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STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF BRINZOLAMIDE IN BULK DRUGS

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ABSTRACT

A stability-indicating isocratic RP-HPLC method was developed and validated for the determination of Brinzolamide in bulk drugs using Hemochrom C18 column (150mm×4.6 mm, 5µm) with mobile phase consisting of 1mM ammonium formate: acetonitrile (76:24 v/v) with a flow rate of 1mL/min (UV detection at 254 nm). Linearity was observed over the concentration range 5-100 µg/mL (r^2=0.9994) with regression equation y=15043x-3093. Brinzolamide was subjected to stress conditions including acidic, alkaline, oxidation, photolysis, and thermal degradation. Brinzolamide is more sensitive towards oxidative degradation. The method was validated as per the ICH guidelines.

KEYWORDS: *Brinzolamide; RP-HPLC; ICH; Validation; Forced degradation.*

1. INTRODUCTION

Brinzolamide is non-competitive reversible carbonic anhydrase (CA) II inhibitor with high specificity. It produces the necessary effect by inhibition an isoenzyme (CA) II in the secretory cells of ciliary processes in the eye. This inhibition of isoenzyme leads to lowering down of intraocular pressure (IOP) by reducing the rate of aqueous humour formation.[1] Chemically Brinzolamide is (4*R*)-4-(ethylamino)-2-(3-methoxypropyl)-1,1-dioxo-3,4-dihydrothieno[3,2-e]thiazine-6-sulfonamide.[2]

According to a review of the literature (Table 1) suggest only few HPLC methods have been used to determine Brinzolamide and other medications simultaneously.[3],[4],[5],[6] The goal of this research is to develop a simple, precise, and accurate mass compatible method for determining Brinzolamide and to use it in stability studies as well.

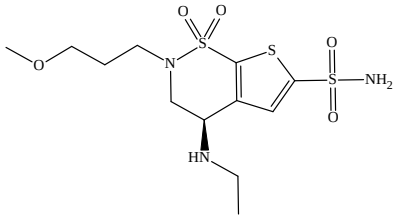

Figure 1: Structure of Brinzolamide.

Table 1: Comparison of the performance characteristics of the present method with the published methods.

Sr.no	Method/Reagent	Linearity	Remarks	Ref
1.	HPLC/Acetonitrile: 0.05 M phosphate buffer at the ratio of (30:70, v/v)	5-50 µg/ml	Simultaneous	[3]
2.	HPLC/Triethylamine phosphate buffer: Acetonitrile: Methanol (70:20:10, v/v)	40-140 µg/ml	Simultaneous	[4]
3.	HPLC/Acetonitrile: Potassium dihydrogen phosphate buffer (40:60, v/v)	100-500 µg/ml	No stability studies/UV-Vis detector/No mass compatibility	[5]
4.	LC-QTOF MS/Methanol and 10 mM Ammonium formate (90:10, v/v)	0.5-20 µg/ml	Dried blood spots	[6]
5.	HPLC/ Acetonitrile: 1mM Ammonium formate (24:76, v/v)	5-100 µg/ml	Stability indicating method (PDA detector), Mass compatible.	Present work

1. EXPERIMENTAL

1.1 ***Chemicals and reagents***

Brinzolamide was obtained from FDC Limited (Mumbai, India). Acetonitrile (HPLC grade), ammonium formate, sodium hydroxide, hydrochloric acid and hydrogen peroxide were purchased from Merck (India). All chemicals were of analytical grade.

1.2 ***HPLC Instrumentation and conditions***

Chromatographic separation was achieved by using a Waters 2690 Alliance with photodiode array detector and Hemochrom C18 (150mm 4.6 mm, 5µm particle size) column. Isocratic elution was performed using acetonitrile and 1mM ammonium formate (24:76, v/v) with flow rate 1 mL/min and injection volume of 10µL into the HPLC system.

Brinzolamide standard stock solution (1000 mg/mL) (Solution A) was prepared by accurately weighing 25 mg of Brinzolamide in a 25 mL volumetric flask and making up to volume with Acetonitrile. 10ml of Standard stock solution was pipetted out and transferred to 100ml volumetric flask and was diluted up to 100ml with Acetonitrile resulting in solution of 100µg/ml (Solution B).

1.3 ***Analytical Method Validation***[7],[8]

The developed method was validated according to standard ICH guideline for system suitability, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

The system suitability was checked by injecting three different injections of 50µg/ml. Various parameters like Tailing factor, No. of theoretical plates, Peak area were checked according to USP criteria.

Linearity test solutions for assay were prepared from stock solution B in the range of 5-100µg/ml. Each concentration measurement was done in triplicate. Linearity was evaluated by calculating the mean of area as a function of analyte concentration and a graph of Area under curve v/s Concentration of Brinzolamide was plotted.

