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Original Research Article

SIMULTANEOUS DETERMINATION OF BRIMONIDINE TARTRATE AND TIMOLOL MALEATE IN COMBINED PHARMACETICAL DOSAGE FORM USING TWO DIFFERENT GREEN SPECTROPHOTOMETRIC METHODS

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Abstract: Two simple, precise and accurate spectrophotometric methods have been developed and validated for the simultaneous Determination of Brimonidine tartrate (BRT) and Timolol maleate (TIM) in bulk and pharmaceutical formulations. Namely Simultaneous equation (method 1), Q-absorbance ratio (method 2). In method 1, both the drugs exhibit good linearity over the concentration range of 4-20 mg/mL and 1 to 50 mg/mL of Brimonidine tartrate and Timolol maleate at 255nm (r^2 =0.9998 and 0.9998 respectively) and 295nm (r^2 =0.999 and 0.999 respectively) wavelengths respectively. Method 2, involves the formation of Q-absorbance equation using the absorptivity values at 271nm (isoabsorptive point) (r^2 =0.999 and 0.9998 respectively) and 295nm (λmax of Timolol maleate) (r^2 =0.9998 and 0.999 respectively) and Beer's Lambert's law was obeyed over the concentration range of 4-20 mg/mL and 10-50 mg/mL respectively. The proposed methods were validated according to ICH guidelines for evaluation of accuracy, precision, sensitivity. These are economically viable methods that do not require any prior separation procedure and costly solvents.

Keywords: Brimonidine tartrate, Timolol maleate, Simultaneous equation method, Q-ratio method, Validation.

Introduction

Brimonidine tartrate (BRT) [5-bromo-6 (2-imidazolidinylideneamino) quinoxaline L-tartrate] is a selective alpha-2 adrenergic agonist[1]. It is usually given in eye drop form. Its ocular hypotensive effect is due to its ability to decrease aqueous humor production and increase uveoscleral outflow. Its selectivity towards alpha-2 adrenergic receptors and its neuroprotective activity on retinal ganglionic cells makes it as an important therapeutic agent for the treatment of open angle glaucoma. Its superiority in treating glaucoma in cardiopulmonary patients makes this drug as a better alternative to timolol. Its use in ischemic neuropathy has also been reported[2].

Timolol maleate (TIM) [(S)-1-[(1,1-

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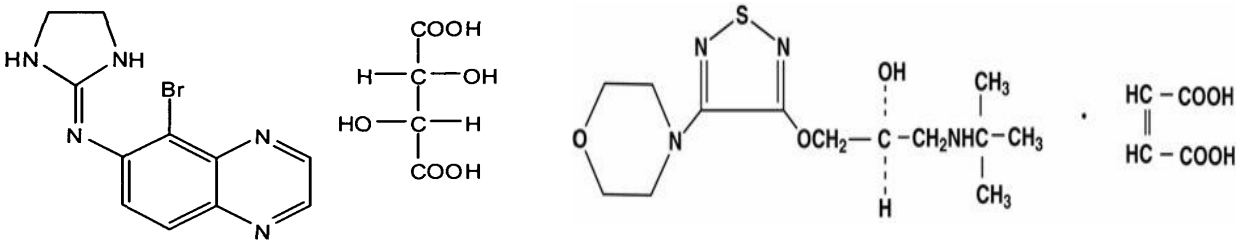
dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5- thiadiazol -3 -yl] oxy]-2-propanol, (Z)-2-butenedioate] is a nonspecific β- adrenergic blocker[3,4]. It was the first β-blocker to be used as an antiglaucoma agent. None of the newer β-blockers were found to be more effective than timolol. In its oral form it is used to treat high blood pressure and prevent heart attacks, and occasionally to prevent migraine headaches. In its ophthalmic form it is used to treat open-angle and occasionally secondary glaucoma[5].



(a) (b)

Fig. I: Chemical structures of (a) Brimonidine tartrate (b) Timolol maleate

Literature review reveals that there are many analytical methods like spectrophotometric, HPLC, and HPTLC, reported for the determination of Timolol maleate individually and in combination with other drugs like Latanoprost, Brinzolamide and Dorzolamide HCl[6-11]. For Brimonidine tartrate several methods have described for quantifying in different samples and using various analytical techniques such as HPLC, UPLC-MS, and HPTLC individually and in combination with other drugs[12-14].

