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Analytical Method Development and Validation for Simultaneous Estimation of Timolol Maleate and Brimonidine Tartrate in Bulk and Marketed Ophthalmic Formulation

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ABSTRACT:

A new, simple, precise, accurate and sensitive High Performance Thin Layer Chromatographic method has been developed for the estimation of Timolol Maleate and Brimonidine Tartrate in bulk and marketed ophthalmic formulation. The determination was made at 268 nm for Timolol Maleate and Brimonidine Tartrate over the concentration range of 500-1500 ng/spot and 200-600 ng/spot respectively. The mean recovery of Timolol Maleate and Brimonidine Tartrate was found 99.40% and 99.14% respectively. The LOD was found to be 65.33 ng/spot and 48.20 ng/spot for Timolol Maleate and Brimonidine Tartrate respectively. The LOQ was found to be 197.97 ng/spot and 146.08 ng/spot for Timolol Maleate and Brimonidine Tartrate respectively. Chloroform: Methanol: Ammonia (30%) (9:1:0.1 v/v/v) was selected as a mobile phase. The validation of method was carried out as per ICH Guidelines.

Keywords: Timolol Maleate, Brimonidine Tartrate, High Performance Thin Layer Chromatographic Method, Validation, Combigan Eye-Drops.

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INTRODUCTION:

Analysis of pharmaceutical product is very important as it concerned with quality of life. Timolol Maleate and Brimonidine Tartrate are used separately and in combination for the treatment of Glaucoma. Timolol Maleate blocks both β -1 and β -2 adrenergic receptors, reduces intraocular pressure by reducing aqueous humor production or possibly outflow; reduces blood pressure by blocking adrenergic receptors and decreasing sympathetic outflow, produces a negative chronotropic and inotropic activity through an unknown mechanism. Brimonidine Tartrate is an alpha adrenergic receptor agonist. It has a peak ocular hypotensive effect occurring at two hours post-dosing. Fluorophotometric studies in animals and humans suggest that brimonidine tartrate has a dual mechanism of action by reducing aqueous humor production and increasing uveoscleral outflow. Till date no High Performance Thin Layer Chromatographic method was reported for quantitative estimation of Timolol Maleate and Brimonidine Tartrate. The present manuscript describes simple, accurate, precise and sensitive High Performance Thin Layer Chromatographic Method for the estimation of Timolol Maleate and Brimonidine Tartrate in bulk and marketed ophthalmic formulation.

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Camag Linomat IV (Semiautomatic Spotting device)

Camag Twin trough chamber (10 x10 cm²)

(www.jpsbr.org)

MATERIAL AND METHODS

Instruments

Camag Linomat IV (Semiautomatic Spotting device)

Camag Twin trough chamber (10 x10 cm²)

Camag TLC Scanner-3

Camag CATS4 Software

Hamilton Syringe (100 µl)

ELICO Li614 pH analyser

All weighing were done on electronic analytical balance (Wensar Dab220).

Chemicals and Reagents:

Timolol Maleate and Brimonidine Tartrate bulk powders were obtained from FDC pvt. Ltd., India. The formulation Combigan was procured from the local market. All other chemicals used were of analytical grade. Calibrated glass wares were employed throughout the work.

Selection of a Mobile Phase:

Chloroform: Methanol: Ammonia (30%)(9:1:0.1 v/v/v) was selected as a mobile phase for Timolol Maleate and Brimonidine Tartrate.

Preparation of Standard Stock Solution

A mixed standard solution of Timolol Maleate (125 µg/ml) and Brimonidine Tartrate (50 µg/ml) was prepared by accurately weighing Timolol Maleate (13.7 mg) dissolving in methanol and Brimonidine Tartrate (10 mg) dissolving in water and diluted to 10 ml with methanol and water respectively in the same volumetric flask. After this take 2.5 ml solution of Timolol Maleate solution and 1 ml solution of Brimonidine Tartrate in 10 ml volumetric flask and dilute up to 10 ml with methanol. 5 ml from this solution was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol.

Preparation of Working Solutions

The sample consisted of different concentrations of standard Timolol Maleate solution ranging from 500-1500 ng/spot and standard Brimonidine Tartrate solution ranging from 200-600 ng/spot. The solutions were prepared by pipetting out 4, 6, 8, 10 and 12 µl of the stock solution containing Timolol Maleate (125 µg/ml) and Brimonidine Tartrate (50 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol.

