Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 5 Issue 5 May 2021

Research Article

Chemo-metric Assisted UV-Spectrophotometric Methods for the Simultaneous Estimation of Brimonidine Tartrate and Timolol Maleate

Mukthinuthalapati Mathrusri Annapurna* and Yenda Manishankar

Department of Pharmaceutical Analysis and Quality Assurance, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, India

***Corresponding Author:** Mukthinuthalapati Mathrusri Annapurna, Department of Pharmaceutical Analysis and Quality Assurance, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, India.

Received: March 29, 2021

Published: April 30, 2021

Abstract

Two Chemo-metric assisted UV-spectrophotometric methods have been developed for the simultaneous determination of Timolol maleate and Brimonidine tartrate in ophthalmic dosage forms. Timolol is a beta blocker used for the patients suffering from ocular hypertension and glaucoma and Brimonidine tartrate is a an alpha-2 adrenergic receptor agonist. Simultaneous equation method and absorbance ratio method were proposed for the simultaneous determination of Timolol maleate and Brimonidine tartrate in ophthalmic preparations. The two methods were validated and were found to be linear over 1 - 60 µg/mL for Timolol maleate and 1 - 40 µg/mL for Brimonidine tartrate. The two proposed methods find application in the quality control of pharmaceuticals.

Keywords: Timolol Maleate; Brimonidine Tartrate; Spectrophotometry; Simultaneous Equation Method; Q-Analysis; Validation

Introduction

Timolol [1] is chemically (S)-1-(tert-butylamino)-3-[(4-morpholin-4-yl-1, 2, 5-thiadiazol-3-yl) oxy] propan-2-ol. Timolol (Figure 1) is used as an anti-hypertensive and has molecular formula, $C_{13}H_{24}N_4O_3S$ with molecular weight 316.42 g/mol (pKa 9.21). Timolol (TML) is a beta-adrenergic antagonist and levo isomer is more active. Brimonidine tartrate [2] is chemically known as 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl) quinoxalin-6-amine with molecular formula, $C_{11}H_{10}BrN_5$ and molecular weight 292.14 g/mol. Brimonidine tartrate (BRM) is used to treat open-angle glaucoma or ocular hypertension. Brimonidine tartrate (Figure 2) is used for treating ocular hypertension. Brimonidine tartrate is an α_2 adrenergic agonist that acts by the activation of G protein-coupled receptor [3].

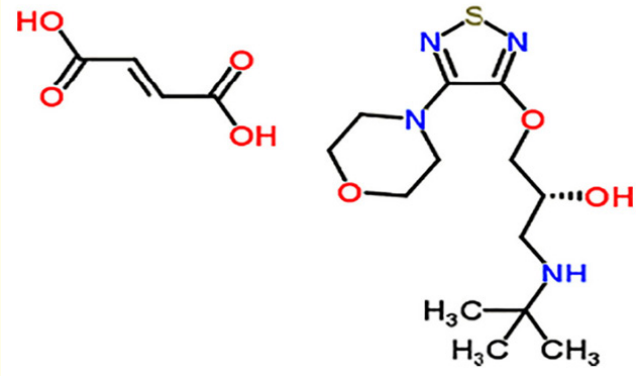

Figure 1: Structure of timolol maleate.

The combination of Timolol maleate and Brimonidine tartrate has a very good combined effect. Analytical techniques such as HPTLC [4], HPLC [5,6] and spectrophotometric [7-12] methods were developed for the simultaneous determination of Timolol

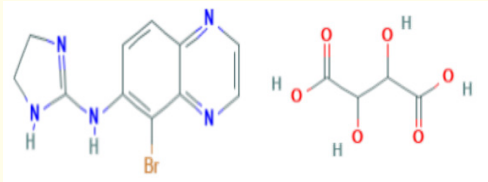

Figure 2: Structure of brimonidine tartrate.

maleate and Brimonidine tartrate in pharmaceutical dosage forms. Two UV spectrophotometric methods have been developed and validated [13] as per ICH guidelines for the simultaneous determination of Timolol maleate and Brimonidine tartrate in pharmaceutical formulations.

Materials and Methods

A double beam UV-VIS spectrophotometer (Shimadzu Model No. UV-1800) with a pair of 10 mm path length matched quartz cells was used for the study. All the drug sample solutions were scanned 200-400 nm with medium scanning speed.

