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Development and Validation of Stability Indicating RP- HPLC Method for the Determination of Brinzolamide in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

A proven stability indicating approach that is straightforward, exact, precise, and specific was created for the assessment of Brinzolamide in the form of an ocular suspension. At room temperature, the chromatographic separation was accomplished using a standard Phenomenex C18 150 mm (4.6 x 150 mm, 5µm). A mobile phase comprising buffer, 0.1% formic acid – methanol (65:35 v/v) injected over the column at a flow rate of 1 ml/min and an injection volume of 10µl was used to achieve the separation. Using a UV detector, the analyte at 254 nm was found. The International Conference on Harmonization was complied with while validating the technique performance. Brinzolamide was found to have a retention duration of 3.497 minutes. The percentage recovery that was attained was 99.88 for brinzolamide. Brinzolamide's regression equation is y = 20013x-10108. When the stability indicating method was used under different stress scenarios, the degradants had no effect on the effectiveness of brinzolamide. The new RP-HPLC method yielded great resolution, which increases the method's reliability. It was also highly specific, exact, sensitive, and stable suggesting. Thus, the described method can be implemented in companies for routine quality control testing.

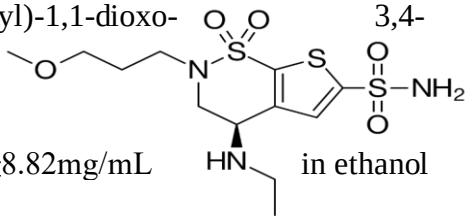
Key Words: *Brinzolamide, RP-HPLC, C_{18} Column, Methanol, 0.1% formic acid, % recovery.*

INTRODUCTION

In most cases, precise analytical measurements at very low concentrations using a range of instruments are necessary in modern analytical chemistry. As a result, understanding the equipment that is now utilized in chemical analysis is crucial to ensuring future advancement in a number of scientific domains. This covers a range of chemical specialties, including environmental sciences, biotechnology, medicinal chemistry, pharmaceutical chemistry, and biochemistry. Only with a deeper understanding of the concepts behind both measurement and separation-achieving instruments will it be feasible to make the best use of equipment and generate more relevant data that can be consistently evaluated.[1]

DRUG PROFILE[2-4]

IUPAC Name : (4R)-4-(ethylamino)-2-(3-methoxypropyl)-1,1-dioxo-3,4-dihydrothienol[3,2-e]thiazine-6-sulfonamide.



Molecular Formula: $C_{12}H_{21}N_3O_5S_3$
Molecular Weight: 383.5 g/mol
Solubility: Insoluble in water; ≥15.05mg/mL in DMSO; ≥8.82mg/mL in ethanol with gentle warming and ultrasonic

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pKa: 5.9 & 8.5
Therapeutic category: Carbonic anhydrase inhibitor
Melting point: 131ºC

METHOD

Diluent: Based up on the solubility of the drugs, diluent was selected; 0.1% Formic acid as diluent.

Chromatographic conditions: Numerous trails were run in order to choose the ideal chromatographic conditions, and the best trail was chosen for the optimized procedure.

Preparation of 0.1% formic acid buffer: 1ml of formic acid solution was diluted to 1000ml to get 0.1% formic acid.

Preparation of Standard stock solutions: A dry 10 ml volumetric flask containing 10 milligrams of brinzolamide was filled. After adding mobile phase to the volumetric flask to three-quarters of its capacity, the resultant solution was sonicated for five minutes. After adding mobile phase to fill the volumetric flask to capacity, a 0.45µ membrane filter was used to filter the mixture. Following that, the filtrate was utilized as the main standard stock solution, containing 1000 ppm of brinzolamide.

Preparation of Standard working solutions: Working standards in the concentration range of 2–10µg/ml were prepared by taking aliquots from the standard stock solution.

Preparation of Sample stock solutions: A 1% w/v Brinzolamide suspension that was on sale was purchased. In a spotless, dry 100 ml volumetric flask, 1 ml of the suspension was added, and the remaining volume was filled with HPLC water.

Preparation of Sample working solutions: After 30µl of the filtered sample stock solution from the previously produced solution was added to a 5-milliliter volumetric flask, made up with buffer, and verified, 10µl of injection was added to the HPLC.

Preparation of Sample stock solutions: A 1%w/v Brinzolamide suspension that was on sale was purchased. In a 100 ml dry volumetric flask, 1 ml of the suspension was taken and the volume was adjusted with HPLC water to the appropriate level.

Preparation of Sample working solutions: After 30µl of the filtered sample stock solution from the previously produced solution was added to a 5-milliliter volumetric flask, made up with buffer, and verified, 10µl of injection was added to the HPLC.

