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Research Article

DEVELOPMENT AND VALIDATION OF SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF BRINZOLAMIDE

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ABSTRACT

Introduction: Brinzolamide (BRZ), one of the carbonic anhydrase inhibitors, is effective in reducing intraocular pressure for glaucoma therapy mainly through decreasing the production of the aqueous humor. There are few literatures giving the insight on simultaneous estimation of brinzolamide with brimonidine but standard independent UV spectrophotometric method of estimation of brinzolamide has not been reported till time. Aim: This research was focused to develop a suitable method of validation of BRZ and acquire consistent, reliable and accurate data on linearity, detection limit, quantification limit, accuracy and precision using a UV spectrophotometer. Methodology: The UV spectrum of Brinzolamide was recorded by scanning 10 µg/ml concentration of brinzolamide prepared in ethanol within the wavelength region of 400-200 nm against ethanol as blank. Study of accuracy, precision and limit was carried for the validation of process. Result and Discussion: The absorbance maxima was recorded and found to be 254 nm. Initially, the linearity of the method was performed in a range of 4-12 µg/ml and the method showed good linearity with regression of $y = 0.0231x – 0.016$ ($R^2 = 0.9992$), where x and y were Brinzolamide concentration and UV absorbance at 254 nm respectively. Intraday and interday precision revealed % relative standard deviation of 0.664% and 0.618% respectively. Method accuracy showed average % accuracy value of 101.12%. Limit of detection and limit of quantification were 0.334 µg/ml and 1.013 µg/ml respectively. Conclusion: The proposed method is simple and could be regarded as an economically viable technique in the routine quality control analysis of BRZ.

Keywords: Brinzolamide, validation, absorbance, UV spectrum.

INTRODUCTION

Glaucoma is the term used for a group of ophthalmic disorders characterized by an increase in intraocular pressure (IOP), which results in damage to the optic disc and visual field disturbances leading to an imbalance between the production and drainage of aqueous humor[1]. Brinzolamide (BRZ), one of the carbonic anhydrase inhibitors, is effective in reducing IOP for glaucoma therapy mainly through decreasing the production of the aqueous humor[2]. Brinzolamide (BRZ), chemically *(R)* – 4- (ethylamino)-3,4-dihydro-2-(3-methoxypropyl)-2H thienol (3,2-e) -1,2-thiazine-6-sulphonamide 1,1-dioxide, (Figure 1) is a highly specific, non-competitive reversible carbonic anhydrase inhibitor, which is indicated for patients with ocular hypertension or open-angle glaucoma, a condition associated with an elevated intraocular pressure in the eye. Inhibition of carbonic anhydrase in the ciliary processes of the eye decreases aqueous humor secretion, presumably by slowing the formation of bicarbonate ions with subsequent reduction in sodium and fluid transport resulting in the reduction of intraocular pressure[3].

Validation is an analytical procedure providing the performance aspect of the procedure meets the necessary requirements prerequisite for the deliberate analytical practice. The results from method validation can be used to determine the quality, reliability and consistency of analytical results[4]. Literature survey reveals that few methods such as UV spectrophotometry, HPLC and HPTLC are reported for simultaneous estimation of brinzolamide with brimonidine[3,5]. Also, UV derivative spectrophotometric methods have been reported for the determination of timolol and brinzolamide[6,7]. But no UV spectrophotometric method for single estimation of brinzolamide has been reported till time. So there is an instant need of a standard method which fulfills all requirements of validation according to ICH guidelines. The present study involves development and validation for a simple, sensitive, accurate and precise UV method for determination of brinzolamide alone.

MATERIALS AND METHODS

Material

The standard drug Brinzolamide was purchased from Sigma-Aldrich Corporation (USA) and rest solvents of analytical grade were purchased from Merck & Co. (USA).

Instrumentation

Thermo Fischer Evolution 121 (Thermo Fischer Scientific, USA) was used for the development of an analytical method in the wavelength range of 200-400 nm.

