (Al-Ghannam, 2006; Abdine et al., 2002; Manjunatha et al., 2008; Basavaiah et al., 2009 and Shahdousti et al., 2008) owing to its simplicity, speed, selectivity and sensitivity.

In the present communication, we report the development of three accurate and precise extraction-free spectrophotometric methods based on the chloroform soluble ion–pair complexes between RTB with sulphonphthalein dyes BPB, BCP and BTB. The absorbance measurements were made at 425 nm (BPB, BCP) or 420 nm with BTB without extraction. The methods were applied to the determination of RTB in tablets. No interference was observed from the tablet additives. The methods provide rapid and economic procedures, and more sensitive compared to the previously reported spectrophotometric methods (Shanmukha kumar et al., 2010, 2011; Dannana Gowry and Marothu Vamsi, 2007; Ramzia et al., 2011).

2. Experimental

2.1. Instrument

All the absorbance measurements were made using a Systronics model 106 digital spectrophotometer provided with 1 cm matched quartz cells.

2.2. Materials

Pharmaceutical grade rizatriptan benzoate (RTB) was received from Jubilant Life Sciences, Mysore, India, as a gift and used as received. The following formulations were obtained from commercial sources and subjected to analysis: Rizora-10 and Rizora-5 from Intas Pharmaceuticals Ltd., Ahmedabad, India.

2.3. Reagents and chemicals

All the reagents and solvents used were of analytical-reagent grade. Bromophenol blue (BPB), bromocresol purple (BCP) and bromothymol blue (BTB) (all from Loba Chemie Ltd., Mumbai, India) solutions were prepared daily as 0.1% BPB and BCP in dichloromethane and 0.025% BTB in chloroform.

Table 1 Comparison of the propo sed and the existing visible spectrophotometric methods.

Sl. No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range ($\mu g\ ml^{-1}$) 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	Less sensitive, involves extraction step	Shanmukha Kumar et al. (2010)
	(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	Less sensitive, heating required, multi-step reaction	
2.	(a) 2,6-dichloro quinone-4-chlorimide (DCQC)	Oxidative coupling product measured	610	5–25 NA	NA	Less sensitive	Dannana Gowry and Marothu Vamsi (2007)
	(b) 1,2-napthoquinone-4-sulfonic acid(NQS)	Color produced by replacement of imino group of RTB by sulfonate group of NQS measured	480	15–75 NA	NA	Less sensitive	
	(c) Brucine, sodium metaperiodate	Oxidative coupling product measured	530	8–40 NA	NA	Less sensitive	
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	Shanmukha Kumar et al. (2011)
	(b) Folin–Ciocalteu reagent, sodium hydroxide	Reduced FC-reagent was measured	610	2–10 ($\varepsilon = 3.5 \times 10^3$)	5	Less sensitive	
	(c) Alizarin red	Extracted ion–pair complex was measured	425	4–20 ($\varepsilon = 5.4 \times 10^3$)	2	Less sensitive, involves extraction step	
4.	7,7,8,8-tetracyanoquinodimethane (TCNQ)	Charge transfer complex measured	744	10–100 NR	15	Less sensitive, heating required	Ramzia et al. (2011
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	Proposed methods
	(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		

NR: Not reported; NA: Not available.

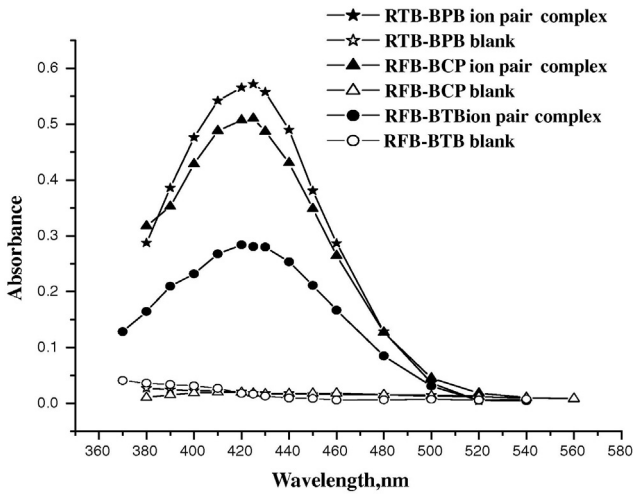

Figure 2 Absorption spectra of RTB–BPB (method A), RTB–BCP (method B) and RTB–BTB (method C) ion–pair complexes.

