

UV-Spectrophotometry - Multicomponent Mode of Analysis for Simultaneous Estimation of Brinzolamide and Brimonidine Tartrate in Bulk and Ophthalmic Formulation

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ABSTRACT

Brinzolamide (BRZ) and Brimonidine Tartrate (BT) in combination are available as an ophthalmic suspension in the ratio of 5:1. A simple, reproducible and efficient method for the simultaneous determination of BRZ and BT in Bulk and Ophthalmic formulation has been developed. The absorbance was assessed at two wavelengths i.e. 252.40 nm (λ max of BRZ) and 246 nm (λ max of BT) in methanol. In this method, BRZ and BT executed linearity in the concentration range of 5-35 μ g/mL and 3-18 μ g/mL, respectively at their respective λ max. The developed method was found to be accurate, precise and rugged as marked by small values of % RSD according to ICH guidelines.

1. Introduction

Brinzolamide (BRZ), chemically is (R)-4-(ethyl amino)-3,4-dihydro-2-(3-methoxypropyl)-2 H-thieno [3, 2-e]-1,2-thiazine-6-sulphonamide, 1-dioxane (**Figure 1a**). It is a recent active compound which is helpful only for topical usage in the management of glaucoma [1, 2]. **Brimonidine Tartrate (BT)**, 5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl) quinoxalin-6-amine; (2R,3R)-2,3-dihydroxybutanedioic acid (**Figure 1b**). It is α 2-adrenoreceptor agonists which lowers intra-ocular pressure (IOP) in patients with open-angle glaucoma or ocular hypertension (IP 2014, Merck index 2001). Literature review revealed few analytical methods

such as UV-Spectrophotometry [4, 6], HPLC [5, 7, 11], HPTLC [8, 6], LC-MS/MS [9] and Stability- Indicating UPLC [10] methods for measurement of BRZ and BT alone and in combination with other drugs. To our knowledge, in literature UV-Spectrophotometry- Multicomponent mode of analysis has not been reported so far for simultaneous determination BRZ and BT in pharmaceutical formulation. Therefore, the novelty of present work is to establish new [3, 12, 15], cost-effective, sensitive, precise and accurate method for the simultaneous estimation of BRZ and BT in combined ophthalmic dosage form by using multi-component mode of analysis [13, 15], 2018).

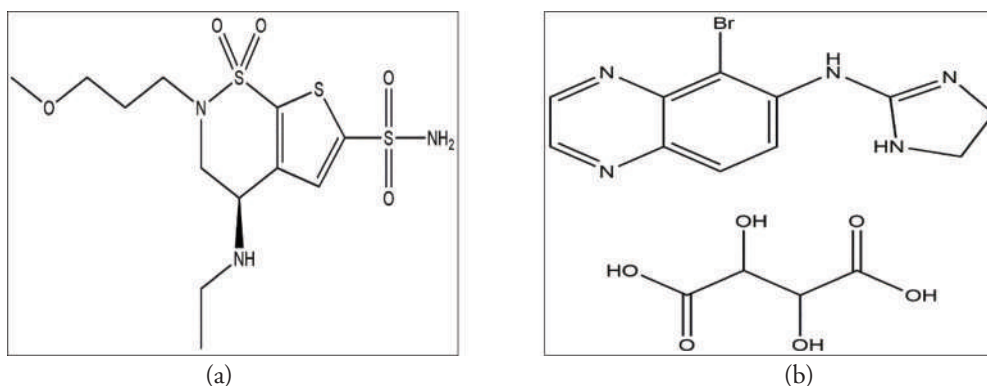


Figure 1. Chemical Structure of (a) Brinzolamide and (b) Brimonidine Tartrate

2. Materials and Methods

2.1 Chemicals and Reagents

Pure Brinzolamide (BRZ) and Brimonidine tartrate (BT) were procured as a gift samples from Amneal Pharmaceutical Ltd. Ahmadabad, India and Alembic Pharmaceutical Ltd., Vadodara, India. AR grade methanol was obtained from Merck Ltd., Worli, India.

2.2 Instrumentation

A double beam UV-VIS spectrophotometer (UV-1601, Shimadzu, Japan) with 10 mm quartz cells was used. Analytical balance (Shimadzu AUX 120) was used for weighing purpose.

2.3 Selection of Common Solvent

Methanol (AR) was selected as common solvent for developing spectral features of both the drugs. The selection of the solvent was made after assessing the solubility in different solvents.

