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DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF BRIMONIDINE TARTRATE & TIMOLOL MALEATE IN COMBINATION

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ABSTRACT

The objective of present study was to develop simple, accurate, precise, cost effective and reliable analytical method for estimation of Brimonidine Tartrate & Timolol Maleate in combination. The method of analysis is derivative spectroscopy to eliminate spectral interference by measuring absorbance of Brimonidine Tartrate & Timolol Maleateat 251.5 & 228 nm for first derivative spectroscopy mode respectively. The results of analysis were validated as per ICH guidelines. The results of recovery studies and precision were found to be within limits.

KEYWORDS: Brimonidine Tartrate& Timolol Maleate, First Derivative Method.

INTRODUCTION

Brimonidine Tartrate is chemically [5- bromo-6(2-imidazolidinylideneamino) quinoxaline L-tartrate]&Timolol Maleate [(S)-1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morphonlinyl)-1,2,5-thiadiazol -3-yl]oxy]-2-propanol,(Z)-2-butenedioate].[1] Timolol Maleate and Brimonidine Tartrate are used separately and in combination for the treatment of Glaucoma.[2] Timolol Maleate blocks both β-1 and β-2 adrenergic receptors, reduces intraocular pressure by reducing aqueous humor production or possibly outflow; reduces blood pressure by blocking adrenergic receptors and decreasing sympathetic outflow, produces a negative chronotropic and inotropic activity through an unknown mechanism. Brimonidine Tartrate is an alpha adrenergic receptor agonist. It has a peak ocular hypotensive effect occurring at two hours post-dosing. Fluorophotometric studies in animals and humans suggest that brimonidine

tartrate has a dual mechanism of action by reducing aqueous humor production and increasing uveoscleral outflow. Number of chromatographic methods like HPTLC[3] and HPLC[4,5,6] methods are reported. Literature survey reveals that spectrophotometric methods like Simultaneous Equation Method[7], Absorbance Ratio Method[8] & Area Under Curve[9] are reported. But no first order derivative spectroscopy method was reported for estimation of Brimonidine Tartrate &Timolol Maleate.

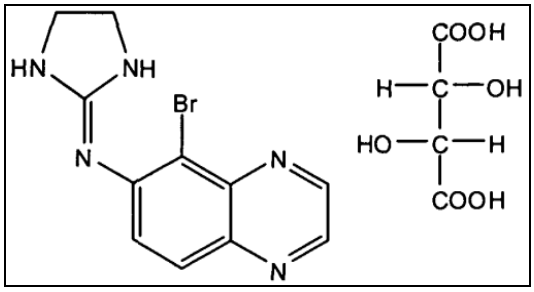

Fig.1 Structure of Brimonidine Tartrate

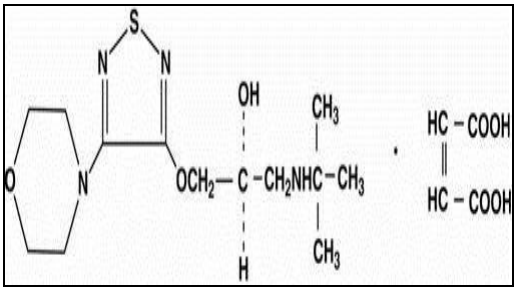

Fig.2 Structure of Timolol Maleate

MATERIAL AND METHODS

Instruments

Double beam UV- Vis spectrophotometer (JASCO V -730) with matched pair of 1cm quartz cells was used to record spectra of all solutions. The spectra were recorded at spectral band width of 1.0 nm, scanning speed 100 nm/min and data pitch of 0.5 nm. All weighing were done on electronic analytical balance (Shimadzu AY 120).

Chemicals and Reagents

TimololMaleate and BrimonidineTartrate working standards were obtained from Micro Labs pvt. Ltd., India., Distilled water.

Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving, accurately measured 10mg of Brimonidine Tartrate &Timolol Maleate separately in water and the volume was made up to 10 ml (stock solution 1000µg/ml).

