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R E S E A R C H A R T I C L E

Simultaneous Estimation Method Development and Validated on Timolol Maleate and Brimonidine Tartrate by Using HPLC

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A B S T R A C T

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Timolol Maleate and Brimonidine Tartrate, in its pure form as well as in tablet dosage form. Chromatography was carried out on an Agilent C18 (4.6×250mm) 5µ column using a mixture of Buffer: Acetonitrile (75:25) as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 270nm. The retention time of the Brimonidine Tartrate and Timolol Maleate was 2.0, 3.3±0.02min respectively. The method produce linear responses in the concentration range of 11.25-45µg/ml of Brimonidine Tartrate and 37.5-150µg/ml of Timolol Maleate. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Brimonidine Tartrate, Timolol Maleate, RP-HPLC, validation.

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C O N T E N T S

1. Introduction

A relatively selective alpha-2 adrenergic receptor agonist. Brimonidine Tartrate ophthalmic solution 0.2% is indicated for lowering intraocular pressure in patients with open angle glaucoma or ocular hypertension. Glaucoma is a slowly progressive optic neuropathy disease in this there is loss of retinal ganglion cells, excavation of the optic

nerve head with varying degree of visual impairment or blindness Brimonidine tartrate ophthalmic solution 0.2% 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 [1].

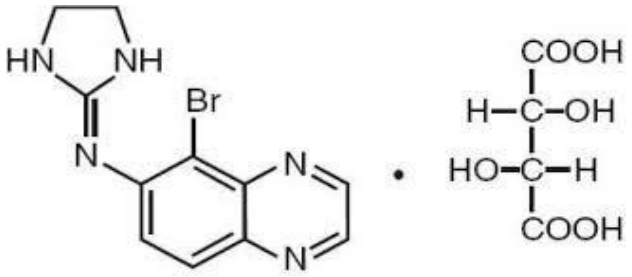

Fig 1: Chemical structure of Brimonidine Tartrate

Timolol maleate is a beta1 and beta2 (non-selective) adrenergic receptor blocking agent that does not have significant intrinsic sympathomimetic, direct myocardial depressant, or local anesthetic (membrane-stabilizing) activity. Beta-adrenergic receptor blockade reduces cardiac output in both healthy subjects and patients with heart disease. In patients with severe impairment of myocardial function, beta-adrenergic receptor blockade may inhibit the stimulatory effect of the sympathetic nervous system necessary to maintain adequate cardiac function. Beta-adrenergic receptor blockade in the bronchi and bronchioles results in increased airway resistance from unopposed parasympathetic activity. Such an effect in patients with asthma or other bronchospastic condition ispotentiallydangerous.[2]

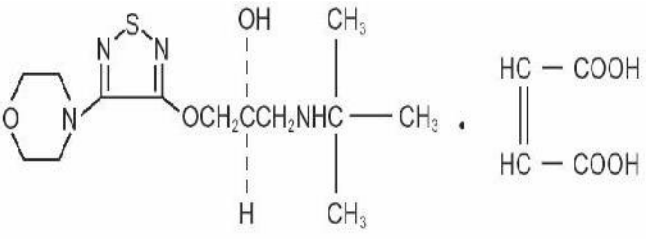

Fig 2: Chemical structure of Timolol Maleate

2. Materials and Methods

Chromatographic conditions

A perominence isocratic HPLC system (waters 2695 HPLC with auto sampler and PDA Detector) column Agilent C18 (4.6 x 250mm, 5µm). A 10µL Rheodyne injection syringe was used for sample injection. HPLC grade Water and Acetonitrile were used for the preparing the mobile phase. A freshly prepared Buffer : Acetonitrile(75:25) was used as the mobile phase. The solvents was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 0.8mL/min., column temperature was maintained at room temperature and the detection of the drug was carried out at 270nm.

Preparation of mobile phase

Buffer: Accurately weighed 2.72gm of Potassium dihyrogen ortho phosphate taken in a 1000ml volumetric flask. About 900ml of milli-Q water was added and sonicated to dissolve and finally made up the volume with water. Then PH was adjusted to 4.3 with dil. Orthophosphoric acid solution.

Diluent preparation: Mobile phase as diluent.

Preparation of standard stock solution:

Standard stock solution of Brimonidine tartrate:

Accurately 2.25 mg of Brimonidine tartrate was weighed into a clean and dry 10ml volumetric flask, dissolved with sufficient volume of diluent and sonicate for 5min.The volume made up to 10ml with diluent (225µg/ml).

Standard stock solution of Timolol Maleate:

Accurately 7.5 mg of Timolol was weighed into a clean and dry 10 ml volumetric flask, dissolved with sufficient volume of diluent and sonicate for 5min.The volume made up to 10ml with diluent (750µg/ml).