Very few analytical methods like Ratio difference spectroscopic and TLC densitometry were reported for estimation of BRT and TIM in combined pharmaceutical dosage forms[15, 16]. Hence an attempt has been made to develop spectrophotometric methods for simultaneous estimation of BRT and TIM in combined dosage forms.

Materials and Methods

Instruments

A Shimadzu model UV-1800 double beam UV-Visible Spectrophotometer, attached to a computer software UV probe 2.34, with a spectral width of 1 nm and pair of 1 cm matched quartz cells was used. Shimadzu analytical balance was used throughout practical. Class 'A' volumetric glassware were used.

Materials

The working standards of BRT and TIM were gifted from Micro labs Ltd, Bangalore, and FDC Ltd, Goa respectively. The ophthalmic formulation of BRT and TIM (Label claim: Brimonidine tartrate 2mg and Timolol maleate IP 5mg), Combigan (Piramal Enterprises Ltd, MP) was purchased from the local market. Acetonitrile and water (HPLC grade) were obtained from E. Merck Ltd Mumbai, India; Phosphate buffer- pH-3.6 was obtained from Sd Fine chemicals Pvt Ltd Mumbai, India.

Preparation of Standard stock solution

Accurately weighed standard drug of BRT (10 mg) and TIM (10 mg) were transferred to a separate 100 mL volumetric flask, dissolved in 20 mL Dist. Water by shaking manually for 5 min. The Volume was adjusted with the same up to mark to give final concentrations of BRT (100mg/mL) and TIM (100mg/mL).

Preparation of Calibration curve (method 1)

From the Standard solutions of BRT (0.4, 0.8, 1.2, 1.6, and 2.0 mL) and standard solutions of TIM (1.0, 2.0, 3.0, 4.0 and 5.0 mL) was pipette out in to a separate series of 10 mL volumetric flask. The volume was adjusted to the mark with Dist. Water and mixed.

The absorbances of the solutions were measured at 255nm and 295nm against taking Distilled water as a blank. The absorptivity coefficients of each drug at both wavelengths were determined and substituted in their equation to obtain concentration of both drugs. The concentration of each compound in the mixture was calculated from the following simultaneous equations[17] .

CBRT = A2ay1 - A1ay2 / ax2ay1 - ax1ay2 … 1.

CTIM = A1ax2 - A2ax2 / ax2ay1 - ax1ay2 … 2.

Where, C_{BRT} and C_{TIM} are concentration of BRT and TIM respectively; A1 and A2 are absorbance of mixture at 255nm and 295nm respectively; ax_1 and ax_2 are absorptivity coefficient of BRT at 255nm and 295nm respectively; ay_1 and ay_2 are absorptivity coefficient of TIM at 255.0 nm and 295.0 nm respectively.

Preparation of Calibration curve (method 2)

From the Standard solutions of BRT (0.4, 0.8, 1.2, 1.6, and 2.0 mL) and standard solutions of TIM (1.0, 2.0, 3.0, 4.0 and 5.0 mL) was pipette out in to a separate series of 10 mL volumetric flask. The volume was adjusted to the mark with Dist. Water and mixed.

The over line spectrum of BRT and TIM, one wavelength was selected for the estimation of both drugs, which is known as iso-absorptive point (at 271nm) and one was λmax (295nm) of one drug. The dilutions of standard and sample solutions were prepared. The Absorptivity values were determined at 271nm. The method employs Q values and the concentrations of drugs in sample solution were determined by using following formula[17],

$$C_X = \frac{(Q_M - Q_Y) \times A_1}{(Q_X - Q_Y) \times aX_1} \quad \text{AND} \quad C_Y = \frac{A_1}{aX_1 - C_X}$$

Where, A_1 s& A_2 are the absorbance of the mixture at 271nm & 295nm respectively; aX_1 and aY_1 are absortivities of BRT and TIM respectively at 271nm; aX_2 and aY_2 are absortivities of BRT and TIM respectively at 295nm; $QM=A_2/A_1$, $QX= aX_2/ aX_1$ and $QY= aY_2/ aY_1$.

Validation of Methods[18]

Proposed methods were validated in accordance with ICH guidelines Q2 (R1) for evaluation of various parameters; linearity, limit of detection, limit of quantification, precision and accuracy.

