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Investigation and optimization of the use of spectrophotometry for the assay of rabeprazole sodium with *in situ* bromine and two dyes as reagents

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ABSTRACT

Two simple and sensitive spectrophotometric methods are described for the assay of rabeprazole sodium (RPS) in pure and tablets using bromate-bromide as the bromination reagent in acid medium, and two dyes as subsidiary reagents. The two methods are based on the bromination of RPS by a known excess of *in situ* generated bromine followed by determination of unreacted bromine by reacting with a fixed amount of methyl orange (method A) or indigo carmine (method B) and measuring the absorbance at 508 or 610nm. In both methods, the amount of bromine reacted corresponds to the amount of RPS. The experimental conditions for the assay have been optimized. In two methods, the absorbance is found to increase linearly with the concentration of RPS at the respective wavelengths. Beer's law is obeyed over the ranges 0.40-2.80 and 0.80-5.60μg mL^{-1} for method A and method B respectively and the respective molar absorptivity values are 1.29×10^5 and 5.38×10^4 l mol^{-1} cm^{-1}. The limits of detection and quantification are reported for both methods. The statistical analysis of the methods was validated according to the present ICH guidelines. The proposed methods were applied to the analysis of tablet form of RPS and the results tallied well with the label claim. No interference was observed from the concomitant substances normally added to tablets.

KEYWORDS

Rabeprazole sodium;
Spectrophotometry;
Bromate-bromide;
Dyes;
Tablets.

INTRODUCTION

Rabeprazole sodium (RPS) is chemically known as 2-({[4- (3-methoxy propoxy)-3-methyl-2-pyridinyl] methyl}sulfiyl)-1H-benzimidazole sodium ($C_{18}H_{20}N_3NaO_3S$) is shown in figure 1. Rabeprazole sodium represents the newest class of antisecretory reagents that are well known for their proton pump (H/ K-ATPase) inhibitor activity, most profoundly diminishing gastric acid secretion and thus, lowering the luminal concentration of hydrogen ions. It has recently been demonstrated that rabeprazole sodium is the only proton pump inhibitor among tested (omeprazole, lansoprazole) that augments gastric mucin content[1]. It has proven efficacy in healing, symptom relief and prevention of relapse peptic ulcers and gastrooesophageal reflux disease. It is an important alternative to H_2 antagonists and an additional treatment option to other proton pump inhibitors in the management of acid related disorders. In view of the

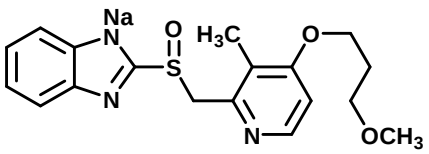

Figure 1 : Structure of rabeprazole sodium

$$BrO_3^- + 5\,Br^- + 6H^+ \longrightarrow 3\,Br_2 + 3\,H_2O$$

$$RPS + \text{Known excess of } Br_2 \longrightarrow \text{Oxidation product of RPS} + \text{Unreacted } Br_2$$

$$\text{Unreacted } Br_2 + \text{Fixed amount of dye} \longrightarrow \text{Oxidation product of dye} + \text{Unreacted dye (Colored)}$$

Scheme 1

great importance and wide use of rabeprazole sodium, different analytical methods have been reported for its determination which includes high performance liquid chromatography[2-4], liquid chromatography coupled with tandem mass spectrometry[5], capillary electrophoresis[6]. As far as sensitive and economical methods of assay are concerned, few spectrophotometric methods have been reported for the quantification of RPZ based on extractable ion pair complexes[7], oxidative coupling with 3-methyl 2-benzothiazolinone hydrazone hydrochloride and 1-chloro-2, 4-dinitrobenzene[8], derivative spectrometry[9] and UV-spectrophotometry[10,11] have been used to determine pharmaceuticals in dosage forms. So far no spectrophotometric method based on oxidation with bromate-bromide mixture have been reported for determination of rabeprazole sodium in tablet forms. Two simple and sensitive spectrophotometric methods for the analysis of RPS from pharmaceutical dosage forms are reported here. The methods utilize bromate-bromide mixture and methyl orange and indigo carmine as reagents, which have successfully been used for the sensitive spectrophotometric determination of many bioactive substances[12-18]. The proposed methods have the advantages of speed and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for industrial quality control.