Determination of Absorbance Maxima

1ml of stock solutions of Brimonidine Tartrate &Timolol Maleate was diluted to 10 ml to get 100 µg/ml solutions. The absorbance of resulting solution was measured against respective blank solution (distilled water) in the UV region of 200-400 nm. Zero order shows absorbance at 247 nm and first order shows absorbance at 251.5 nm for Brimonidine Tartrate.

Similarly zero order shows absorbance at 295 nm and first order shows absorbance at 228 nm for Timolol Maleate.

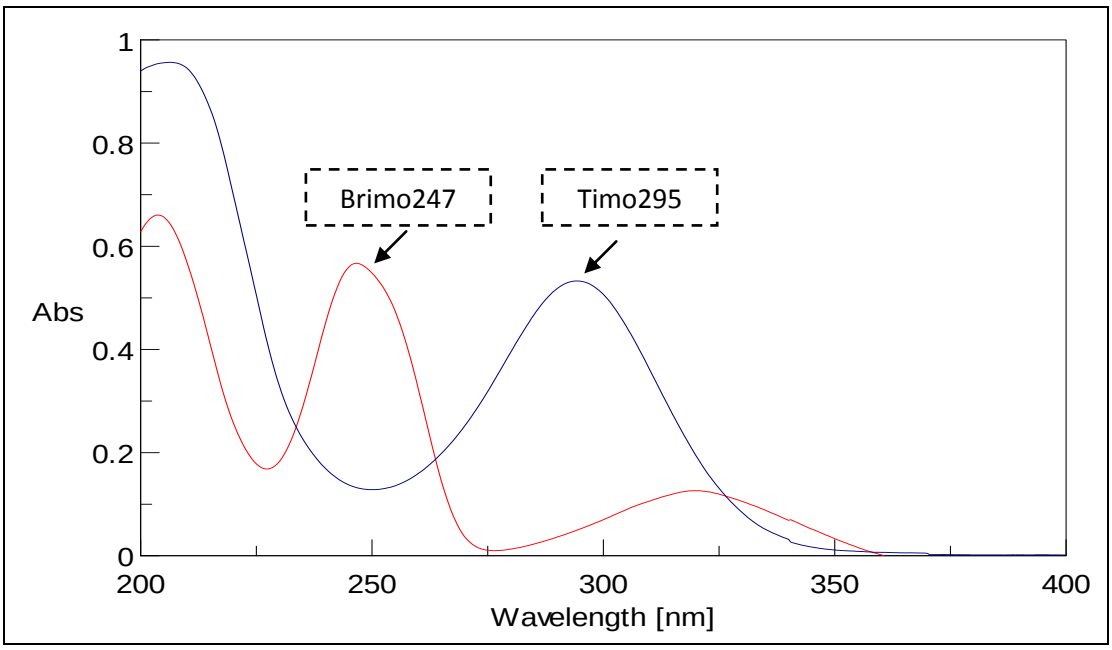

Fig 3: Overlay Zero order spectrum of Brimonidine Tartrate &Timolol Maleate.

