Stability Indicating HPTLC Method For The Simultaneous Estimation Of Brimonidine Tartrate And Timolol Maleate

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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 Brimonidine Tartrate and Timolol Maleate in combination. The determination was made at 281 nm for Brimonidine Tartrate &Timolol Maleate. Chloroform: Methanol: Ammonia in ratio of (9: 0.2: 0.1) v/v/v was the optimized mobile phase for estimation of combination. The validation of method was carried out as per ICH Guidelines.*

Key Words: *Brimonidine & Timolol, HPTLC, Stability indicating.*

1. INTRODUCTION:

Brimonidine Tartrate is chemically [5- bromo-6(2-imidazolidinylideneamino) quinoxaline L-tartrate]&Timolol Maleate [(S)-1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morphonlinyl)-1,2,5- thiadiazol -3-yl]oxy]-2-propanol,(Z)-2-butenedioate] (1). Timolol Maleate and Brimonidine Tartrate are used separately and in combination for the treatment of Glaucoma (2). 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. Number of chromatographic methods like HPTLC (3) and HPLC (4,5,6) methods are reported. Literature survey reveals that spectrophotometric methods like Simultaneous Equation Method (7), Absorbance Ratio Method (8) & Area Under Curve (9) are reported. But no stability indicating HPTLC method was reported for estimation of Brimonidine Tartrate & Timolol Maleate.

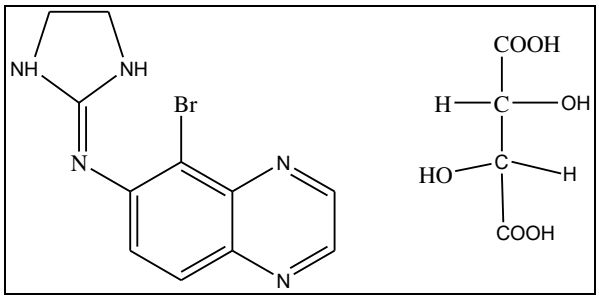

Fig.1 Structure of Brimonidine Tartrate

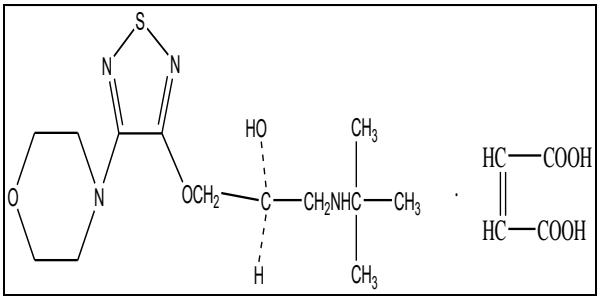

Fig.2 Structure of Timolol Maleate

2. MATERIAL AND METHODS :

Instruments

HPTLC

Camag Linomat 5 (Semiautomatic Spotting device)
Camag Twin trough chamber (10 x10 cm)
Camag TLC Scanner-3
Camag WINCATS Software (version 1.4.3.6336)
Hamilton Syringe (100 µl)
All weighing were done on electronic analytical balance. (Shimadzu AY 120)

Chemicals and Reagents:

Timolol Maleate and Brimonidine Tartrate working standards were obtained from Micro Labs pvt. Ltd., India. , Distilled water. Chloroform, Methanol & Ammonia were purchased from LOBA Chemie (Mumbai).

Selection of a Mobile Phase:

Chloroform: Methanol: Ammonia (30%) (9:0.2:0.1 v/v/v) was selected as a mobile phase for Brimonidine Tartrate & Timolol Maleate.

Preparation of Standard Stock Solution:

Standard stock solution was prepared by dissolving, accurately measured 10mg of Brimonidine Tartrate &Timolol Maleate separately in water and the volume was made up to 10 ml (stock solution 1000µg/ml).

Preparation of Working Solutions

The sample spotted was of different volumes of standard Brimonidine Tartrate solution and standard Timolol Maleate solution ranging from 200 - 1000 ng/spot. The solutions were prepared by pipetting out, 2ml of the stock solution containing Brimonidine Tartrate & (5 ml) Timolol Maleate from 1000 ug/ml stock solution was mixed & diluted to 10 ml & used for spotting.