Linearity

Calibration curves were plotted over a concentration range of 4-20 mg/mL and 10-50 mg/mL for BRT and TIM respectively. The calibration curves were constructed by plotting absorbances Vs concentrations.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n=6) of BRT and TIM (4 mg/mL and 10 mg/mL respectively) without changing the parameters for the simultaneous equation and Q-ratio method.

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by analyzing corresponding responses in triplicate on the same day and on 3 different days, by using standard solutions of BRT and TIM at 12 mg/mL and 30 mg/mL respectively. Results were reported in terms of RSD.

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) of the drug was derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline

The LOD may be expressed as:

$$DL = \frac{3.3\,\sigma}{S}$$

Where, σ= the standard deviation of the response S = the slope of the calibration curve The LOQ may be expressed as:

$$QL = \frac{10\,\sigma}{S}$$

Where, σ= the standard deviation of the response S = the slope of the calibration curve

Accuracy (Recovery study)

The accuracy of the methods was determined by calculating recoveries of BRT and TIM by the standard addition method. Known amounts of standard solutions of BRT and TIM were added at 80,100 and 120 % levels to prequantified sample solutions of BRT and TIM (4 and 10 mg/mL respectively). The amounts of BRT and TIM were estimated by applying the obtained values to the simultaneous equation and Q-ratio method.

Analysis of BRT and TIM in Pharmaceutical dosage form

In pharmaceutical dosage form, both drugs BRT and TIM in ratio of 1:2.5. The absorbance was measured at 255nm and

295nm (Simultaneous equation) and 271and 295nm (Q- ratio) for quantification of BRT and TIM respectively. The amounts of BRT and TIM present in sample solutions were determined by fitting the response into the simultaneous equation and Q-ratio method for BRT and TIM.

Results and Discussion

Method 1: Simultaneous equation method

The standard solutions of BRT and TIM were prepared separately in Dist.water. They were scanned in the wavelength range of 200-400 nm. Data were recorded at an interval of 1 nm. Maximum absorbance was obtained at 255nm and 295nm for BRT and TIM respectively. Select these two analytical wavelengths for determination of BRT and TIM respectively shown in figure II. These two wavelengths can be employed for the determination of BRT and TIM without any interference from their combined pharmaceutical dosage form.

Method 2: Q-ratio method

The standard solutions of BRT and TIM were prepared separately in Dist.water. They were scanned in the wavelength range of 200-400 nm. Data were recorded at an interval of 1 nm. using over line spectrum of BRT and TIM, one wavelength was selected for the estimation of both drugs, which is known as iso absorptive point (at 271nm) shown in fig. II and one was λmax (295.0 nm) of one drug. The dilutions of standard and sample solutions were prepared. The Absorptivity values were determined at 271nm. The method employs Q values and the concentrations of drugs in sample solution were determined by using the formula.