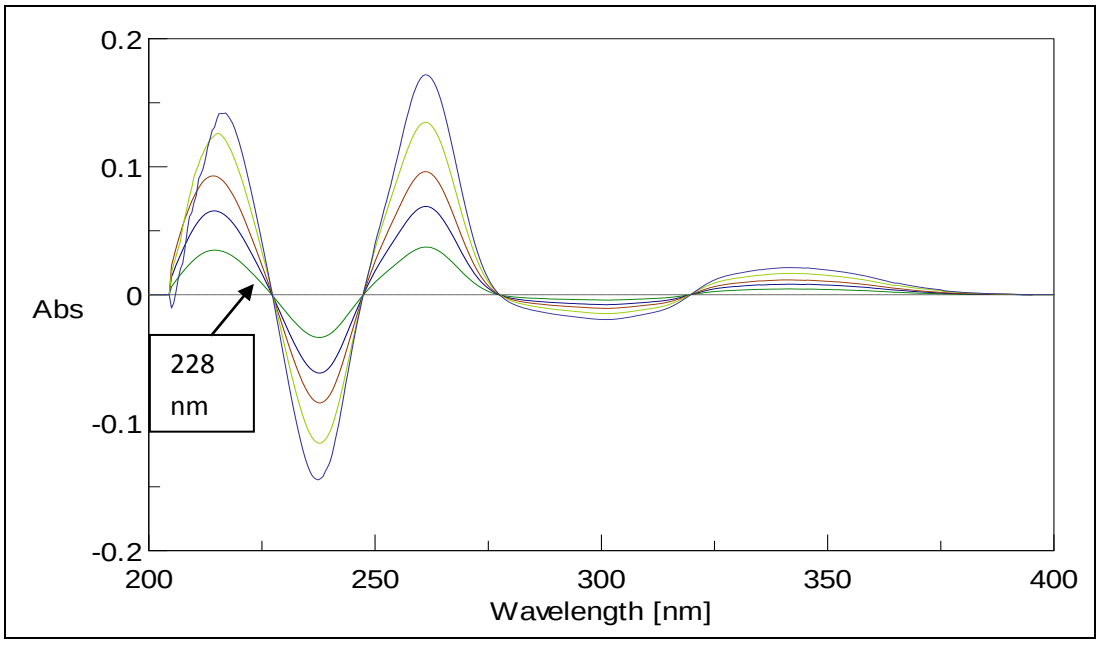

Fig 4: Overlay First order spectrum of Brimonidine Tartrate.

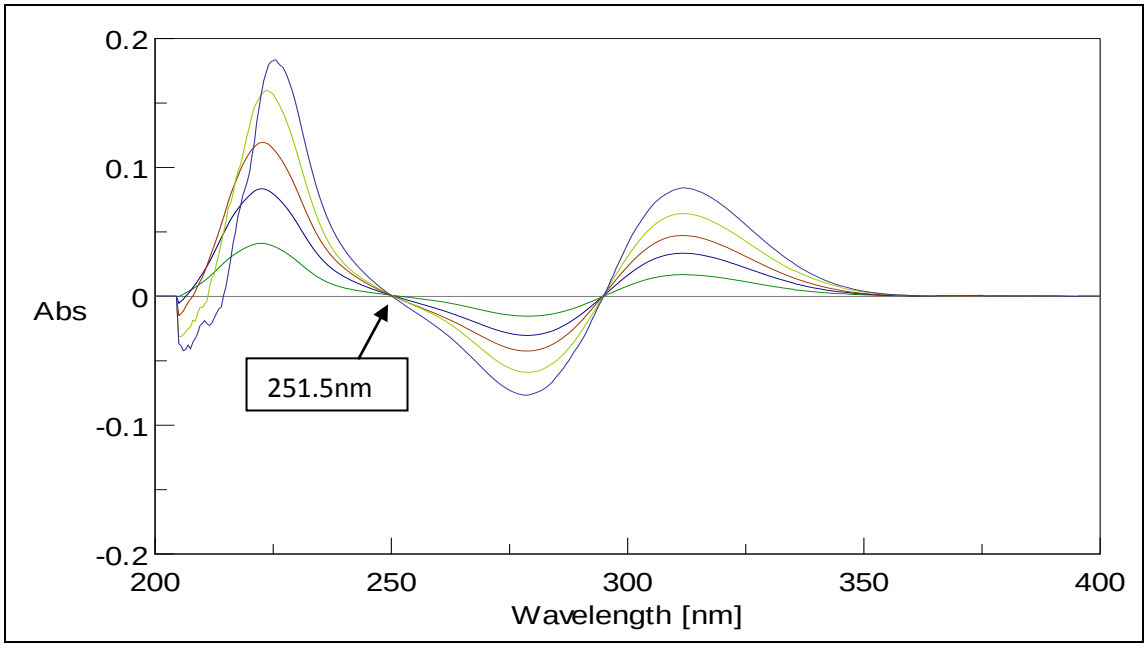

Fig 5: Overlay First order spectrum of Timolol Maleate.

