Available online on 15.01.2020 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Analytical method development and validation for the determination of Brinzolamide by RP-HPLC

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ABSTRACT

Brinzolamide is inhibitor of carbonic anhydride and is highly specific and non-competitive. The aim of the present study is to develop a simple, precise, accurate, sensitive RP-HPLC method for the determination of bulk drug. The objective of the method validation is to demonstrate whether the method was suited for the intended purpose. The method was validated as per the ICH guidelines. The method was validated for linearity, precision (repeatability, intermediate precision), accuracy, specificity, robustness, ruggedness, limit of detection and limit of quantification. Cosmosil (4.6X250mm, 5 μ) column was used for separation. The selected wavelength for Brinzolamide was 254 nm. The mobile phase consists of Acetonitrile: Potassium dihydrogen phosphate buffer (40:60). Flow rate was delivered at 1.0 mL/min. Appropriate dilutions of standard stock solutions were prepared to get desired concentrations in the range of 100-500 mcg/ml. The equation of standard curve was $y = 441.8x + 1132$ and $R^2 = 0.998$. The RT obtained was 6.6167 minutes.

Keywords: Brinzolamide, UV spectroscopy, RP-HPLC, ICH

Article Info: Received 03 Nov 2019; Review Completed 21 Dec 2019; Accepted 29 Dec 2019; Available online 15 Jan 2020



Cite this article as:

Tiwari B, Shirsat MK, Kulkarni A, Analytical method development and validation for the determination of Brinzolamide by RP-HPLC, Journal of Drug Delivery and Therapeutics. 2020; 10(1):92-96 <http://dx.doi.org/10.22270/jddt.v10i1.3845>

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INTRODUCTION:

Brinzolamide is inhibitor of carbonic anhydride and is highly specific and non-competitive. Chemically Brinzolamide is (4R)-4-(ethylamino)-2-(3-methoxypropyl)-1,1-dioxo-2H,3H,4H-1λ⁶-thieno[3,2-e][1,2]thiazine-6-sulfonamide. Carbonic anhydrase is a type of enzyme that can be found in several body tissues including eye. It is responsible for catalysation of the reversible reaction indulged in carbon dioxide hydration and carbonic acid dehydration. In human beings, carbonic anhydrase is present in form of various isoenzymes among which the most active one is carbonic anhydrase II. During the ciliary processes of eye when carbonic anhydrase is inhibited it results in reduction of secretion of aqueous humour, most probably by decreasing the creation of bicarbonate ions with further decrease in transportation of sodium and fluid. This result in decrease of intraocular pressure.

Literature review suggest few HPLC, HPTLC, spectroscopic, Stability indicating HPLC determinations were performed.¹⁻¹³ The aim of the present study is to develop a simple, precise, accurate, sensitive HPLC method for the determination.

After, doing in depth study in the present research work, it was found that the present research work is having various advantages over the previous work. The advantages include less retention times of the component, with good resolution. The % RSD of robustness was found to be less. The results obtained from the validation suggest that the method was found to be precise, accurate, linear and robust enough and the method was also found to be economical.

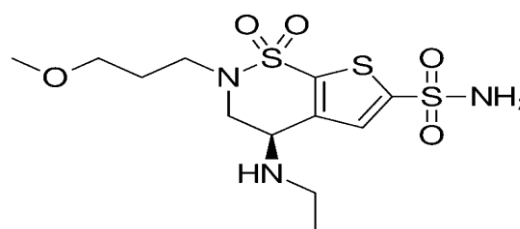


Figure 1: Structure of Brinzolamide

EXPERIMENTAL WORK:

Chemicals and reagents: HPLC grade acetonitrile, methanol, from Spectro Chemie. Ammonium acetate, acetic acid of analytical grade was used. Millipore grade water was used. The reference standard samples of Brinzolamide was provided was provided by Ajanta Pharma.

