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Kinetic Spectrophotometric Method for Quantitation of Rizatriptan Benzoate in Pharmaceutical Formulations

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Authors' contributions

This work resulted from the collaboration between all authors. Author SM performed the literature search, laboratory experiments, data analysis, designed and managed the study, wrote the first draft of the manuscript and communicated it to the journal. Author KR provided the instrumental facilities and valuable discussion. Authors MAV and NN read and approved the final manuscript.

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ABSTRACT

A simple, sensitive and accurate kinetic spectrophotometric method for the determination of Rizatriptan Benzoate in pure and dosage form is described. The method is based on spectrophotometrically monitoring the oxidation of the drug by a known excess of Chloramine-B in HCl medium using initial rate, fixed time and variable time method in HCl medium. The oxidized product exhibits a maximum absorption at 490nm. The apparent molar absorptivity and Sandell's sensitivity values are in the range 2.97×10^4 to 0.0198 respectively. The proposed method has been successfully applied to the determination of RTB in commercial pharmaceutical formulations. Statistical comparison of the results with well established reported method shows agreement in terms of precision and accuracy.

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1. INTRODUCTION

Analytical assay methods have been adopted in recent years for the determination of drugs in pharmaceuticals to ensure the quality of bulk drug materials.

Also, determination of drugs in biological fluids has become essential in pharmaceutical analysis. Techniques like LC-MS are used to analyse pharmacokinetic parameters in the plasma samples [1]. Metabolites of the drug in the rat urine has been identified using GC-MS and LC-MS [2,3]. LC-NMR [4], CE-ICP-MS [5] and CE-MS [6] have become common in the analysis of pharmaceuticals. However, all these techniques suffer certain drawbacks in terms of long term reproducibility, instrument expertise and cost effectiveness.

UV spectrophotometry, though a traditional technique, finds its use in the analysis of pharmaceutical dosage forms owing to its simplicity, speed and precision. In recent years, using this technique, quantisation of drugs are carried out mainly based on redox reactions. [7,8,9].

In this context, UV spectrophotometry has been chosen to determine Rizatriptan benzoate chemically describe as N, N-dimethyl-5-(1H-1,2,4-triazol-1-yl methyl)-1H-indole-3-ethanamine monobenzoate, belonging to a class of migraine medications "Triptans" also known as serotonin 5-hydroxytryptamine 1 receptor subtype agonist which works by narrowing the intracranial dilated blood vessels and relive the symptoms of migraine. RTB is estimated in the dosage forms [10] using extractive spectrophotometric method with bromocresol green as an ion-pair complexation reagent [11], methyl orange and ligand 2,2-Bipyridyl as complexing agent to give a chromogen [12], derivative spectrophotometric method [13], HPTLC method [14], zero order UV spectroscopic method [15] and HPLC method [16].

Many of the above methods suffer from limitations such as usage of toxic dyes, derivatising the spectra or expensive experimental setup. Thus, there is still a need to develop simple, sensitive and cost effective method to determine RTB in the dosage form.

Chloramine-B(CAB), an important member of the class N-haloamines, finds its extensive use in the study of kinetics of drugs [17,18] and dyes [19,20]. Recently, quantitative determination of drugs is carried out by one of the derivative of CAB [21]. The same derivative is used in indirect spectrophotometric methods, with the help of iodine-starch [22] and indigocarmine [23] in acidic medium.

The present work is a direct spectrophotometric method based on the formation of coloured oxidation product in acid medium and subsequent measurement of absorbance. This new procedure is simple and can be used without the aid of expensive instruments.

2. EXPERIMENTAL

2.1 Instruments

All the UV-spectrophotometric measurements were made on Shimadzu UV 1700 PC UV-vis spectrophotometer provided with 1cm matched quartz cells.

2.2 Reagents and Chemicals

All the reagents and solvents used were of analytical grade. Pharmaceutical grade RTB was received from Apotex India. Ltd as a gift sample and was used without further purification. The formulated tablets of RTB such as Maxalt-RPD (from MSD) and Rizact (from Cipla) were purchased from the local market. Chloramine-B was obtained by Merck, India and the purity was checked by iodometric determination of chlorine. An aqueous solution of CAB was prepared, standardized iodometrically and preserved in a brown bottle to prevent its photochemical deterioration. Double distilled water and a mixture of 1:1 double distilled water and analytical grade methanol obtained from Merck, India was used as a solvent throughout the kinetic investigation.

