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SPECIFIC STABILITY INDICATING RP-HPLC PHOTODIODE ARRAY BASED METHOD FOR ESTIMATION OF BRIMONIDINE TARTRATE AND TIMOLOL MALEATE FOR THE TREATMENT OF GLAUCOMA

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ABSTRACT

Simple, accurate, precise, rapid and economical methods were developed for simultaneous estimation of Brimonidine tartrate and Timolol maleate in pharmaceutical dosage form. Brimonidine tartrate and Timolol maleate are used to treat glaucoma. Brimonidine is an alpha-2 adrenergic agonist. Timolol maleate is a non-selective beta-adrenergic receptor antagonist. The wavelengths maxima selected for quantification were 247.0 nm for Brimonidine tartrate and 295.0 nm for Timolol maleate. A RP-HPLC method was developed for simultaneous estimation of both drugs on ODS HYPERSIL C-18 column [250 mm,4.6 mm, 5 µm] column using Water: Methanol: Triethylamine in the ratio of 50:50:0.2 with flow rate and detection wavelength being 1 ml/min and 266.0 nm respectively. Linearity range for Brimonidine tartrate and Timolol maleate solutions found to be 10-24 µg/ml and 25-60 µg/ml respectively. The retention time of brimonidine tartrate and timolol maleate was found to be 3.515 and 5.573 respectively. The column temperature was kept ambient. Linearity was observed in the concentration range of 10-24 µg/ml (r = 0.999) and 25-60µg/ml (r = 0.999) Brimonidine tartrate and Timolol maleate respectively. Forced degradation studies were conducted according to the ICH guidelines and the drug product was found to be stable in all conditions. Hence, the method could be successfully applied for routine analysis of Brimonidine Tartrate and Timolol Maleate in combined ophthalmic dosage form.

Keywords: Brimonidine tartrate, Timolol maleate, Stability, RP-HPLC, Validation

INTRODUCTION:

Brimonidine tartrate (BRT) [5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate] **[1]** is used to treat glaucoma. Brimonidine is an alpha-2 adrenergic agonist. Alpha 2 agonists, by activating the G protein-coupled receptor, inhibit adenylate cyclase activity **[2, 3]**. This decreases the production of cAMP and therefore the aqueous humor of the ciliary body **[3]**.

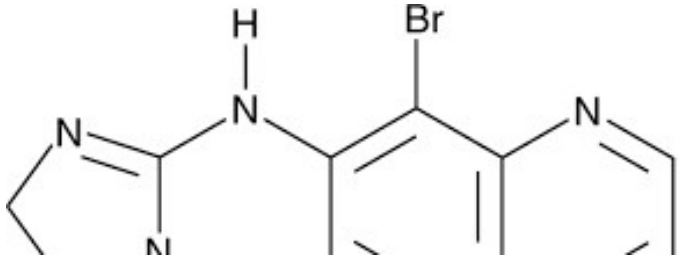

Figure 1: Structure of Brimonidine Tartrate

Timolol maleate (TIM) [(S)-1-tert-butylamino-3-(4-morpholino-1,2,5-thiadiazol-3-yloxy) propan-2-ol hydrogen maleate] **[4]** is a non-selective beta-adrenergic receptor antagonist. Timolol maleate prevents stimulation of beta1-adrenergic in heart muscles and beta2-adrenergic in lungs, blood vessels, uterus at receptor sites. It may decrease water production, which reduce intraocular pressure (IOP) **[2, 3]**. Stability Indicator Method (SIM) is defined as a validated analytical procedure for the precise and accurate measurement of active ingredients (pharmaceuticals or medicinal products) free of impurities, excipients and degradation products during processing **[5]**.

