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Novel analytical spectrophotometric methods for the Quantification of Brimonidine tartrate -An α adrenergic agonist

*** Mukthinuthalapati Mathrusri Annapurna, Reddy Narendra and Manas Ranjan Pattanaik**
Department of Pharmaceutical Analysis and Quality Assurance,
GITAM Institute of Pharmacy, GITAM University, Visakhapatnam, India
*** Corresponding author: e-mail: mathrusri2000@yahoo.com**

ABSTRACT

Four new spectrophotometric methods have been developed for the assay of Brimonidine tartrate in pharmaceutical dosage forms. The first method was developed in phosphate buffer pH 6.0 (Method A) showing absorption maxima at 247 nm and in the second method, borate buffer pH 9.0 (Method B) was used (λ_{max} 257 nm). Linearity was observed over the concentration range 0.1-50 µg/mL for both the methods with regression equations 0.0656x -0.0121 and 0.0606x + 0.0038 for method A and B respectively. First derivative spectrophotometric methods (C and D) were developed in phosphate buffer pH 5.0 and sodium acetate pH 4.0 in which Brimonidine tartrate obeys Beer Lambert's law 1-40 and 0.2-30 µg /ml with regression equations 0.0034x - 0.0007 and 0.0064x + 0.0004 respectively. The proposed spectrophotometric methods were validated and can be applied for the determination of Brimonidine tartrate in pharmaceutical formulations.

Key words: Brimonidine tartrate, Phosphate buffer (pH 6.0), Phosphate buffer (pH 5.0), Sodium acetate buffer (pH 4.0), Borate buffer (pH 9.0), Validation.

1. INTRODUCTION

Brimonidine tartrate (BMD) (The Merck Index, 2006) chemically 5-Bromo-*N*- (4,5-dihydro-1*H*-imidazol-2-yl) quinoxalin-6-amine 2,3-dihydroxybutanedioic acid has a molecular formula $C_{15}H_{16}BrN_5O_6$ and molecular weight 442.1221 g/mol. Brimonidine tartrate (Figure 1) is an alpha adrenergic receptor agonist licensed for the reduction of intra-ocular pressure in open angle glaucoma or ocular hypertension in patients for whom beta blockers are inappropriate. It may also be used as adjunctive therapy when intra-ocular pressure is inadequately controlled by other anti-glaucoma therapy (Carol, 1999).

Literature survey reveals that spectrophotometric (Prakash, 2010), fluorimetric (Sunita, 2013), HPLC (Arun, 2011; Shirke, 2002; Nagaraju, 2014; Narendra, 2014; Madhavi, 2009; Jiang, 2009) UPLC (Manohar, 2011), TLC (Pritam, 2011), HPTLC (Mahajan, 2010), LC/MS/MS (Jiang, 2009) and HILIC (Ali, 2009) methods were published for the determination of Brimonidine tartrate in pharmaceutical formulations and biological fluids. As there is only one spectrophotometric method till now the authors have made an attempt to develop two zero order and two first derivative spectrophotometric methods in four different buffer solutions for the determination of Brimonidine tartrate in ophthalmic solutions and validated as per ICH guidelines.

2. MATERIALS AND METHODS

2.1. Instrumentation: A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Shimadzu).

2.2 Chemicals and reagents: Analytical grade methanol (Merck), disodium phosphate (Na_2HPO_4) (Merck), mono potassium phosphate (KH_2PO_4) (Merck), boric Acid, sodium hydroxide, glacial acetic acid were used. Brimonidine tartrate was obtained as eye drops with brand names Brimodin P (Labelled claim: 1.5mg/5mL; Cipla limited, India.) and Alphagan (Labelled claim: 2 mg/10mL; Allergan India Ltd, India).

2.3. Preparation of phosphate buffer pH 6.0: 25.0 ml of 0.2 M potassium dihydrogen phosphate (KH_2PO_4) and 2.8 ml of 0.2 M sodium hydroxide were taken in to a 100 ml volumetric flask and made up to volume with water.

2.4. Phosphate buffer pH 5.0: 6.8 g of potassium dihydrogen phosphate was dissolved in water in a 1000 ml volumetric flask and the pH was adjusted to 5.0 using 10 M potassium hydroxide.

2.5. Borate buffer pH 9.0: 6.20 g of boric acid was dissolved in 500 mL volumetric flask with water. This solution was transferred into a 1000ml volumetric flask along with 41.5 mL of 1M sodium hydroxide and diluted with water to 1000 mL to adjust pH 9.0.

2.6. Sodium acetate buffer pH 4.0: 2.86 ml of glacial acetic acid was taken and 1.0 ml of a 50 % w/v solution of sodium hydroxide was added in a 1000 ml volumetric flask, finally water was added up to volume and mix.

