ORIGINAL ARTICLE

# Multi Component Mode and Derivative Spectrophotometric Methods for the Simultaneous Determination of Timolol maleate and Brimonidine Tartrate

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## Abstract

**Introduction:** New multicomponent spectrophotometric methods have been established for the simultaneous determination of timolol maleate and brimonidine tartrate in pharmaceutical formulations (eye drops). Timolol is a propanolamine derivative having non-selective beta-adrenergic antagonist action with antihypertensive action. Brimonidine is used to treat glaucoma, or ocular hypertension and timolol can be used as an antihypertensive and anti-glaucoma agent. **Materials and Methods:** Shimadzu ultraviolet (UV)-1800 model UV-visible spectrophotometer double beam was used for the present study. The three new methods were developed using phosphate buffer pH 7.0. **Results and Discussion**: The absorption maxima were found at 295 nm for timolol maleate and 247 nm for brimonidine tartrate, respectively. Linearity was observed 1–120 µg/ml for timolol maleate and 1–60 µg/ml for brimonidine tartrate and the three methods were validated as per the ICH guidelines. **Conclusions:** The three methods can be used for the determination of timolol maleate and brimonidine tartrate in eye drops.

**Key words:** Brimonidine tartrate, first derivative spectrophotometry, multicomponent mode, simultaneous equation method, timolol maleate, validation

## INTRODUCTION

Timolol maleate is an anti-glaucoma agent and its levo-isomer is more active.[1] Brimonidine is used to treat glaucoma or ocular hypertension.[2,3] It is an adrenergic agonist, and the combination of timolol maleate and brimonidine tartrate is widely used in the market. Very less liquid chromatographic methods,[4-6] spectrophotometric methods[7-11] and high-performance thin-layer chromatography[12] methods have been established in the literature for the simultaneous determination of timolol maleate and brimonidine tartrate in eye drops. In the present study, the authors have proposed three simple analytical techniques, i.e., simultaneous equation, first derivative and multicomponent mode methods for the simultaneous determination of timolol maleate and brimonidine tartrate [Figure 1] in eye drops. And the methods were validated.[13]

## MATERIALS AND METHODS

### Chemicals and reagents

Stock solutions of both timolol and brimonidine were prepared in methanol and dilutions were made with phosphate buffer pH 7.0. The phosphate buffer pH 7.0 was prepared as per the procedure is given in IP 1996.The combination of timolol maleate and brimonidine tartrate is available with brand name Combigan (Allergen Plc, India) as eye drops containing timolol maleate 0.5% and brimonidine tartrate 0.2%, respectively.

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Shimadzu ultraviolet (UV)-visible double beam spectrophotometer Model No. UV-1800 was used for the present study. All the chemicals were procured from Merck (India), and all are of AR grade. Solutions were scanned with medium scanning speed in 200–400 nm range.

### Method development

Three spectrophotometric methods - simultaneous equation method (Method I), simultaneous first derivative method ($D_1$) (Method II), and multicomponent mode (Method III) were developed for the simultaneous determination of timolol maleate and brimonidine tartrate.

#### *Method I: Simultaneous equation method*

Adequate dilute solutions of timolol maleate and brimonidine tartrate were prepared from their stock solutions separately with phosphate buffer (pH 7.0) and scanned in UV region (200–400 nm) as their solutions were colorless. The absorption spectra so obtained have shown $\lambda_{max}$ at 295 nm for timolol maleate and 247 nm for brimonidine tartrate. These two wavelengths were selected for the simultaneous equation method. The absorptivity (ε) values were calculated from their individual spectra of all the linearity range and incorporated in simultaneous equation from which individual concentrations of timolol maleate and brimonidine tartrate were determined. Calibration curves were drawn by plotting the concentration of the drug solution on the X-axis and the corresponding absorbance on the Y-axis for both Timolol maleate and Brimonidine tartrate.