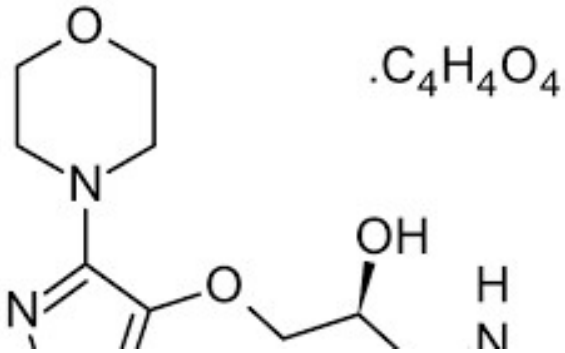

Figure 2: Structure of Timolol Maleate

Literature survey reveals that many methods have been reported for individual BRT and TIM estimation by stability indicating RP-HPLC and spectrophotometric methods **[6-12]**. Only one method is reported in the literature for stability-indicating assay of

BRT and TIM in combination by using a buffer in mobile phase **[13]**.

However, we attempted to construct a BRT and TIM stability-indicating assay method utilising a simple mobile phase of water and methanol, which provided greater accuracy and resolution. The purpose of this study was to create a simple, inexpensive, precise, and specific stability-indicating assay technique in the simplest mobile phase for rapid detection of BRT and TIM in combined pharmaceutical formulation. According to ICH criteria, the devised approach was validated **[14]**.

MATERIALS AND METHODS:

Instrumentation:

Analysis was performed on a chromatographic system of Hitachi HPLC system separation module connected to PDA detector. The system attach to computer program EZstart. The chromatographic separation was achieved on ODS HYPERSIL C-18 column [250 mm, 4.6 mm, 5 µm]. Shimadzu analytical balance was used throughout practical. Class 'A' volumetric glasswares were used.

Chemicals and reagents:

Brimonidine tartrate and Timolol maleate were obtained as a gift sample from Sun Pharma Advanced Research Centre, (SPARC) Vadodara. All the solvents and chemicals used were of analytical grade.

Method development:

Preparation of mobile phase:

Mobile phase is prepared by mixing 50 ml of methanol, add 50 ml of water and 0.2 ml of Triethanolamine. Filter it through 0.45 µm glass filter paper.

Preparation of standard stock solutions:

Brimonidine tartrate 100 mg and Timolol maleate 100 mg was transferred to 100 ml volumetric flask and dissolved in mobile phase. Make up the volume to the mark with mobile phase to obtain standard stock solution of BRM (1000 µg/ml) and TIM (1000 µg/ml). It is further diluted to get 100µg/ml of BRM and 100 µg/ml of TIM. Stock solution was filtered through a 0.45 µm glass fiber filter paper. The working standard solution of BRM and TIM was prepared from suitable aliquots of stock solution **[15]**.

Preparation of Calibration curve:

From the Standard solutions of BRM (10-24 µg/ml) and standard solutions of TIM (25-60 µg/ml) was pipette out in to a separate series of 10 ml volumetric flask. The volume was adjusted to the mark with mobile phase and mixed.

The pure drug solution of BRM and TIM were injected individually into HPLC system

and allow run in different mobile were tried in order to find the optimum conditions for the separation of BRM and TIM. It was found that mobile phase containing Water: Methanol: Triethanolamine (50:50:0.2 v/v), at a flow rate of 1 ml/min with detection at 266 nm gave satisfactory results with sharp well defined and resolved peaks with minimum tailing as compared to other mobile phases. Under these conditions the retention times were typically 3.51 min for BRM and 5.57 min for TIM.

Analysis of BRM and TIM in Pharmaceutical dosage form

In pharmaceutical dosage form, both drugs BRM and TIM in ratio of 5:2. This solution was further diluted with mobile phase to obtain mixed sample solutions in the Beer's and Lamberts range.

Forced Degradation study

Procedure Brimonidine tartrate and Timolol Maleate ophthalmic drops was stressed with following mentioned conditions and solutions were prepared with respective stressed samples and each sample solution was injected into the Chromatographic system as per methodology.