Procedure:

1 ml of standard stock solution of Brimonidine tartrate (225µg/ml) and1 ml of standard stock solution of Timolol (750 µg/ml) are transferred in to a 10 ml volumetric flask and the volume made with diluents. The resulting solution was sonicated for 10 min and injected and chromatograms were recorded. A Standard solution was prepared by using Brimonidine tartrate and Timolol maleate working standards as per the test method the prepared solution was injected six times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD of peak areas from six replicate injections.

Sample solution preparation:

5 tablets were weighed and average weight was determined and powdered. An amount of powder equivalent to average weight of 5 tablets (equivalent to 25mg of Brimonidine Tartrate and 500mg of Timolol Maleate) was weighed and transferred into a 100 ml volumetric flask. 60ml of diluent was added and sonicated for 25 min to ensure complete dissolution. Then the solution was filtered and further the volume was made up with diluent. From this solution, 0.2ml was taken into a 10 ml volumetric flask and made up the volume with diluent.

Method validation[3-12]

Linearity:

The linearity of the method was demonstrated over the concentration range of 11.25-45ppm of the Brimonidine Tartrate target concentration and 37.5-150ppm of Timolol Maleate. Different concentrations of the pure drug were injected into the chromatographic system. Calibration curve of Brimonidine Tartrate and Timolol Maleate was constructed by plotting peak area versus applied concentration of Brimonidine Tartrate and Timolol Maleate. The obtained results shown an excellent correlation between peak area and concentration of pure drug within the concentration range & it has shown in fig: 3 and 4. The correlation coefficient for the average area at each level versus concentration of analyte was calculated and is presented in Table: 1&2 and their calibration parameters were shown in Table: 3&4.

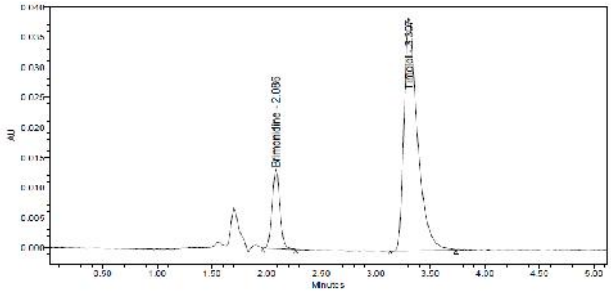

Fig 3: Optimized Chromatogram of Brimonidine Tartrate and Timolol Maleate

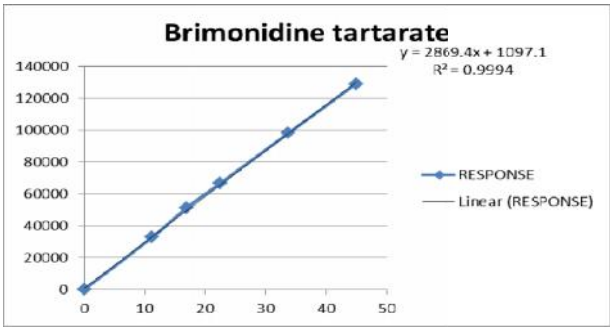

Fig 4: Calibration curve of Brimonidine Tartrate

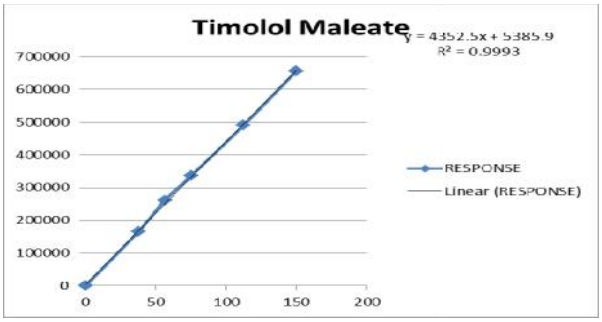

Fig 5: Calibration curve of Timolol Maleate

Precision method

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution was made and the response factor of drug peak and% RSD were calculated and present in Table 5&6. The chromatogram was shown in Fig 4. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and response factor of drug peak and %RSD were calculated shown in Table5&6. From the data obtained, the developed method was found to be precise.