Instrumentation and analytical conditions:

The analysis was carried out by using Younglin (S.K) Gradient System UV Detector, 4.6X250mm cosmosil column, 20ml loop size HPLC (With UV/Vis Detector. Other instrumentation includes Double beam UV- Visible spectrophotometer Simadzu UV-1800, digital balance (metler tolado), vacuum pump (Gelman science), pH meter (poloman).

Chromatographic conditions:

Cosmosil (4.6X250mm, 5 μ) column was used for separation.

Preparation of Standard stock solution:

100 mg of Brinzolamide was weighed in to 100 ml volumetric flasks. The drug was dissolved in 40 ml of solvent, and shaken manually for 10 min. The volume was made up to the mark with solvent and the final strength obtained was 1000 μ g/ml.

Preparation of sample solution:

1, 2, 3, 4, 5ml of the standard stock solution of Brinzolamide were transferred separately into five 10ml volumetric flasks and volume was made up to mark to get the final concentrations of 10, 20, 30, 40, 50 μ g/ml of Brinzolamide. The chromatograms were recorded and a standard curve was plotted by taking concentrations on x-axis and area under curve on y-axis. The equation of standard curve was generated to determine the concentration of drug in pure and degradation products.

Validation procedure:

The objective of the method validation is to demonstrate whether the method was suited for the intended purpose. The method was validated as per the ICH guidelines. The method was validated for linearity, precision (repeatability, intermediate precision), accuracy, specificity, robustness, ruggedness, limit of detection, limit of quantification. A calibration graph was constructed by taking six different concentrations, ranging from 50 – 150 μ g/mL. The peak area was calculated, and calibration curve was constructed by taking peak area and concentration on both the axis. The linearity was evaluated by linear regression analysis. The precision studies were demonstrated by two parameters inter day and intraday precision. Intraday precision was performed by injecting six replicated injections to the chromatographic system on the same day and calculated the %RSD. The inter day precision was performed by injecting six replicated injections at two consecutive days. From the peak area of the chromatograms, the %RSD was calculated. The accuracy was determined by adding a known amount of the standard to the sample, and the percentage recovery was estimated. The robustness was determined by incorporating deliberate changes into the method conditions like the change in flow rate, pH, and gradient. Ruggedness was performed by carrying out the proposed method with two different analysts.

RESULTS AND DISCUSSION:

Selection of wavelength:

UV spectrum was obtained by preparing a solution by taking diluents and scanned between 200 to 400 nm. Brinzolamide shows λ_{max} at 254 nm. So, it is selected as a detection wavelength.