2.4. Standard stock solution

A stock standard solution of 200 μg ml^{-1} of RTB was prepared by dissolving accurately weighed 20 mg of pure drug in chloroform and diluting to the mark with chloroform in a 100 ml calibrated flask. This solution was diluted appropriately with chloroform to get working concentrations of 20, 25 and 30 μg ml^{-1} for use in methods A, B and C, respectively.

2.5. Recommended methods

2.5.1. BPB method

Varying aliquots of standard RTB solution in chloroform equivalent to 0.8–16.0 μg ml^{-1} (0.2–4.0 ml of 20 μg ml^{-1}) were accurately transferred into a series of 5 ml calibrated flasks and the total volume in each flask was brought to 4 ml by adding chloroform. After the addition of 1 ml of 0.1% BPB solution in dichloromethane, the content was mixed well and the absorbance of the ion–pair complex was measured at 425 nm against a reagent blank similarly prepared without adding RTB solution.

2.5.2. BCP method

Aliquots of 0.2–4.0 ml RTB standard solution in chloroform (25 μg ml^{-1}) equivalent to 1.0–20.0 μg ml^{-1} were measured accurately and transferred into a series of 5 ml calibrated flasks. The total volume in each flask was brought to 4 ml by adding chloroform. To each flask, 1 ml of 0.1% BCP solution in dichloromethane was added and mixed well. The absorbance of the resulting yellow colored chromogen was measured at 425 nm against reagent blank.

2.5.3. BTB method

Into a series of 5 ml calibration flasks, aliquots (0.2–4.0 ml) of standard RTB solution (30 μg ml^{-1}) equivalent to 1.2–24.0 μg ml^{-1} RTB were accurately transferred and the total volume in each flask was brought to 4 ml by adding chloroform. To each flask 1 ml of 0.025% BTB solution in chloroform was added and mixed well. The absorbance of the yellow colored ion–pair complex was measured at 420 nm against the reagent blank.

In all the methods, a calibration graph was prepared by plotting the increasing absorbance values *versus* concentration of RTB. The concentration of RTB was read from the calibration graph or computed from the respective regression equation derived using Beer's law data.

2.5.4. Procedure for tablets

Twenty tablets containing RTB were weighed and pulverized into a fine powder. An amount of tablet powder equivalent

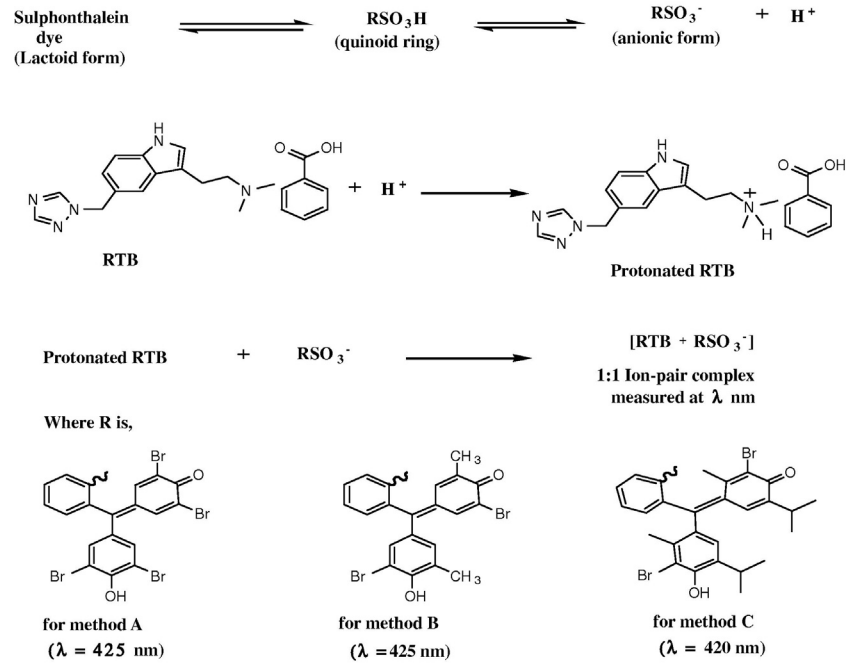

Figure 3 The probable mechanism for the formation of ion–pair complexes with BPB, BCP and BTB in methods A, B and C, respectively.

to 10 mg of RTB was weighed into a 100 ml calibrated flask, 50 ml of chloroform added and the mixture was shaken for 20 min, then the volume was made up to the mark with the same solvent, mixed well and filtered using Whatman No. 42 filter paper. The first 10 ml portion of the filtrate was discarded and the subsequent portion was diluted appropriately with chloroform to get the working concentrations (20, 25 and 30 μg ml^{-1} RTB) and subjected to analysis following the procedures described above.