The accuracy was performed by standard addition method. Recovery study was performed at three concentration levels (80%, 100%, 120%) and % recovery was calculated. The study was performed in triplicates and mean %RSD was determined at 32, 40 and 48 µg/ml.

Intra-day precision was performed by preparing 3 different test concentrations (40,70 and 100µg/ml) and assaying for three times (n=3) at 3 different intervals on the same day. Inter-day precision was performed by analysing samples for three consecutive days. The %RSD values were calculated.

LOD and LOQ were analysed based on signal to noise ratio (n=3) as described in ICH Q2(R1) guidelines.

The robustness of the developed method was established by introducing small changes in the HPLC conditions which included 1) Changes in the flow rate (0.8ml/min & 1.2ml/min) & 2) Changes in the % organic mobile phase composition (22 and 26). Robustness of the method was studies using three replicates at a concentration level of 55 µg/ml of Brinzolamide.

1.4 ***Forced Degradation Studies***[9]–[11]

The study was intended to ensure the effective separation of Brinzolamide and its degradation peaks at the retention time of Brinzolamide. Forced degradation studies were carried out to develop a stability indicating and specific method. All solutions for use in stress studies were

prepared at an initial concentration of 1mg/mL of Brinzolamide. Before injection all the samples were diluted with acetonitrile to give a final concentration of 60µg/ml.

Acid hydrolysis was carried out in 1M HCl and alkaline degradation was conducted using 1M NaOH. The neutralization of the solutions was carried out using the same strength of the acid/base. Solutions for oxidative stress studies were prepared using 3% H2O2 at a concentration of 1mg/mL and diluted accordingly with the mobile phase. For thermal stress testing, the drug solution (1mg/mL) was heated in thermostat at 60^0C for 30min, cooled and used. The drug solution (1mg/mL) for photostability testing was exposed to sunlight for 4h and analysed.

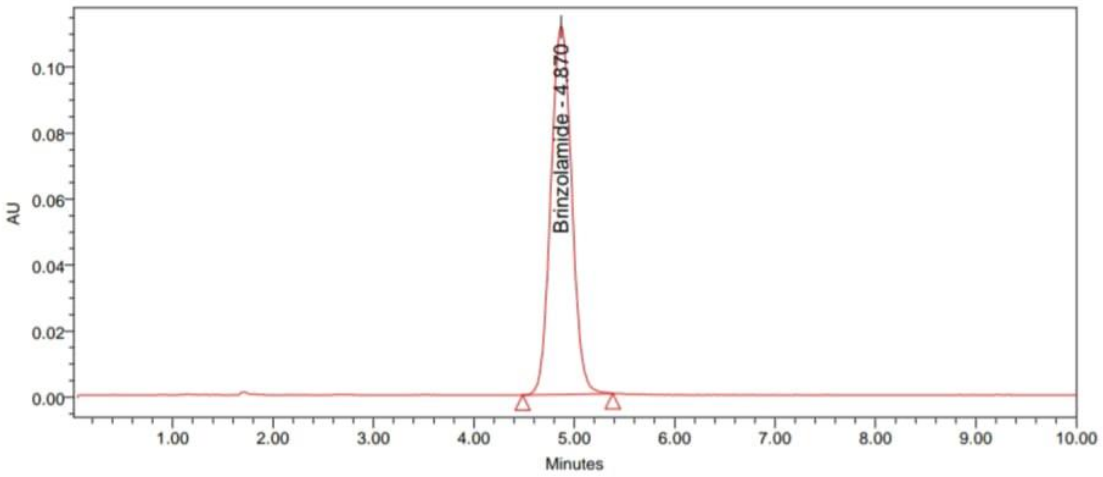

Figure 2: Chromatogram of standard Brinzolamide in optimised conditions.

Table 2: Linearity of Brinzolamide.

Sr. No	Concentration (µg/ml)	*Mean peak area ± SD (n=3)	*%RSD
1.	5	71390 ± 752	1.05
2.	25	374538 ± 6805	1.82
3.	40	593094 ± 11051	1.86
4.	55	840547 ± 15315	1.82
5.	70	1044790 ± 18112	1.73
6.	85	1255812 ± 3658	0.29
7.	100	1514428 ± 16457	1.09

*Mean of three replicates.

Table 3: Accuracy study of Brinzolamide

Level	Amount added (µg/ml)	*Amount found (µg/ml)	*% Recovery	%RSD
80%	32	31.74	99.13	0.71
100%	40	40.31	100.79	0.37
120%	48	47.59	99.14	0.46

*Mean of three replicates.

Table 4: Inter day and Intra day precision studies of Brinzolamide.

