

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF BRINZOLAMIDE AND BRIMONIDINE TARTRATE

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Abstract

Two simple, accurate, sensitive and economical UV Spectrophotometric methods have been developed and subsequently validated for the simultaneous determination of Brinzolamide and Brimonidine tartrate. Method (A) is second derivative zero crossing UV spectroscopy, method (B) is divisor ratio first derivative spectroscopy. Linearity range for both methods were 20-40 µg/ml and 4-8 µg/ml for brinzolamide and brimonidine tartrate respectively. For method A values of limit of detection (LOD) were 0.8145µg/ml, and 0.9059 µg/ml and the values of limit of quantitation (LOQ) were 2.449 µg/ml and 2.745 µg/ml for brinzolamide and brimonidine tartrate respectively. For method B, LOD values were 0.4935 µg/ml and 1.2131 µg/ml and LOQ values were 1.4805µg/ml and 3.3639µg/ml for brinzolamide and brimonidine tartrate, respectively. The precision values were less than 2% RSD and Accuracy was found to be 98%-102%.

Keywords: Brinzolamide, Brimonidine tartrate, second derivative zero crossing, divisor ratio first derivative spectroscopy

Introduction:

Brinzolamide (BRZ), [(R)-(+)-4-Ethylamino-2-(3-methoxypropyl)-3, 4-dihydro-2H Thieno [3,2-e]-1,2-thiazine-6-sulfonamide-1,1-dioxide], (fig. 1) is a new active substance which is useful only for topical use in the treatment of glaucoma. Brimonidine tartrate (BT) is (5-bromo-6- (2-imidazolidinylideneamino) quinoxaline L-tartrate) (fig. 2) is a α_2 -adrenoreceptor agonist. It is used to lower intra-ocular pressure in patients with open-angle glaucoma or ocular hypertension. The IOP lowering efficacy of Brimonidine tartrate ophthalmic solution diminishes over time in some patients. This loss of effect appears with a variable time of onset each patient and should be closely monitored. BRZ is official in USP. USP describe its chromatographic estimation [1]. There are UV [2], and HPLC [3] method available for simultaneous estimation of BRZ and timolol. Several analytical methods based on UV [4], RP-HPLC [5], HPTLC [6], LC/MS/MS [7] and stability indicating method by UPLC [8] was reported for the determination of BT alone. RP-HPLC [9] and TLC-densitometry [10] methods are reported in literature for estimation of BT and timolol melete. The absence of literature provides the need for developing a sensitive, new, economical, precise and accurate method for the simultaneous estimation of BRZ and BT from combined ophthalmic preparation.

Materials and Method

Instrument

A dual beam UV-Visible spectrophotometer (Shimadzu, Japan), model UV-1700 having two matched quartz cells of 1 cm light path and UV probe software (Shimadzu version 2.34).

Materials

Brimonidine (BRZ) was procured from Swapnroop Drug Pvt.Ltd. Aurangabad, Maharashtra, India. Brimonidine tartrate (BT) was obtained as a gift sample from Alembic Pharmaceutical Ltd., Vadodara, Gujarat, India. All reagents used in experiment were analytical grade and distilled water was prepared in laboratory.

Preparation of standard solutions

Stock solutions of 1000 µg/ml of BRZ and BT were prepared by dissolving accurately Weighed quantity of 10 mg and 15.15 mg in to 10 ml volumetric flasks, dissolved and Diluted up to mark with water: methanol (50:50) respectively. Further dilution was performed with water: methanol (50:50) to prepare working standard solutions containing 100 µg/ml of BRZ and BT each.

Preparation of sample solution

Laboratory prepared suspension which contains 1% BRZ and 0.2% BT, from that 10 ml of suspension were taken into a dried 100 ml volumetric flask. About 70 ml of water: methanol (50:50) was added to it and sonicated for 10 minutes. Cooled to room temperature and diluted to volume with the same and mix well. This solution was filtered through Whatman filter paper.

Methods

Method A: Zero Crossing Second Derivative Spectrophotometry: The solutions of standard BRZ and BT were prepared in the range of 20-40 µg/ml and 4-8 µg/ml respectively. The absorption spectra of the solutions of BRZ and BT were recorded in the range of 200 nm to 400 nm and transformed to second derivative with $\Delta\lambda = 8\text{nm}$ and scaling factor 100.