2.5.5. Placebo blank analysis

A placebo blank of the composition: talc (35 mg), starch (25 mg), acacia (20 mg), methyl cellulose (30 mg), sodium citrate (20 mg), magnesium stearate (25 mg) and sodium alginate (25 mg) was transferred to a 50 ml calibration flask, 30 ml of chloroform was added and the mixture was shaken for 20 min. Then the volume was made up to the mark with the same solvent, mixed well and filtered using Whatman No. 42 filter paper and subjected to analysis using the procedures described above.

2.5.6. Synthetic mixture analysis

To the placebo blank of the composition described above, 10 mg of RTB was added and homogenized, transferred to a 100 ml calibrated flask and the solution was prepared as described under "Procedure for tablets", and then subjected to analysis by the procedures described above.

2.5.7. Procedure for stoichiometric relationship

Job's method of continuous variations of equimolar solutions was employed: 2.55×10^{-5} M each of RTB in chloroform and BPB in dichloromethane (Method A) solutions, 3.19×10^{-5} M each of RTB in chloroform and BCP in dichloromethane (Method B) solutions and 5.1×10^{-5} M each of RTB and BTB in chloroform (Method C) solutions were prepared separately. A series of solutions was prepared in which the total volume of RTB and reagent was kept at 5 ml. The drug and reagent were mixed in various complementary proportions (0:5, 1:4, 2:3,..., 5:0, inclusive) and completed as directed under the recommended procedures. The absorbance of the resultant ion–pair complex was measured at 425 nm in methods A and B, and 420 nm in method C, respectively.

3. Results and discussion

RTB was found to react with the dyes in dichloromethane or chloroform medium to produce an intense and stable ion–pair complex. Chemically, the structure of RTB possesses an aliphatic tertiary amino group and features its basic nature. This structure suggests the possibility of utilizing an acidic dye as chromogenic reagent. In dichloromethane or chloroform RTB does not absorb in the visible region. The dyes employed have insignificant absorbance (Fig. 2). The formation of intense yellow colored product with an absorption maximum at 425 or 420 nm is due to an opening of lactoid ring and subsequent formation of quinoid group (Ashour et al., 2006). It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulfonic acid group, the quinoid body must predominate. The more basic aliphatic tertiary amine, due to the two electron releasing methyl groups, gets protonated prior to other aromatic amines. Finally, protonated RTB forms ion–pair with the anionic form of the dye. The possible reaction pathway is shown in Fig. 3.

3.1. Optimization of variables and method development

3.1.1. Effect of solvent

In order to select a suitable solvent for preparation of the reagent solutions used in the study, the reagents were prepared separately in different solvents such as 1,4-dioxane, chloroform, acetonitrile, acetone and dichloromethane, and the reaction of RTB with BPB, BCP or BTB was followed. In methods A and B as shown in Fig. 4, dichloromethane was best suited for the preparation of BPB and BCP solutions, respectively. The chloroform solvent was found to be the ideal solvent for preparation of BTB for method C (Fig. 4). Similarly, the effect of the diluting solvent was studied for all methods and the results showed that none of the solvents except chloroform formed sensitive and stable colored species in methods A, B and C. Therefore, chloroform was used for dilution throughout the investigation.

3.1.2. Effect of dye concentration

The influence of the concentration of BPB, BCP and BTB on the intensity of the color developed at the selected wavelength was studied (Fig. 5). One milliliter each of 0.1% BPB and BCP or 0.025% BTB was sufficient to produce maximum and reproducible color with minimum blank absorbance.