2.3 Procedure for the Preparation of Stock Solution

Standard RTB was weighed and transferred to 10 ml volumetric flask and made up to the mark to give a solution of 4×10^{-4} M. Working solutions were prepared when required by diluting 5 ml of

this solution to 10 ml resulting in a solution 2×10^{-4} M.

2.4 Procedure for the Preparation of Dosage form Sample Solution

Twenty tablets containing RTB were weighed and pulverized in to fine powder. A required quantity of powder equivalent of RTB was transferred to 10 ml calibrated flasks, made up to the mark with double distilled water to get a solution of concentration of 4×10^{-4} M. This is filtered through whatmann filter paper no.42. 5ml of the tablet extract was diluted to 10 ml to give a working solution of concentration 2×10^{-4} M. Different aliquots of working solutions was then subjected to spectrophotometric procedure.

2.5 Procedures for the Determination of RTB

2.5.1 Initial rate method

Aliquots of standard RTB solutions ranging from $0.2-2 \times 10^{-4}$ M were transferred in to a series of flasks. A fixed concentration 1×10^{-3} of CAB was added in the presence of 1×10^{-4} HCl to each of the flasks, and was diluted up to 10 ml. The contents were mixed well and their absorbance was measured at 490nm as a function of time.

The logarithm of the reaction rate 'k' obtained by initial rate method were plotted as a function of logarithm of RTB concentration. The amount of drug was calculated from the calibration graph.

2.5.2 Fixed time method

In this method, absorbance difference caused by the oxidant (1×10^{-3} M) over the concentration range of $0.2-2.0 \times 10^{-3}$ M of RTB in HCl (1×10^{-4} M)

medium was monitored, at regular intervals of 5 minutes. The difference in absorbance between $t_1=1$ min and $t_2= 6, 11, 16, 21, 26$ minutes were computed and plotted against the concentration to get the corresponding linear regression equations.

2.5.3 Variable time method

A fixed concentration of oxidant (1×10^{-3} M) was added to different concentration of RTB ($0.2 - 2.0 \times 10^{-3}$ M) in HCl (1×10^{-4} M) medium. The time required to reach the maximum absorbance at 490 nm was recorded for each concentration. The equation of calibration graph was determined.

3. RESULTS AND DISCUSSION

3.1 Stoichiometric Relationship

Job's method of continuous variation [24] was employed. A plot of mole ratio between the drug and oxidant Vs absorbance was drawn using standard equimolar solutions of drug and oxidant in 1:1 methanol-water mixture.

A series of solutions consisting of fixed total number of moles of oxidant and drug but in which the complementary proportions (0.2:1.8, 0.3:1.7, 0.4:1.6.....) are varied and made up to 2ml calibrated flasks. The absorbance of the resulting solutions were measured at 490nm at the end of 10 minutes at 298 K when the reaction has reached near completion, against reagent blank treated similarly. (Fig. 1) displays a maximum absorbance of 3.45 (ie the ratio 2.33:1.00) which indicates the formation of 2:1 (CAB-RTB) oxidized product.

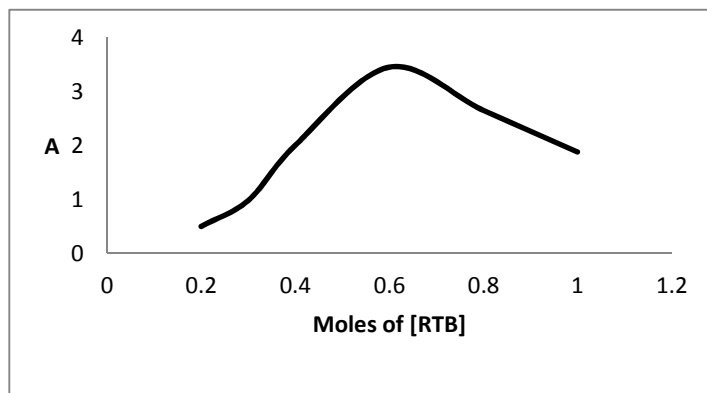


Fig. 1. Job's plot of continuous variation graph for RTB – CAB reaction product

The interaction of RTB and CAB was also investigated, by limiting logarithmic method [25] by varying the concentration of RTB [2×10^{-3} - 1×10^{-2} M] keeping CAB fixed [0.2×10^{-3}]. Then by varying concentration of CAB [0.5×10^{-4} - 0.5×10^{-3} M] keeping RTB fixed [2×10^{-2} M]. Upon plotting the logarithm of concentration and absorbance, two straight lines were obtained (Fig 2) with slopes in the ratio 1:2 indicating the same

ratio was involved in the reaction of RTB and CAB.