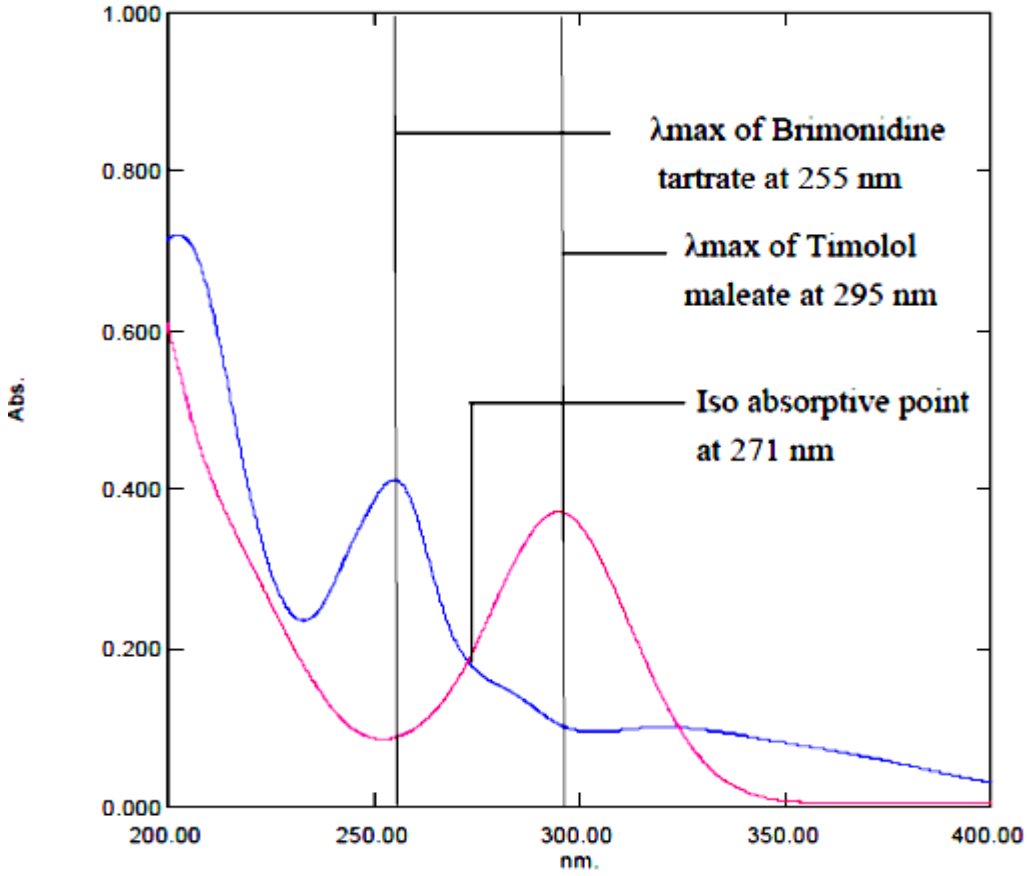


Fig. II: Overlain spectra of Brimonidine tartrate and Timolol maleate

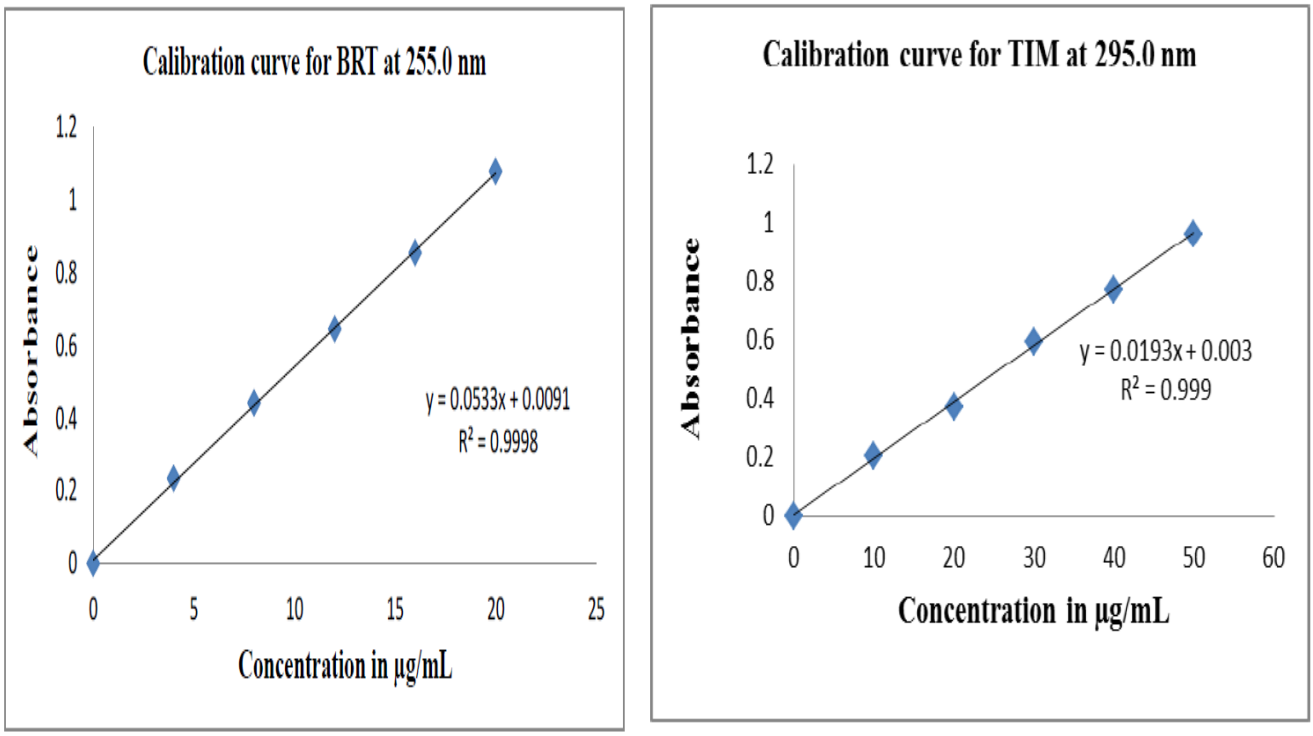


Fig. III: Calibration curve for Brimonidine tartrate at 255 nm and Timolol maleate at 295 nm by Simultaneous Equation Method.

