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# New spectrophotometric methods for the simultaneous determination of Brimonidine and Timolol in Eye drops

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**ABSTRACT**

Two new validated spectrophotometric methods have been proposed for the simultaneous determination of Brimonidine and Timolol in ophthalmic preparations. Simultaneous equation method and Q-Analysis were chosen for the determination of Brimonidine and Timolol in borate buffer. Linearity was observed 1-60 µg/ml for Timolol and 1-40 µg/ml for Brimonidine. Both the methods were validated and can be used for the simultaneous determination of Brimonidine and Timolol in eye drops.

**KEY WORDS:** Brimonidine, Timolol, Spectrophotometry, Simultaneous equation method, Q-Analysis Validation.

## 1. INTRODUCTION

Brimonidine (CAS No. 59803-98-4) is chemically known as 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)quinoxalin-6-amine with molecular formula, C11H10BrN5 and molecular weight 292.14 g/mol. Brimonidine (BRM) is freely soluble in water and soluble in methanol with pKa 7.78. Brimonidine (Figure 1a) is used to treat open-angle glaucoma or ocular hypertensiona. Brimonidine (The Merck Index, 2006) is indicated for the lowering of intraocular pressure in patients with open-angle glaucoma or ocular hypertension. Brimonidine is an $\alpha_2$ adrenergic agonist that acts by the activation of G protein-coupled receptor. This G protein-coupled receptor inhibits the activity of adenylate cyclase. The $\alpha_2$ agonist results in vasoconstriction of blood vessels and vasoconstriction reduces the aqueous humour flow (Toris, 1999). Timolol (CAS No. 26839-75-8) is chemically *(S)*-1-(*tert*-butylamino)-3-[(4-morpholin-4-yl-1, 2, 5-thiadiazol-3-yl) oxy] propan-2-ol. Timolol (TML) can be used as an antihypertensive, antiarrhythmic, antiangina, and antiglaucoma agent. It has molecular formula, $C_{13}H_{24}N_4O_3S$ and molecular weight 316.42 g/mol (pKa 9.21). Timolol (The Merck Index, 2006) is a beta-adrenergic antagonist and the levo isomer is the more active. Timolol (Figure 1b) is used for the treatment of migraine disorders, tremor. The combination of Brimonidine and Timolol have a rapid onset of action, with peak ocular hypotensive effect seen at two hours post-dosing for Brimonidine and one to two hours for Timolol. Very few spectrophotometric (Desai, 2014; Vinayaka, 2015; Hiral, 2014) and liquid chromatographic methods (Arun, 2011; Abdullah, 2014) for the simultaneous determination of BRM and TML. The authors have proposed two spectrophotometric methods for the determination of BRM and TML and the methods were validated (ICH guidelines, 2005).

## 2. MATERIALS AND METHODS

**Chemicals and reagents:** The stock solutions of both Brimonidine and Timolol were prepared in methanol and a series of solutions were prepared on dilution with borate buffer (pH 9.0) for the construction of calibration curve. The combination of Brimonidine and Timolol is available with brand name Combigan (Allergen Plc, India) as eye drops containing Brimonidine tartrate 0.2% and Timolol 0.5%.

**Instrumentation:** UV-1800 double beam UV-VIS spectrophotometer (Shimadzu) with a pair of 10mm path length matched quartz cells is used for the study. All the sample solutions were scanned 200-400 nm with medium scanning speed.

**Procedure:** In simultaneous equation method (Method A), the absorption maxima of the two drugs were selected and in Q-analysis (Method B) one of the drugs absorption maxima and the isosbestic point were chosen for the calculation purpose. Brimonidine shows absorption maxima ($\lambda_{max}$) at 257 nm Timolol at 295 nm respectively. The absorptivity values were calculated at 257 nm and 295 nm for both Brimonidine and Timolol from their individual spectra in simultaneous equation method. Two isosbestic (isoabsorptive) points were observed at 288.5 and 315 nm in the overlay absorption spectrum. For Q-Analysis, the absorption maxima ($\lambda_{max}$ = at 257 nm) of Brimonidine and the isosbestic point 288.5 nm were chosen for the calculation purpose.

**Validation:**

**Linearity:** 1-60 µg/ml Timolol and 1-40 µg/ml Brimonidine solutions were prepared from the stock solutions separately and scanned against the reagent blank i.e. borate buffer pH 9.0 and the absorbance as well as the absorptivity values were calculated at the selected wavelengths for both the methods. A graph was drawn by taking the concentration of the drug solution on the x- axis and the corresponding absorbance values on the y-axis at the selected wavelengths.