Chromatographic Condition:

Stationary phase: Precoated silica gel G60 F_{254}aluminium sheets 10x10 cm, layer thickness 0.2mm.
Activation: TLC plates prewashed with Methanol
Mobile phase: Chloroform: Methanol: Ammonia (9:0.2:0.1 v/v/v)
Temperature: Room temperature
TLC chamber saturation Time: 10 min
Migration distance: 6 cm
Detection: Densitometrically using a UV detector at 281 nm
Band width: 6 mm
Space between 2 bands: 5mm
Spraying rate: 2 µl/sec
Scanning parameters:
Slit dimension: 5 x 0.20 mm
Wavelength of detection: 281 nm
Lamp: Deuterium

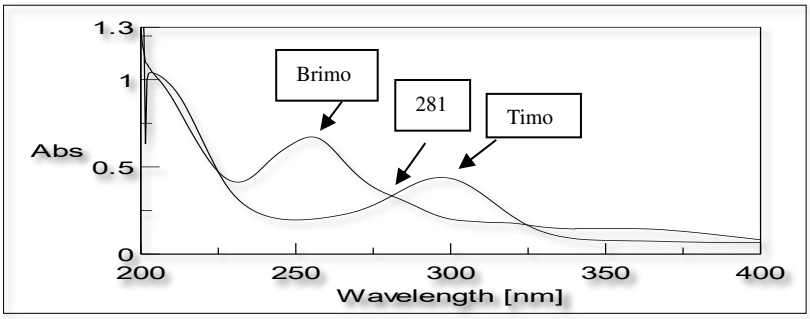

Fig 3. Overlay spectrum of Brimonidine Tartrate &Timolol Maleate

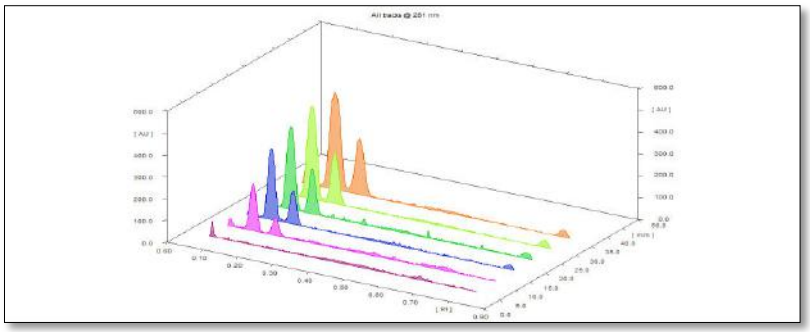

Fig 4 :Densitogram of Brimonidine Tartrate &Timolol Maleate (200 – 1000 ng/band)

Table 1. : Linearity of Brimonidine Tartrate &Timolol Maleate

Sr. no.	Conc (ng/band)	Rf		Area	
		Timo	Brimo	Timo	Brimo
1.	**200**	0.11	0.17	3449.8	1810.7
2.	**400**	0.11	0.17	4352.6	2830.3
3.	**600**	0.11	0.17	5542.6	3521.6
4.	**800**	0.11	0.17	7282.7	4283.2
5.	**1000**	0.11	0.17	8490.6	5241.2

3. FORCED DEGRADATION STUDIES [9]

To provide an indication of the stability-indicating ability and specificity of the proposed method forced degradation studies were performed. Hydrolysis under acidic, alkaline & neutral & oxidative & thermal and photolytic degradation studies were conducted on Brimonidine Tartrate &Timolol Maleate with the objective of obtaining 10-30% degradation.

Acid degradation:

2 ml of working standard solution of Brimonidine Tartrate &Timolol Maleate (1000 µg/ml) was mixed with 2 ml of 1N HCl. The 6 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition. Sample aliquots was kept for overnight degradation & volume was made up with distilled water upto 10 ml & examined.