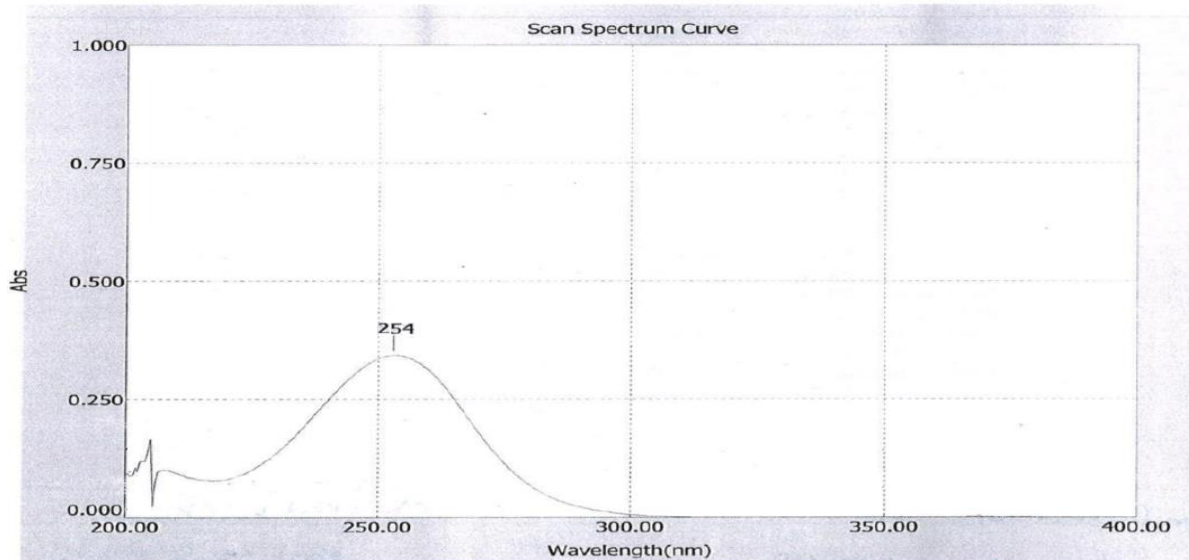


Figure 2: Spectrum of Brinzolamide

Development and optimization of the HPLC method:

For getting an optimized chromatographic condition, A Cosmosil (4.6X250mm, 5 μ) column was used for separation. The mobile phase consists of Acetonitrile: Potassium dihydrogen phosphate buffer (40:60). Flow rate was

delivered at 1.0 mL/min with detection wavelength at 254 nm. A 20 μ L was injected to the chromatographic system with ambient temperature. Acetonitrile: Potassium dihydrogen phosphate buffer (40:60) was the optimized mobile phase selected for experimentation. The RT obtained was 6.6167 minutes.

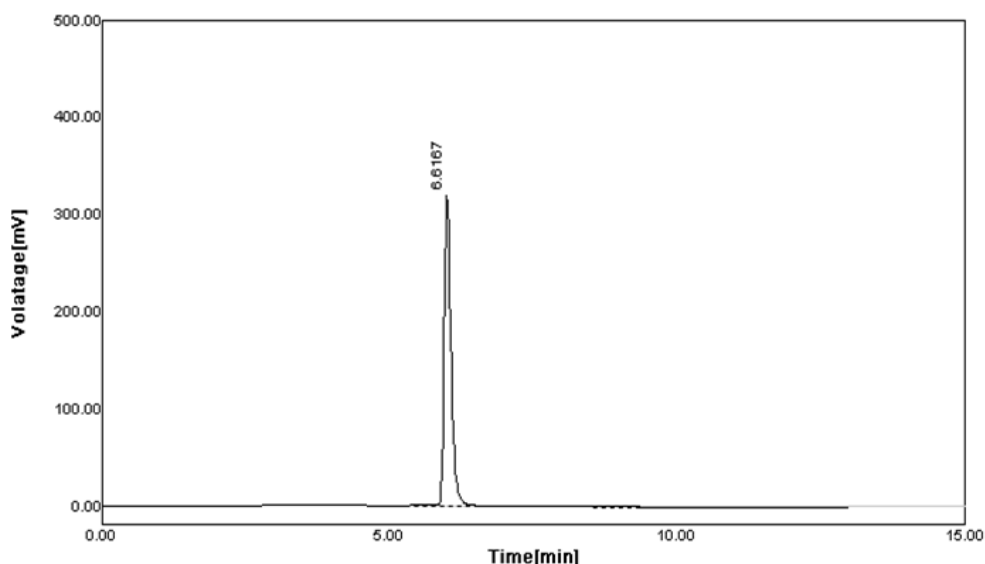


Figure 3: RT of Brinzolamide

Method validation:

Linearity:

Appropriate dilutions of standard stock solutions were prepared to get desired concentrations in the range of 100-

500 mcg/ml. Then the concentrations were plotted against the area under curves to get the equation of standard curve, as presented in Table 1.

Table 1: Dilutions for linearity study

Stock solution (µl)	Diluted (ml)	Final concentration
100	10	10
200	10	20
300	10	30
400	10	40
500	10	50