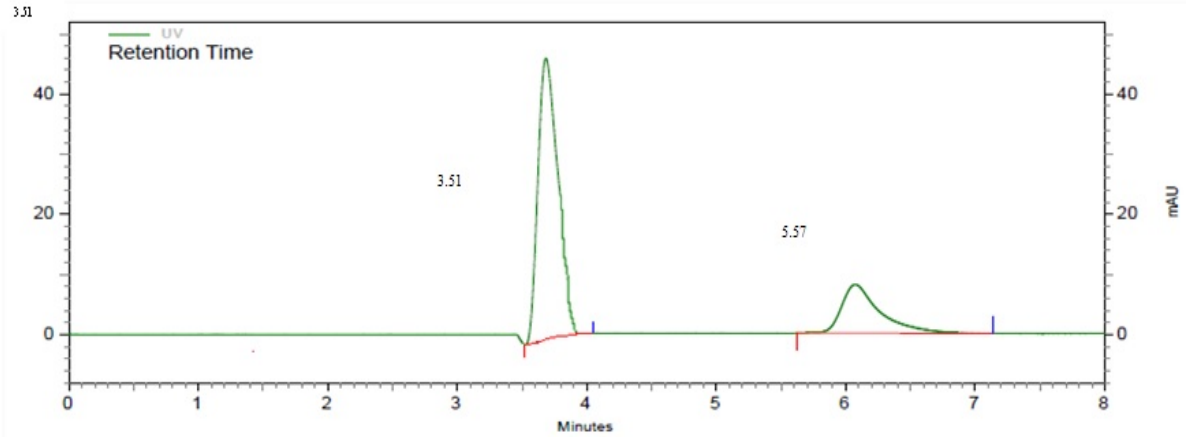

Figure 3: Chromatogram of (BRM) Brimonidine tartrate (tR 3.51 min) and (TIM) Timolol maleate (tR 5.576 min)

Oxidation Degradation Studies: To 1 ml of stock solution of Brimonidine and Timolol, 1 ml of 3% hydrogen peroxide was added separately. The solutions were kept for 30 min at room temperature. For HPLC study, the resultant solution was diluted to obtain 16µg/ml & 40 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

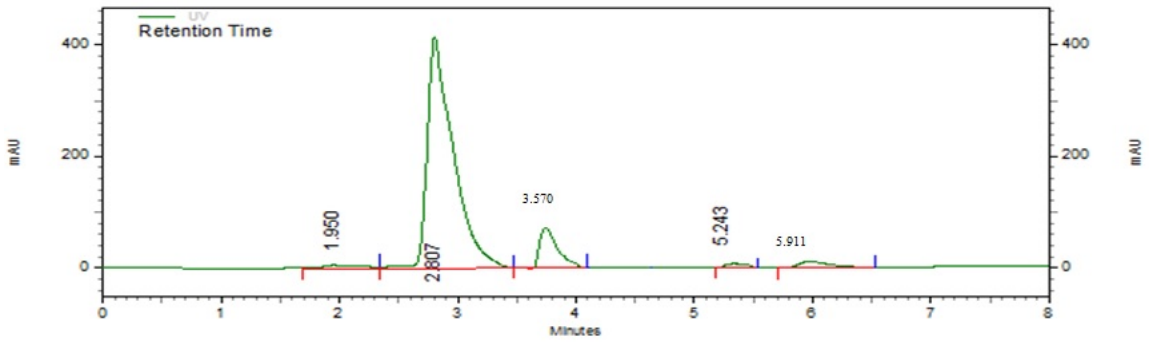

Figure 4: Chromatogram of Formulation in 3% H_2O_2

Acid Degradation Studies: To 1 ml of stock solution Brimonidine and Timolol, 1 ml of 0.1N Hydrochloric acid was added and kept for 30mins at room temperature. The resultant solution was diluted to obtain 16 µg/ml & 40 µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded.