2.7. Preparation of stock and sample solutions: The standard solution of Brimonidine tartrate was prepared by dissolving accurately about 25 mg of the Brimonidine tartrate with methanol in a 25 mL volumetric flask.

The stock solution was further diluted with phosphate buffer (pH 6.0), sodium acetate buffer (pH 4.0), borate buffer (pH 9.0), phosphate buffer (pH 5.0),for method A (0.1-50 µg/ml), method B (0.1-50 µg/ml), method C (1-40 µg/ml), method D (0.2-30 µg/ml) respectively as per the requirement.

2.8. Procedure for preparation of calibration curve: A series of drug solutions 0.1-50 µg /ml for both method A and B and 1-40 and 0.2-30 µg /ml for method C and D respectively were scanned (200-400 nm) against their reagent blank i.e. phosphate buffer (pH 6.0) for method A, borate buffer (pH 9.0) for method B; phosphate buffer (pH 5.0) for method C and sodium acetate buffer (pH 4.0) for method D and the absorption spectra were recorded. The absorption maximum (λ_{max}) was observed at 247 nm and 257 nm for method A and B respectively and the absorbance was recorded against each concentration. The absorption spectra so obtained for method C and D were converted in to first derivative spectra by the inbuilt software of the instrument and the resulting spectrum shows both maxima and minima and therefore the magnitude of the amplitude was recorded against concentration for method C and D.

A graph was drawn by taking the concentration of the drug solutions on the x-axis and the corresponding absorbance values on the y-axis for method A and B whereas the amplitude values were plotted against concentration for method C and D.

2.9. Assay of marketed formulations of Brimonidine tartrate (eye drops): Brimonidine tartrate is available as eye drops with brand names Brimodin P (Labelled claim: 1.5mg/5mL; Cipla limited, India.) and Alphagan (Labelled claim: 2 mg/10mL; Allergan India Ltd, India) and procured from the local pharmacy store. The contents of each brand of Brimonidine tartrate equivalent to 10 mg was extracted with methanol, sonicated and made up to volume with methanol in a 10 mL volumetric flasks (1 mg/mL) and filtered. The dilutions were made with the respective reagents from this stock for method A, B and C and D and the percentage recovery was calculated.

2.10. Precision and accuracy: The precision and accuracy studies were performed as per the ICH guidelines.The absorbance of six replicates (20 µg/ml) for Method A and B as well as the derivative absorbance of six replicates C and D were noted and the % RSD was calculated.

Accuracy was evaluated by the percent recovery studies and % RSD in which addition of 80%, 100%, and 120% of pure sample solutions were added to the pre-analysed formulation solution.

3. RESULTS AND DISCUSSION

New spectrophotometric methods were developed for the determination of Brimonidine tartrate in pharmaceutical preparations i.e. eye drops. Brimonidine tartrate has shown absorption maxima (λ_{max}) at 247 and 257 nm in phosphate buffer pH 6.0 (Method A) and borate buffer pH 9.0 (Method B) and the corresponding absorption spectra were shown in Figure 2 and 3.

First derivative spectrophotometric methods (C and D) were developed in phosphate buffer pH 5.0 and sodium acetate pH 4.0 and the corresponding derivative spectra were shown in Figure 4 and 5. Brimonidine tartrate has shown zero crossing points at 229.95, 247.69, 278.03, 32081 and 380.06 nm with maxima at 237.28 and minima at 261.85 nm in Figure 4 (Method C) and therefore the amplitude has been taken against the concentration for the construction of the calibration curve (Table 1). Similarly in method D the amplitude was taken on the y axis against concentration for the calibration curve.

Currently available marketed formulations were collected from the local pharmacy store and extracted with the corresponding reagents used for method A, B, C, D and the percentage recovery was calculated (Table 2). Beer's law was obeyed over the concentration range 0.1-50 µg/mL for both the methods with regression equations 0.0656x -0.0121 and 0.0606x + 0.0038 for method A and B respectively. Brimonidine tartrate also obeys Beer Lambert's law 0.1-40 and 0.2-30 µg/ml with regression equations 0.0034x - 0.0007 and 0.0064x + 0.0004 respectively for method C and D respectively. The optical characteristics of the proposed methods were shown in Table 3.

The % RSD values in precision studies were found to be 0.28, 0.34, 0.18 and 0.42 for method A, B C and D respectively (RSD <2%) indicating that the method is more precise. The % RSD values in accuracy studies were found to be 0.189, 0.426 and 0.546 for method A, B C and D respectively (RSD <2%) indicating that the method is more accurate.