Apparatus

All absorption spectra were made using UV-Vis-NIR spectrophotometer (Simadzu 1601, Japan) equipped with 1 cm matched quartz cells by using a personal computer loaded with the UV-PC 3.9 software package. An electronic micro balance (Sartorius MC 5, Germany) was used for weighing the solid materials.

Materials and reagents

All solvents and reagents used were of analytical grade. Double-distile water was used throughout the investigation. A stock solution equivalent to 1000μg mL^{-1} $KBrO_3$ containing a large excess of KBr was prepared by dissolving accurately weighed 100 mg of $KBrO_3$ (Qualigens, Mumbai, India) and 1.0g of KBr (Reidel Chemicals, India) in water and diluting to 100mL in a volumetric flask. The above solution was diluted appropriately with water to get 10 and 30μg mL^{-1} $KBrO_3$ for use in method A, and method B respectively. To prepare 50μg mL^{-1} methyl orange for method A, first, a 500 μg mL^{-1} dye solution was prepared by dissolving accurately weighed 59mg of dye (S.d. Fine Chem., Mumbai, India, 85% dye content) in water and diluting to 100mL in a calibrated flask. It was diluted to 10-fold to obtain the required concentration. For method B, a 1000μg mL^{-1} stock standard solution was first prepared by dissolving accurately weighed 112 mg of dye (S.d. Fine Chem., Mumbai, India, 90% dye content) in water and diluting to volume in a 100mL calibrated flask. The solution was then diluted 5- fold to get the working concentration of 200μg mL^{-1}. Hydrochloric acid (~5M) was prepared by diluting required volume of concentrated acid (S.d.fine-chem Ltd., Mumbai, India) to 100mL with water for two methods.

Standard RPS was procured from Dr. Reddy's laboratories, Hyderabad, India. A stock standard solution containing 200μg mL^{-1} of RPS was prepared by dissolving accurately weighed 20 mg of pure drug in a 100mL of calibrated flask with double distil water. The solution was further diluted with distil water to get working concentrations of 20μg mL^{-1} of RPS for two methods.

Method using methyl orange (method A)

Aliquots (0.2 to 1.4mL) of standard RPS solution (20μg mL^{-1}) corresponding to 0.4-2.8μg mL^{-1} were transferred into a series of 10mL calibrated flasks. To each flask was added 1mL of 5M hydrochloric acid followed by 1.5mL of bromate-bromide mixture (10μg mL^{-1} w.r.t $KBrO_3$). The content was mixed well and the flasks were set aside for 25 min with occasional shaking. Finally, 1.5mL of 50μg mL^{-1} methyl orange solution was added to each flask, diluted to the mark with water and the absorbance of solution was mea-

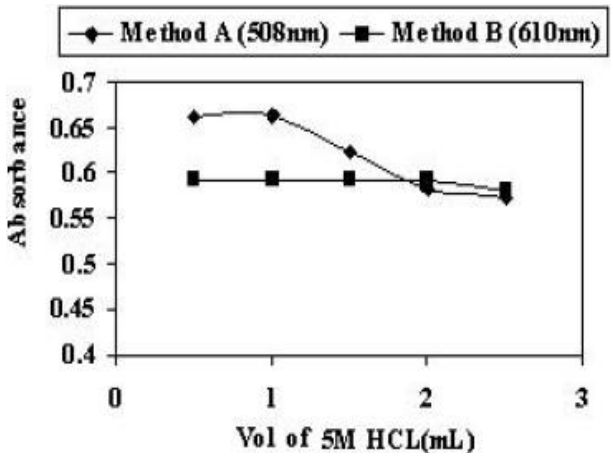

Figure 2 : Effect of acid on 2.0μg mL^{-1} and 4.0μg mL^{-1} of RPS for method A and method B respectively