Preparation of phosphate buffer (pH 2.0) solution

0.136 grams of potassium di hydrogen phosphate was weighed accurately and dissolved in about 800 ml of water in a 1000 ml volumetric flask. The pH of the solution was adjusted to 2.0 with the help of hydrochloric acid and sufficient water was added to make up to volume after sonication.

Preparation of stock solutions

Stock solutions were prepared in two different volumetric flasks by dissolving 25 mg of each of Timolol maleate and Brimonidine tartrate in methanol and there by diluting the stock solutions as per requirement with phosphate buffer pH 2.0.

Procedure

Two methods were proposed for the simultaneous determination of Timolol maleate and Brimonidine tartrate i.e. simultaneous equation method (Method A) and Q-analysis or Absorbance ratio method (Method B). A 10 μg/ml solution of both Timolol maleate and Brimonidine tartrate were prepared initially from the stock solutions and the absorption maxima (λ_{max}) was noted. The absorption spectra of Timolol maleate and Brimonidine tartrate were overlaid and shown in figure 3.

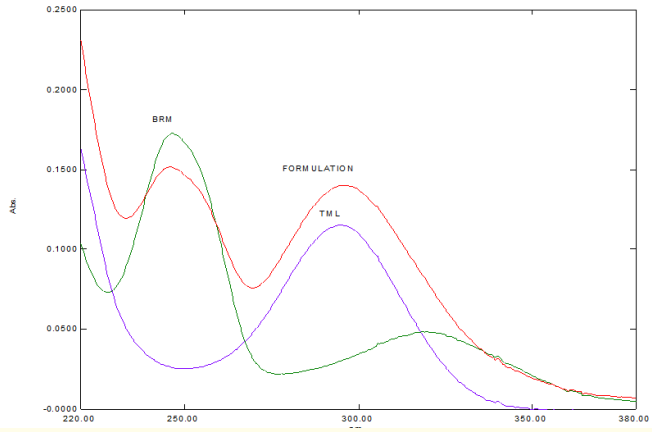

Figure 3: Absorption spectrum of timolol maleate (TML) (5 µg/ml), Brimonidine tartrate (BRM) (2 µg/ml) and formulation (Eye drops) (TML: BRM 5: 2) in phosphate buffer pH 2.0.

Simultaneous equation method (Method A)

In simultaneous equation method the absorption maxima of both Timolol maleate and Brimonidine tartrate were selected. Brimonidine tartrate has shown absorption maxima (λ_{max}) at 246 nm and Timolol maleate has shown absorption maxima (λ_{max}) at 295 nm respectively. The absorptivity values (ε) were calculated from the absorbance values for both the drugs Timolol maleate and Brimonidine tartrate at 246 nm and 295 nm for all the solutions prepared for the linearity study from their individual spectra and substitute in the simultaneous equations.

Q-analysis or Absorbance ratio method (Method B)

In Q-analysis or absorbance ratio method the wavelength of the absorption maxima (λ_{max}) of one of the drugs and the wavelength of the isosbestic point were selected for the calculation purpose. Two isosbestic (iso-absorptive) points were observed at 268.23 and 319.82 nm from the overlay absorption spectrum of Timolol maleate and Brimonidine tartrate. For Q-Analysis method, the absorption maxima of Brimonidine tartrate (λ_{max} = at 246 nm) and the isosbestic point 268.23 nm were selected for the calculation purpose.

Method validation [13]

Linearity

1-60 *µg*/ml Timolol maleate and 1-40 *µg*/ml Brimonidine tartrate solutions were prepared separately from their individual stock solutions and the solutions were scanned against the reagent blank i.e. phosphate buffer pH 2.0. The absorbance values and thereby the absorptivity values were calculated at the selected wavelengths for both Method A and Method B. A calibration curve was drawn by taking the concentration of each of the drug solutions individually 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) on the same day and on different days and the percentage relative standard deviation (% RSD) was calculated. Accuracy studies were carried out by standard addition method. A fixed concentration of formulation solution was spiked with 80%, 100% and 120% of pure drug solutions and the % recovery was calculated.