Table.1. Linearity for the derivative spectrophotometric methods of Brimonidine tartrate

Conc. (µg/ml)	Absorbance at λ (nm)					
	Method C			Method D		
	237.28 (Maxima)	261.85 (Minima)	Amplitude	237.51 (Maxima)	261.56 (Minima)	Amplitude
0.2	-	-	-	0	0.001	0.001
1	0.002	0.003	0.005	0.003	0.005	0.008
2	0.004	0.006	0.01	0.005	0.007	0.012
3	0.007	0.01	0.017	-	-	-
4	0.010	0.013	0.023	-	-	-
5	0.013	0.015	0.028	-	-	-
10	0.028	0.034	0.062	0.031	0.037	0.068
20	0.056	0.067	0.123	0.057	0.069	0.126
30	0.085	0.102	0.187	0.089	0.106	0.195
40	0.114	0.137	0.251	-		

Table.2. Assay of Brimonidine tartrate (Eye drops)

Brand	Labeled Amount (mg)	*Amount obtained (mg)				% Recovery*			
		Method				Method			
		A	B	C	D	A	B	C	D
I	1.5	1.491	1.493	1.496	1.498	99.40	99.53	99.73	99.86
II	2.0	1.985	1.989	1.991	1.993	99.25	99.45	99.55	99.65

***Each value is average of three determinations**

Table.3. Optical characteristics of Brimonidine tartrate by the proposed methods

Parameters	Method A	Method B	Method C	Method D
Beer-Lambert's limits (µg /ml)	0.1-50	0.1-50	1-40	0.2-30
λ_{max} /Amplitude range (nm)	247	257	237.28-261.85	237.51-261.56
Molar extinction coefficient (Litre/mol.cm)	1.92224×10^5	1.805394×10^5	-	-
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.0125	0.01315	-	-
Slope	0.0656	0.0606	0.0034	0.0064
Intercept	0.0121	0.0038	0.0007	0.0004
Correlation coefficient	0.9999	0.9999	0.9998	0.9993

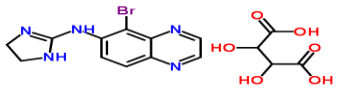


Figure.1.Chemical structure of Brimonidine tartrate (BMD)

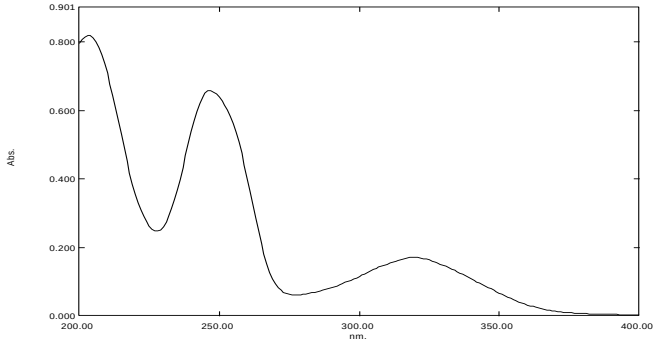


Figure. 2. Absorption spectrum of Brimonidine tartrate (10 µg/ml) in phosphate buffer (pH 6.0)

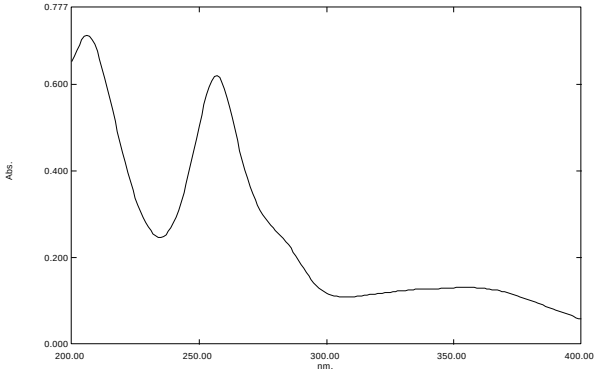


Figure. 3. Absorption spectrum of Brimonidine tartrate (10 µg/mL) in borate buffer (pH 9.0)

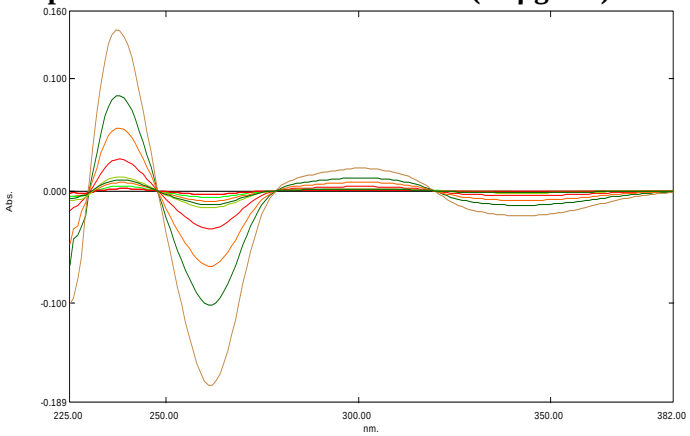


Figure. 4. Absorption spectrum of Brimonidine tartrate (10 µg/mL) in phosphate buffer (pH 5.0)