Concentration of Brinzolamide	*Inter-Day	%RSD	*Intra-Day	%RSD
40µg/ml	40.28±0.36	0.89	40.07±0.13	0.34
70 µg/ml	70.16±0.13	0.19	70.29±0.09	0.13
100 µg/ml	100±0.43	0.43	99.66±0.66	0.67

*Mean of three replicates.

Table 5: LOD of Brinzolamide.

Sr. No	Parameter	LOD
1.	*Height of noise(h)	88
2.	*Height of Brinzolamide peak(H)	316
3.	*S/N= 3	3.59

*Mean of three replicates.

Table 6: LOQ of Brinzolamide.

Sr. No	Parameter	LOQ
1.	*Height of noise(h)	101
2.	*Height of Brinzolamide peak(H)	1142
3.	*S/N= 3	11.30

*Mean of three replicates.

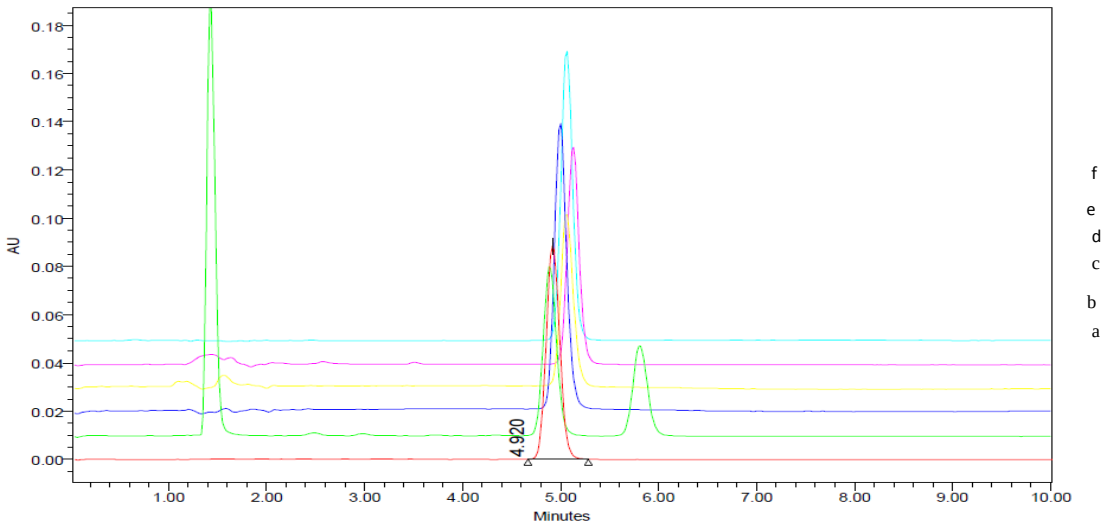

Figure 3: Representative chromatograms of Brinzolamide (60 µg/ml) a) Untreated b) Oxidative c) Alkaline d) Acidic e) Photolytic and f) Thermal degradations.

Table 7: Forced degradation studies of Brinzolamide.

Sr.no	Degradation conditions	Retention time of degradation products (min)	Brinzolamide % Degradation
1	Acid hydrolysis	---	No degradation
2	Base hydrolysis	---	No degradation
3	Oxidative degradation	5.809	21%
4	Photolytic degradation	---	No degradation
5	Thermal degradation	---	No degradation
	Total Degradation		21%

2. RESULTS AND DISCUSSION

The representative chromatogram for Brinzolamide is shown in the (Fig. 2). In the present method the drug was found to be linear in the range of 5-100 µg/ml (Table 2) and the calibration curve was described by the equation y= 15043x – 3093 with correlation coefficient of 0.9994. The method was validated as per the ICH guidelines. The validation parameters were within the limits. The number of theoretical plates were 3101 (more than 2000) and tailing factor was 1.19 (less than 2) for the Brinzolamide peak. The % recovery in accuracy studies was found to be 99.13- 100.79% (Table 3). The % RSD for precision was found to be 0.19- 0.89% (Interday) and 0.13- 0.67% (Intraday) (Table 4). The LOD (Table 5) was found to be 0.25 µg/ml and LOQ (Table 6) was found to be 0.75 µg/ml. The %RSD values for robustness studies was found to be less than 2% indicating that the method is robust. Brinzolamide shows significant degradation in oxidative stress conditions (Table 7). 21% of the drug was decomposed under oxidative conditions with a peak at 5.8 min and the initial peak at 1.5 min indicates the peroxide peak (Figure 3).

3. CONCLUSION

The developed RP-HPLC method was found to be simple, accurate, precise, sensitive, robust and mass compatible method for determination of Brinzolamide in its bulk forms.

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