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Development and Validation of High Performance Liquid Chromatographic Method for Estimation of Brimonidine Tartrate as bulk drug and in Ophthalmic Formulation

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Abstract : Glaucoma is complex disease characterized by ocular hypertension with a progressive visual loss that could resist in blindness due to damage occurred to optic nerve. Brimonidine tartrate is commonly used drug in glaucoma therapy which is selective alpha 2 adrenergic agonist. The reverse phase high performance liquid chromatographic method was developed and validated for estimation of brimonidine tartrate in bulk drug and pharmaceutical dosage form. Better separation was achieved on Kromasil C 18 (250 mm X 4.6 mm i.d., 5 µm particle size) column using isocratic elution program with mobile phase citric acid monohydrate buffer : water: methanol (30:50:20 v/v/v) and pH 3 was maintained by using triethylamine. The flow rate was 1.0 ml/min. Elute was detected at 246 nm and it effectively separated at retention time of 6 minutes. The developed method was successfully validated according to ICH guidelines. The method was validated for linearity, accuracy, specificity, precision and robustness. The LOD and LOQ was 1.47 and 4.47 µg/ml respectively. The optimized and validated method can be used for estimation of brimonidine tartrate in bulk and in ophthalmic formulation.

Key words : Brimonidine Tartrate, Reversed phase-HPLC.

Introduction

Glaucoma is complex disease characterized by ocular hypertension with a progressive visual loss that could resist in blindness due to damage occurred to optic nerve. There are several drugs are available to treat these condition. Brimonidine tartrate is used in the treatment of open-angle glaucoma or ocular hypertension. It is selective alpha-2 adrenergic receptor agonist. Chemically it is 5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate[1].

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No significant HPLC method reports were found for estimation of Brimonidine Tartrate in pharmaceutical formulation while few HPTLC, HPLC, LC-MS, HILIC (Hydrophilic interaction liquid chromatography) methods reported for the estimation of Brimonidine Tartrate in blood serum and in ocular fluids[2-6].

The aim of study is to developed and validates simple, specific, sensitive, accurate and precise HPLC method for determination of Brimonidine Tartrate in ophthalmic formulation as per International Conference on Harmonization (ICH) guidelines[7-14].

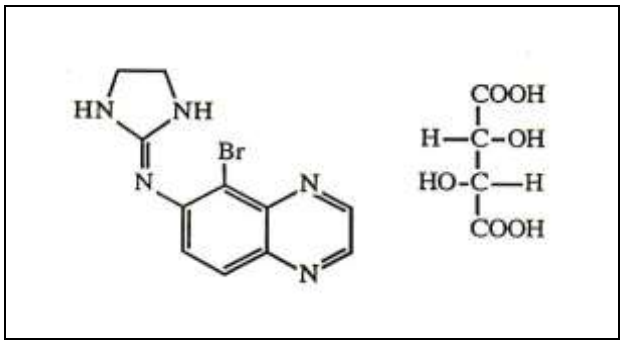

Figure 1. Structure of Brimonidine Tartrate

Experimental

Apparatus

A Shimadzu RP-HPLC instrument (LC -20 AD as per CFR 21*)* equipped with an photodiode array detector, manual injector with 20 µl loop, and Kromasil C18 column (250 mm × 4.6 mm id, 5 µm particle size) and LC- solution software was used. Contech CB-50 analytical balance and ultra sonic cleaner (Spetralab, UCB-40) were used during the study.

Reagents and materials

Brimonidine Tartrate was received as gift sample from Cipla Ltd., (Mumbai, Maharashtra). HPLC grade methanol (Qualigens), citric acid monohydrate buffer was of AR grade. Water for RP-HPLC was prepared by double glass distillation and filtered through nylon membrane filter 0.45 µm (Pall Lab Sci).

Chromatographic conditions

Kromasil C18 Column (250 mm × 4.6 mm id, 5 µm particle size) was used at ambient temperature. The mobile phase consisted of citric acid monohydrate buffer: water : methanol (30: 50:20 v/v/v) and pH 3 was maintained by triethylamine. Flow rate was 1.0 ml/min. The mobile phase was filtered through a 0.45 µm membrane filter and degassed before used. The elution was monitored at 246 nm and injection volume was 20 µl.

Preparation of solutions

0.01 M Citric Acid Monohydrate Buffer

Accurately weighed Citric acid monohydrate (1.05 gm) was transferred to a beaker and dissolved in double distilled water (500 ml). Further pH adjusted to three by using triethylamine.

Preparation of Standard Stock Solution

Standard stock solution of brimonidine tartrate was prepared by dissolving 100 mg drug in 100 ml methanol (1000 ug/ml). Further 1 ml was diluted to 10 ml with methanol to obtain a working standard having a concentration 100 µg/ml. nm. The standard solutions of brimonidine tartrate were scanned in the range of 200-

400 nm against buffer solution as a blank. Brimonidine tartrate showed maximum absorbance at 246 nm. Further optimized wavelength at 246 nm.