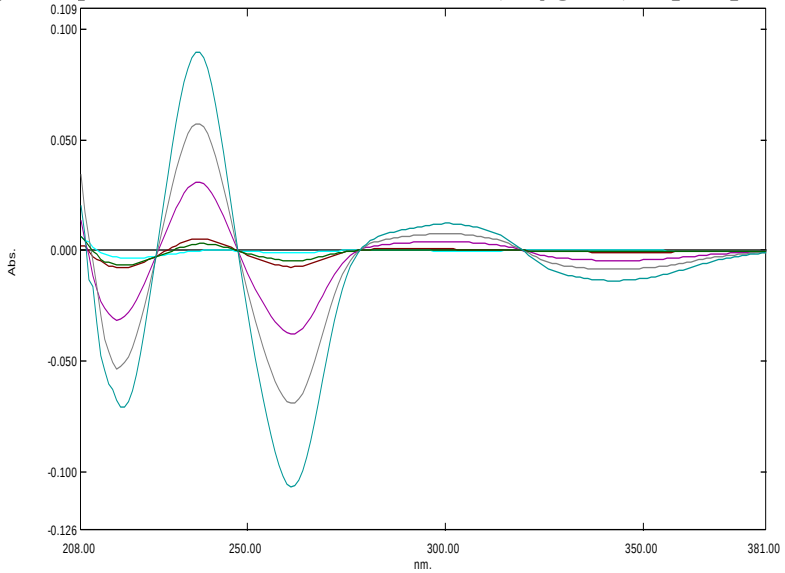
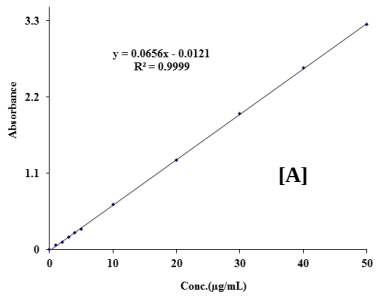
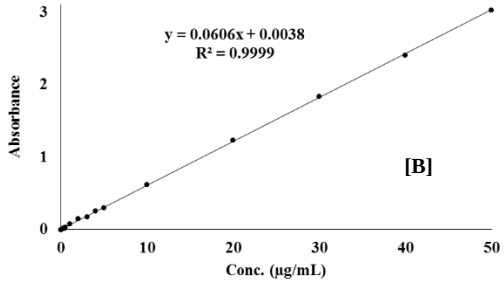


Figure. 5. Absorption spectrum of Brimonidine tartrate (10 µg/mL) in sodium acetate buffer (pH 4.0)





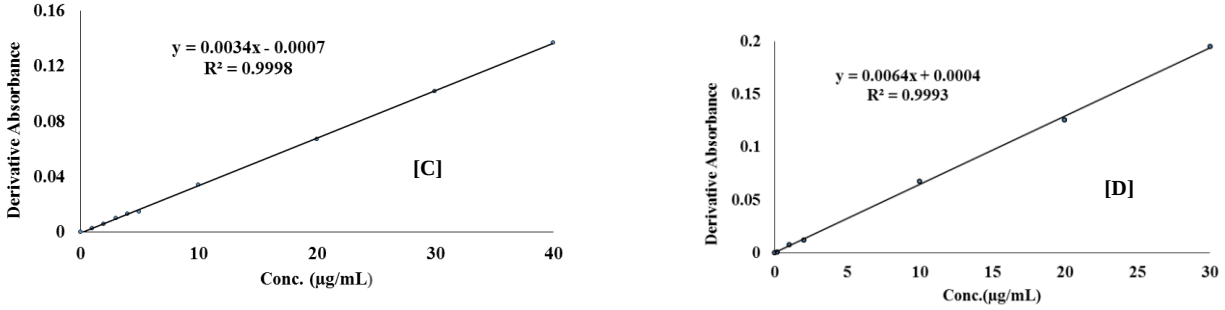


Figure. 6. Calibration curves of Brimonidine tartrate
A) phosphate buffer pH 6.0 B) borate buffer C) phosphate buffer pH 5.0 and D) sodium acetate buffer

4. CONCLUSION

The analytical methods devoped for the determination of Brimonidine tartrate are precise and accurate and can be applied for the determination of pharmaceutical formulations successfully.

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