3.1.3. Effect of reaction time and stability

The optimum reaction time for the development of color at ambient temperature (30 ± 2 °C) was studied and it was found

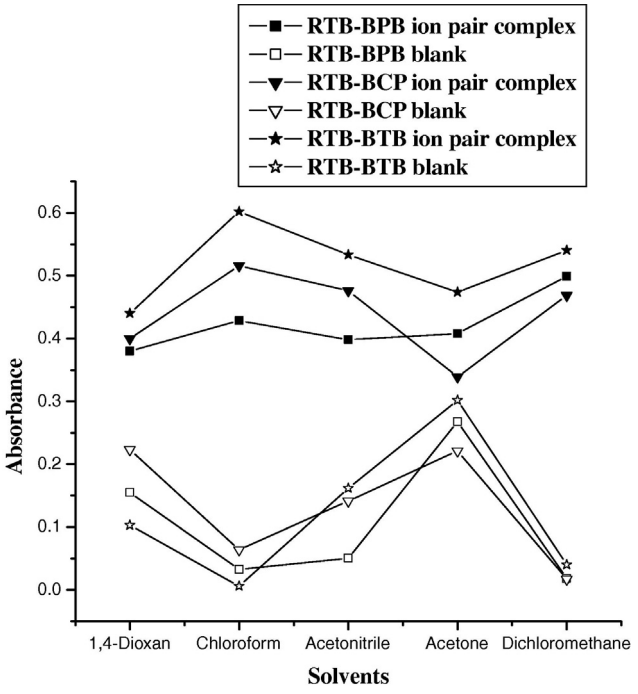

Figure 4 Effect of solvents on the formation of RTB–BPB complex (8 μg ml^{-1} RTB), RTB–BCP complex (10 μg ml^{-1} RTB) and RTB–BTB complex (12 μg ml^{-1} RTB).

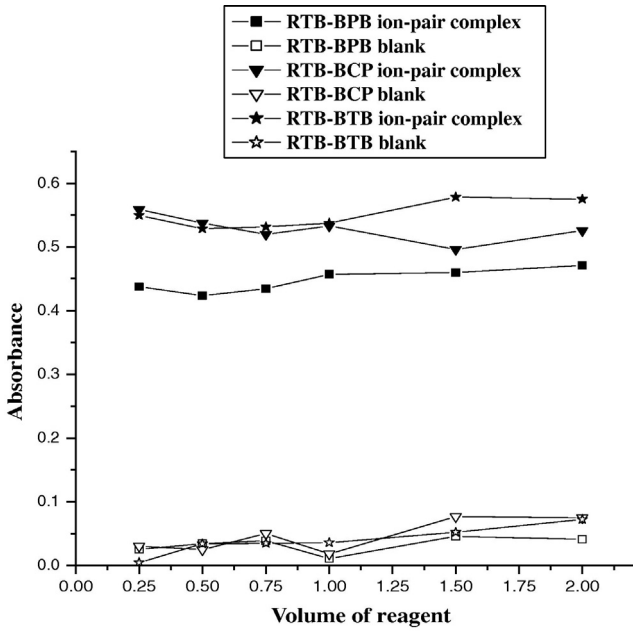

Figure 5 Effect of reagent concentration on the formation RTB–BPB complex (8 μg ml^{-1} RTB), RTB–BCP complex (10 μg ml^{-1} RTB) and RTB–BTB complex (12 μg ml^{-1} RTB).

that a 5 min standing time was sufficient for the complete formation of ion–pair complexes in all the methods. The formed color was stable for 1 h in methods A and B and it was stable more than 12 h in method C.

3.1.4. Study of composition of ion–pair complex and its conditional stability constant

The composition and conditional stability constant of the RTB–BPB or RTB–BCP or RTB–BTB complex formed were evaluated by applying Job's method of continuous variations (Douglas and Donald, 1971) using equimolar concentrations of RTB (prepared by following the general procedure) and the dye. The concentration of dye used in method A was 2.55×10^{-5} M, method B was 3.19×10^{-5} M, whereas concentration of RTB and dye is 5.10×10^{-5} M in method C. The experiments were performed by mixing equimolar solutions of drug and reagent by maintaining the total volume at 5.0 ml. The plots of the mole ratio between drug and reagent *versus* the absorbance values were prepared (Fig. 6), and the results revealed that the formation of ion–pair complex between drug and reagent followed a 1:1 reaction stoichiometry.

The conditional stability constant (K_f) of the ion–association complex was calculated from the continuous variation data using the following equation (Erk, 2003):

$$K_f = \frac{A/A_m}{[1 - A/A_m]^{n+2} C_M (n)^n}$$

where A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_M is the mole concentration of drug at the maximum absorbance and n is the stoichiometry which BPB/BCP/BTB ion associates with RTB. The log K_f values were found to be 7.75, 6.47 and 7.19 for BPB method, BCP method, and BTB method, respectively.