**Precision and Accuracy:** The intra-day and inter-day precision studies were performed at three different concentration levels (10, 20 and 40 µg/mL) and the %RSD was calculated. Accuracy studies were carried out for both the methods A and B (80%, 100%, and 120%) and the % recovery was calculated.

**Assay of Brimonidine and Timolol:** The combination of Brimonidine and Timolol is available with brand name Combigan (Allergen Plc, India) as eye drops containing Brimonidine tartrate 0.2% and Timolol 0.5%. The eye drops preparation was procured from local pharmacy store and extracted with methanol and then assayed after dilution with borate buffer with the two methods.

## 3. RESULTS AND DISCUSSION

Two new spectrophotometric methods, simultaneous equation method (Method A) and Q – Analysis (Method B) were proposed for the simultaneous determination of Brimonidine and Timolol in borate buffer pH 9.0. Timolol and Brimonidine obeys Beer-Lambert's law 1-60 µg/ml and 1-40 µg/ml in both the methods (Figure 3A and 3B) with linear regression equations $y = 0.0221x + 0.0012$ ($R^2 = 0.9995$) and $y = 0.0636x - 0.0013$ ($R^2 = 0.9992$) for Timolol and Brimonidine respectively. Table.1, compares the previously published methods in detail with the present method.

**Simultaneous equation method (Method A):** The overlay absorption spectrum of Brimonidine and Timolol and their formulation was shown in Figure 2. The specific absorptivity value of any drug is the absorbance of 1%, i.e. g/100ml solution. The absorptivity values were calculated and substituted in the simultaneous equation given below. A1 and A2 represents the absorbance of the formulation solution at 257 nm and 295 nm respectively;

$C_{BRM}$ and $C_{TML}$ are the concentrations of Brimonidine and Timolol (g/100ml)

At 257 nm, $A_1 = 607.7\ C_{BRM} + 46.34\ C_{TML}$

At 295 nm, $A_2 = 138.1\ C_{BRM} + 221.51\ C_{TML}$

$ax_1$ = Absorptivity of BRM at 257nm = 607.7

$ax_2$ = Absorptivity of BRM at 295nm = 138.1

$ay_1$ = Absorptivity of TML at 257nm = 46.34

$ay_2$ = Absorptivity of TML at 295nm = 221.51

**Absorbance ratio method (Q Analysis) (Method B):** Two isosbestic points were observed at 288.5, 315 nm in the overlay absorption spectrum and assay was performed at 315 nm and 257 nm. The absorptivity values obtained at the selected wavelengths were substituted in the given equation

$Cx = Qm-Qy/Qx-Qy \times A_1/ax_1$

$Cy = Qm-Qx/Qy-Qx \times A_2/ay_1$

Cx = Concentration of Brimonidine

Cy = Concentration of Timolol

$A_1$= Absorbance at isoabsorptive wavelength 288.5 nm.

$A_2$ = Absorbance at wavelength 257 nm.

$ax_1$ = Mean absorptivity of Brimonidine at 288.5 nm. = 205.81

$ay_1$ = Mean absorptivity of Timolol at 257 nm. = 46.34

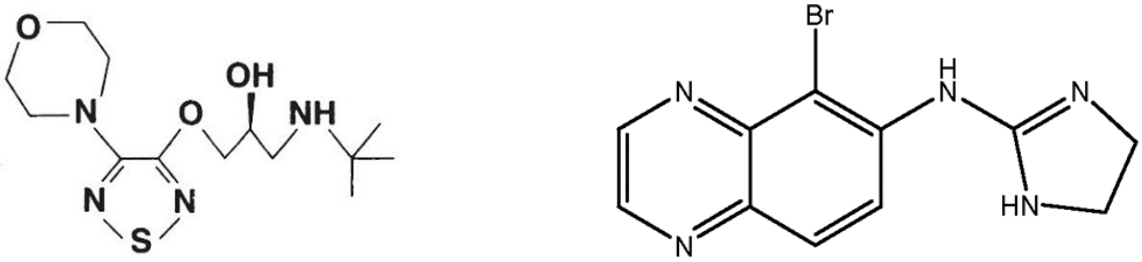
Qm = Ratio of absorbance of formulation solution at 288.5 & 257 nm.