Assay of brimonidine tartrate and timolol

The combination of both Timolol maleate and Brimonidine tartrate is available with brand names Brimocom Eye Drop (Cipla Ltd, India), Brimopress T Eye Drops (Centaur Pharma, India), Combigan eye drops (Allergan plc, India) and Iotim B Drops (FDC Ltd, India) with label claim: Brimonidine tartrate 0.2% w/v and Timolol maleate 0.5% w/v (Brimonidine tartrate: 2 mg/ml and Timolol maleate: 5 mg/ml). Two different brands of these eye drops (Total volume 5 ml) were procured from the medical store and the active constituents were extracted using methanol in to a volumetric flask and sonicated. The extracted solution was filtered and the filtrate was diluted with phosphate buffer pH 2.0 as per the requirement and assay was performed with the two proposed methods. The absorption spectra of Timolol maleate and Brimonidine tartrate were overlaid and shown in figure 3.

Results and Discussion

Two new spectrophotometric methods, simultaneous equation method (Method A) and Absorbance ratio method (Method B) were proposed for the simultaneous determination of Timolol maleate and Brimonidine tartrate in phosphate buffer pH 2.0. The review of literature of the previously published analytical methods were compared with the present proposed methods and summarized in table 1.

Reagents/Mobile phase (v/v)	Linearity (µg/mL)	λ_{max} (nm)	Observation	Reference
Liquid chromatographic methods				
Chloroform: Methanol: Ammonia (30%) (9:1:0.1)	500-1500 ng/spot (TML) 200-600 ng/spot (BRM)	268	HPTLC	[4]
Phosphate buffer: Acetonitrile (65:35)	10-20	295	HPLC	[5]
Ammonium acetate : Methanol (40:60)	10-60	254	HPLC	[6]
Spectrophotometric methods				
Phosphate buffer (pH 7.0)	1-120 (TML) 1-60 (BRM)	295 247	Simultaneous equation method First Derivative method Multi-component mode method	[7]
Distilled water	1-50 (TML) 4-20 (BRM)	255 295 271 295	Simultaneous equation method Q absorbance equation method	[8]
Borate buffer (pH 9.0)	1-60 (TML) 1-40 (BRM)	257 251.5	First Derivative method Ratio Derivative Spectroscopy Multi-component mode method	[9]

Distilled water	2-14 (TML) 5-35 (BRM)	247 295	Simultaneous equation method Q absorbance ratio method	[10]
Borate buffer (pH 9.0)	1-60 (TML) 1-40 (BRM)	295 257	Simultaneous equation method Q-analysis	[11]
Distilled water	2-50 (TML) 2-14 (BRM)	290 244	Simultaneous equation method First Derivative method Ratio first derivative method	[12]
Phosphate buffer (pH 2.0)	1-60 (TML) 1-40 (BRM)	295 246	Simultaneous equation method Absorbance ratio method	Present work

Table 1: Review of published analytical methods of timolol maleate and brimonidine tartrate.

Method validation

Linearity

Timolol maleate and Brimonidine tartrate obeys Beer-Lambert's law and have shown linear response over the concentration range 1-60 *µg*/ml and 1-40 *µg*/ml respectively (Table 2) for both the methods with linear regression equations y = 0.0223x + 0.0068 (R^2 = 0.9997) (Figure 4) and y = 0.0676x + 0.0007 (R^2 = 0.9993) (Figure 5) for Timolol maleate and Brimonidine tartrate respectively.

Simultaneous equation method (Method A)

From the individual absorption spectra of Brimonidine tartrate and Timolol maleate the absorptivity values were calculated from the linearity table and substituted in the simultaneous equation. The specific absorptivity value of any drug is the absorbance of 1%, i.e. g/100ml solution. A_1 and A_2 represents the absorbance of the formulation solution at 246 nm and 295 nm respectively.