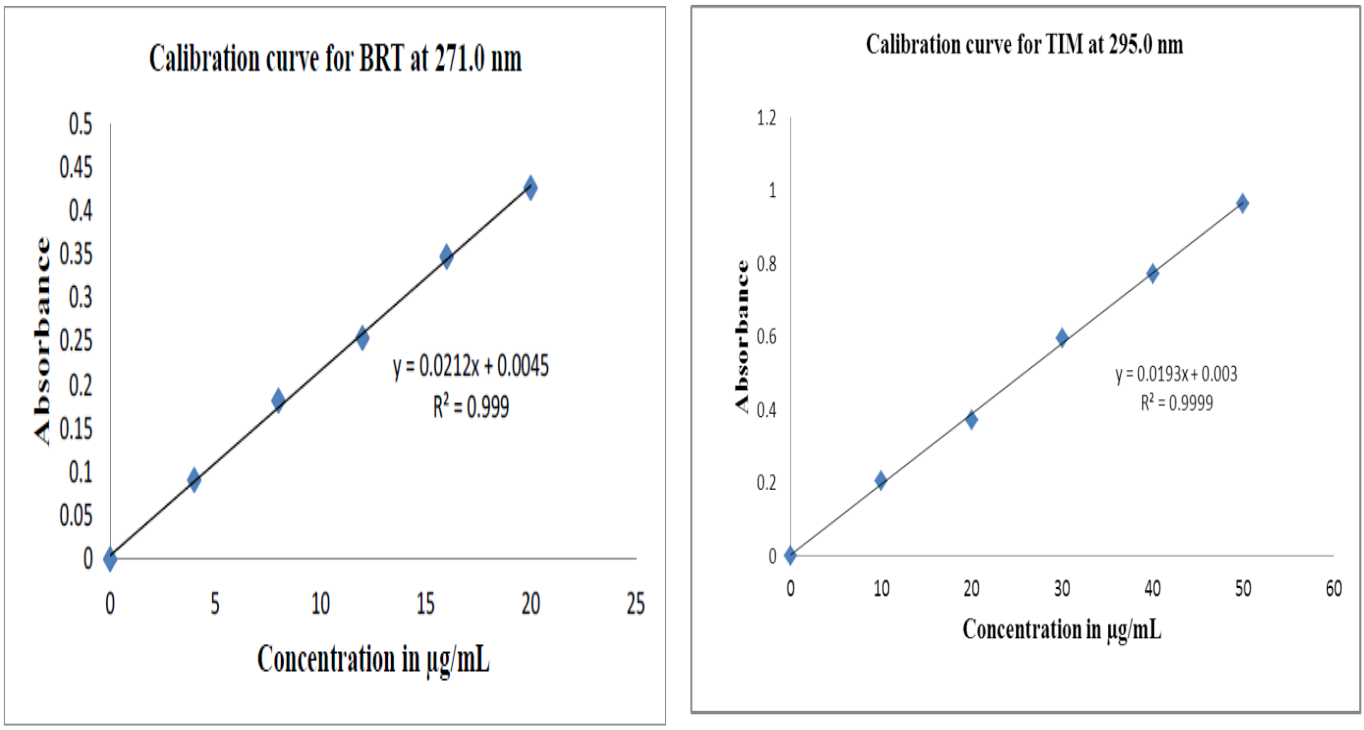


Fig. IV: Calibration curve of Brimonidine tartrate at 271 nm and Timolol maleate at 295 nm by Q-Ratio method.

Table I gives regression analysis data for the proposed methods. The precision data for the both methods is given in **Table II** and **Table-III** gives Recovery study performed by spiking the standard solution at 80, 100 and 120%, less than 2% RSD indicate the recovery study was acceptable.