2.4 Preparation of Stock Standard Solution and determination of λ_{max}

Stock standard solutions of BRZ and BT were prepared separately by dissolving 10 mg into 100 mL methanol to

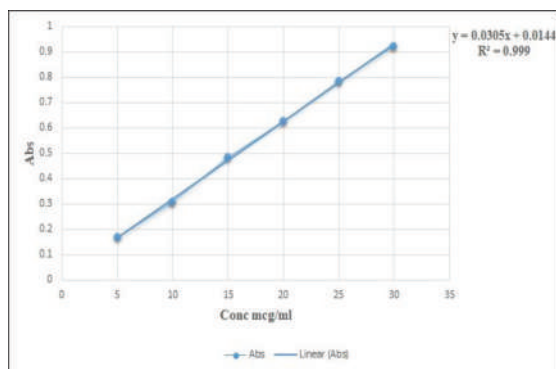
obtain concentrations 100 $\mu\text{g}/\text{mL}$ of each. From these stock solutions, working standard solutions having concentration 10 $\mu\text{g}/\text{mL}$ of BRZ and 10 $\mu\text{g}/\text{mL}$ of BT were prepared by proper dilutions. They were scanned in the UV- region i.e. 400 - 200 nm.

2.5 Study of Linearity Curves

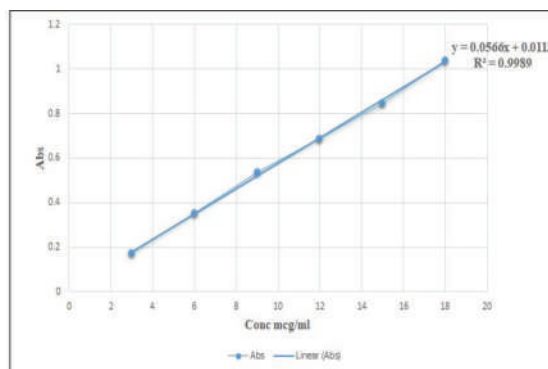
An appropriate volume of BRZ and BT in the range of 0.5 – 3.5 mL and 0.3 – 1.8 mL were transferred into series of separate 10 mL volumetric flasks and volume was made up to mark with methanol to get concentrations in the range of 05 – 35 $\mu\text{g}/\text{mL}$ and 03 – 18 $\mu\text{g}/\text{mL}$, respectively. The absorbance of BRZ and BT was measured at 252.40 nm and 246 nm, respectively. Calibration curves were plotted as concentrations versus absorbance, given in **Figure 2a (BRZ)** and **2b (BT)**.

2.6 Multicomponent Mode of Analysis

Six mixed standard solutions of BRZ and BT in the ratio of 1:5 were prepared in methanol as shown in **Table 1**. All the mixed standard solutions were scanned over the range of 400 - 200 nm, in the multicomponent mode, using two sampling wavelength 252.40 nm (λ_{max} of BRZ) and 246 nm (λ_{max} of BT). The overlain spectra are shown in **Figure 3**. The data from these scans were used to determine the concentrations of two drugs in ophthalmic solution.



(a)



(b)

Figure 2. (a) Calibration curve for BRZ (b) Calibration curve for BT

Table 1. Mixed Standards of BRZ and BT

Drugs	I	II	III	IV	V	VI
BRZ ($\mu\text{g}/\text{mL}$)	0	15	25	30	35	35
BT ($\mu\text{g}/\text{mL}$)	7	3	5	6	7	0

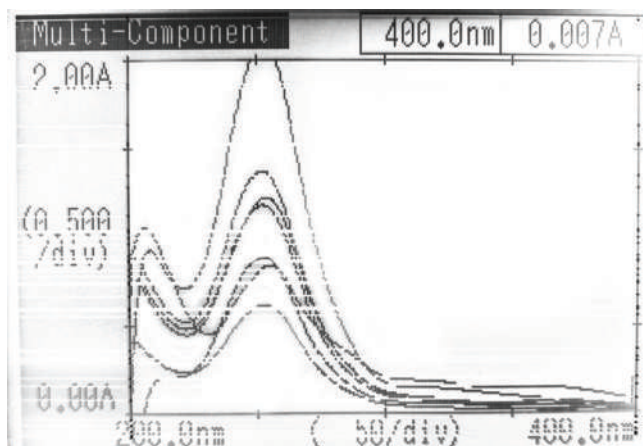


Figure 3. An overlain Spectra of Brinzolamide and Brimonidine Tartrate

2.7 Analysis of Marketed Formulation

For assay of marketed formulation, a volume of suspension equivalent to 10 mg of BRZ and 2 mg of BT accurately transferred into 100 mL volumetric flask containing 50 mL methanol, sonicated for 20 min and volume was made upto the mark with same solvent and filtered through Whatmann filter paper (no.41). Aliquot portion 1.0 mL was transferred into 10 mL volumetric flask and volume was adjusted to mark with the same solvent. The sample solution was scanned over the range 400 - 200 nm, in the multicomponent mode; using two sampling wavelength 252.40 (λ max of BRZ) and 246 nm (λ max of BT). The percent label claim was calculated and results are presented in **Table 2**.