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

At 246 nm, $A_1 = 680.2\ C_{BRM} + 49.26\ C_{TML}$

At 295 nm, $A_2 = 98.3\ C_{BRM} + 221.8\ C_{TML}$

ax_1 = Absorptivity of Brimonidine tartrate at 246nm = 680.2

ax_2 = Absorptivity of Brimonidine tartrate at 295nm = 98.3

ay_1 = Absorptivity of Timolol maleate at 246nm = 49.26

ay_2 = Absorptivity of Timolol maleate at 295nm = 221.8

Absorbance ratio method (Q Analysis) (Method B)

Two isosbestic (iso-absorptive) points were observed at 268.23 and 319.82 nm from the overlay absorption spectrum of Timolol maleate and Brimonidine tartrate. For Q-Analysis method, the absorption maxima of Brimonidine tartrate (λ_{max} = at 246 nm) and the isosbestic point 268.23 nm were selected for the calculation purpose. The absorptivity values obtained at the selected wavelengths were substituted in the given equation:

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

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

Cx = Concentration of Brimonidine tartrate

Cy = Concentration of Timolol maleate

A_1= Absorbance at iso-absorptive wavelength 268.23 nm.

A_2 = Absorbance at wavelength 246 nm.

ax_1 = Mean absorptivity of Brimonidine tartrate at 268.23 nm. = 115.3

ay_1 = Mean absorptivity of Timolol maleate at 246 nm. = 49.26

Qm = Ratio of absorbance of formulation solution at 268.23 and 246 nm.

Qx = Ratio of absorptivity of Brimonidine tartrate at 268.23 and 246 nm. = 0.849

Qy = Ratio of absorptivity of Timolol maleate at 268.23 and 246 nm. = 4.502

$Cx = Qm\text{-}4.502/0.849\text{-}4.502 \times A_1/115.3$

$Cy = Qm\text{-}0.1851/4.502\text{-}0.849 \times A_2/49.26.$

Conc. (µg/ml)	Timolol maleate	Brimonidine tartrate
1	0.0308	0.0794
2	-	0.1726
5	0.1154	0.3196
10	0.2418	0.6451
20	0.4474	1.3607
30	0.6611	1.9915
40	0.8792	2.7352
50	1.1193	-
60	1.3302	-

Table 2: Linearity of timolol maleate and brimonidine tartrate.

*: Mean of three replicates.

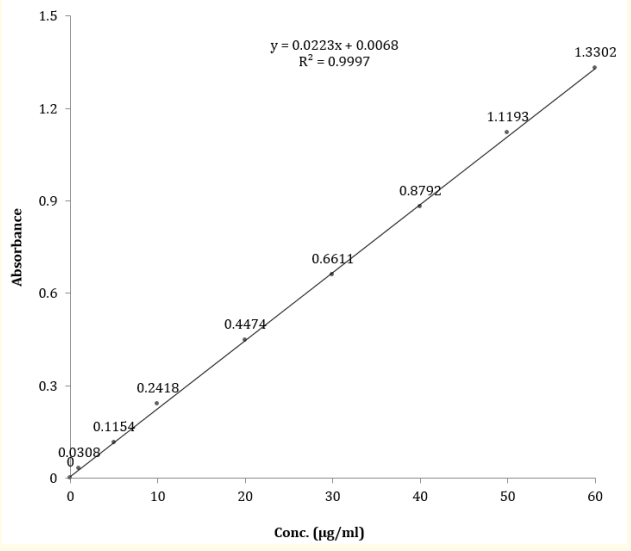

Figure 4: Calibration curve of timolol maleate.

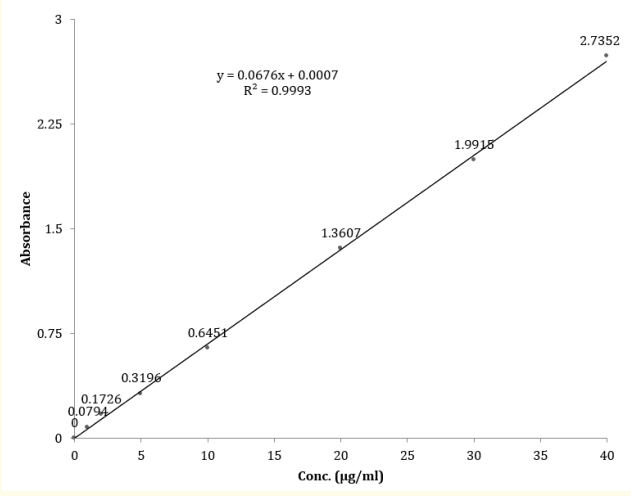

Figure 5: Calibration curves of brimonidine tartrate.