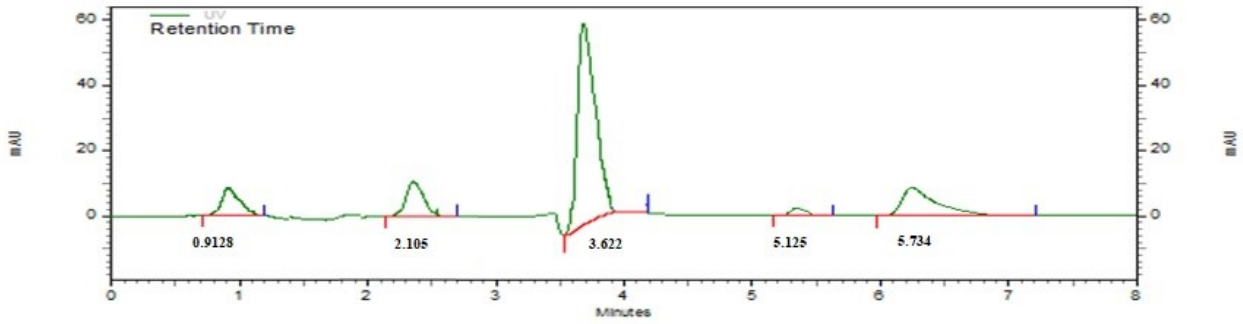

Figure 5: Chromatogram of Formulation in 0.1 N acid

Alkali Degradation Studies: To 1 ml of stock solution Brimonidine and Timolol, 1 ml of 0.1N sodium hydroxide was added and refluxed for 30mins at room temperature. The resultant solution was diluted to obtain 16µg/ml & 40 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded.

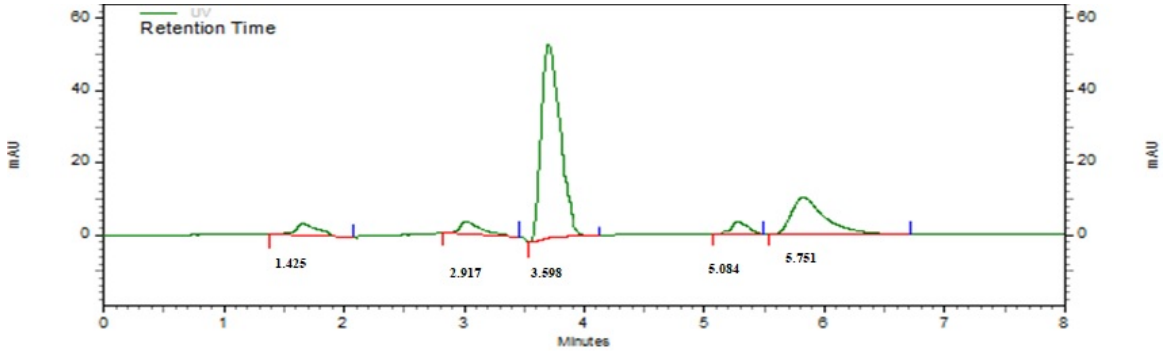

Figure 6: Chromatogram of Formulation in 0.1 N alkali

Photolytic Degradation Studies: To 1 ml of Brimonidine and Timolol stock solution were kept in UV chamber at 366nm. The resultant solution was diluted to obtain 16µg/ml & 40 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded.

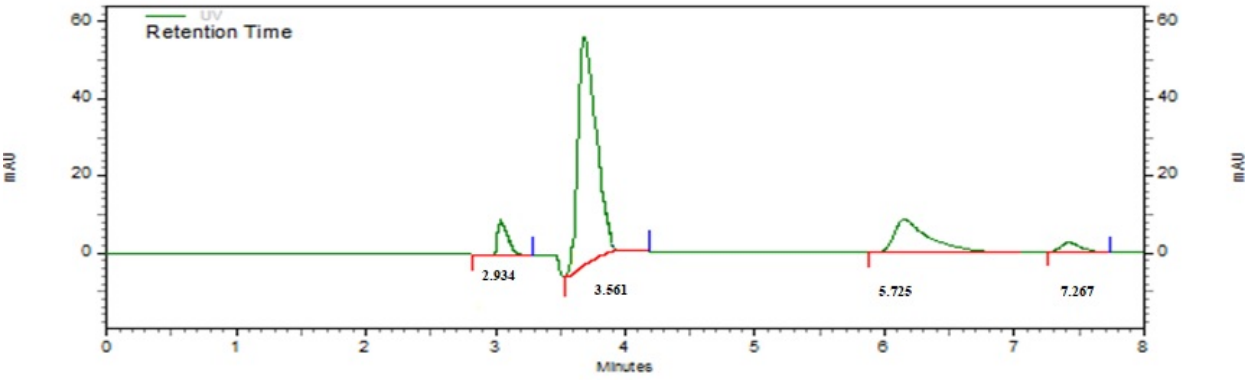

Figure 7: Chromatogram of Formulation in Photolytic degradation

Thermal Degradation Studies: To 1 ml of Brimonidine and Timolol stock solution were kept in hot air oven at 50℃ for 1 hr. The resultant solution was diluted to obtain 16 µg/ml & 40 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded.