Method Validation[7-13]

Calibration curve

ICH recommends that for establishment of calibration curve, a maximum of five concentrations should be used. To set calibration curve, stock solution of drug (100 µg/ml) was further diluted with the help of diluents in concentration range of 40-80 µg/ml. The samples were injected in triplicate into RP-HPLC. A standard plot of peak area v/s concentration of drug in µg/ml was plotted. Correlation coefficient and regression equation were obtained from the calibration curve.

Accuracy (% Recovery)

To check the accuracy of the method, recovery studies were carried out by addition of formulation to pre-analyzed sample solution at three different levels 80, 100 and 120 %. Chromatogram was obtained and the peak areas were noted. At each level of the amount, three determinations were carried out. Accuracy of given analytical method were calculated.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of Quantification (LOQ) for Brimonidine Tratrate was derived by calculating signal-to-noise ratio (S/N, i.e. 3.3 for LOD and 10 for LOQ) and using following equation as per International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ = the standard deviation of the responses and S = Slope of calibration curve.

Specificity and System Suitability

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradants, matrix etc. One blank and one standard preparation were injected and chromatograms were recorded which is further calculated for system suitability parameters.

Robustness

The robustness of the developed RP-HPLC method were carried out by small deliberate variations in the optimized method. The effect of change in flow rate (± 0.2 ml/min), mobile phase composition (± 3ml) and wavelength (± 2nm) were studied.

Intra-day and Inter-day precision

Intra-day precision was carried out on same day using same HPLC system and same column. The intra-day precision was determined by analyzing six replicates of Brimonidine Tartrate solution (60 µg/ml) on the same day while inter-day precision was determined by analysing corresponding standard on two different days over a period of one week.

Result and Discussion

The several mobile phase compositions were tried to optimized RP-HPLC parameters. A satisfactory separation and good peak symmetry was found in a mixture of citric acid monohydrate buffer pH 3, water and methanol (30:50:20) at 1.0 ml/min flow rate. As it is shown in Figure 2, the optimum wavelength for detection was set at 246 nm at which much better detector responses for Brimonidine Tartrate was obtained. The retention time was 6 min as reported in Figure 3.

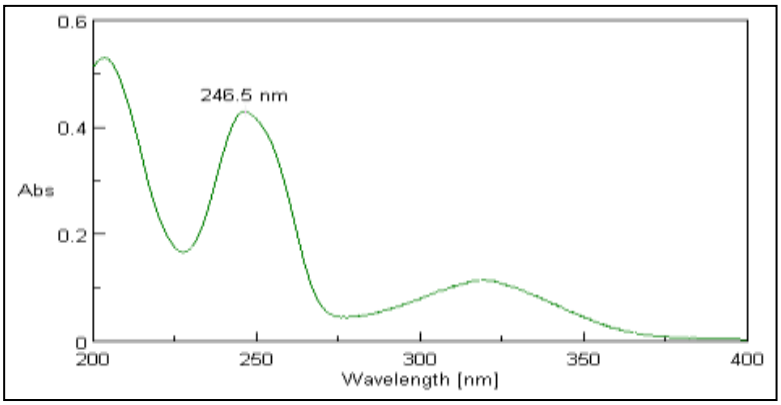

Figure 2 . Maximum detection wavelength of Brimonidine Tartrate

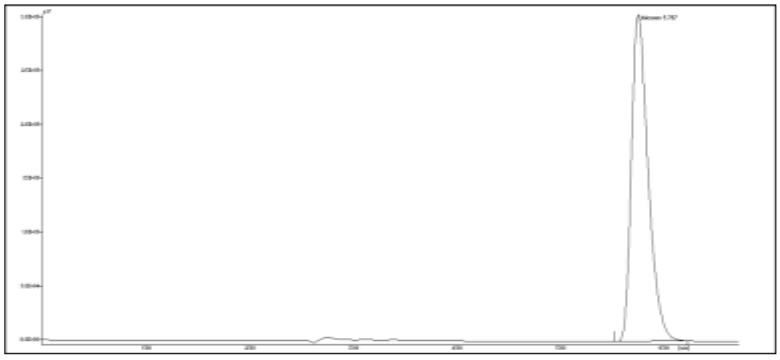

Figure 3. Typical RP- HPLC Chromatogram Brimonidine Tartrate with corresponding retention time

Chromatographic conditions are outlined in Table 1.