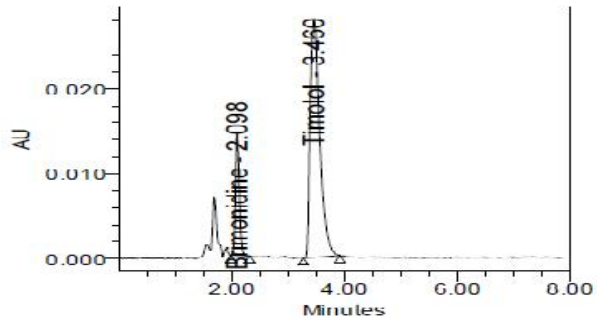

Fig 6: Chromatogram of precision

Accuracy

A study of recovery of Brimonidine Tartrate and Timolol Maleate from spiked placebo was conducted at three different spike levels i.e.50%, 100% and 150% samples were prepared with Brimonidine Tartrate and Timolol Maleate raw material equivalent to about the target initial concentration of Brimonidine Tartrate and Timolol Maleate. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in Table 7&8. The mean recoveries of Timolol Maleate spiked were found to be in the range of 99.4% - 99.7% and Brimonidine Tartrate from spiked were found to be in the range of 100.5-99.5%.

LOD and LOQ:

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The residual standard deviation of the regression lines and slope of the calibration curves were used to calculate the LOD and LOQ.

System suitability: System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 30µg/ml. The results given in Table9 were within acceptable limits.

3. Results and Discussion

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of Brimonidine Tartrate and Timolol Maleate in bulk dug and pharmaceutical dosage form by using the most commonly employed Agilent C-18 column with PDA-detection. The run time was set at 8min and the retention time for Brimonidine Tartrate and Timolol Maleate was 2.0, 3.3±0.2min respectively. Each sample was injected 5 times and the retention times were same. When the concentrations of Brimonidine Tartrate and Timolol Maleate and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r2=0.999) was observed between the concentration of Brimonidine Tartrate and Timolol Maleate and the respective peak areas in the range 11.25-45µg /ml of Brimonidine Tartrate and 37.5-150µg/ml of Timolol Maleate. The regression equation was used to estimate the amount of Brimonidine Tartrate and Timolol Maleate, either in tablet formulations or in validation study (precision and accuracy). For the proposed RP-HPLC method, characteristic parameters were shown in Table: 2.To analyse tablet formulations, RP-HPLC method has been developed. Brimonidine Tartrate and Timolol Maleate tablets were analyzed as per the procedure described above. The low % RSD values (≤ 2) indicated that the method was precise and accurate. The mean recoveries found in the range of 99% – 100.5%. No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

Table 1: System suitability studies of Brimonidine Tartrate and Timolol Maleate by RP-HPLC method

Property	Brimonidine Tartrate Values	Timolol Maleate Values	Required limits
Retention time (R_t)	2.0±0.02	3.3±0.02	RSD ≤ 2%
Theoretical plates (N)	4354	3593	N > 2000
Tailing factor	1.09	1.6	T≤ 2000

Table 2: Linearity results for Brimonidine Tartrate

Conc. (µg / ml)	11.25	16.875	22.5	33.75	45
Avg. area	32792	51117	66671	98068	129167
Correlation	0.999				

Table 3: Linearity results for Timolol Maleate

Conc. (µg / ml)	37.5	56.25	75	112.5	150
Avg. area	165345	261074	336199	490441	656253
Correlation	0.999				

Table 4: Precision results for Brimonidine Tartrate

Sl no	Concentration (µg /ml)	Intraday precision (area)	Interday precision (area)
1	22.5	64556	66314
2	22.5	64156	68541
3	22.5	64765	68400
4	22.5	64845	67050
5	22.5	64267	67309
6	22.5	64202	65374
Mean		64465	67165
Std Dev		298.84	1215.3
% RSD		0.4	1.8

Table 5: Precision results for Timolol Maleate

Sl no	Concentration (µg /ml)	Intraday precision (area)	Interday precision (area)
1	75	331754	328283
2	75	330189	329880
3	75	331936	333612
4	75	338506	327857
5	75	331691	328063
6	75	333451	330024
Mean		3303.7	2463.0
Std Dev		336630	332447
% RSD		0. 9	0.7

Table 6: Accuracy results for Brimonidine Tartrate

Level %	Recovery (%)	Mean Recovery	SD	RSD (%)
50%	100.16	99.36	0.6963	0.70
	99.03			
	98.90			
100%	99.80	100.44	0.5512	0.55
	100.78			
	100.72			
150%	101.46	100.98	0.4404	0.44
	100.88			
	100.59			

Table 7: Accuracy results for Timolol Maleate

Level %	Recovery %	Mean Recovery	SD	RSD (%)
50%	99.9			
	99.09			

	99.72	99.57	0.4253	0.43
100%	101.39	100.89	1.8260	1.81
	102.42			
	98.87			
150%	98.85	99.13	0.3375	0.34
	99.03			
	99.50			