Qx = Ratio of absorptivity of Brimonidine at 288.5 & 257 nm. = 0.1851

Qy = Ratio of absorptivity of Timolol at 288.5 & 257nm. = 4.7801

$Cx = Qm-4.7801/0.1851-4.7801 \times A_1/205.81$

$Cy = Qm-0.1851/4.7801-0.1851 \times A_2/46.34$




**(a)** **(b)**

**Figure.1. Chemical structures of Timolol (a) Brimonidine (b)**

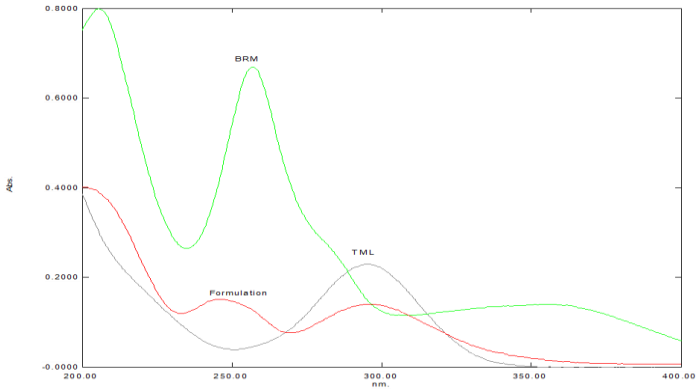
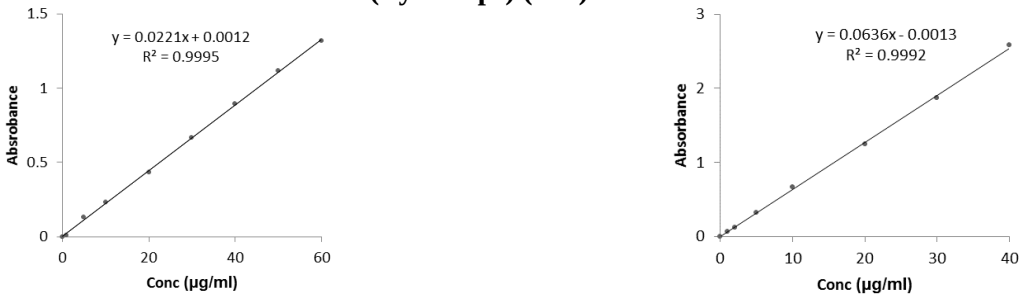



**Figure.2. Absorption Spectrum of Timolol (5 µg/ml), Brimonidine (2 µg/ml) and Timolol: Brimonidine formulation (Eye drops) (5: 2) in borate buffer**




**(a)** **(b)**

**Figure.3. Calibration curves of Timolol (a) and Brimonidine (b)**

**Table 1. Details of published analytical methods of Timolol and Brimonidine**

| **Method** | **Reagents/ Mobile phase** | **Linearity (µg/mL)** | **$\lambda_{max}$ (nm)** | **References** |
|---|---|---|---|---|
| Spectrophotometric | Distilled water | 2-50 (TML)<br>2-14 (BRM) | 290<br>244 | Desai, 2014 |
| Spectrophotometric | Distilled water | 4-20 (TML)<br>10-50 (BRM) | 255<br>295 | Vinayaka, 2015 |
| Spectrophotometric | Distilled water | 2-14 (TML)<br>5-35 (BRM) | 247<br>295 | Hiral, 2014 |
| HPLC | Phosphate buffer: Acetonitrile (65:35, v/v) | 10-20 | 295 | Arun, 2011 |
| HPLC | Ammonium acetate : Methanol (40:60, v/v) | 10-60 | 254 | Abdullah, 2014 |
| Spectrophotometric | Borate buffer (pH 9.0) | 1-60 (TML)<br>1-40 (BRM) | 295<br>257 | Present work |

**Table.2. Assay of Timolol and Brimonidine**

| **Formulation brand** | **Drug** | **Label claim (mg)** | ***Amount found** | | ***% Recovery** | |
|---|---|---|---|---|---|---|
| | | | Method A | Method B | Method A | Method B |
| Combigan Eye drops | Timolol<br>Brimonidine | 5<br>2 | 4.96<br>1.97 | 4.97<br>1.98 | 99.2<br>98.5 | 99.4<br>99.0 |

## 4. CONCLUSION

The two spectrophotometric methods is simple, precise and accurate for the routine simultaneous determination of Timolol and Brimonidine in pharmaceutical formulations successfully.

## 5. ACKNOWLEDGEMENT

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