



# Validated Spectrophotometric Method for Estimation of Brimonidine Tartrate in Bulk and Pharmaceutical Formulation

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## ABSTRACT:

Brimonidine Tartrate is  $\alpha$ -adrenergic agonist used to treat open-angle glaucoma, ocular hypertension (as eye drop) and rosacea (as a gel). Various methods for analysis of same are available but are time consuming and expensive. Various simultaneous method of Brimonidine Tartrate with another drug is available. Here we have developed new, precise, simple spectrophotometric method for estimation of Brimonidine Tartrate from bulk. Medium prepared were selected Distilled Water. It showed absorption maxima at 254 nm. This method was validated according to ICH guidelines. The drug obeyed Beers law and showed good correlation. The linearity was observed between 0–25  $\mu\text{g/mL}$ . There was no significant difference in the intraday and interday analysis of Brimonidine Tartrate determined. The results of analysis were validated with respect to recovery, linearity; Limit of detection and limit of quantitation were found to be satisfactory.

**KEYWORDS:** Brimonidine Tartrate, Recovery, UV-Visible Spectrophotometer.

## INTRODUCTION:

Brimonidine Tartrate is an imidazole derivative compound that act as  $\alpha$ -adrenergic agonist used for the treatment of open-angle glaucoma. The ocular hypotensive effect of this molecule is because of its ability to decrease aqueous humor production. Chemically, it is 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl) quinoxalin-6-amine 2,3-dihydroxybutanedioate OR 5-bromo-6-(2-imidazolidinylidene-amino) quinoxaline

*L*-tartrate. It is white or slightly yellowish or slightly brownish powder. It is freely soluble in water, dimethyl sulphoxide and methanol and practically insoluble in anhydrous ethanol and toluene.<sup>1,2</sup>

It is official in Indian Pharmacopoeia<sup>3</sup>. Several analytical techniques have been reported for Brimonidine Tartrate with combination i.e., RP High performance liquid chromatography<sup>4</sup>, simultaneous estimation by UV<sup>5</sup>, HPLC<sup>6</sup> and HPTLC method for simultaneous estimation<sup>7</sup>. From this some methods are costlier and time consuming.

The main purpose of this investigation is to develop and validate spectrophotometric method which is simple, rapid, precise and for estimation of Brimonidine Tartrate from bulk. This method could also be easily used in routine analytical work and for dissolution studies at very low concentration of Brimonidine Tartrate.

## **MATERIAL AND METHOD:**

### **Drugs and Chemicals:**

Brimonidine Tartrate was obtained as a gift sample from FDC Roha, Raigad. Brimodin Eye Drops purchased from local market for analysis of Brimonidine Tartrate.

### **Instrument Used:**

The UV-Visible Double Beam Spectrophotometer [SYSTRONICS (INDIA) LIMITED AU - 2702] was used.

### **Method:**

#### **Determination of $\lambda$ max:**

Weighed amount of Brimonidine Tartrate was dissolved in Distilled Water to obtain 100 µg/ml solution. This solution was subjected to scanning between 200-400 nm and an absorption maximum was determined.

#### **Standard Stock Solution:**

Brimonidine Tartrate (10 mg) was accurately weighed and transferred to 100 ml of volumetric flask. It was then dissolved in some amount of Distilled Water. The volume was made up to 100 ml with distilled water.

#### **Linearity and Calibration:**

The aliquots of standard solution were diluted serially with sufficient distilled water to obtain the concentration range of 0 to 25 µg/ml. A calibration curve for Brimonidine Tartrate was obtained by measuring the absorbance at the  $\lambda$  max of 254 nm. Statistical parameters like the slope, intercept, coefficient of correlation, standard deviation, Relative standard deviation and error were determined.

### Precision (Repeatability):

To check the degree of precision of the method, suitable statistical evaluation was carried out by inter and intra-day calibration. The standard deviation, coefficient of variation (COV) and standard error was calculated.

### Recovery Studies:

Recovery studies were performed to judge the accuracy of the method. 1 mL of standard formulation (100 µg/mL) was taken in three 10mL volumetric flask and to it added 1 mL, 2 mL and 3 mL of working standard solution (100 µg/mL) respectively and made the volume to mark. The respective absorbance at 254 nm was recorded against the blank. The amount of added concentration was determined from the absorbance values obtained and percent recovery was determined for each formulation.

### LOD and LOQ:

The limit of detection (LOD) and limit of quantitation (LOQ) were determined based on the standard deviation of response of the calibration curves. The standard deviation of y-intercept and slope of calibration curve were used to calculate the LOD and LOQ.