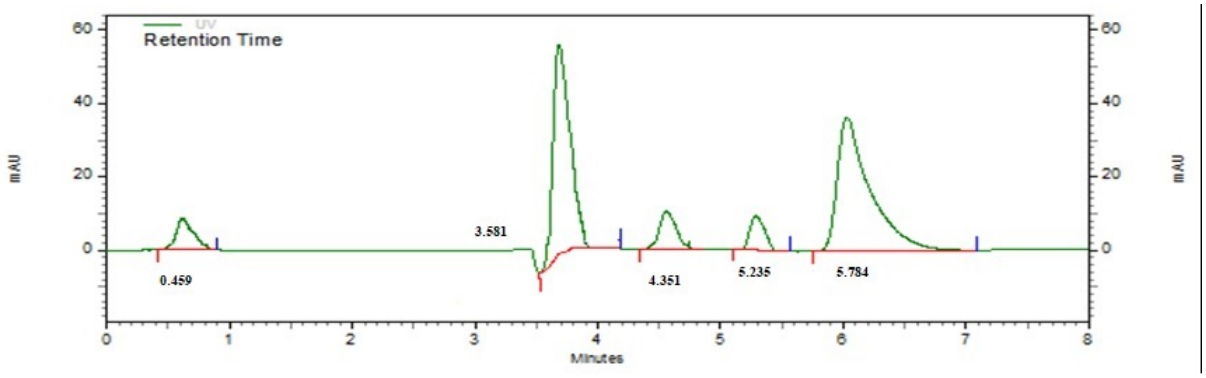

Figure 8: Chromatogram of Formulation in Thermal degradation

RESULTS AND DISCUSSION:

The results of validation studies on simultaneous estimation method developed for Brimonidine tartrate and Timolol maleate in the current study involving Water: Methanol: Triethanolamine (50:50:0.2 v/v), as the mobile phase for HPLC are given **Table 1**.

Linear correlation was obtained between absorbance versus concentrations of BRM and TIM in the ranges of 10-24 µg/ml and 25-60 µg/ml, respectively. The linearity of

the calibration curve was validated by the high values of correlation coefficient of regression.

Forced Degradation Studies

The results of the forced degradation studies indicated the specificity of the method that has been developed. The BRM and TIM were stable in all stress conditions. The results of forced degradation studies are shown in **Table 2**.

Method precision

The % RSD values for Brimonidine tartrate were found to be 1.94%. The RSD values for Timolol maleate were found to be 0.4386%. Relative standard deviation was less than 2 %, which indicates that the proposed method is repeatable **Table 3**.

LOD and LOQ:

The LOD and LOQ were separately determined based on the calibration curves for Brimonidine tartrate and Timolol maleate. The LOD and LOQ were found to be 0.130µg/ml and 0.395µg/ml for BRM and 0.1805µg/ml and 0.547µg/ml for TIM respectively.

Robustness:

The standard deviation of the peak areas was calculated for each parameter and the %RSD was found to be less than 2 %. Results shows low values of %RSD signify the robustness of the method shown in **Table 4**.

Accuracy:

The recovery experiments were performed by the standard addition method. The mean recoveries were 100.19 % and 99.7 % for BRM and TIM respectively. The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in **Table 5**.

Assay of pharmaceutical dosage form:

The proposed validated methods were successfully applied for the determination of BRM and TIM in their combined dosage forms. Results are given in **Table 6**.

System Suitability parameters:

System suitability parameters were studied to verify the optimum conditions. The system suitability test was performed as per USP guidelines on the chromatograms. Different parameters were evaluated such as retention time, resolution, tailing factor, theoretical plates. The results obtained are summarized in **Table 7**.