Chromatographic Condition:

Stationary phase: Precoated silica gel G60 F254 aluminium sheets 10X10 cm², layer thickness 0.2mm.

Activation: TLC plates prewashed with Methanol and activated in oven at 60°C for 10 mins.

Mobile phase: Chloroform: Methanol: Ammonia (30%) (9:1:0.1 v/v/v)

Temperature: Room temperature

TLC chamber saturation Time: 15 min

Migration distance: 6 cm

Detection: Densitometrically using a UV detector at 268 nm

Spotting parameters:

Band width: 5mm

Space between 2 bands: 5mm

Spraying rate: 10 µl/sec

Scanning parameters:

Slit dimension: 4X0.45 mm

Wavelength of detection: 268 nm

Lamp: Deuterium

Measurement mode: Absorption/Reflection

The solution of Timolol Maleate and Brimonidine Tartrate were applied to silica gel G60 F254 TLC plates (10 cm × 10 cm) by means of applicator under the chromatographic condition mentioned above. The plate was developed in a twin-trough chamber previously saturated for 15 min with the mobile phase. TLC plate was dried in a current of air. Scanning was performed in the reflectance-absorption mode using a UV detector in the range of 200-400 nm. Both components show reasonable good response at 268 nm. So 268 nm was selected as an analytical wavelength.

Assay of Pharmaceutical dosage form

1 ml solution of eye drops was taken in a 10 ml volumetric flask and was diluted up to the mark with distilled water + methanol (2:8). The solution obtained was 500 µg/ml of Timolol Maleate and 200 µg/ml of Brimonidine Tartrate.

From this transfer 5 ml of the above stock solution in 10 ml volumetric flask and make up to the mark with distilled water+ methanol (2:8). So the resulted solution contains 250µg/ml of Timolol Maleate and 100µg/ml of Brimonidine Tartrate.

From this stock solution 3 μ l solution was applied as a band and the plate was then developed, dried and analyzed. The experiment was repeated 3 times.

Method Validation

Method validation was performed following ICH guidelines. The proposed method has been extensively validated in terms of linearity, accuracy and precision, limit of detection, limit of quantification and system suitability parameter.

Linearity (Calibration curve)

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 500-1500 ng/spot and 200-600 ng/spot for Timolol Maleate and Brimonidine Tartrate respectively (n=3).

The calibration curves were constructed by plotting area v/s. concentrations. The linear regression equation were $y = 2.633x + 1923$. ($R^2 = 0.994$) and $y = 6.572x + 4404$. ($R^2 = 0.998$) for Timolol Maleate and Brimonidine Tartrate respectively.

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the prequantified sample preparation at three different concentration levels 50 %, 100 % and 150 %, taking in to consideration percentage purity of added drug sample. The amount of Timolol Maleate and Brimonidine Tartrate was estimated by applying obtained values to the respective regression line equations. Each concentration was analysed 3 times and average recovery was measured.

Precision

Precision is the measure of either the degree of reproducibility or of repeatability of analytical method. It is indication of random error. The precision of an analytical method is usually expressed as the standard deviation, relative standard error or coefficient of variance of a series of measurements.

I. Repeatability

Precision performed under the same conditions (same apparatus, same analyst within the short intervals of time and using identical reagents) using the same sample.

(a) Repeatability of measurement of scanner: (%RSD<1%, n=6)

A mixed standard solution (8 μ l) was spotted on precoated TLC plate. The plate was developed, dried and analyzed as described. Area of spot was measured six times and %RSD of obtained data was calculated.

II. Intraday reproducibility:

A variation of results within same day is called intraday variation. It was determined by repeating calibration curve 3 times on same day.

III. Interday reproducibility:

Variation of results amongst day is called interday variation. It was determined by repeating calibration curve daily for 3 different days.

Limit of Detection and Limit of Quantification

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the $3.3 \times (SD/Slope)$ and $10 \times (SD/Slope)$ criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slope of the calibration curve.

RESULT AND DISCUSSION

A reliable method was developed for the estimation of the Timolol Maleate and Brimonidine Tartrate in bulk and marketed ophthalmic formulation by High Performance Thin Layer Chromatography. It was performed in concentration range of 500-1500ng/spot and 200-600ng/spot for Timolol Maleate and Brimonidine Tartrate respectively at 268 nm. The correlation coefficient was found to be $R^2 = 0.994$ and $R^2 = 0.998$ for Timolol and Brimonidine respectively. The mean recovery of Timolol Maleate and Brimonidine Tartrate was found 99.40% and 99.14% respectively. The LOD was found to be 65.33ng/spot and 48.20ng/spot for Timolol Maleate and Brimonidine Tartrate respectively. The LOQ was found to be 197.97ng/spot and 146.08ng/spot for Timolol Maleate and Brimonidine Tartrate respectively. The proposed method was precise and accurate, which can be applied for the analysis of Timolol Maleate and Brimonidine Tartrate in marketed formulation.