#### *Method II: Simultaneous first derivative method ($D_1$)*

The individual zero-order absorption spectra of TIL and BMN were converted into their first-order derivative spectra with the help of inbuilt software. The first-order derivative spectra of TIL have shown zero crossing points (ZCP) at 251.8 and 295 nm and that of BMN at 227.2, 247.38, and 277.66 nm. TIL was quantified from the minima at 227.2 nm (ZCP of BMN) and that of BMN from maxima at 295 nm (ZCP of TIL). A graph was drawn by taking the drug concentration (TIL or BMN) on the X-axis and the corresponding derivative absorbance on the Y-axis.

#### *Method III: Multicomponent mode*

In this multicomponent mode method, a minimum of three solutions containing both TIL and BMN in different ratios were taken and scanned (200–400 nm). Along with these solutions, an unknown ratio of mixture of TIL and BMN was also scanned, and the inbuilt software directly gives the concentration of the individual components, i.e., TIL and BMN present in the unknown (or formulation solutions). For the present study, eight standard solutions containing timolol maleate and brimonidine tartrate were prepared in different ratios such as 5:5, 10:10, 10:20, 20:10, 30:40, 50:20, 60:40, and 150:60 in phosphate buffer pH 7.0 and scanned in multicomponent mode.

### Method validation

#### *Linearity*

1–120 µg/mL TIL and 1–60 µg/mL BMN solutions were prepared from their individual stock solutions separately and scanned against the reagent blank, i.e., phosphate buffer (pH 7.0). The absorbance, as well as the absorptivity values, was calculated at the selected wavelengths for Method I as described in the procedure. A linearity 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 whereas for Method II derivative absorbance was plotted against the concentration of the drug solution. For Method III, the concentration of the drug solutions is obtained directly from the inbuilt software.

#### *Precision and accuracy studies*

The intraday and interday precision studies were performed at three different levels (10, 20, 40, and 80 µg/mL) and accuracy studies were carried out by standard addition method (80%, 100%, and 120%) and the percentage recovery was calculated.

#### *Assay of timolol maleate and brimonidine tartrate*

The combined dosage form, i.e., eye drops is available with brand name Combigan (Allergen Plc, India) containing timolol maleate 0.5% and brimonidine tartrate 0.2% and the eye drops were procured from the local pharmacy store and extracted with methanol. Later dilutions were made with phosphate buffer solution for all the three methods, and percentage recovery was calculated using the linear regression equation.

## RESULTS AND DISCUSSION

Three spectrophotometric methods were developed for the simultaneous determination of timolol maleate, and brimonidine tartrate in pharmaceutical formulations in phosphate buffer solution (pH 7.0) and the methods were validated as per the ICH guidelines. Many of the spectrophotometric methods were developed in distilled water, and the authors have proposed a new technique, i.e., using phosphate buffer (pH 7.0) and a brief comparative study of spectrophotometric methods so far developed in the literature with the present proposed methods was given in Table 1.

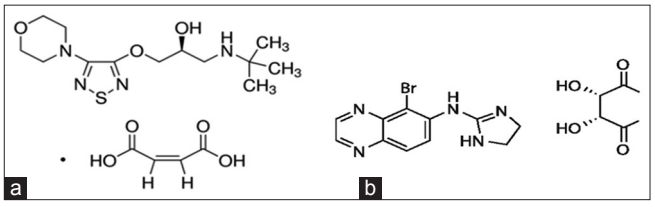


**Figure 1:** Chemical structures of (a) timolol maleate (b) brimonidine tartrate

### Method I: Simultaneous equation method

The overlay zero-order absorption spectra [Figure 2a] have shown $\lambda_{max}$ at 295 nm for timolol maleate and 247 nm for brimonidine tartrate. The absorptivity values were calculated from the absorbance taken for all the solutions at 295 nm and 247 nm for both TIL and BMN and the absorptivity values were substituted in the simultaneous equation given below.

$A_1$ and $A_2$ represent the absorbance of the combined mixture solution at 295 nm and 247 nm, respectively; $C_{TIL}$ and $C_{BMN}$ are the concentrations of TIL and BMN (g/100 ml), respectively.