3.2. Method validation

3.2.1. Analytical parameters

A linear relation was found to exist between absorbance and the concentration of RTB in the ranges given in Table 2. The calibration graph in each case is described by the equation:

$$Y = a + bX$$

where Y = absorbance, a = intercept, b = slope and X = concentration in μg ml^{-1} obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, the limits of detection and quantification calculated as per the current ICH guidelines (ICH, 2005) are compiled in Table 2 and are indicative of the sensitivity of the methods. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae:

$$\text{LOD} = 3.3\sigma/s \quad \text{and} \quad \text{LOQ} = 10\sigma/s$$

where σ is the standard deviation of five reagent blank determinations and s is the slope of the calibration curve.

3.2.2. Accuracy and precision

The accuracy and precision of the methods were evaluated by performing seven replicate analyses on pure drug solution at three different concentration levels (within the working range). The relative error (RE %), an indicator of accuracy (Table 3) was within 1.33 and within day precision, also called the repeatability, expressed as relative standard deviation (RSD %) was less than 1.25 indicating high accuracy and repeatability of the methods. The reproducibility of the methods also known as the day-to-day precision was evaluated by performing replicate analyses on pure drug solution at three levels over a period

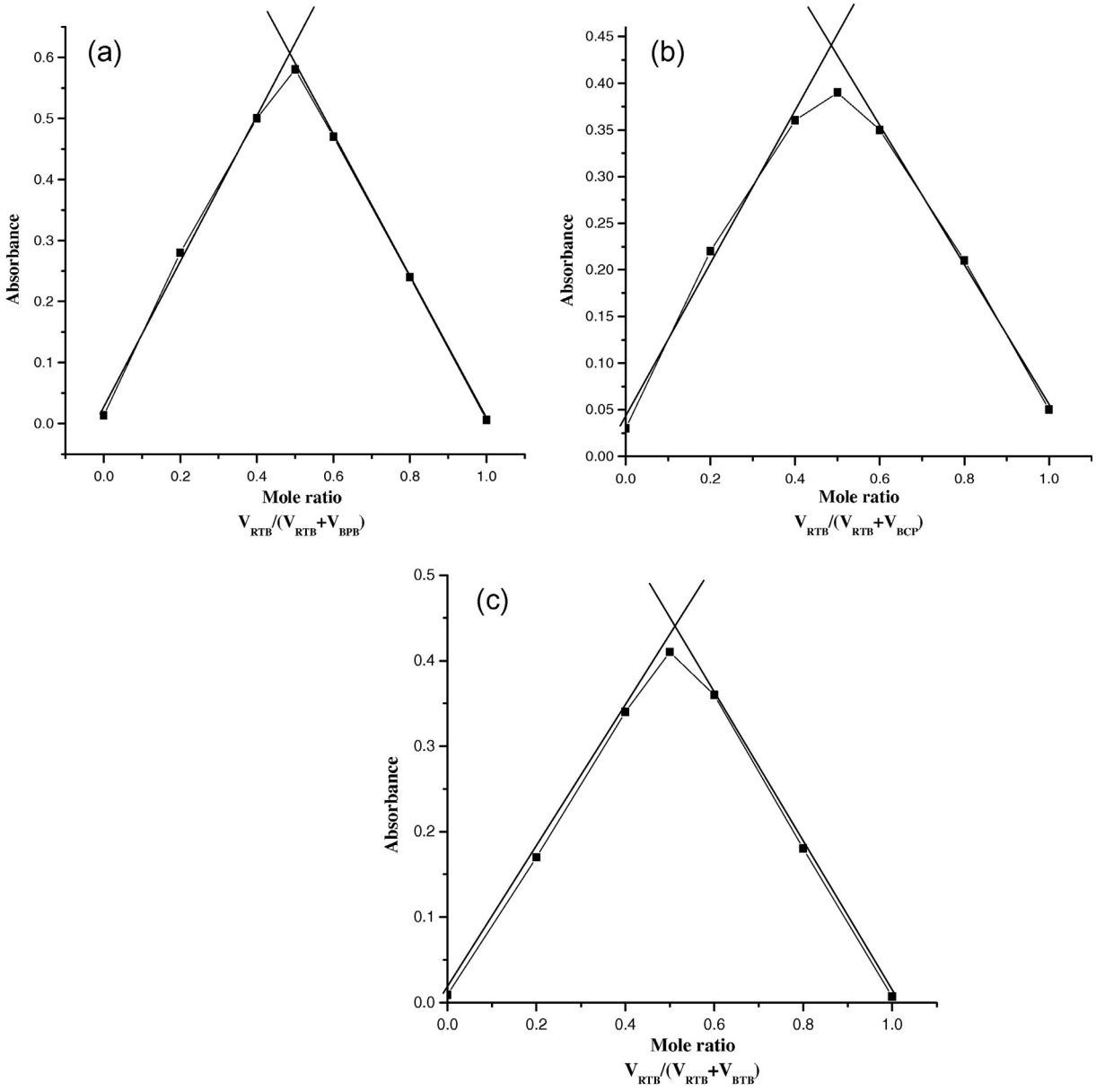

Figure 6 Job's plots obtained for (a) 2.55×10^{-5} M RTB and BPB ion–pair complex (b) 3.19×10^{-5} M RTB and BCP ion–pair complex and (c) 5.1×10^{-5} M RTB and BTB ion–pair complex.

Table 2 Regression and analytical parameters.

Parameter	BPB method	BCP method	BTB method
λ_{max} (nm)	425	425	420
Beer's law limits (μg ml^{-1})	0.8–16.0	1.0–20.0	1.2–24.0