### RESULTS:

The UV scan of standard solution between 200 – 400 nm showed the absorption maxima at 254 nm, shown in **fig. 1**. The Beer's law was verified from the calibration curve by plotting a graph of concentration vs absorbance. The plot is shown in **fig. 2**. Regression analysis showed very good correlation. The calibration plot shows straight line equation which is clear by the regression analysis equation  $Y = mX + C$ . (Where Y is absorbance, m is the slope and X is the concentration of Brimonidine Tartrate in mcg/mL) as obtained by the least square method. The results thus obtained are depicted in **Table No. 1**. No significant variations were observed on interday and intraday analysis was depicted in **Table No. 2** and **Table No. 3**. The results of recovery studies of formulations were studied and are shown in. **Table No. 4**.

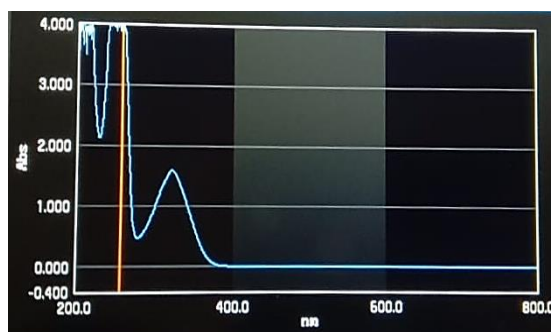
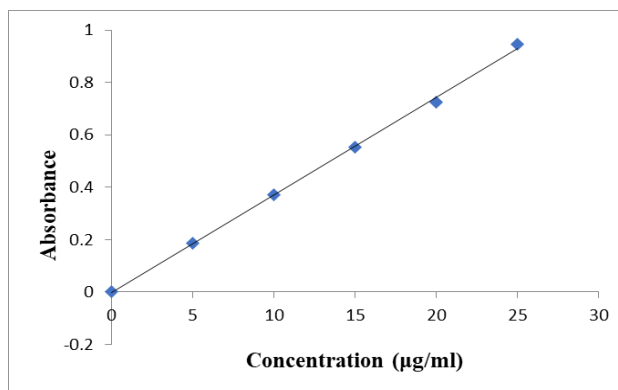


Figure No. 1: Spectrum of Brimonidine Tartrate



**Figure No. 2: Calibration Curve of Brimonidine Tartrate in Distilled Water**

**Table 1. Optical Characteristics and Precision**

Absorption Maxima	254 nm
Beer's law limit	0-25
Coefficient of Correlation	0.99948
Regression equation	$Y=0.03733x-0.0022$
Slope	0.03733
Y-intercept	-0.0022
LOD and LOQ	0.8355 and 2.5320 µg/ml

**Table 2. Intraday Variability of Brimonidine Tartrate**

Conc. (µg/ml)	Absorbance	%RSD
5	0.18733±0.009452	5.018
15	0.55333±0.026083	4.701
25	0.947±0.03	3.262

Each value is average± SD (n=3)

**Table 3. Interday Variability of Brimonidine Tartrate**

Conc. (µg/ml)	Absorbance	%RSD
5	0.20011± 0.011	5.50
15	0.5784±0.0253	4.37
25	0.9734±0.0229	2.35

Each value is average± SD (n=3)

**Table 4. Recovery Study of Brimonidine Tartrate**

Sr. No.	Standard Amount (µg/ml)	Amount of Standard added (µg/ml)	Theoretical Amount (µg/ml)	Amount Recovered	% Recovered
1	10	10	20	19.290	98.144
2	10	20	30	29.620	
3	10	30	40	39.721	

**DISCUSSION:**

The spectrum of Brimonidine Tartrate in Distilled water showed the absorption maxima at 254 nm. The statistical analysis of data obtained for the calibration curve of Brimonidine Tartrate in pure solution indicated a high level of precision for the proposed method, as evidenced by low value of coefficient of variation. The coefficient of correlation was highly significant. The linearity range was observed between 0 – 25 µg/ml. Coefficient of correlation was found to be 0.99948. The plot clearly showed a straight line passing through origin ( $Y=0.03733x-0.0022$ ). The interday and intraday study was validated by low values of % RSD and standard error, indicating accuracy and precision of the methods. Excellent recovery studies (98.144%) further prove the accuracy of the method. The LOD value was observed 0.8355 µg/ml and LOQ value was 2.5320 µg/ml.

**CONCLUSION:**

From the study it can be concluded that the method described in this paper for the determination of Brimonidine Tartrate from bulk and formulation is simple, accurate, sensitive and reproducible. The proposed method utilizes inexpensive solvents. The proposed method could be applied for routine analysis in quality control laboratories.

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