Precision and accuracy

The precision studies were performed on the same day (Intra-day) and on three different days (Interday) (Intra-day) at three different concentration levels (10, 20 and 40 µg/mL). The % RSD was found to be 0.18-0.81 (Intra-day) and 0.65-1.20 (Interday) for Method A and 0.23-0.78 (Intra-day) and 0.76-0.91 (Interday) for Method B for Timolol maleate. The % RSD was found to be 0.51-0.67 (Intra-day) and 0.81-1.10 (Interday) for Method A and 0.42-0.73 (Intra-day) and 0.61-0.92 (Interday) for Method B for Brimonidine tartrate. The % RSD in precision studies is found to be less than 2 (Table 3) indicating that the methods are precise.

The % RSD in accuracy was found to be 0.91-1.07 (Method A) and 0.84-1.01 (Method B) for Timolol maleate with a % recovery 98.54 -99.66 and 98.72-99.21 for Method A and Method B respectively. The % RSD in accuracy was found to be 0.88-1.16 (Method A) and 0.97-1.25 (Method B) for Brimonidine tartrate with a % recovery 98.61 -99.42 and 98.10-99.59 for Method A and Method B respectively. The % RSD in accuracy studies is found to be less than 2 (Table 4) indicating that the methods are accurate.

Assay of brimonidine tartrate and timolol

Two different brands of eye drops (Total volume 5 ml) containing the combination of both Timolol maleate and Brimonidine tartrate were extracted using methanol and diluted with phosphate buffer pH 2.0 and the assay was performed with the above two proposed methods. The absorption spectra of the formulation (Eye drops) containing Timolol maleate and Brimonidine tartrate (5: 2) was shown in figure 3. The % recovery (Table 5) was calculated from the absorptivity values and found to be 98.6 - 99.2 for Method A and 98.4 - 99.0 for Method B for Timolol maleate and that of Brimonidine tartrate were found to be 98.5 - 99.0 for Method A and 96.5 - 98.0 for Method B respectively.

Conclusion

The authors have developed two spectrophotometric methods for the simultaneous determination of Timolol maleate and Brimonidine tartrate in pharmaceutical formulations and the methods are validated. These two methods are simple, precise and accurate and can be successfully applied for the routine quality control of pharmaceuticals in industries.

Drugs	Conc (µg/ml)	Intra-day precision				Inter-day precision			
		*Conc. obtained (µg/ml) ± SD (RSD)		*% Recovery		*Conc. obtained (µg/ml) ± SD (RSD)		*% Recovery	
		Method A	Method B	Method A	Method B	Method A	Method B	Method A	Method B
TML	10	9.93 ± 0.02 (0.18)	9.95 ± 0.023 (0.23)	99.3	99.5	9.94 ± 0.06 (0.65)	9.93 ± 0.076 (0.76)	99.4	99.3
	20	19.86 ± 0.3 (0.54)	19.84 ± 0.129 (0.65)	99.3	99.2	19.82 ± 0.19 (0.98)	19.88 ± 0.169 (0.85)	99.2	99.4
	40	38.9 ± 0.31 (0.81)	39.93 ± 0.312 (0.78)	97.25	99.83	39.5 ± 0.47 (1.20)	39.58 ± 0.36 (0.91)	98.75	98.95
BRM	10	9.98 ± 0.05 (0.51)	9.97 ± 0.042 (0.42)	99.8	99.7	9.93 ± 0.09 (0.98)	9.96 ± 0.079 (0.79)	99.3	99.6
	20	19.84 ± 0.10 (0.52)	19.91 ± 0.137 (0.69)	99.2	99.55	19.78 ± 0.21 (1.10)	19.96 ± 0.122 (0.61)	98.9	99.8
	40	39.4 ± 0.26 (0.67)	39.63 ± 0.289 (0.73)	98.5	99.08	39.2 ± 0.31 (0.81)	39.73 ± 0.366 (0.92)	98.0	99.33

Table 3: Precision studies of timolol maleate and brimonidine tartrate.

*: Mean of three replicates.