Alkaline degradation:

2 ml of working standard solution of Brimonidine Tartrate &Timolol Maleate (1000 µg/ml) was mixed with 2 ml of 1N NaOH. The 6 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition. Sample aliquots was kept for overnight degradation & volume was made up with distilled water upto 10 ml & examined.

Oxidative degradation:

2 ml of working standard solution of Brimonidine Tartrate &Timolol Maleate (1000 µg/ml) was mixed with 2 ml of 30 % H_2O_2. The 6 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition. Sample aliquots was kept for overnight degradation & volume was made up with distilled water upto 10 ml & examined.

Neutral degradation:

2 ml of working standard solution of Brimonidine Tartrate &Timolol Maleate (1000 µg/ml) was mixed with 2 ml of distilled water. The 6 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition. Sample aliquots was kept for overnight degradation & volume was made up with distilled water upto 10 ml & examined.

Thermal Degradation

Dry heat studies were performed by keeping drug sample in oven (100^0 C) for a period of 8 hours. Sample was cool & withdrawn, dissolved in distilled water and diluted to get 10ml. 6µL of the resultant solution was then applied at TLC plate and densitogram was developed.

Photolytic degradation

The photo degradation stability study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/m2 and separately to cool white fluorescent light of NLT 1.2 million Lux-Hr. After exposure accurately weighed 10 mg of drug was transferred to 10 mL of volumetric flask obtain 1000µg/ml. 6 µL of the resultant solution was then applied at TLC plate and densitogram was developed.

Table 2. Forced degradation studies of Brimonidine Tartrate &Timolol Maleate

Sr.No.	Degradation condition	% Recovery (brimo)	% Recovery (timo)	r (s,m)	r(e,m)
1.	**Acid Hydrolysis** (RT 24hrs) (1 N HCl)	93.86	86.50	0.999	0.993
2.	**Base Hydrolysis** (RT 24hrs) (1 N NaOH)	88.94	88.12	0.989	0.991
3.	**Oxidation** (RT 24hrs)(30 % H_2O_2)	79.15	84.16	0.995	0.993
4.	**Neutral** (distilled water) (6 hrs)	94.14	87.56	0.985	0.994
5.	**Photolytic Degradation** (Exposure to UV light for 200 watt hours)	94.05	90.46	0.995	0.999
6.	**Fluroscence Degradation** (Exposure to light for 1.2 million lux hours)	96.50	88.12	0.999	0.996
7.	**Thermal degradation** (Exposure to dry heat at 100ºC for 8 Hrs)	89.69	84.05	0.994	0.998

4. METHOD VALIDATION (10)

Linearity& Range

Five different concentrations of Brimonidine Tartrate & Timolol Maleate in the range of (200-1000 ng/band) were prepared from standard stock solution of 1000 µg/ml of both the drugs and was spotted & analysed. Linearity equation of Brimonidine Tartrate was found to be y = 4.1154 x + 1076.7, r^2 = 0.995 & of Timolol Maleate was found to be y = 6.427 x + 1985.6, r^2 = 0.985 respectively.

Specificity:

The specificity of the method was checked by peak purity profiling studies of stress degradation samples. The peak purity values was found to be more than 0.990. This indicates there is non interference of any other impurity or degradation product. Thus, the method is said to be specific.

Precision:

The precision was evaluated with respect to both repeatability and intermediate precision. Repeatability was evaluated by a minimum of 9 determinations covering the specified range for the procedure (e.g. 3 concentrations/ 3 replicates each) for the test solution of the drugs Brimonidine Tartrate (200 ng/band) &Timolol Maleate (500 ng/band). The studies were repeated for three different days to determine intermediate precision. Peak areas of the drugs were determined and % RSD was calculated.