Table 2. Analysis of Marketed Ophthalmic formulation

Drugs	Label claimed [mg]	% Amount found [n = 6]	\pm SD	% RSD
BRZ	10	100.58	1.13	1.12
BT	2	100.94	0.50	0.49

SD-Standard Deviation

% RSD-Relative standard deviation

n- Number of determination (6)

3. Validation of Method

The method was validated in terms of accuracy, precision, repeatability and ruggedness as per the ICH guidelines [13].

3.1 Accuracy/Recovery Studies

The accuracy was demonstrated at three different levels. To the pre-analyzed sample solution of 15 μ g/mL of BRZ and 3 μ g/mL of BT, a known quantity of drug standards of BRZ and BT were added at 80, 100 and 120 % level and re-analysed using proposed method. The analysis was repeated for three times at each level.

3.2 Precision (Intra-day and Inter-day Variations)

Intra-day precision was determined by analyzing the 20, 25 and 30 μ g/mL of BRZ and 4, 5, and 6 μ g/mL of BT solutions for three times in the same day and Inter-day precision was determined by analyzing 20, 25 and 30 μ g/mL of BRZ and 4, 5 and 6 μ g/mL of BT of drug solutions daily for three consecutive days.

3.3 Repeatability Studies

Repeatability was determined by analyzing 15 μ g/mL of BRZ and 3 μ g/mL of BT for six times.

3.4 Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions.

3.5 Sensitivity

Sensitivity of the proposed method was estimated in terms of Detection Limit (DL) and Quantitation Limit (QL). The DL and QL were calculated by the use of the equation,

$$DL = 3.3 \times \frac{ASD}{S} \text{ and } QL = 10 \times \frac{ASD}{S}$$

where, 'ASD' is Average standard deviation of absorbance of the drug (n = 3), taken as a measure of noise, and 'S' is the slope of the corresponding calibration curve.

4. Results and Discussion

BRZ and BT in combination are available as an ophthalmic suspension in the ratio of 5:1. A simple, reproducible and efficient method for the simultaneous determination of BRZ and BT in Bulk and Ophthalmic formulation has been developed. The measurement of absorbance at two wavelengths i.e. 252.40 nm, λ max of BRZ and 246.0 nm, λ max of BT in methanol were performed. In this method BRZ and BT followed linearity in the concentration range of 5-35 μ g/mL

for BRZ and 3-18 µg/mL for BT at their respective λ max. The proposed method was applied for pharmaceutical formulation. The % label claim for BRZ and BT was found to be 100.58 % and 100.94 %, respectively. The amount of drug estimated by method was found to be in good agreement with the label claim. The method was validated for accuracy, precision and ruggedness. Accuracy of the method was checked by recovery studies at three different levels i.e. 80 %, 100 % and 120 %. The % recovery of BRZ and BT was found to be 98.36 – 99.11% and 99.82 – 100.0 %, respectively; the % RSD values less than 2 indicative of accuracy of the method. The method was found to be precise as indicated by the inter-day, intra-day and repeatability analysis; showing % RSD less than 2. The results did not show any statistical difference between operators suggesting that method developed was rugged. The summary of validation parameters is presented in **Table 3**.

Table 3. Validation parameters of BRZ and BT by proposed methods

Parameter	BRZ	BT
Linearity		
Linearity range (µg/mL)	5-35	3-18
Linearity equation	$Y = 0.0305X + 0.0144$	$Y = 0.0035X + 0.0014$
Correlation coefficient (r^2)	0.9959	0.9990
% Recovery		
% RSD	0.25	0.57
Precision [% RSD]		
Intra-day [n=3]	0.82	0.41
Inter-day [n=3]	1.12	1.164
Repeatability [n=6]	0.27	0.74
Ruggedness [% RSD]		
Analyst-I	0.25	0.34
Analyst-II	0.54	0.42
Sensitivity		
DL	0.12	0.22
QL	0.38	0.65

% RSD-Relative standard deviation
DL-Detection Limit
QL-Quantitation Limit

5. Conclusion

The developed method is simple, economic, accurate and precise and can be used for routine simultaneous analysis of BRZ and BT from its pharmaceutical formulation.

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7. Conflict of Interest

The authors declare no potential conflict of interest.

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Abbreviations

- BRZ-Brinzolamide
- BT-Brimonidine Tartrate
- UV- Ultra Violet
- VIS- Visible
- µg/mL- Micro Gram/ Milliliter
- DL-Detection Limit
- QL-Quantification Limit
- ASD-Average Standard Deviation
- % RSD-Percentage Relative Standard Deviation
- SD-Standard Deviation
- ICH-International Council on Harmonization