Determination of concentration range

For preparation of different concentrations, aliquots of stock solution of suitable concentrations of Brimonidine Tartrate & Timolol Maleate were transferred into a series of 10 ml volumetric flasks and volumes were made up to mark with distilled water. Five different concentrations were prepared in the range of 10-50µg/ml and the absorbances were measured at 251.5 nm in first derivative mode for Brimonidine Tartrate against solvent blank. Similarly five different concentrations were prepared in the range of 25-125µg/ml and the absorbances were measured at 228 nm in first derivative mode for Timolol Maleate against solvent blank. The obtained absorbance values are plotted against the concentrations to get the calibration graph.

METHOD VALIDATION[10]

Linearity& Range

Five different concentrations of Brimonidine Tartrate (10- 50 µg/ml) &Timolol Maleate (25 – 125 µg/ml) were prepared from standard stock solution of 1000 µg/ml of both the drugs and was analysed in first derivative mode. Linearity equation of Brimonidine Tartrate was found to be y = 0.058x -0.0025, r^2= 0.999 & ofTimolol Maleate was found to be y = 0.020x + 0.093 r^2 = 0.989 respectively.

Specificity

Results of solution showed that there is no interference of excipients when compared with the working standard solution. Thus, the method is said to be specific.

Precision

The precision was evaluated with respect to both repeatability and intermediate precision. Repeatability was evaluated by taking absorbance of five replicate samples of test solution of the drugs Brimonidine Tartrate (10 µg/ml) & Timolol Maleate (25 µg/ml). The studies were repeated for three different days to determine intermediate precision. Peak areas of the drugs were determined and % RSD was calculated.

Table 1: Interday Precision for Brimonidine Tartrate & Timolol Maleate.

Sr. No.	Drug	Conc(ug/ml)	Interday	Mean	SD	% RSD
1.	**Brimonidine Tartrate**	10 ug/ml	251.5	0.012612	0.000127	1.00
2.	**Timolol Maleate**	25 ug/ml	228	0.036848	0.000103	0.28

Table 2: Intraday Precision for Brimonidine Tartrate &Timolol Maleate.

Sr. No.	Drug	Conc(ug/ml)	Intraday	Mean	SD	% RSD
1.	**Brimonidine Tartrate**	10 ug/ml	251.5	0.012938	0.000115	0.88
2.	**Timolol Maleate**	25 ug/ml	228	0.000487	0.000118	1.24

Accuracy

The accuracy of the method was assessed by the recovery studies at three different concentrations by the addition of known amount of standard to the test solutions. The drugs were spiked at three different levels i.e., 80 %, 100% & 120 %. The recovery was calculated by slope and intercept of the linearity plot of drugs. The results obtained for accuracy are presented in the table.

Table 3: Accuracy for Brimonidine Tartrate &Timolol Maleate.

Drug	Level % of Accuracy	% Recovery
Brimonidine Tartrate	80	98.23
	100	99.08
	120	97.45
Timolol Maleate	80	97.56
	100	96.23
	120	98.52

Limit of detection (LOD) and Limit of Quantitation (LOQ)

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S where, σ = standard deviation of the y-intercept of linearity equations and S = slope of the calibration curve of the analyte.

Table 4: LOD & LOQ for Brimonidine Tartrate & Timolol Maleate.

Drug	LOD	LOQ
Brimonidine Tartrate	0.33 µg/ml	1.00 µg/ml
Timolol Maleate	0.13 µg/ml	0.40 µg/ml

Robustness

Robustness was evaluated by deliberate variation in parameter like wavelength. Robustness was studied at a concentration of 10µg/ml for Brimonidine Tartrate & 25µg/ml for Timolol Maleate. The results were found to be unaffected by small changes like 0.5 nm in wavelength.

CONCLUSION

Estimation of Brimonidine Tartrate & Timolol Maleate was achieved by UV method. Calibration curve was plotted by using concentration Vs absorbance in the first order derivative mode. From the calibration curve it was found that both the drugs obey beer's law & the precision of the method was studied by making repeated analysis. The recovery studies were also carried out to ensure the accuracy of the method by adding known concentration of pure drug to a preanalysed formulation. All the above parameters combined with simplicity and ease of operation ensures that the application of proposed method for estimation Brimonidine Tartrate & Timolol Maleate was found to be useful with high accuracy, precision. It can be used for routine analysis & in bulk and pharmaceutical preparation.

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