3.2 Reaction Mechanism

The predicted mechanism is shown in scheme 1 for the reaction between RTB-CAB in the presence of HCl [26].

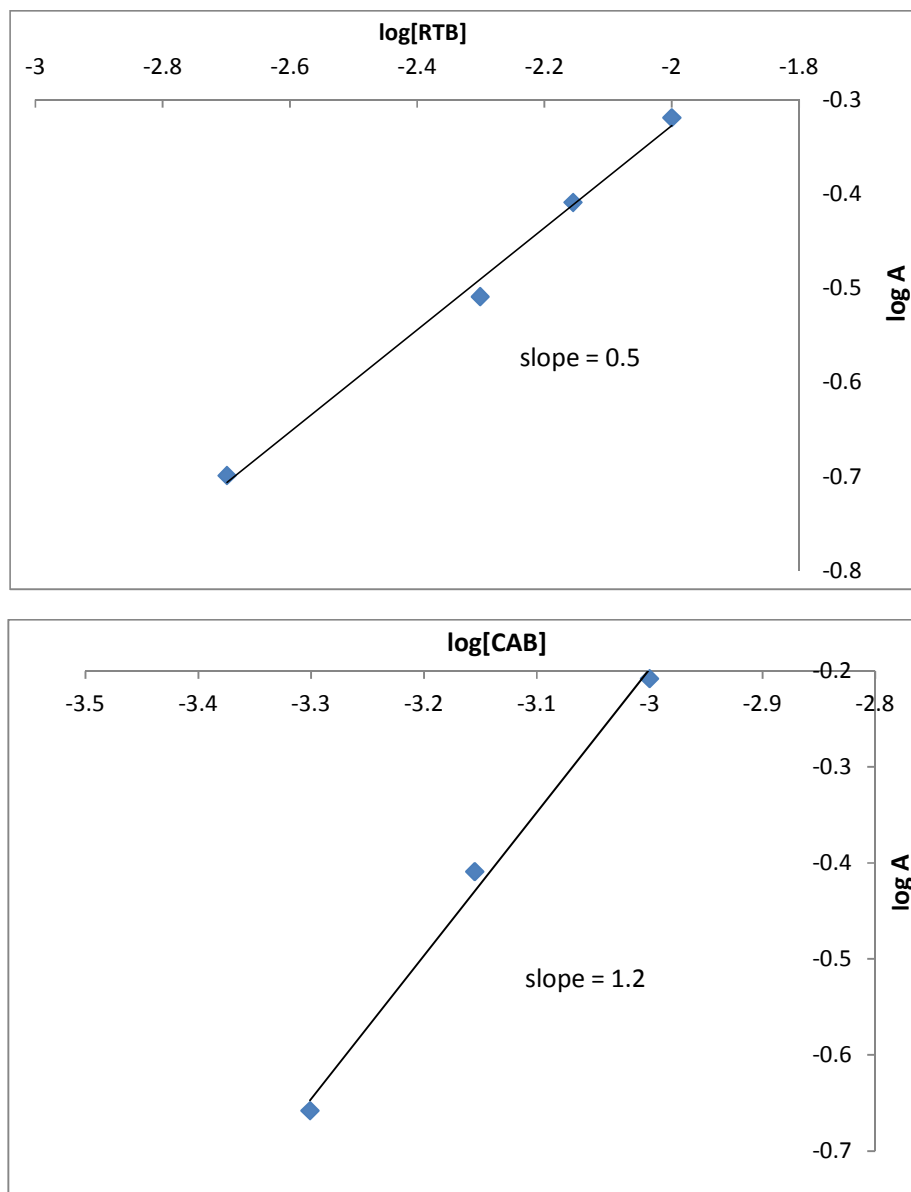
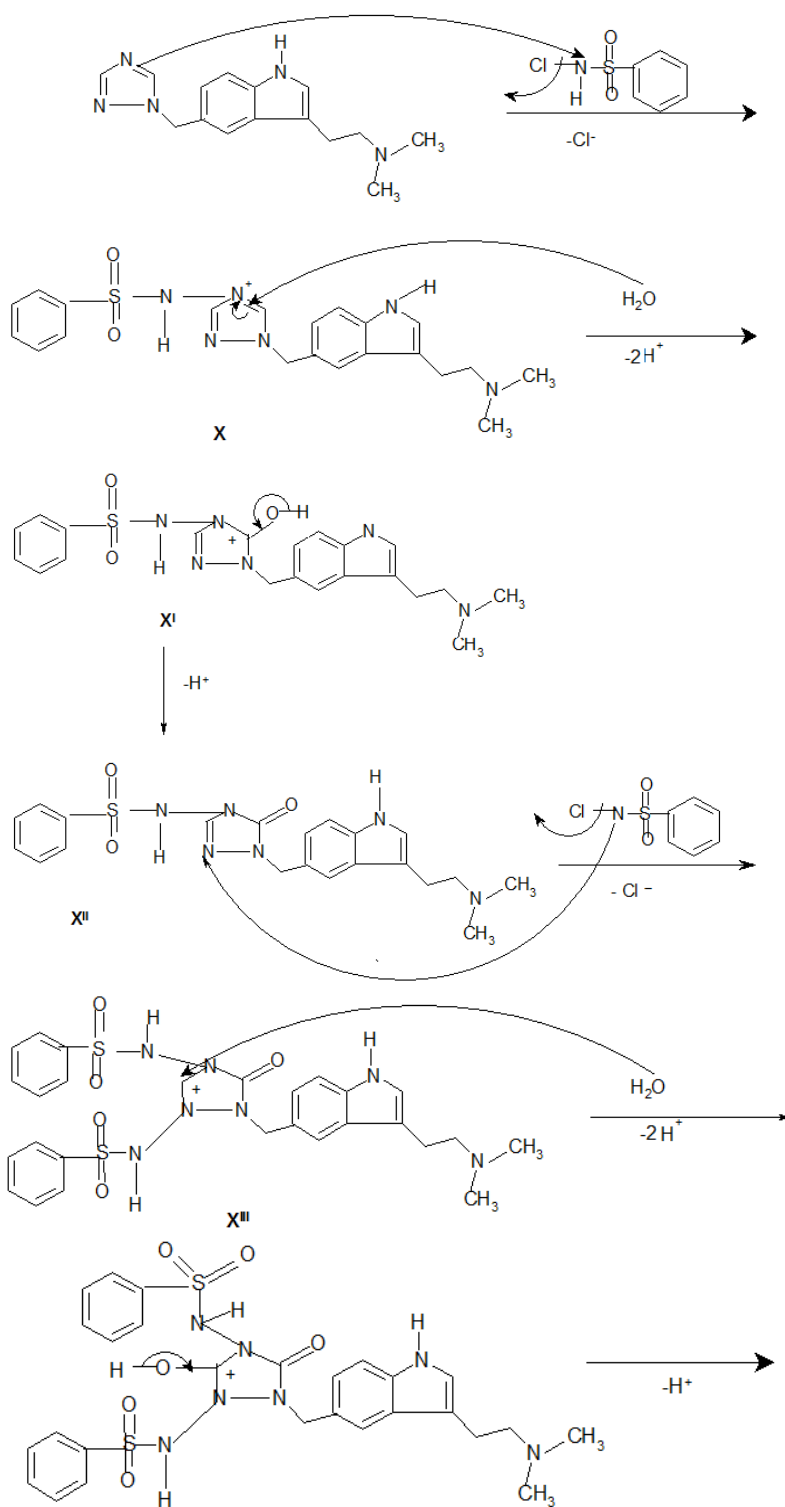
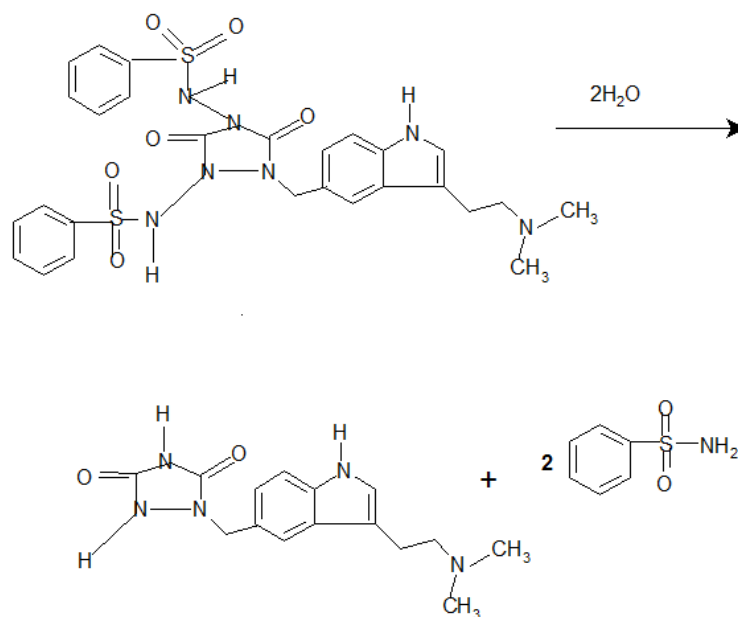