Molar absorptivity (l mol^{-1} cm^{-1})	1.76×10^4	1.96×10^4	1.63×10^4
Sandell sensitivity[a] (μg cm^{-2})	0.0222	0.0198	0.0240
Limit of detection (μg ml^{-1})	0.13	0.11	0.09
Limit of quantification (μg ml^{-1})	0.39	0.34	0.27
Regression equation, Y[b]			
Intercept (a)	−0.0150	0.0018	−0.0005
Slope (B)	0.0502	0.0504	0.0418
Correlation coefficient (r)	0.9996	0.9997	0.9999
Standard deviation of intercept (S_a)	0.00603	0.00641	0.00359
Standard deviation of slope (S_b)	0.00067	0.00057	0.00027

[a] 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.

[b] $Y = a + bX$, 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 intra-day and inter-day precision and accuracy.

Method	RTB taken (μg ml^{-1})	Intra-day (n = 7)			Inter-day (n = 5)		
		RTB found (μg ml^{-1})[a]	%RSD[b]	%RE[c]	RTB found (μg ml^{-1})[a]	%RSD[b]	%RE[c]
BPB method	4.00	4.04	0.40	1.07	4.07	0.69	1.33
	8.00	8.06	0.32	0.79	8.11	0.41	0.87
	12.00	12.14	0.16	1.18	12.15	0.34	1.25
	5.00	5.04	0.35	0.71	4.98	0.58	0.92
BCP method	10.00	9.92	1.25	0.77	9.96	0.92	0.89
	15.00	14.92	0.64	0.50	15.03	1.06	0.67
	6.00	5.94	0.55	0.98	5.98	0.76	1.13
BTB method	12.00	12.04	0.90	0.31	12.09	1.12	0.51
	18.00	17.95	0.56	0.28	18.03	0.83	0.36

[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 ruggedness	
		Parameters altered			
		Reagent volume (ml)[a] RSD (%) (n = 3)	Reaction time[b] RSD (%) (n = 3)	Inter-analysts[a] RSD (%) (n = 4)	Inter-cuvettes[a] RSD (%) (n = 4)
BPB method	4.00	1.21	1.01	1.76	1.38
	8.00	0.98	1.22	1.31	1.17
	12.00	1.13	0.96	1.03	0.99
BCP method	5.00	1.23	1.44	0.98	1.43
	10.00	1.11	1.24	1.37	1.54
	15.00	1.09	1.16	1.59	1.39
BTB method	6.00	1.19	1.03	0.96	1.53
	12.00	0.95	1.26	1.44	1.09
	18.00	1.30	1.23	1.06	1.21

[a] In all methods, the volume of reagent was 0.8, 1.0 and 1.2 ml.
[b] The reaction time was 4, 5 and 6 min for methods A, B and C.