Table 8: Characteristic parameters of Brimonidine Tartrate and Timolol Maleate for the proposed RP-HPLC method

Parameters	**Brimonidine Tartrate**	**Timolol Maleate**
Calibration range (µg/ml)	11.25-45 of Brimonidine Tartrate	37.5-150 µg/ml of Timolol Maleate
Detection wavelength	270nm	270nm
Mobile phase	Buffer and Acetonitrile (75:25)	Buffer and Acetonitrile (75:25)
Retention time	2.086±0.02	3.307±0.02
Regression equation(Y*)	Y=2869x+1097	Y=4352x – 5385
Slope (m)	2869	4352
Intercept (c)	1097	5385
Correlation coefficient (r^2)	0.999	0.999
Intraday precision (%RSD*)	0.2	0.4
Interday precision (% RSD*)	0.7	0.21
Limit of detection (mcg/ ml)	1.26	4.08
Limit of quantitaion (mcg/ml)	3.82	12.3

4. Conclusion

The proposed RP-HPLC method was also validated for intra and inter-day variation. When the solution containing 22.5µg/ml of Brimonidine Tartrate and 75µg/ml of Timolol Maleate was repeatedly injected on the same day, the % RSD in the peak area for six replicate injections was found to be 0.1% for Brimonidine Tartrate and 0.8% for Timolol Maleate. Also the inter day variation (6 days and six injections) was found to be 0.7% for Brimonidine Tartrate and 0.2% for Timolol Maleate. The results are presented in Table: 3. The % RSD values were within 2 and the method was found to be precise. It can concluded the proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Brimonidine Tartrate and Timolol Maleate and can be reliably adopted for routine quality control analysis of Brimonidine Tartrate and Timolol Maleate in Bulk and its pharmaceutical formulations.

5. References

[1] C. Sonanis, A.P Rajput, Development and validation of a new stability indicating analytical method for the determination of Brimonidine Tartrate in drug substances and drug products using UPLC, IJPRS, Volume 3, issue 1, 2011.

[2] Madhavi.A, Naidu.A, Subbarao D.V, Srinivasu.P, Development and validation of new LC method for the analysis of Brimonidine Tartrate and related compounds.

[3] Mohammed shahid ali, Aamer Roshanli Khatri, Muhammad Imran, Mhori mohisn(2009),A stability- indicating assay of brimonidine Tartrate and stress testing using HILIC.

[4] Shirke RR, Pai N (2002) Rp-hplc determination of brimonidine tartrate in brimonidine tartrate eye drops Ind. Drugs 39 :484-486, 2002.

[5] Prakash Bhargav, Pandurang Desshapande, Saurabh Pandey,Sanjeev Chandran,Development and validation of stability indicating U.V spectrophotometric method for the estimation of brimonidine tartrate in pure form and Preformulation Studies, Der Pharmacia Lettre, Scholarship Research library, 2010, 2(3), page 106-122.

[6] Mahajan Ananad, Athensla Fonseca,Gandhi santosh V,Deshpande padmanabh B,development and validation of high performance thin layer chromatograph method for the estimation of brimonidine tartrate as Bulk drug in ophthalmic solutions, IJPTR, vol.2,No.3,pp 1376-1379, July-Sept 2010.

[7] Pritam jain, Rahul khatal, Sanjay surana,Development and validation of first order U.V spectrophotometric method for the determination of Brimonidine tartrate in bulk and in formulation.

[8] Jiang S, Chappa AK, Proksch JW (2009) A rapid and sensitive LC/MS/MS assay for the quantitation of brimonidine in ocular fluids and tissues. J of Chr B 877: 107-114.

[9] ERKN (2003) Rapid and sensitive HPLC method for the simultaneous determination of dorzolamide hydrochloride and timolol maleate in eye drops with diode-array and UV detection. Pharmazie 58: 491-493.

[10] Acheampong A, Tang L, Diane DS (1995) Measurement of brimonidine concentrations in human plasma by a highly sensitive gas chromatography/ mass spectroscopic assay. J Pharma Biomed Anal. 3:995-1002.

[11] Tzovolou DN, Lamari F, Mela EK, Gartaganis SP, Karamanos NK (2000) Capillary electrophoretic analysis of brimonidine in aqueous humor of the eye and blood sera and relation of its levels with intraocular pressure. Biomed Chr 14 : 301-305.

[12] Pritam S. Jain, Rahul N. Khatal, Hardik N. Jivani and Sanjay J. Surana, Development and Validation of TLC-densitometry Method for Simultaneous Estimation of Brimonidine tartrate and Timolol maleate in Bulk and Pharmaceutical Dosage Form J Chromatograph Separat Techniq 2:113. 1000113, 2011.