Fig. 2. Limiting logarithmic plots for molar reactivity of RTB and CAB





N,N-dimethyl-5-(1H-1,2,4-triazol-3,5-dione-1-yl methyl)-Benzenesulphonamide
1H-indole-3-ethanamine

Scheme 1. Detailed mechanistic scheme for the oxidation of RTB by CAB in acid solutions

3.3 Quantitation Methods

$$\text{Log } K = \log k + n \log C$$

3.3.1 Initial rate method

Initial rate of the reaction was determined by measuring the slope of initial tangent to the absorbance-time curves (Fig. 3).

The order of RTB was determined by plotting the logarithm of initial rate of the reaction with molar concentration of RTB (Fig. 4).

Regression analysis for the values were performed by fitting the data to the following equation

A straight line with slope ≈ 1 confirmed the reaction was of first order. The curve was linear and Beer's law was obeyed over the concentration range of $0.2 - 2.0 \times 10^{-4}$ M with an apparent molar absorptivity of 2.14×10^4 l/mol/cm and sandell's sensitivity 0.0057. The limit of detection (LOD) and limit of quantification (LOQ) were calculated and found to be 0.244 and 0.741 respectively.

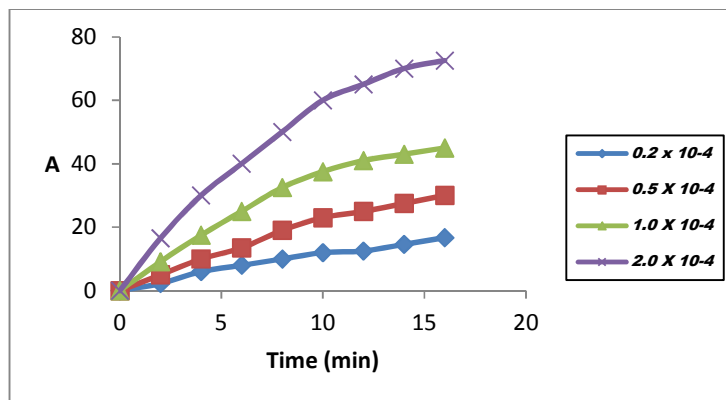


Fig. 3. Absorbance versus time graph for the reaction between RTB and CAB in acid medium