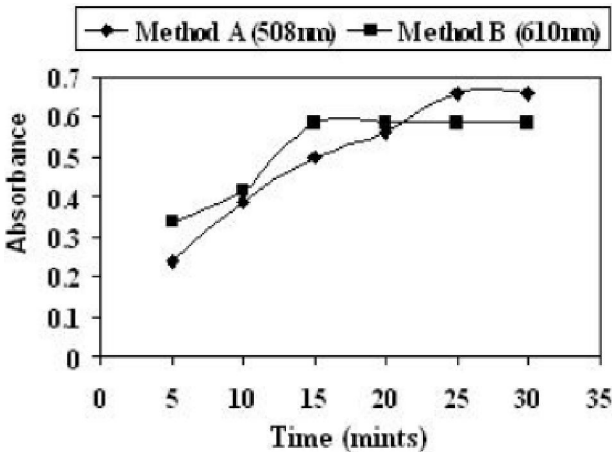

Figure 3 : Effect of time on 2.0μg mL^{-1} and 4.0μg mL^{-1} of RPS for method A and method B respectively

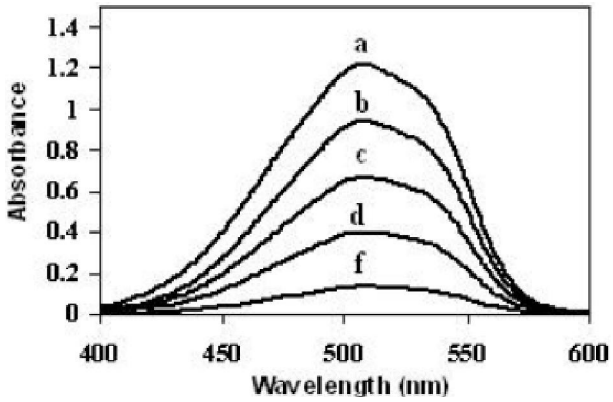

Figure 4 : (a) Blank (without RPS) (b) 2.8μg mL^{-1} (c) 2.0 μg mL^{-1} (d) 1.2 μg mL^{-1} (e) 0.2μg mL^{-1} of RPS measured against water

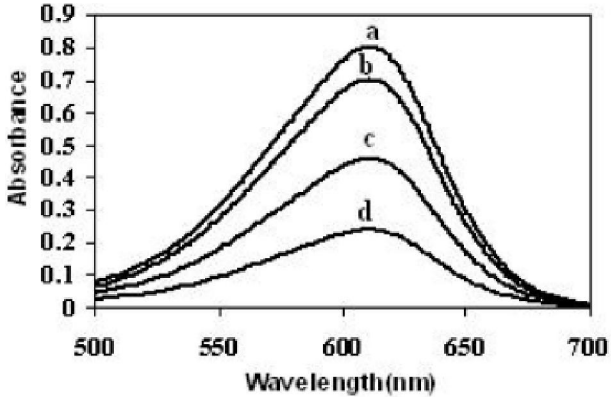

Figure 5 : (a) Blank (without RPS) (b) 4.8μg mL^{-1} (c) 3.2μg mL^{-1} (d) 1.6μg mL^{-1} of RPS measured against water

sured at 508nm against distilled water after 3 min.

Method using indigo carmine (method B)

Aliquots (0.4 to 2.8mL) of standard RPS solution (20μg mL^{-1}) corresponding to 0.8-5.60μg mL^{-1} were transferred into a series of 10mL calibrated flasks. To each flask was added 1mL of 5M hydrochloric acid followed by 1.5mL of bromate-bromide mixture (30μg mL^{-1} w.r.t $KBrO_3$). The content was mixed well and the flasks were set aside for 20 min with occasional shaking. Finally, 1.0mL of 200μg mL^{-1} indigo carmine solution was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 610nm against distilled water after 3 min.

In either spectrophotometric method, a calibration graph was prepared by plotting the absorbance versus the concentration of RPS. Unknown concentration was read from the calibration graph or calculated from the respective regression equation derived using the Beer's law data.