Selection of Analytical wavelength

In order to ascertain the wavelength of maximum absorption (λmax) of BRZ, a stock solution of 100 mg/ml was prepared by taking 10 mg of drug in 100 ml of ethanol. Different solution of drugs (1 mg/ml, 10 mg/ml and 100 mg/ml) in ethanol were scanned using UV spectrophotometer (Thermo Fischer Evolution 121) within the wavelength region of 400 - 200 nm against ethanol as blank. The resulting spectra were shown as in Figure 2

& absorption curve showed characteristic absorption maxima at 254 nm for the drug.

Standard Preparation

Accurately weighed 1 mg of standard Brinzolamide was dissolved in 10 ml of ethanol (standard stock solution). From this standard stock solution, the aliquots of different concentration were prepared by suitable dilution varying in between 4-12 µg/ml using ethanol. The absorbance of subsequent concentration was measured at 254 nm as given in Table 1. Using the data, the calibration curve for the drug, BRZ was prepared as given in Figure 3. These diluted solutions were analyzed for linearity, precision, accuracy, limit of Detection (LOD) and limit of Quantification (LOQ).

METHOD VALIDATION

Linearity

Linearity is the capacity to obtain results directly proportional to the analyte concentration in a sample within a determined range[8]. Drug followed Beer-Lambert's law at concentration levels ranging between 4-12 µg/ml. The calibration curve is shown in Figure 3: regression coefficient (R^2) was found to be 0.999. The investigation was carried on three different days using UV-VIS spectrophotometry technique at 254 nm[9,10].

Precision

The precision of the developed methods was studied by performing intra-day and inter-day precision studies. The intraday precision was determined by performing measurements of 4µg/ml concentration of brinzolamide (BRZ) on the same day at different time interval. The inter-day precision of the method was checked by repeating the study on three consecutive days and the percentage relative standard deviation (%RSD) was calculated[9,11] as given in table 2 and table 3.

Accuracy

Accuracy is defined as the closeness of agreement between the actual (true) value and an analytical value obtained by applying a test method for a number of times. Brinzolamide solutions were prepared in ethanol with concentrations of 2, 7 and 15 µg/ml, which corresponds to the minimum, medium and maximum values of the previously determined standard calibration curve. The samples were analyzed and concentrations were recalculated from the calibration curve. Assays were performed in triplicate on two different days[9,12] as given in table 4.

Limit of detection (LOD)

Limit of Detection (LOD) is the lowest analyte concentration in a sample that can be calculated with precision and accuracy (ICH 2005). Limit of Detection (LOD) was based on the standard deviation of the response and the slope of the corresponding curve using the following equation:

$$LOD = \frac{3.3\ X\ \sigma}{S}$$

Where σ is the standard deviation of the absorbance of the sample (0.0023) and S is the slope (0.023) of the standard curve[10]. Thus, LOD was calculated to be 0.334 µg/ml.

Limit of quantification (LOQ)

Limit of Quantification (LOQ) is the lowest analyte concentration in a sample which can be quantitatively determined with suitable precision and accuracy (ICH 2005). Limit of Quantification (LOQ) was based on the standard deviation of the response and the slope of the corresponding curve using the following equation:

$$LOQ = \frac{10\ X\ \sigma}{S}$$

Where σ is the standard deviation of the absorbance of the sample (0.0023) and S is the slope (0.023) of the standard curve[10]. Thus, LOQ was calculated to be 1.013 µg/ml.

TABLE 1: CONCENTRATION VS ABSORBANCE TABLE FOR LINEARITY STUDY OF BRZ

Concentration (µg/ml)	Absorbance (λ_{max} = 254 nm)
4	0.078
6	0.119
8	0.169
10	0.216
12	0.260

TABLE 2: INTRA-DAY PRECISION

Time (hr)	Theoretical Conc. (µg/ml)	Abs	Conc. found (µg/ml)	Mean	Standard Deviation (SD)	% RSD (SD/MEAN X 100)
0	4	0.086	4.43	4.361	0.029	% RSD = 0.664
3	4	0.088	4.521			
6	4	0.079	4.13			

TABLE 3: INTER-DAY PRECISION

Time (day)	Theoretical Conc. (µg/ml)	Abs	Conc. found (µg/ml)	Mean	Standard Deviation (SD)	% RSD (SD/MEAN X 100)
Day 1	4	0.078	4.086	4.202	0.026	% RSD = 0.618
Day 2	4	0.079	4.13			
Day 3	4	0.085	4.391			