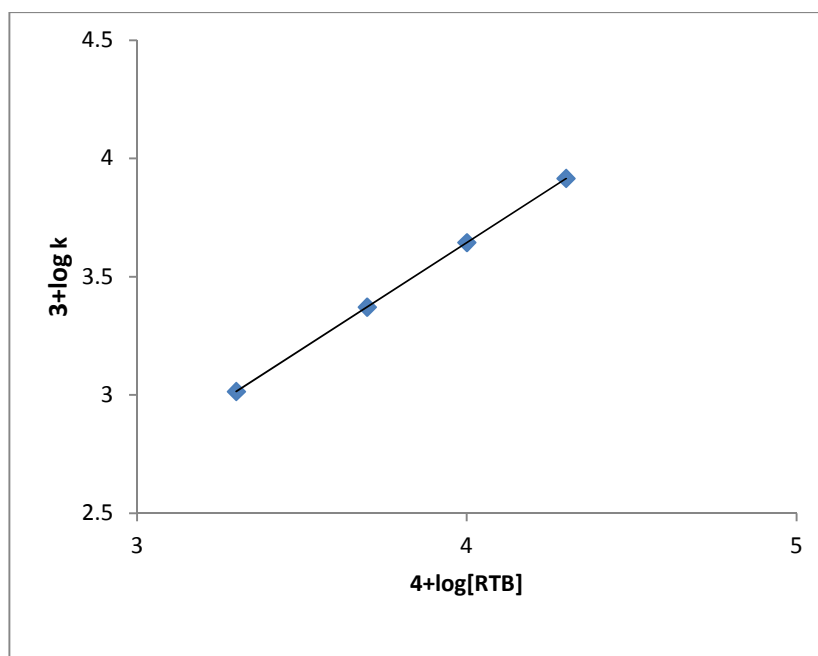


Fig. 4. Plot of log k of the reaction for different concentration of RTB

3.3.2 Fixed time method

In this method, the change in the absorbance of coloured solutions was measured at regular intervals of 5 minutes (Table 1).

It is observed that slope increases with time and the most acceptable linearity was obtained when calibration graph were plotted by considering the difference in absorbance between 1 and 16 minutes with the most acceptable value of r. Hence a fixed time of 15 minutes was chosen as the most suitable interval for the measurement. The values of LOD and LOQ are found to be 0.110 and 0.340 respectively.

Table 1. Results of calibration curve for RTB by fixed time method

Δt (min)	Calibration equation	Correlation co-efficient
5	$\Delta A = 0.08 + 0.14 C$	0.993
10	$\Delta A = 0.04 + 0.26 C$	0.995
15	$\Delta A = 0.01 + 0.53 C$	0.993
20	$\Delta A = 0.05 + 1.0 C$	0.999
25	$\Delta A = 0.07 + 1.32 C$	0.998

3.3.3 Variable time method

The time required to reach the maximum absorbance was recorded for varying concentration of RTB (Table 2). Reciprocal of

time ($1/\Delta t$) Vs initial concentration was plotted and the equations of calibration graphs was found to be $1/\Delta t = 1.39 C + 0.187$ with correlation co-efficient $r = 0.979$. The values of LOD and LOQ are found to be 0.090 and 0.27 respectively.

Table 2. Results of calibration curve at 490 nm using variable time method

Δt (min)	$1/\Delta t$ (s ⁻¹)	[Drug] x 10 ⁻⁴
30	0.56×10^{-3}	0.2
20	0.83×10^{-3}	0.5
13	1.28×10^{-3}	1.0
07	2.38×10^{-3}	1.5

3.4 Validation of the Proposed Methods

3.4.1 Accuracy and precision

Performing recovery experiments through standard addition method, a known amount of pure drug was added to preanalysed dosage forms at different concentration levels (50%, 100%, 150%) and the mixtures were analysed by the proposed methods. The results are shown in (Table 3). However, there was no interference by the formulation excipients.

Table 3. Standard addition method for the determination of RTB in commercial tablets

Formulations	Initial rate method				Fixed time method				Variable time method			
	% addition	RTB added 10 ⁻⁴ M	RTB recovered 10 ⁻⁴ M	% recovery	% addition	RTB added 10 ⁻⁴ M	RTB recovered 10 ⁻⁴ M	% recovery	% addition	RTB added 10 ⁻⁴ M	RTB recovered 10 ⁻⁴ M	% recovery
Maxalt- RPD	50	0.5	0.495	99.49	50	0.5	0.493	98.62	50	0.5	0.496	99.22
	100	1.0	0.992	99.66	100	1.0	0.998	99.82	100	1.0	0.994	99.40
	150	1.5	1.481	98.79	150	1.5	1.508	100.53	150	1.5	1.488	99.62
Rizact	50	0.5	0.482	96.40	50	0.5	0.495	99.49	50	0.5	0.498	99.60
	100	1.0	0.994	99.40	100	1.0	0.993	99.30	100	1.0	0.992	99.66
	150	1.5	1.494	99.60	150	1.5	1.404	97.60	150	1.5	1.482	98.79

Table 4. Intraday and interday assays to test the precision of Initial rate, fixed time and variable time methods