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		
			BPB method	BCP method	BTB method
Rizora-10	10	100.17 ± 0.61	99.76 ± 0.97 t = 0.80 F = 2.53	100.8 ± 1.03 t = 1.21 F = 2.85	102.0 ± 1.32 t = 3.16 F = 3.37
Rizora-5	5	99.89 ± 0.77	101.1 ± 1.12 t = 1.86 F = 2.51	101.1 ± 1.14 t = 2.02 F = 2.19	99.31 ± 0.78 t = 1.18 F = 1.03

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

Table 6 Results of recovery study by standard addition method.

Tablets studied	BPB method				BCP method				BTB method			
	RTB in tablets ($\mu g\ ml^{-1}$)	Pure RTB added ($\mu g\ ml^{-1}$)	Total found ($\mu g\ ml^{-1}$)	Pure RTB recovered[a], 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	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	3.99	2.0	6.01	101.0 ± 1.37	5.04	2.5	7.55	102.0 ± 0.48	6.12	3.0	9.17	102.0 ± 2.12
10	3.99	4.0	8.08	102.3 ± 0.66	5.04	5.0	10.09	101.8 ± 0.56	6.12	6.0	12.14	100.3 ± 0.87
	3.99	6.0	10.05	101.0 ± 0.48	5.04	7.5	12.52	100.3 ± 0.50	6.12	9.0	15.31	102.1 ± 0.52
Rizora	4.04	2.0	6.11	103.5 ± 0.90	5.06	2.5	7. 54	101.6 ± 0.83	5.96	3.0	9.04	102.7 ± 0.49
5	4.04	4.0	8.15	102.8 ± 0.33	5.06	5.0	10.15	103.0 ± 0.57	5.96	6.0	12.01	100.8 ± 0.87
	4.04	6.0	10.13	101.5 ± 0.48	5.06	7.5	12.71	102.8 ± 0.51	5.96	9.0	15.12	101.8 ± 0.30

[a]Mean value of three determinations.

of five days, preparing all solutions afresh. The day-to-day RSD values (Table 3) were less than 2% reflecting the usefulness of the methods in routine analysis.

3.2.3. Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of dye (1 ± 0.2 ml) and contact time (5 ± 1 min), and the effect of the changes were studied on the absorbance of the ion–pair complex. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as %RSD ($\leqslant$1.44%). 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 0.96–1.76 indicating acceptable ruggedness. These results are presented in Table 4.

3.2.4. Effect of co-formulated substances

The effect of co-formulated substances was tested by placebo blank and synthetic mixture analyses. A convenient aliquot of the placebo blank solution was subjected to analysis according to the recommended procedures. There was no interference by the inactive ingredients as indicated by the near blank absorbance in all the methods.

The analyses of synthetic mixture solution yielded percent recoveries which ranged between 98.68–101.31 and with standard deviation of 0.57–1.21. The results of this study indicated that the inactive ingredients did not interfere in the assay.

3.2.5. Application to the analysis of tablets

In order to evaluate the analytical applicability of the proposed methods to the quantification of RTB in commercial tablets, the results obtained by the proposed methods were compared with those of the reference method (Altinoz et al., 2002) by applying Student's *t*-test for accuracy and *F*-test for precision. The reference method consisted of the measurement of the absorbance of the tablet extract in water at 225 nm. The results (Table 5) show that the Student's *t*- and *F*-values at 95% confidence level are less than the theoretical values, indicating that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

3.2.6. Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure RTB at three levels (50%, 100% and 150% of that found in tablet powder) and the total was determined by the proposed methods. The percent recovery of pure RTB added was in the range 100.3–103.5% with standard deviation of 0.30–2.12 (Table 6) indicating that the recovery was good, and that the co- formulated substance did not interfere in the determination.

4. Conclusions

This paper describes for the first time the application of extraction-free ion–pair complexation reaction for the quantification of RTB in pharmaceutical formulations. Compared with the

existing visible spectrophotometric methods, the proposed methods are simple, selective, cost-effective, free from auxiliary reagents and more sensitive as can be seen from the molar absorptivity values. Moreover, the proposed methods are free from tedious experimental steps such as heating or extraction step unlike the previously reported methods (Shanmukha kumar et al., 2010, 2011; Dannana Gowry and Marothu Vamsi, 2007; Ramzia et al., 2011). The most attractive feature of these methods is its relative freedom from interference by the usual tablet diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical formulations. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. Hence, recommended methods are well suited for the assay and evaluation of drug in quality control laboratories.

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