TABLE 4: ACCURACY

Concentrations (µg/ml)				Standard Deviation (SD)	% RSD (SD/MEAN X 100)	Accuracy
Initial	Day 1	Day 2	Mean			
2 µg/ml	2.028	2.071	2.049	0.0008	0.039	102.47±2.31
7 µg/ml	6.999	7.187	7.093	0.016	0.225	100.65±0.908
15 µg/ml	14.985	15.086	15.035	0.004	0.026	100.23±0.224

Average % Accuracy = (102.47 + 100.65 + 100.23)/3 = 101.12%

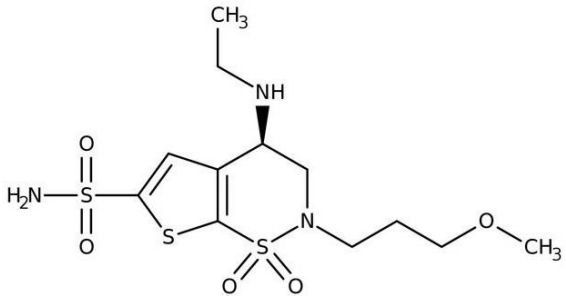

Figure 1: Structure of Brinzolamide (BRZ)

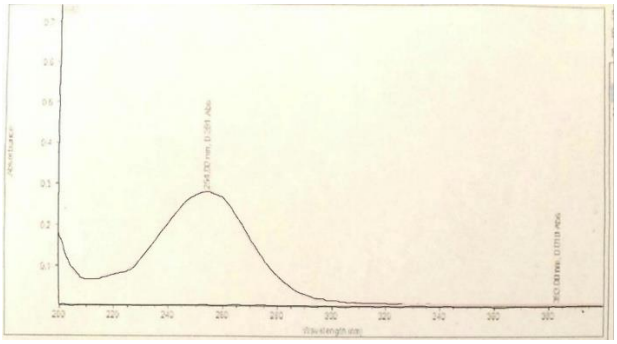

Figure 2: UV-spectrum of Brinzolamide in ethanol

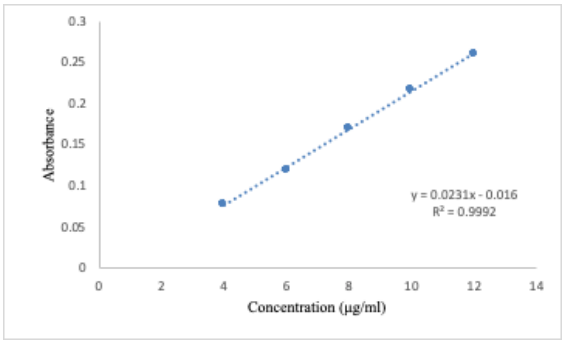

Figure 3: Calibration curve of Brinzolamide at 254nm

RESULT AND DISCUSSION

The Brinzolamide was found to be soluble in ethanol. The λmax of the drug was found to be 254 nm as shown in Figure 2. From the result obtained from Table 1, it was found that brinzolamide obeys linearity within the concentration range of 4-12 µg/ml and the coefficient correlation was found to be 0.9992. The regression of the curve was y = 0.0231x – 0.016 as shown in Figure 3 where x and y were Brinzolamide concentration and UV absorbance at 254 nm respectively. The detection and quantitation limits as LOD and LOQ were calculated and these were found to be 0.334 and 1.013 µg/ml respectively. The precision (measurements of intra-day and inter-day) results showed (Table 2 and 3) significant reproducibility with percent relative standard deviation (% RSD) is below 2.0. This indicated that the method is highly precise. The percent recovery value (Table 4), which was higher than 100%, indicates the accuracy of the method.

CONCLUSION

An Ultraviolet spectroscopy method for determination of the concentration of brinzolamide was validated as per ICH guidelines and it meets to specific acceptance criteria in respect of linearity, accuracy, sensitivity, intraday and inter-day precision. Hence, the proposed method is simple and could be regarded as economically viable techniques in the routine quality control analysis of BRZ.

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