Method B: Ratio Derivative Spectrophotometry:

The solutions of standard BRZ and BT were prepared in the range of 20-40 µg/ml and 4-8 µg/ml respectively. The absorption spectra of the solutions of BRZ and BT were recorded in the range of 200 nm to 400. The spectra of BRZ and BT were divided by one standard spectrum of 6µg/ml of BT and 30µg/ml of BRZ respectively to give ratio spectra. These ratio spectra were then derivatised to first order. Ratio derivative spectra are free from interference by divisor drug.

Validation

Developed methods were validated in accordance with ICH guidelines [11] for evaluation of various parameters; linearity, limit of detection, limit of quantification, precision, accuracy.

Linearity

From working standard solution of BRZ and BT concentration range of 20-40 µg/ml and 4-8 µg/ml were prepared respectively. BRZ and BT shows linear absorbance in concentration range of 20-40 µg/ml and 4-8 µg/ml respectively for both methods

Precision

Precision of the developed methods was studied by performing intra-day and inter-day precision studies. The intra-day precision was determined by performing three measurements of different concentration on the same day at different time interval. The inter-day precision of method was checked by repeating the study on three consecutive days and % R.S.D. was calculated.

Accuracy

Accuracy was determined by the standard addition method. This study was performed by addition of known amounts of BRZ and BT to a known concentration of laboratory prepared ophthalmic suspension. It was used for checking the interference from excipients used in the dosage forms.

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

The LOD and LOQ of the proposed methods were calculated from the standard deviation (σ) of the response and the Slope of the calibration curve (S) in accordance to the equations:

$$\text{LOD} = 3.3 \times \sigma/S \text{ and}$$

$$\text{LOQ} = 10 \times \sigma/S.$$

Results and Discussion

Method A

In this method zero order spectra were converted to second order spectra. At 267.6 nm BRZ having zero crossing point and BT can be estimated. At 284.3 nm BT having zero crossing point and BRZ can be determined. (Fig. 3)

Method B

This method has various advantages of easy measurements on separate peaks, higher values of analytical signals, and no need to work at zero cross over point. The effect of divisor concentration on the analytical parameters such as slope, intercept and correlation coefficient was also tested. The chosen divisor concentration gave good results for the slope, intercept and correlation coefficient of calibration graphs as well as for selectivity. The ratio spectra of different BRZ obtained by dividing zero order spectra of standard solution of 6 $\mu\text{g/ml}$ of BT and then these ratio spectra were converted to first order spectra with the interval of $\Delta\lambda = 8 \text{ nm}$ and scaling factor 100. Similarly The ratio spectra of different BT obtained by dividing zero order spectra of standard solution of 30 $\mu\text{g/ml}$ of BRZ and then these ratio spectra were converted to first order spectra with the interval of $\Delta\lambda = 8 \text{ nm}$ and scaling factor 100. Division and derivatization were done by UV probe (version 2.34 shimadzu) software. (fig. 4)

Validation of method

Summary of validation parameter given in table 1 and table 2

Conclusion

From the obtained results, proposed methods are simple, rapid, sensitive, accurate, precise and does not require any prior separation procedure of BRZ and BT. Hence, the proposed method could be regarded as economically viable techniques in the routine quality control analysis of BRZ and BT either alone or in combination with a relatively inexpensive instrumentation. Above data shows there were no interference of excipients.