Table 3: Interday Precision for Brimonidine Tartrate &Timolol Maleate

Conc. (ng/ml)	Mean Area		SD		%RSD	
	Brimo	Timo	Brimo	Timo	Brimo	Timo
200	1566.66	4273.13	29.05	25.45	1.85	0.59
400	2932.5	6184.13	19.94	199.98	1.93	0.68
600	4112.03	7890.46	52.04	101.18	1.26	1.28

Table 4: Intraday Precision for Brimonidine Tartrate &Timolol Maleate

Conc (ug/ml)	Mean Area		SD		%RSD	
	Brimo	Timo	Brimo	Timo	Brimo	Timo
200	2063.53	4443.23	23.88	50.10	1.15	1.12
400	2849.66	4646.86	42.98	40.48	1.50	0.87
600	3943.06	6374.2	36.04	81.03	0.91	1.27

Assay:

Assay was performed on blend of bulk drugs plus excipients. It was determined by extrapolation of peak area & it was found to be Brimonidine Tartrate – 97.53 % &Timolol Maleate – 99.78 %.

Accuracy:

The accuracy of the method was assessed by the recovery studies at three different concentrations by the addition of known amount of standard to the test solutions. The drugs were spiked at three different levels i.e., 80 %, 100% & 120 %. The recovery was calculated by slope and intercept of the linearity plot of drugs. The results obtained for accuracy are presented in the table.

Table 5: Accuracy for Brimonidine Tartrate &Timolol Maleate

Drug	Level % of Accuracy	% Recovery	% RSD
Brimonidine Tartrate	**80**	98.98	1.38
	100	102.88	0.73
	120	99.86	0.36
Timolol Maleate	**80**	98.89	1.44
	100	101.23	0.48
	120	98.04	1.17

Limit of detection (LOD) and Limit of Quantitation (LOQ):

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S where, σ = standard deviation of the y-intercept of linearity equations and S = slope of the calibration curve of the analyte.

Table 6: LOD & LOQ for Brimonidine Tartrate &Timolol Maleate

Drug	LOD	LOQ
Brimonidine Tartrate	30.94 ng/band	93.76 ng/band
Timolol Maleate	48.70 ng/band	147.59 ng/band

Robustness:

Robustness of the method was determined by carrying out the analysis under conditions during which Saturation time (20 mins) ± 5 min., Mobile phase composition, Time from spotting to development (immediate),

Detection Wavelength and the effects on the peak area was noted. The %RSD values of all robustness parameters were examined and found to be within the limit of 2%, showed that the proposed method was robust.

Table 7: Summary of Validation Studies

Sr. no.	Validation Parameters		Brimonidine Tartrate		Timolol Maleate	
1.	Specificity		Specific		Specific	
2.	Linearity Range		y = 4.1154 x + 1076.7 r^2 = 0.995 (200- 1000 ng/ml)		y = 6.427 x + 1985.6 r^2 = 0.985 (200- 1000 ng/ml)	
3.	Precision	Interday	**200**	1.85	**200**	0.59
			400	1.93	**400**	0.68
			600	1.26	**600**	1.28
		Intraday	**200**	1.15	**200**	1.12
			400	1.50	**400**	0.87
			600	0.91	**600**	1.27
4.	Assay		97.53 %		99.78 %	
5.	Accuracy	**80 %**	98.98 %		98.89 %	
		100 %	102.88 %		101.23 %	
		120 %	99.86 %		98.04 %	
6.	Limit of Detection (LOD)		30.94 ng/ml		48.70 ng/ml	
7.	Limit of Quantitation (LOQ)		93.76 ng/ml		147.59 ng/ml	
8.	Robustness		Robust		Robust	

5. CONCLUSION:

Literature survey revealed that few HPLC and UV methods have been reported for estimation of Brimonidine tartrate. No HPTLC Stability indicating method has been reported for estimation of Brimonidine tartrate & Timolol maleate. The present study was aimed at development of HPTLC technique for determination Brimonidine tartrate &Timolol maleate as bulk drugs.

These drug showed 10- 20 % degradation in acidic, alkali, neutral, oxidation, thermal & Photolytic conditions. The HPTLC method proved to be simple, less expensive, fast, accurate, precise and robust and thus can be used for routine analysis of Brimonidine tartrate in ophthalmic solutions.

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