At 295 nm, $A_1 = 206.5\ C_{TIL} + 81.37\ C_{BMN}$

At 247 nm, $A_2 = 46.28\ C_{TIL} + 575.27\ C_{BMN}$

Absorptivity of TIL at 295 nm =206.5; Absorptivity of TIL at 247nm = 46.28

Absorptivity of BMN at 295 nm = 81.37;.Absorptivity of BMN at 247 nm = 575.24

Linearity was observed 1–120µg/ml for Timolol maleate and 1–60 µg/ml for brimonidine tartrate with linear regression equations y = 0.0211 x - 0.0082 ($R^2$ = 0.9999) [Figure 2b] and y = 0.0565 x + 0.0157 ($R^2$ = 0.9995) [Figure 2c] for TIL and BMN, respectively.

### Method II: Simultaneous first derivative method

The overlay first-order derivative spectrum of TIL and BMN was shown in Figure 3a. Linearity was observed over the concentration range 1–120 µg/mL and 1–60 µg/mL for TIL and BMN, respectively. A straight line graph was obtained with regression equations y = 0.0014 x - 0.0013 ($R^2$ = 0.9995) [Figure 3b] and y = 0.0003 x - 0.0002 ($R^2$ = 0.9995) [Figure 3c] for TIL and BMN, respectively.

### Method C: Multicomponent mode

In multicomponent mode, method timolol maleate and brimonidine tartrate were directly determined from the inbuilt software.

### *Precision and accuracy studies*

The percentage relative standard deviation (RSD) range for intraday and interday precision was found to be 0.86–0.91 and 0.98–1.02 (Timolol maleate); 0.76–0.89 and 0.97–1.13 (brimonidine tartrate) in all Methods I-III indicating that the three methods are precise. The percentage RSD in accuracy studies was also found to be <2 in all the methods proving that the Methods I-III are accurate [Table 2].

### *Assay of timolol maleate (TIL) and brimonidine tartrate (BMN)*

Combigan eye drops available in the local pharmacy store were procured and extracted with methanol followed by filtration and dilution with phosphate buffer and analyzed by the above methods. The percentage recovery obtained in the assay studies was shown in Table 3.The marketed formulation (eye drops) TIL:BMN (5:2) has shown good percentage recovery for both TIL and BMN and the results were given in Table 3.

## CONCLUSION

The three validated spectrophotometric methods are simple, precise, accurate, and very easy to perform the routine analysis of combined dosage forms of timolol maleate and brimonidine tartrate.

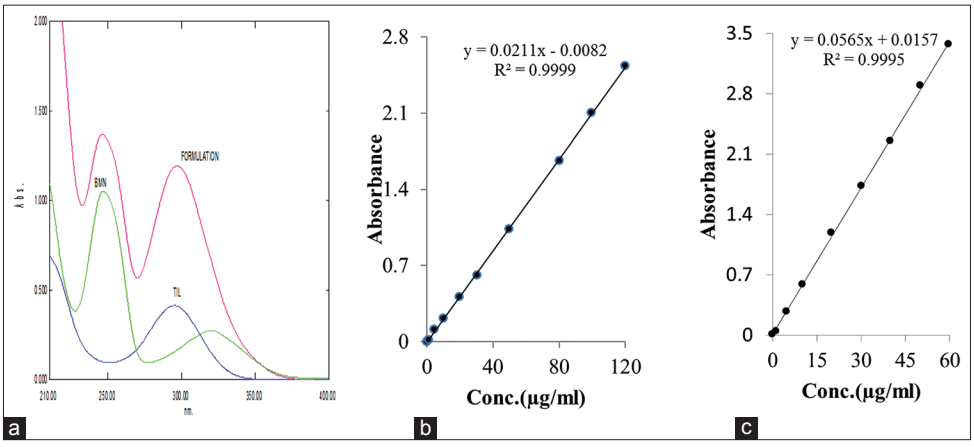


**Figure 2:** (a) Overlay zero-order absorption spectra of timolol maleate (20 µg/mL) brimonidine tartrate (20 µg/mL) and formulation (eye drops) in phosphate buffer (pH.7.0), (b) calibration curve of timolol maleate, and (c) calibration curve of brimonidine tartrate