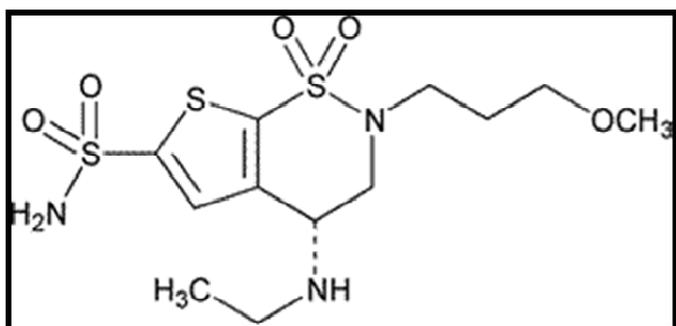


Fig. 1 BRINZOLAMIDE

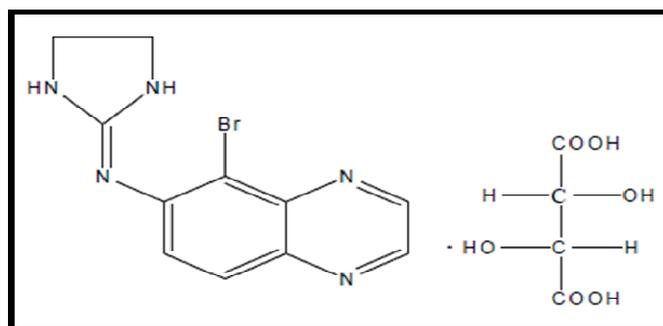


Fig. 2 BRIMONIDINE TARTRATE

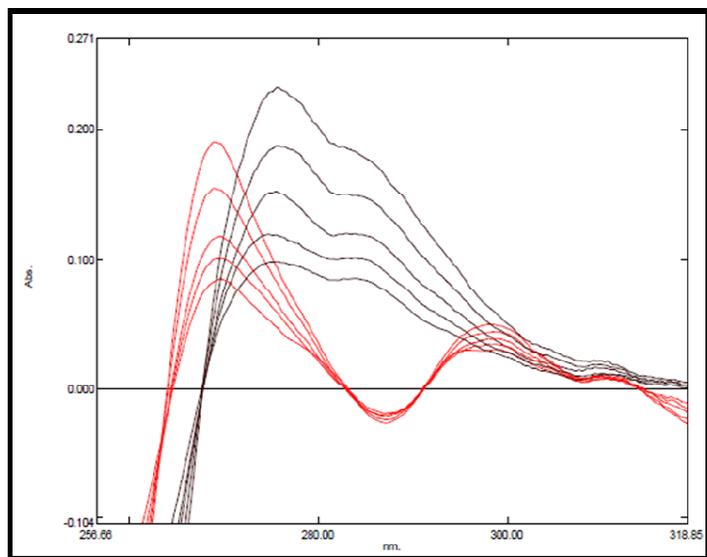


Fig. 3 Zero crossing second derivative spectrum

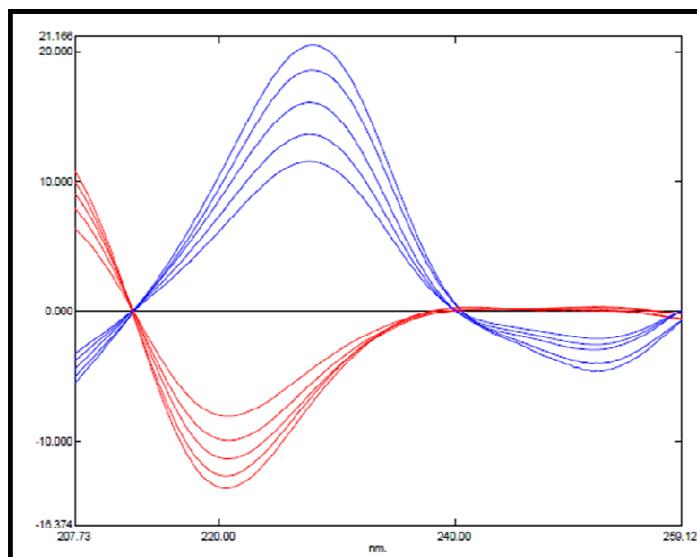


Fig. 4 Divisor ratio first derivative spectrum

Table-1 Summary of validation parameter

Parameter	Method A		Method B	
	BRZ	BT	BRZ	BT
Analytical	284.3	267.6	227.7	220.7
Beer's range	20-40	4-8	20-40	4-8
Slope	0.012	0.032	0.525	0.875
Intercept	0.002	0.033	0.969	1.492
Correlation	0.9997	0.9976	0.9993	0.9978
Intraday	0.5643	1.2467	0.7862	0.8999
precision(% RSD)				
Interday	0.6928	1.3362	0.8442	1.0478
Precision(%RSD)				
LOD(µg/ml)	0.8165	0.9059	0.4935	1.2131
LOQ(µg/ml)	2.449	2.745	1.4805	3.3639

Table-2 Result of recovery study by developed method

Method	% Added	Conc. Actual (µg/ml)		% RECOVERY ± SD	
		BRZ	BT	BRZ	BT
A	50	15	3	98.933±0.208	98.667±0.451
	100	15	3	98.88±0.430	102.066±0.294
	150	15	3	99.146±0.929	98.377±0.648
B	50	15	3	100.25±0.357	100.72±0.139
	100	15	3	101.08±0.869	101.334±0.898
	150	15	3	99.428±0.327	98.911±0.726

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