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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR ESTIMATION OF BRIMONIDINE TARTRATE AS BULK DRUG AND IN OPHTHALMIC FORMULATION

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ABSTRACT

The optimized reverse phase high performance liquid chromatographic method was developed for estimation of Brimonidine Tartrate in bulk drug and pharmaceutical dosage form. Chromatography was performed on Kromasil C 18 (250 mm X 4.6 mm i.d. , 5 µm particle size) column 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 5.96 min. The LOD and LOQ was 1.47 and 4.47 µg/ml respectively. A linear response was observed over the concentration range 40-80 µg/ml for Brimonidine Tartrate. Thus the proposed HPLC method was found accurate, specific, precise, robust and reproducible.

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INTRODUCTION:

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. 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 (Rahore, 2010, Phogat, 2011, Jiang, 2009, Sethi 2001).

The aim of study was to develope 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 (ICH, 2005).

MATERIAL AND METHODS:

Apparatus

A Shimadzu RP-HPLC instrument (LC -20 AD as Per 21 CFR) 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.

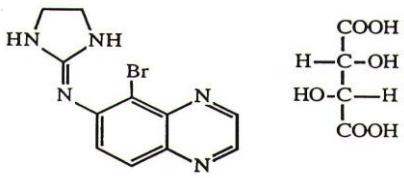

Figure 1: Structure of Brimonidine Tartrate

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 and 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

Citric Acid Buffer

Accurately weighed Citric acid monohydrate (1.05 gm) was transferred to a beaker (500 ml) and dissolved in double distilled water (500 ml).

The mobile phase was citric acid monohydrate, pH 3: water: methanol (30: 50:20)

Preparation of Standard Stock Solution

Standard stock solution of Brimonidine Tartrate was prepared by dissolving 10 mg of drug in10 ml of methanol to get the concentration of 1 mg/ml from which 1 ml was further diluted to 10 ml with methanol to obtain a working standard having a concentration of 100 µg/µl.

Determination of wavelength of maximum absorbance

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 246nm. So the wavelength selected for the determination of Brimonidine Tartrate was 246 nm.

Method Validation (ICH, 2005)

Calibration curve (Linearity)

Calibration curve was plotted over concentration range of 40-80 ug/ml for Brimonidine Tartrate. Accurately measured standard stock solution of Brimonidine Tartrate (4, 5, 6, 7 and 8 ml) was transferred to a series of 10 ml volumetric flask and volume in each flask was adjusted 10 ml with mobile phase. Resulting solution were injected into the column and the peak area obtained at flow rate of 1.0 ml per minute for Brimonidine Tartrate. Calibration curve was constructed for Brimonidine Tartrate by plotting peak area versus concentration at 246 nm. Each reading was average of three determinations.

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.

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 was injected and chromatograms were recorded which is further calculated for system suitability parameters.

Robustness

Robustness studies were carried out by examining the effect of small, deliberate variation of the analytical conditions on the peak areas of the drug. Factors varied were volume of mobile phase (± 3 ml), wavelength (±2 nm) and flow rate (± 0.2 ml/min). One factor at a time was changed to study the effect.

Intra-day and Inter-day precision

The intra-day precision was determined by analyzing standard solution of Brimonidine Tartrate at 60 ug/ml concentration for six replicates on the same day while inter-day precision was determind by analysing corresponding standard on two different days over a period of one week.

RESULT AND DISCUSSION:

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of citric acid monohydrate buffer pH 3, methanol and water (30:20:50) at 1.0 ml/min flow rate. As it was 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 5.962 min as reported in Figure 3.

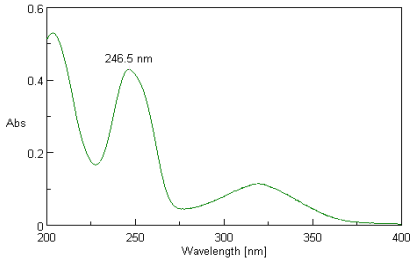

Figure 2: Maximum detection wavelength of Brimonidine Tartrate

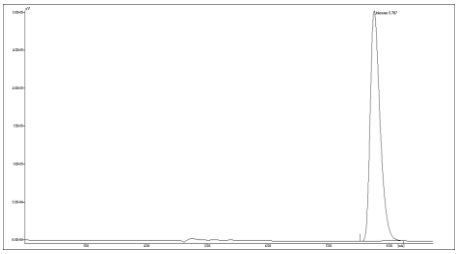

Figure 3: Typical RP- HPLC Chromatogram Brimonidine Tartrate (40 ug /ml) with corresponding retention time.

Chromatographic conditions are outlined in Table 1. The calibration graph for Brimonidine Tartrate was constructed by plotting the peak area versus their corresponding concentrations; good linearity was found over the range 40-80 µg/ml. The calibration graph is shown in Figure 4.

Table 1: Chromatographic conditions

Parameter	Observation
Mobile phase	0.01 Mol/L Citric acid monohydrate: Methanol : Water (30:20:50) 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 µl
Run time	10 Minutes
Retention time	5.96 mins

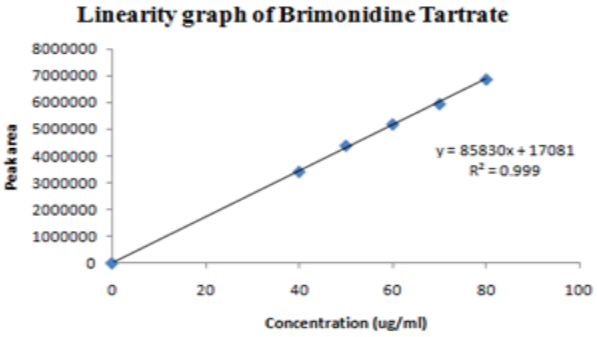

Figure 4: Calibration Curve of Brimonidine Tartrate

The proposed method has been applied to the assay of Brimonidine Tartrate in pharmaceutical dosage form. The results obtained indicate 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. Data of recovery study is shown in Table 4. 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

System suitability Parameters	Observation	Acceptance Criteria
Peak Area	3419885	-
Tailing Factor	1.49	NMT 2
Column Efficiency	5861	NLT 2500
% R.S.D.	0.7425	NMT 2

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

Parameter	Observation
Calibration range	40-80 µg/ml
Detection limit	1.477 µg/ml
Quantitation limit	4.476 µg/ml
Slope	85830
Intercept	17081
Correlation coefficient	0.999
Intraday RSD, %	0.7425
Interday RSD, %	Day 1 -0.7425 Day 2- 0.4202

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

Amount taken (µg/ml)	Amount added (µg/ml)	Amount found	% Recovery
25	20	45.17	100.39
25	25	49.48	98.96
25	30	54.18	98.52

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

Experiment	% RSD	Theoretical plates	Tailing factor
(+) Wavelength	0.244	5713	1.50
(-) Wavelength	0.167	5992	1.49
(+)Flow rate	0.549	5760	1.40
(-) Flow rate	1.300	6091	1.61
(+) Mobile phase volume	0.303	7065	1.46
(-) Mobile phase volume	0.653	5041	1.51

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