**Table 1:** Comparison of present spectrophotometric methods with the reported methods

| Reagents | Method | Linearity (µg/mL) | Remarks | Ref |
|---|---|---|---|---|
| Borate buffer (pH 9.0) | First derivative method<br>Ratio first derivative method<br>Multicomponent mode method | 1–60 (TIL)<br>1–40 (BMN) | Buffer is used | 7 |
| Distilled water | Simultaneous equation method Q-ratio method | 4–20 (BMN)<br>1–50 (TIL)<br>4–20 (BMN)<br>10–50 (TIL) | Low linearity range | 8 |
| Distilled water | Simultaneous equation method<br>First derivative method<br>Ratio first derivative method | 2–14 (BMN)<br>2–50 (TIL) | Low linearity range | 9 |
| Distilled water | First derivative method<br>Ratio first derivative method | 2–35 (BMN)<br>5–85 (TIL) | Low linearity range | 10 |
| Distilled water | Simultaneous equation method<br>Q-ratio method | 2–14 (BMN)<br>5–35 (TIL) | Low linearity range | 11 |
| Phosphate buffer (pH 7.0) | Simultaneous equation method<br>First derivative method<br>Multicomponent mode method | 1–60 (BMN)<br>1–120 (TIL) | Buffer is used wide linearity range | Present method |

**Table 2:** Accuracy study of timolol maleate (TIL) and brimonidine tartrate (BMN)

| Drugs | Spiked concentration (µg/ml) (%) | Formulation concentration (µg/ml) | Method I | | Method II | | Method III | |
|---|---|---|---|---|---|---|---|---|
| | | | %RSD | %*Recovery | %RSD | %*Recovery | %RSD | %*Recovery |
| TIL | 0.8 (80) | 1 | 1.13 | 99.56 | 0.67 | 99.4 | 0.97 | 98.24 |
| | 1 (100) | 1 | 1.11 | 99.09 | 0.82 | 98.37 | 1.07 | 99.37 |
| | 1.2 (120) | 1 | 0.99 | 98.64 | 0.94 | 98.87 | 0.67 | 99.97 |
| BMN | 4 (80) | 5 | 1.33 | 98.57 | 1.04 | 99.22 | 1.34 | 99.57 |
| | 5 (100) | 5 | 0.21 | 99.87 | 1.25 | 99.61 | 1.57 | 98.91 |
| | 6 (120) | 5 | 1.13 | 99.20 | 0.97 | 98.12 | 1.09 | 99.02 |

*Mean of three replicates. RSD: Relative standard deviation

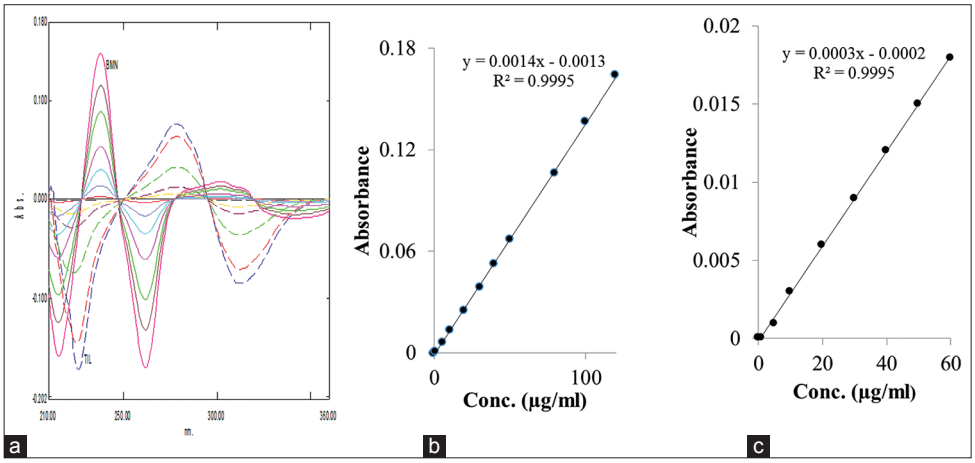


**Figure 3:** (a) Overlay first derivative absorption spectra of timolol maleate and brimonidine tartrate, (b) calibration curve of timolol maleate, and (c) calibration curve of brimonidine tartrate