Proposed method	Assay	Amount 10 ⁻³			
		Taken	Found±SD	% Recovery	RSDa
Initial rate	Intra day	2.0	2.026± 0.042	100.20	0.428
		5.0	4.996±0.118	99.99	0.336
		10.0	10.013±0.070	100.02	0.122
	Interday	2.0	1.992±0.092	99.92	0.922
		5.0	4.996±0.192	99.98	0.548
		10.0	10.048±0.126	100.09	0.226
Fixed time	Intraday	2.0	2.013±0.088	100.11	0.845
		5.0	5.122±0.155	100.33	0.455
		10.0	10.-51±0.134	100.09	0.243
	Interday	2.0	1.975±0.134	99.74	1.341
		5.0	5.051±0.166	100.15	0.482
		10.0	10.051±0.165	100.05	0.488
Variable time	Intraday	2.0	1.990±0.042	99.90	0.422
		5.0	4.987±0.052	99.95	0.136
		10.0	10.040±0.058	100.05	0.108
	Interday	2.0	2.008±0.071	100.05	0.700
		5.0	5.011±0.102	100.03	0.297
		10.0	10.020±0.070	100.03	0.124

^aMean of four independent analysis

Solutions of four different concentrations of RTB were prepared. Intraday and interday precisions for initial rate, fixed time and variable time methods were measured for three times for pure samples and tablets on three consecutive days. The standard deviation, relative standard deviation obtained in both intraday and inter day assays shown in (above Table 4) are acceptable.

3.4.2 Comparison of the proposed methods with the reported spectrophotometric methods

The present method is advantageous as it involves measurement in visible region unlike the earlier reported methods that involved measurements in ultraviolet region [15]. Though some of the reported methods involves measurement in visible region, they demand elaborate extractive method and usage of toxic dyes [11,12]. The proposed method is simpler than the derivative method which requires derivatising the spectrum into first and second order. Further, some reported methods involve

tedious procedure of developing chromatogram [14] or column with a careful choice of mobile phase [16].

These disadvantages above make the proposed method a specific and precise method for a routine analysis in pharmaceutical industries (Table 5).

3.4.3 Application to the analysis of tablets

The proposed methods were applied to the pharmaceutical formulation of Rizatriptan Benzoate and the results were compared with those of the reference method [27] which consisted of measuring the absorbance of the tablet extract in water at 225 nm. The calculated student's t-test and F-test values at 95% confidence level do not exceed the theoretical ones indicating a good agreement between the results (Table 6) obtained by reference and proposed methods in terms of accuracy and precision.

Table 5. Comparison of the proposed methods with the reported spectrophotometric methods

Method/reagent	Reaction conditions	λ_{max}	Concentration range	LOD	Ref
Oxidation					
Proposed initial rate	pH=3	490 nm	0.2- 2.0 x 10 ⁻⁴ M (or 0.01-0.1 µg/ml)	0.244	This work
Proposed fixed rate	pH=3	490 nm	0.2- 2.0 x 10 ⁻⁴ M (or 0.01-0.1 µg/ml)	0.110	This work
Proposed variable time	pH=3	490 nm	0.2- 2.0 x 10 ⁻⁴ M (or 0.01-0.1 µg/ml)	0.090	This work
Formation of ion-pair complex					
Bromocresol green	pH=3	416 nm	0.5-50 µg/ml	3.33	11
2,2 ¹ -Bipyridyl	pH=1.46	490 nm	10-50 µg/ml	-	12
Methyl orange	pH=7	420	4-20 µg/ml	-	12
Derivative spectroscopy	pH=13	281	0.5-80 µg/ml	-	13
HPTLC method	pH=2-5	225	1-10 µg/ml	50 ng/band	14
Zero order	pH=1	226	1-5 µg/ml	0.0311	15
HPLC	pH=3.4	225	15.032 mg/ml- 80.172 mg/ml	-	16

Table 6. Results of the analysis of tablets by proposed methods

Tablet brand name	Label claim mg/tablet	Found (% of label claim ± SD)		t-test	F-test
		Reference method	Proposed methods		
Maxalt- RPD	5mg	100.98	98.15	1.38	1.24
Rizact	5mg	99.72	99.68	0.93	0.89

Tabulated t-value at 95% confidence level is 2.22; Tabulated F-value at 95% confidence level is 6.05;

^aMean value of four determinations

4. CONCLUSION

The present study reports, a simple kinetic spectrophotometric method for the determination of Rizatriptan Benzoate in the formulation. The proposed initial rate, fixed time and variable time methods can be applied as they are sensitive for analysis at low concentrations. The method is inexpensive, has a very low toxicity level and can be applied in quality control laboratories.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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