Table 1: Analysis Data for Brimonidine tartrate and Timolol maleate

Parameters	Brimonidine Tartrate	Timolol Maleate
Wavelength (nm)	266.0 nm	266.0 nm
Linearity (µg/ml)	10-24 µg/ml	25-60 µg/ml
Regression equation (y= mx + c)	y = 260645x – 24939	y = 29726x – 22840
Correlation Coefficient (r^2)	0.999	0.999
Slope (m)	260645	29726
Intercept (c)	24939	22840

Table 2: Results of forced degradation study

Type of degradation	Conditions	% Degradation	
		BRM	TIM
Acid degradation	0.1 N HCl for 30 min at Room temp.	17.91	14.17
Alkali degradation	0.1 N NaOH for 30 min at Room temp.	18.57	13.12
Oxidative degradation	3% H_2O_2 for 30 min at Room temp.	15.35	11.12
Photolytic degradation	UV- for 30 min at 366 nm	13.25	12.61
Thermal degradation	In hot air oven at 50 °C for 1 hr	9.75	11.05

Table 3: Precision data for Brimonidine tartrate and Timolol maleate

Drug	Conc. (µg/ml)	Intra-day Precision		Inter-day Precision	
		Mean ± S.D	%RSD*	Mean ± S.D	%RSD*
Brimonidine tartrate	12	99.82±0.425	0.428	99.8±0.317	0.318
	16	100.12±0.375	0.374	98.97±1.32	1.33
	20	100.16±0.849	0.847	99.38±1.50	1.495
Timolol maleate	30	99.99±0.237	0.230	100.05±0.96	0.963
	40	100.13±1.24	1.23	101.10±1.25	1.23
	50	99.98±0.40	0.40	100.16±0.411	0.40

*Average of six experiments for repeatability and three experiments for Intra-day, Inter-day.

Table 4: Robustness data for Brimonidine tartrate and Timolol maleate

Factor	Level	Retention time (min)		% RSD*	
		BRM	TIM	BRM	TIM
Flow rate	0.8	4.59	7.22	1.69	0.49
	1.2	2.47	3.17	1.61	0.94
Mobile phase (water: methanol)	55:45	2.96	4.98	0.58	0.418
	45:55	4.066	6.023	0.614	0.66

*Average of three experiments

Table 5: Recovery data for Brimonidine tartrate and Timolol maleate

Drug	Amount Present (µg/ml)	Amount Added (µg/ml)	Total amount (µg/ml)	Amount Recovered* (µg/ml)	Recovery (%)*± RSD
Brimonidine tartrate	10	8	18	18.03	100.19± 0.23
	10	10	20	19.89	99.33± 0.78
	10	12	22	21.92	99.86± 0.28
Timolol maleate	25	20	45	44.80	99.63± 0.372
	25	25	50	50.10	100.26± 0.69
	25	30	55	54.90	99.91± 0.37

*Average of three experiments.

Table 6: Assay data for Brimonidine tartrate and Timolol maleate in pharmaceutical dosage form in eye drops

SR. No	Amount Taken ((µg/ml)		Amount found ((µg/ml)		% Label Claim	
	BRM	TIM	BRM	TIM	BRM	TIM
1	16	40	15.96	39.92	99.75	99.8
2	16	40	15.92	40.64	99.5	101.6
3	16	40	16.22	40.16	101.37	100.4
4	16	40	16.08	40.32	100.5	100.8
5	16	40	15.84	39.36	99	98.4
6	16	40	15.94	40.16	99.625	100.4
Mean			15.993	40.093	99.95	100.233
S.D			0.13545	0.430	0.844	1.076
% RSD			0.846	1.07	0.845	1.073

Table 7: System suitability parameters

System Suitability Parameters	Proposed Method	
	Brimonidine tartrate	Timolol maleate
Retention Time (tR) (min)	3.51	5.57
Capacity Factor (k)	4.84	9.33
Plates number (n)	3248	2357
Tailing Factor (T)	1.65	1.84
Resolution Factor (R)	5.57	

CONCLUSION

For the simultaneous determination of brimonidine tartrate and timolol maleate as a combination medicinal dose, a simple, specific, precise, and accurate RP-HPLC approach was established. This straightforward procedure provides accurate results while predicting the combined dose form in a short-term investigation. As a result, quality control methods developed and validated for simultaneous estimate of both medications can be successfully employed in routine quality control analysis.

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