Procedure for pharmaceutical formulations

Rabeprazole sodium containing ten tablets were weighed and ground into a fine powder. An amount of the powder equivalent to 20mg of RPS was weighed into a 100ml volumetric flask, 50ml water added and shaken thoroughly for about 10 min. The volume was diluted to the mark with water, mixed well and filtered using Whatmann No. 41 filter paper. The filtrate was diluted stepwise to get 20μg mL^{-1} of RPS for use in spectrophotometric methods A and B respectively. A suitable aliquot was then subjected to analysis.

RESULTS AND DISCUSSION

The proposed spectrophotometric methods are indirect and are based on the determination of the residual bromine (*in situ* generated) after allowing the

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TABLE 1 : Analytical and regression parameters of proposed methods

Parameter	Method A	Method B
λ_{max} (nm)	508	610
Beers law limit (μg/mL)	0.4-2.8	0.8-5.6
Molar absorptivity (l mole^{-1} cm^{-1})	1.29×10^5	5.38×10^4
Sandell`s sensitivity (μg/cm^2)	0.003	0.007
Regression equation (Y = a + bC)		
Slope (b)	0.3373	0.1411
Intercept (a)	0.0026	0.0146
Correlation coefficient (r^2)	0.9997	0.9986
Standard deviation of slope (Sb)	0.00254	0.0024
Standard deviation of intercept (Sa)	0.0045	0.00857
Detection limit LOD (μg/mL)	0.044	0.2
Quantification limit LOQ (μg/mL)	0.135	0.6

reaction between RPS and a measured amount of bromine to be complete. The residual bromine was determined by reacting it with a fixed amount of methyl orange and indigo carmine. The methods make use of bleaching action of bromine on the dyes, the discoloration being caused by the oxidative destruction of the dyes. RPS, when added in increasing amounts to a fixed amount of *in situ* generated bromine, consumes the latter proportionally and there occurs a concomitant fall in the amount of bromine. When a fixed amount of dye is added to decreasing amounts of bromine, a concomitant increase in the concentration of dye results. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentration of RPS.

The general reaction scheme of the method for studied drug with in situ bromine is represented as Scheme 1.

Effect of acid

In order to determine the most suitable acid for the reaction, different acids (sulfuric, hydrochloric, nitric, and acetic) were tested. The results revealed that hydrochloric acid was found to be the best medium for the both steps involved in two methods.

The reaction was performed in a series of 10mL volumetric flask containing 2.0 and 4.0μg mL^{-1} of RPS for method A and for method B respectively. It was found that the maximum absorbance was obtained at 1.0ml of 5M HCL. Above this volume, the absorbance decreased for method A where as for Method B the absorbance remained constant (Figure 2). Therefore, a volume of 1.0ml of 5M HCl, was used for all measurements.

TABLE 2 : Evaluation of precision and accuracy

Proposed method	Concentrations (μg/mL)		RSD	R.E	SAE	C.L
	Taken	Found[a]±SD	(%)	(%)		
Method A	0.4	0.39±0.006	1.53	-2.5	0.0026	0.0074
	1.6	1.61±0.037	2.3	0.63	0.017	0.046
	2.8	2.81±0.052	1.85	0.36	0.023	0.065
Method B	0.8	0.81±0.017	2.39	1.25	0.0076	0.021
	3.2	3.21±0.032	0.99	0.31	0.014	0.04
	5.6	5.58±0.059	1.06	-0.36	0.026	0.073

[a]**Mean value of five determinations; RE. Relative error; SD. Standard deviation; SAE. Standard analytical error; RSD. Relative standard deviation; C.L. Confident limit at 95%**

Reaction time and stability of color

The reaction between RPS and insitu bromine was completed at room temperature (25±5°C) within 25 and 20min for method A and method B, respectively (Figure 3). A time span of 3 to 5 min for the reaction between unreacted bromine and dyes in the second step yielded the constant and maximum absorbance. The absorption spectra of the colored species measured at respective wave lengths as shown in figure 4 and 5. The contact time of 25 or 20 min is not critical and any delay up to 30 min in either method had no effect on the absorbance. The measured color in both methods was stable for several hours even in the presence of reaction product.