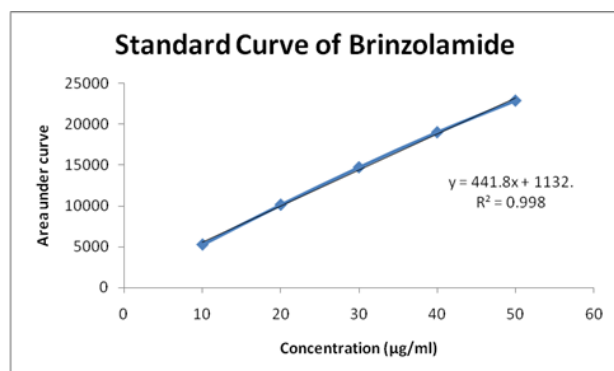


Figure 4: Standard curve of Brinzolamide

Table 2: Linearity Data

S. N.	Conc. in (µg/ml)	Mean AUC (n = 5)	% RSD
1	10	5236	0.453
2	20	10113	0.243
3	30	14705	0.428
4	40	18999	0.651
5	50	22884	0.766

Precision:

Precision is determined at two levels: a) Repeatability b) Intraday and Interday Precision.

Table 3: Precision Data

Parameter	Amount taken ($\mu\text{g/ml}$)	Amount found* ($\mu\text{g/ml}$)	%RSD
System Precision	60	59.94	0.475
Method Precision	60	60.15	0.683

*Mean of Six observations

Table 4: Intraday and Interday Precision Data

Concentration of Brinzolamide	Intraday*	%RSD	Interday *	%RSD
20 $\mu\text{g/ml}$	20.07	0.083	19.92	0.138
40 $\mu\text{g/ml}$	39.99	0.091	39.94	0.129
60 $\mu\text{g/ml}$	60.09	0.074	59.91	0.144

*Mean of three observations

Accuracy:

To check the accuracy of the developed methods, analytical recovery experiment was carried out by standard addition method. Recovery study was performed by adding 80, 100 and 120 % of the test concentration as per ICH guidelines.

Table 5: Accuracy

Sr. No.	Concentration of Drug Added ($\mu\text{g/ml}$)	Concentration of Drug Added ($\mu\text{g/ml}$)	% Recovery \pm SD
1	64	10.4	99.99 \pm 0.006
2	80	13	99.96 \pm 0.018
3	96	15.6	96.95 \pm 0.027

Limit of Quantification (LOQ) and Limit of Detection (LOD):

The limit of quantification (LOQ) is defined as the lower concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantified. The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity. The LOD and LOQ were calculated using following formula

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

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

Where, σ = Standard deviation of the Y- intercepts of the 5 calibration curves, S = Mean slope of the 5 calibration curves.

The LOQ and LOD parameters of Brinzolamide are provided in Table 5.

Table 6: LOD and LOQ parameters

Parameter	Values
S.D. of Intercept*	0.531037
Mean Slope of Calibration Curve*	441.8
LOD ($\mu\text{g/ml}$)	0.00396
LOQ ($\mu\text{g/ml}$)	0.01202

Robustness:

Robustness was studied by observing the change in following parameters, and then observation of each parameter change was done to access their effect on system suitability and assay. Change in mobile phase composition was done by \pm 5.0 ml of organic solvent and the change in the detection wavelength \pm 10 nm was done.

Change in mobile phase composition: The sample solution at test concentration (60 $\mu\text{g/ml}$ of drug was injected thrice with the mobile phase composition changed by \pm 5.0ml of organic solvent from the developed method.

Change in detection wavelength: The sample solution at test concentration (60 $\mu\text{g/ml}$ of drug was injected thrice with the change in detection wavelength by \pm 10 nm from the developed method. The % assay of drug after changes in method parameters were observed.

CONCLUSION:

A precise RP - HPLC method was developed for the determination of Brinzolamide. The shorter run time elutes Erlotinib hydrochloride with good resolution, and symmetry. The method was validated as per the ICH guidelines and the method was found to be simple, precise, linear, accurate, rugged and robust enough.

Table 7: Summary of Validation parameters

Parameters	Brinzolamide
Accuracy	98.95 ±0.427– 99.93 ± 0.226
Precision (%RSD)	
System Precision	0.475
Method Precision	0.683
Intra-day (n = 3)	0.074 - 0.091
Inter-day (n = 3)	0.129 - 0.144
LOD and LOQ	
LOD	0.00396
LOQ	0.01202

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