Drugs	Spiked Conc (µg/ml)	Total Conc. (µg/ml)	Method A			Method B		
			Conc. found (µg/ml)	%*Recovery	%RSD	Conc. found (µg/ml)	%RSD	%*Recovery
TML	8(80%)	18	17.94	99.66	0.92	17.77	0.92	98.72
	10 (100%)	20	19.84	99.19	1.07	19.80	1.01	99.01
	12 120%)	22	21.68	98.54	0.91	21.83	0.84	99.21
BRM	8 (80%)	18	17.80	98.89	1.16	17.86	1.04	99.20
	10 (100%)	20	19.72	98.61	0.98	19.92	1.25	99.59
	12 (120%)	22	21.87	99.42	0.88	21.58	0.97	98.10

Table 4: Accuracy studies of timolol maleate and brimonidine tartrate.

*: Mean of three replicates.

Brand	Drug	Label claim (mg)	*Amount found (mg)		*% Recovery	
			Method A	Method B	Method A	Method B
Brand I	Timolol	5	4.93	4.95	98.6	99.0
	Brimonidine tartrate	2	1.98	1.96	99.0	98.0
Brand II	Timolol	5	4.96	4.92	99.2	98.4
	Brimonidine tartrate	2	1.97	1.93	98.5	96.5

Table 5: Assay of timolol maleate and brimonidine tartrate.

*: Mean of three replicates.

Bibliography

1. Budavari S. "The Merck Index, An Encyclopedia of chemicals, drugs and biologicals". 14th edition, Whitehouse Station, NJ: Merck Research Laboratories Division of Merck and Co., Inc, Monograph number 9444 (2006): 1623.

2. Budavari S. "The Merck Index, An Encyclopedia of chemicals, drugs and biologicals". 14th edition, Whitehouse Station, NJ: Merck Research Laboratories Division of Merck and Co., Inc.; 2006, Monograph number 1375 (2006): 225 (Brimonidine tartrate).

3. Toris C., *et al*. "Acute versus chronic effects of Brimonidine tartrate on aqueous humor dynamics in ocular hypertensive patients". *American Journal of Ophthalmology* 128.1 (1999): 8-14.

4. Suketa KM and Dilip GM. "Analytical method development and validation for simultaneous estimation of Timolol Maleate and Brimonidine tartrate in bulk and marketed ophthalmic formulation". *Journal of Pharmaceutical Science and Bioscientific Research* 4 (2014): 351-356.

5. Arun P., *et al*. "Simultaneous estimation of Brimonidine tartrate and Timolol in nanoparticles formulation by RP-HPLC". *International Journal of Recent Advances in Pharmaceutical Research* 3 (2011): 31-36.

6. Abdullah AE., *et al*. "Development and validation of HPLC method for simultaneous estimation of Brimonidine tartrate and Timolol in bulk and pharmaceutical dosage form". *Journal Chromatograph Separation Technique* 5.3 (2014).

7. Mukthinuthalapati Mathrusri Annapurna., *et al*. "Multi component mode and derivative spectrophotometric methods for the simultaneous determination of Timolol Maleate and Brimonidine tartrate". *Asian Journal of Pharmaceutics* 12.1 (2018): S251-S255.

8. Vinayaka DG., *et al*. "Simultaneous determination of Brimonidine tartrate and Timolol in combined pharmaceutical dosage form using two different green spectrophotometric methods". *Journal of Harmonized Research in Pharmacy* 4.1 (2015): 52-59.

9. Mukthinuthalapati Mathrusri Annapurna., *et al*. "Simultaneous determination of Brimonidine tartrate and Timolol Maleate by first derivative and ratio derivative spectroscopy". *Journal of Analytical and Pharmaceutical Research* 4.6 (2017): 00120.

10. Hiral SP and Hemant MP. "Simultaneous determination of Brimonidine tartrate and Timolol in combined pharmaceutical dosage form using two different green spectrophotometric methods". *World Journal of Pharmacy and Pharmaceutical Sciences* 3.3 (2014): 1330-1340.

11. Mukthinuthalapati Mathrusri Annapurna., *et al*. "New spectrophotometric methods for the simultaneous determination of Brimonidine tartrate and Timolol in Eye drops". *Journal of Chemical and Pharmaceutical Sciences* 10.2 (2017): 786-789.

12. Heta HD and Anandkumari DC. Three simple validated UV spectrophotometric methods for the simultaneous estimation of Timolol and Brimonidine tartrate and their comparison using ANOVA". *International Journal of Pharmaceutical Research and Analysis* 4.3 (2014): 168-177.

13. ICH Validation of analytical procedures: Text and methodology, Q2 (R1), International Conference on Harmonization (2005).