Table 1. Chromatographic conditions

Mobile Phase	0.01 Mol/L Citric acid monohydrate:Water: Methanol (30:50:20) and pH 3 maintained by using triethylamine
Column	Kromasil – C 18 Column (250 mm × 6.5 mm × 5 um)
Flow Rate	1 ml/min
Detection Wavelength	246 nm
Injection Volume	20 ul
Run Time	10 Minutes
Retention Time	6 mins

The calibration graph for Brimonidine Tartrate was constructed by plotting the peak area versus their corresponding concentrations. The linear regression equation was found -

$y = 85830x + 17081$ with correlation coefficient (R^2) 0.999. The calibration graph is shown in Figure 4.

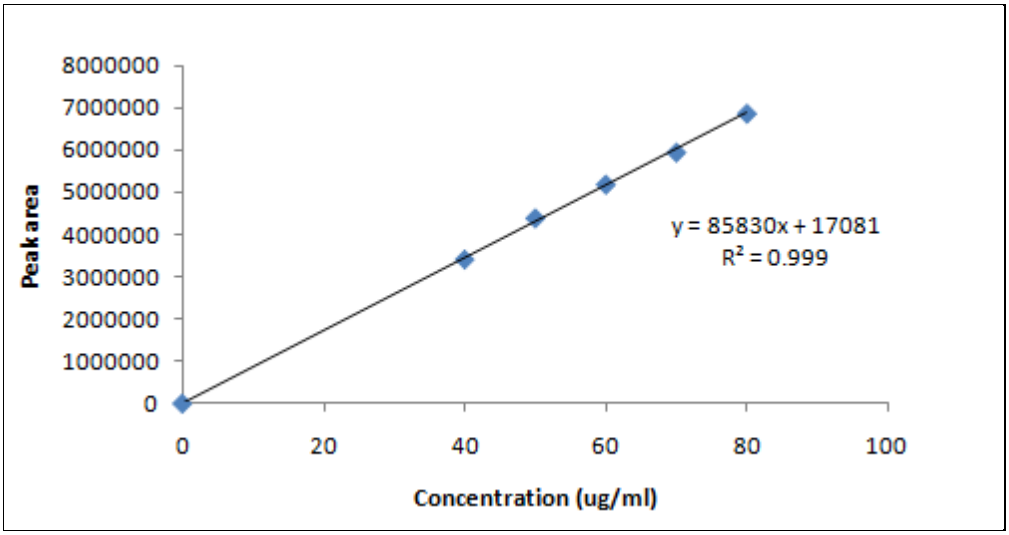

Figure 4 . Calibration Curve of Brimonidine Tartrate

The proposed method has been applied to the assay of Brimonidine Tartrate in pharmaceutical dosage form. The results obtained indicate that the additives present do not interfere with analysis of the studied formulation. System suitability test parameters for Brimonidine Tartrate for the RP-HPLC method are reported in Table 2. The optical and regression characteristics and validation parameters are reported in Table 3. It was estimated that percent RSD was found to be less than 2 in precision study. Data of recovery study is shown in Table 4. Accuracy was found to be within the acceptable limit of 98-102%. Method was confirmed to be unaffected by small deliberate changes in wavelength, flow rate and mobile phase compositions in robustness study. The robustness study is reported in Table 5. On the basis of series of investigation the optimized method can be routinely use for analysis purpose.

Table 2. System suitability testing of the HPLC method.

Sr. No.	System suitability Parameters	Observation	Acceptance Criteria
1	Theoretical Plates	5669	N > 2000
2	Tailing Factor	1.49	Not more than 2
3	Column Efficiency	5861	Not less than 2500
4	% R.S.D.	0.7425	Not more than 2

Table 3. The optical and regression characteristics and validation parameters of HPLC method for analysis of Brimonidine tartrate

Sr. No.	Parameter	Observation
1	Calibration range	40-80 µg/ml
2	Detection limit	1.477 µg/ml
3	Quantitation limit	4.476 µg/ml
4	Correlation coefficient	0.999
5	Intraday Precision (%RSD)	0.7425
6	Interday Precision (%RSD)	Day 1 -0.7425 Day 2- 0.4202

Table 4. Data of recovery study for Brimonidine Tartrate by HPLC method

Sr. No.	**Amount taken** (µg/ml)	**Amount added** (µg/ml)	**Amount Recovered**	**% Recovery**
1	25	20	45.17	100.39
2	25	25	49.48	98.96
3	25	30	54.18	98.52

Table 5. Data of robustness study for Brimonidine Tartrate by HPLC method

Parameter		**% RSD**
Wavelength	248 nm	0.244
	244 nm	0.167
Flow rate	1.2 ml/min.	0.549
	0.8 ml/min.	1.300
Mobile Phase Composition	83:17	0.303
	77:23	0.653

Conclusion

This study leads to conclusion that the proposed method is accurate, precise, simple, sensitive, reproducible and rapid. It can be applied successfully for estimation of Brimonidine Tartrate in pharmaceutical formulation.

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