**Table 3:** Assay of timolol maleate and brimonidine tartrate

| Formulation brand | Drug | Label claim (%) | *Amount found (%) | | | *% Recovery | | |
|---|---|---|---|---|---|---|---|---|
| | | | Method | | | Method | | |
| | | | I | II | III | I | II | III |
| Brand I | TIL and BMN | 0.5 | 0.481 | 0.491 | 0.492 | 96.2 | 98.2 | 98.4 |
| | | 0.2 | 0.196 | 0.198 | 0.194 | 98.0 | 99.0 | 97.0 |

*Mean of three replicates

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## REFERENCES

1. Merck Research Laboratories Division of Merck and Co., Inc. The Merck Index. 14th ed. Monograph Number 9444:1623, (Timolol maleate). Whitehouse Station, NJ: Merck Research Laboratories Division of Merck and Co., Inc.; 2006.
2. Toris C, Camras C, Yablonski M. Acute versus chronic effects of Brimonidine on aqueous humor dynamics in ocular hypertensive patients. Am J Ophthalmol 1999;128:8-14.
3. Merck Research Laboratories Division of Merck and Co., Inc. The Merck Index. 14th ed. Monograph number 1375:225 (Brimonidine). Whitehouse Station, NJ: Merck Research Laboratories Division of Merck and Co., Inc.; 2006.
4. Narendra A, Deepika D, Annapurna MM. Liquid chromatographic method for the analysis of Brimonidine in ophthalmic formulations. Euro J Chem 2012;9:1327-31.
5. Abdullah AE, Hani MH, Lobna MA, Mustafa SM. Development and validation of HPLC method for simultaneous estimation of Brimonidine and Timolol in bulk and pharmaceutical dosage form. J Chrom Sep Tech 2014;5:230.
6. Arun P, Murugesan SK, Nanjaian M. Simultaneous estimation of Brimonidine and Timolol in nanoparticles formulation by RP-HPLC. Int J Rec Adv Pharm Res 2011;3:31-6.
7. Annapurna MM, Sushmitha M, Sevyatha VS. Simultaneous determination of brimonidine tartrate and timolol maleate by first derivative and ratio derivative spectroscopy. J Anal Pharm Res 2017;4:20.
8. Vinayaka DG, Shetty AS, Ahmed M, Kumar SM. Simultaneous determination of Brimonidine and Timolol in combined pharmaceutical dosage form using two different green spectrophotometric methods. Jour Harmo Res Pharm 2015;4:52-9.
9. Heta HD, Anandkumari DC. Three simple validated UV spectrophotometric methods for the simultaneous estimation of Timolol and Brimonidine and their comparison using ANOVA. IntJ Pharm Res Anal 2014;4:168-77.
10. Mohamed SR, Hanan AM, Shereen MT, Mona NS. Simultaneous determination of Timolol maleate and Brimonidine tartarate in their pharmaceutical dosage form. Anal Chem Lett 2014;4:132-45.
11. Hiral SP, Hemant MP. Simultaneous determination of Brimonidine and Timolol in combined pharmaceutical dosage form using two different green spectrophotometric methods. World J Pharm Pharm Sci 2014;3:1330-40.
12. Suketa KM, Dilip GM, Analytical method development and validation for simultaneous estimation of Timolol Maleate and Brimonidine Tartrate in bulk and marketed ophthalmic formulation. J Pharm Sci Biosci Res 2014;4:351-6.
13. ICH Validation of Analytical Procedures: Text and Methodology, Q2 (R1), International Conference on Harmonization; 2005.

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