CONCLUSION

The results of our study indicate that the proposed High Performance Thin Layer Chromatographic method is simple, rapid, precise and accurate. The developed High Performance Thin Layer Chromatographic method was found suitable for determination of Timolol Maleate and Brimonidine Tartrate in

pharmaceutical dosage form without any interference from the excipients. This method is selective for the analysis of Timolol Maleate and Brimonidine Tartrate. It can therefore be conclude that use of the method can save time and money and it can be done with accurate and wide linear range.

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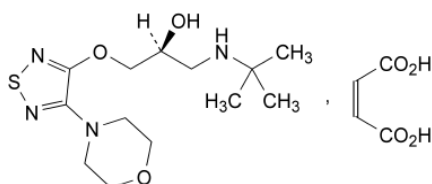


Figure 1: Structure of Timolol Maleate

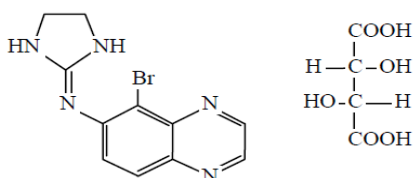


Figure 2: Structure of Brimonidine Tartrate

Table 1: Regression analysis data and summary of validation parameters for the proposed method

SR.NO	PARAMETERS	Timolol Maleate	Brimonidine Tartrate
1	Linearity and Range(µg/ml)	500-1500	200-600
2	Correlation coefficient	0.994	0.998
3	Precision (%RSD)		
	1.Repeatability		
	Scanner	0.20	0.22
	Spotter	1.69	1.09
	2.Intraday	0.82-0.91	0.32-1.31
	3.Interday	1.70-1.89	1.08-1.57
4	Accuracy (%Recovery)	99.40	99.14
5	LOD(µg/ml)	65.33	48.20
6	LOQ (µg/ml)	197.97	146.08
7	Assay	99.28	98.77

Table 2: Recovery data of proposed method

Drug	Amount of Sample (µg/ml)	Amount of standard added (µg/ml)	Total amount (µg)	Total amount found (µg)	Amount Recovered (% Recovery) ±S.D (n=3)	% RSD
Timolol Maleate	500	0	500	495.36	-	-
	500	250	750	744.94	249.57(99.83) ±0.9157	0.122
	500	500	1000	994.00	498.64(99.4) ±3.3801	0.343
	500	750	1250	1237.4	742.18(98.96) ±2.6945	0.217
Brimonidine Tartrate	200	0	200	196.78	-	-
	200	100	300	296.13	99.35(99.35) ±0.1724	0.058
	200	200	400	395.96	198.48(99.24) ±1.0307	0.263
	200	300	500	493.27	296.49(98.83) ±0.6655	0.136

Table 3: Analysis of Timolol Maleate and Brimonidine Tartrate by proposed method

Sr No	Drug	Conc. Added (ng/spot)	Conc. found (ng/spot)	% Conc. found ±S.D (n=3)	% RSD
1	Timolol Maleate	750	744.60	99.28 ± 36.8796	0.9545
2	Brimonidine Tartrate	300	296.32	98.77 ± 41.5394	0.6540

Table 4: System Suitability Parameter

System Suitability Parameter	Timolol Maleate	Brimonidine Tartrate
R _f Value	0.49	0.54
Area±S.D	4726.67±9.47300	6964.63±15.6034

Figure 3: DENSITOGRAM OF TIMOLOL MALEATE (1000 ng/spot) AND BRIMONIDINE TARTRATE (400 ng/spot)