Effect of bromide/bromate mixture and dye

A preliminary experiment was performed to fix the linear range for the bromine (*in situ*) under optimum experimental conditions using the methyl orange (method A) and indigo carmine (Method B). The decrease in absorbance of methyl orange, indigo carmine, experiments was performed using 1.0 ml of 5M hydrochloric acid with varying volumes of bromate-bromide mixture. The decrease in absorbance was found to be linear up to 1.5 of 10μg mL^{-1} of bromine with 1.5 ml of 50μg mL^{-1} methyl orange for method A and 1.5ml 30μg mL^{-1} of bromine with 1.0ml 200μg mL^{-1} of indigo carmine for method B.

Effect of sequence of addition

Drug- acid- bromate/bromide mixture and then dye is the optimum sequence of addition, other sequences

TABLE 3 : Results of recovery experiments by standard addition method

Proposed methods			(%)Recovery[a] ± SD		
Proposed methods	Formulation taken (µg/mL)	Pure drug added (µg/mL)	Rabekind-20 tablets	Rabicer-20 tablets	Razo-20 tablets
Method A	0.8	0.4	99.5±1.1	100.16±1.77	98.75±1.9
		2.0	100.05±1.2	99.83±0.53	100.17±1.68
Method B	1.6	1.6	99.91±1.01	100.31±1.25	100.56±0.9
		4.0	101.02±1.96	100.15±1.43	98.36±1.74

[a] Mean of three determinations

gave lower absorbance values under the same experimental conditions.

Validation of the proposed method

Linearity, limits of detection and quantification: Under the optimum conditions, the calibration graphs correlating the increase in the absorption intensity with the corresponding concentration of the drug were constructed. Regression analysis for the results were as carried out using least-square method. In all cases, Beer's law plots were linear good correlation coefficients as shown TABLE 1. The limits of detection (LOD) and limits of quantitation (LOQ)[19] were determined using the formula: LOD or LOQ = kSDa/b, where k = 3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope.

Precision and accuracy

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of RAB were prepared and analyzed in five determinations. The relative standard deviation as precision and percentage relative error (Er %) as accuracy of the suggested methods were calculated at 95% confidence levels and can be considered satisfactory. The analytical result for accuracy and precision show in TABLE 2 that the methods proposed has good repeatability.

Accuracy and recovery

To check the accuracy and reliability of the methods were ascertained through recovery experiments. To a fixed and known amount of drug in the tablet powder, pure RPS was added at two different levels, and the total content was found by the proposed methods. The recoveries of the pure drug added to the tablet powder were shown in (TABLE 3) the results reveal

TABLE 4 : Results of determination of rabeprazole sodium in pharmaceutical formulations

Samples[#]	Amount per tablet (mg)	% Found* ± SD	
		Method A	Method B
Rabekind[a]	20	100.57±1.6	99.15±1.53
Rabicer[b]	20	99.07±1.45	101.13±1.86
Razo[c]	20	101.2±1.93	100.46±1.38

 # Marketed by: a. Mankind, Delhi; b. Biochem, Mumbai; c. Dr.Reddy's, Hyderabad. *Mean value of five determinations

that the proposed methods are not liable to interference by tablet fillers, excipients and additives usually formulated with pharmaceutical preparations reveal that the average recoveries were in the range 98.36-101.02 % reflecting the high accuracy of the proposed method as indicated by low values of S.D.

Analysis of pharmaceutical formulations

The proposed methods were applied to the determination of RPS in commercial tablets. The applicability of the proposed methods for the assay of this drug in tablets was examined and the results are shown in TABLE 4. Five replicate determinations were made. Satisfactory results obtained for drug are in a good agreement with the label claims (TABLE 4). The average percent recoveries obtained are indicating good accuracy of the methods. The results of analysis of the commercial tablets and the recovery study of the drug suggest that there is no interference from any excipients such as starch, lactose, titanium dioxide, and magnesium stearate, which are present in tablets.

CONCLUSION

The proposed methods provide simple, sensitive and cost effective and are free from such experimental variables as heating or extraction step. The methods depend on the use of simple and cheap chemicals and provide sensitivity comparable to existing methods. Therefore the proposed methods are recommended for the routine quality control analysis of rabeprazole sodium in commercial dosage forms.

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Full Paper

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