Table I: Regression analysis data for the proposed methods

Parameters	Method 1				Method 2			
	BRT		TIM		BRT		TIM	
Wavelength (nm)	255	295	255	295	295	271	295	271
Linearity (mg/mL)	4-20	4-20	10-50	10-50	4-20	4-20	10-50	10-50
Regression equation (Y= mx + c)	0.0533x + 0.0091	0.0114x +0.0041	0.0044x +0.0006	0.0193x +0.003	0.0115x +0.0007	0.0212x + 0.0045	0.0193x +0.003	0.0086x +0.0031
Slope	0.0533	0.0114	0.0044	0.0193	0.0115	0.0212	0.0193	0.0086
Intercept	0.0091	0.0041	0.0006	0.003	0.0007	0.0045	0.003	0.0031
Correlation Coefficient (r^2)	0.9998	0.9997	0.999	0.999	0.9998	0.999	0.999	0.9998
LOD (mg/mL)	0.0971	0.258	0.562	0.155	0.128	0.1599	0.152	0.171
LOQ (mg/mL)	0.264	0.784	1.70	0.470	0.388	0.4542	0.463	0.516

* Average of five determination, LOD=Limit of detection, LOQ=Limit of quantification.

Table II: Precision data for BRT and TIM by proposed methods

Parameters	Method I		Method II	
	BRT	TIM	BRT	TIM
Wavelength (nm)	255	295	271	295
Repeatability (%RSD, n=6)	0.430	0.686	0.753	0.621
Precision (%RSD)				
Interday (n=3)	0.482	0.801	0.714	0.829
Intraday (n=3)	0.581	0.593	0.735	0.538

*n= Number of determination

Table III: Recovery data for BRT and TIM by Proposed methods

Drug	Amount of formulation (mg/mL)	Amount of standard drug added (%)	% Recovery ± %RSD	Amount of formulation (mg/mL)	Amount of standard drug added (%)	% Recovery ± %RSD
	Method I			Method II		
BRT	4	80%	99.56% ± 1.04	4	80%	99.56±0.64
	4	100%	100.75% ± 0.45	4	100%	100.1±1.25
	4	120%	99.90% ± 0.90	4	120%	99.82±1.2
TIM	10	80%	99.90% ± 0.60	10	80%	98.59±0.80
	10	100%	99.21% ± 0.80	10	100%	100.93±1.13
	10	120%	100.73% ± 1.13	10	120%	99.32±1.04

* Average of three determination

Assay of pharmaceutical dosage form (Eye-Drop) by proposed methods

The proposed validated methods were successfully applied for the determination of BRT and TIM in their combined dosage forms. Assay results of the Pharmaceutical dosage (Eye drops) form given in **Table IV**. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine analysis of Brimonidine tartrate and Timolol maleate in pharmaceutical dosage forms.

Table IV: Assay results of the Pharmaceutical dosage (Eye drops) form

Sample No.	Amount present (mg/mL)		Amount obtained (mg/mL)		% Amount obtained		Amount obtained (mg/mL)		% Amount obtained	
			Method I				Method II			
	BRT	TIM	BRT	TIM	BRT	TIM	BRT	TIM	BRT	TIM
1	2	5	1.98	4.96	99.35	99.20	1.98	4.96	99.35	99.20
2	2	5	1.96	4.97	98.45	99.42	1.96	4.97	98.45	99.42
3	2	5	1.97	5.03	98.80	100.64	1.97	5.03	98.80	100.64
4	2	5	2.00	4.98	100.30	99.64	2.00	4.98	100.30	99.64
5	2	5	1.98	5.01	99.45	100.30	1.98	5.01	99.45	100.30
6	2	5	1.99	4.95	99.82	99.08	1.99	4.95	99.82	99.08
Mean			1.975	5.02	99.35	99.71	2.013	5.015	99.35	99.71
SD			0.024	0.028	0.5610	0.8422	0.021	0.016	0.5610	0.8122
%RSD			1.22	0.57	0.5618	0.8162	1.07	0.033	0.5618	0.8162

Conclusion

Based on the results, it can be concluded that the method has linear response in the range of 4–20 and 10–50 mg/mL for Brimonidine tartrate and Timolol maleate. Less than 2 %RSD indicate that UV-spectroscopic methods are accurate and precise.

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and is in good agreement with prepared ratio of the drugs. The additive usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of Brimonidine tartrate and Timolol maleate.

The method can be used for the routine analysis of for Brimonidine tartrate and Timolol maleate in combined pharmaceutical dosage forms.

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