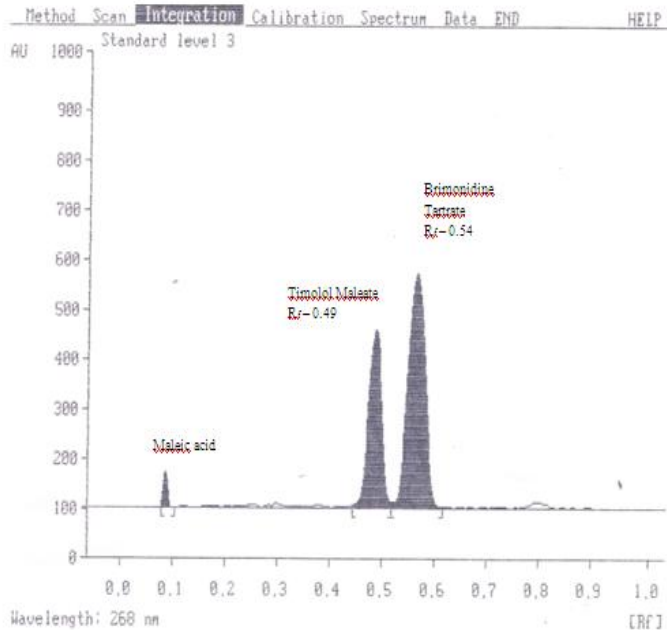


Figure 5: CALIBRATION CURVE FOR STANDARD TIMOLOL MALEATE (500-1500 ng/spot)

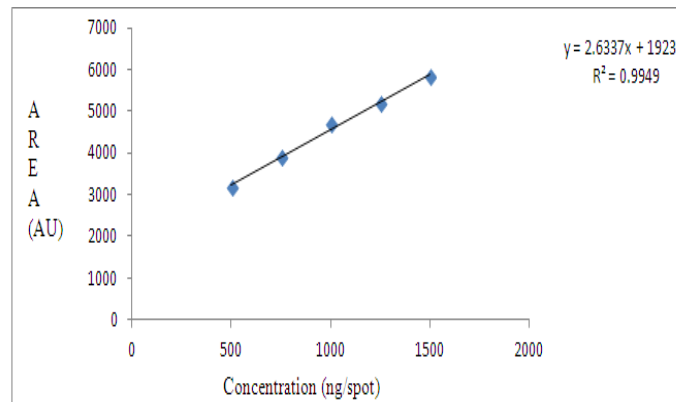


Figure 4: OVERLAY DENSITOGRAM OF TIMOLOL MALEATE (500-1500 ng/spot) AND BRIMONIDINE TARTRATE (200-600 ng/spot)

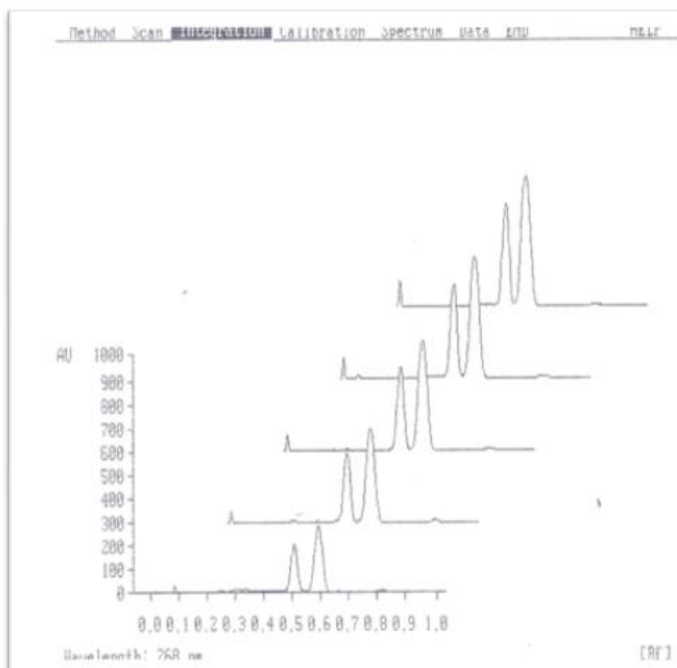
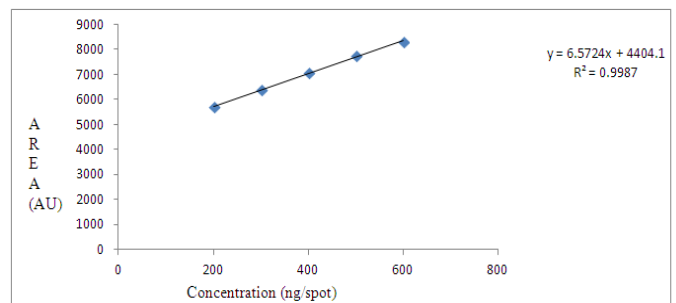


Figure 6: CALIBRATION CURVE FOR STANDARD BRIMONIDINE TARTRATE (200-600 ng/spot)



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