Method Validation: Verifying an analytical procedure's suitability for its intended use is the goal of validation. The typical analytical performance parameters that should be taken into account in the validation of the various types of methods are as follows, according the ICH Q2B guidelines:

1) S
pecificity
2) L
inearity

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3) Accuracy
4) Precision
5) Limit of detection
6) Limit of quantification
7) Robustness
8) System suitability

1.Specificity:
A) Brinzolamide identification: The HPLC system was filled with standard and sample solutions that had been prepared in accordance with the test protocol.[5]
B) Blank interference: A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure.

2. Linearity:
Preparation of Standard stock solutions: Brinzolamide 10 mg was added to a dry 10 ml volumetric flask. The mobile phase was added to the volumetric flask until three-quarters of the way up, and the mixture was then sonicated for five minutes. Following that, a 0.45µ membrane filter was used to filter the mobile phase-filled volumetric flask. Next, the filtrate which included 1000 ppm of brinzolamide was utilized as the main standard stock solution.. [6]

Preparation of Standard working solutions: Working standards in the concentration range of 2–10µg/ml were generated by taking aliquots from the standard stock solution. After injecting each of the aforementioned solutions into the chromatographic apparatus, the peak area was calculated.

3. Accuracy:
Preparation of Standard stock solutions: Brinzolamide 10 mg was added to a dry 10 ml volumetric flask. The mobile phase was added to the volumetric flask until three-quarters of the way up, and the mixture was then sonicated for five minutes. Following that, a 0.45µ membrane filter was used to filter the mobile phase-filled volumetric flask. Next, the filtrate which included 1000 ppm of brinzolamide was utilized as the main standard stock solution.

Procedure: Standard solution, Accuracy-80%, Accuracy-100% and Accuracy-120% solutions were injected in to HPLC system. Amount found and amount added for Brinzolamide, individual recovery and mean recovery values were also calculated. The average % recovery of Brinzolamide, was calculated.

4. Precision:
Preparation of Standard stock solutions: Into a dry 10 ml volumetric flask, 10 mg of brinzolamide was added. Following a 5-minute sonication of the resultant solution, the volumetric flask was filled with mobile phase to a quarter of its total capacity. Following mobile phase addition to the volumetric flask, a 0.45µ membrane filter was used to filter the

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mixture. Brinzolamide at a concentration of 1000 ppm was then added to the filtrate to create the primary standard stock solution.

Procedure: Five injections of the standard solution were made, and each time the area was measured in an HPLC. The region of five replicate injections' %RSD was computed.

5. Robustness: The analysis of aliquots from homogenous batches was done to test the robustness of the suggested approach. Physical parameters such as temperature fluctuations and flow rate and mobile phase composition were varied, but the responses remained within the assay's specified limits.
a) Effect of variation of flow rate: To ascertain the impact of flow rate variation, research was done. The range of the flow rate was 0.9 ml/min to 1.1 ml/min.

b) Effect of variation of mobile phase composition: By altering the mobile phase ratio, a study was carried out to ascertain the impact of change in mobile phase ratio. The mobile phase's organic content was adjusted between 55% and 65%.In addition to the real mobile phase composition in the procedure, a standard solution was generated and analyzed using the varied mobile phase composition. After being prepared, the standard solution was added to the HPLC apparatus.

6. System Suitability Parameters: As per the test protocol, three injections of the Brinzolamide sample solution were made into the HPLC apparatus. Through the computation of the percentage RSD of retention times, tailing factor, theoretical plates, and peak areas from three replicate injections, standard chromatograms were used to assess the system appropriateness characteristics.

7. Degradation Studies:
Acid Degradation Studies: 30µl of 0.1N hydrochloric acid was added to 30µl of brinzolamide stock solution, and the mixture was refluxed for 30 minutes at 60°C. After diluting the resulting solution to yield a 6µg/ml solution, 10µl of the solution was injected into the apparatus, and chromatograms were recorded to evaluate the sample's stability.

Alkali Degradation Studies: To 30µl of stock solution brinzolamide, 30µl of 0.1N Sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 6µg/ml solution and 10µl of the solution was injected into the system and the chromatograms were recorded to assess the stability of sample. [7-8]

5% Hydrogen Peroxide: To 30µlof stock solution brinzolamide, 30µl of 5%hydrogen peroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 6µg/ml solution and 10µl of the solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal Degradation Studies: The standard drug solution was placed in oven at 105º c for 1hr to study dry heat degradation for HPLC study the resultant solution was diluted and 10µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing 6µg/ml to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt

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hours/m^2 in photo stability chamber. For HPLC study.10µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

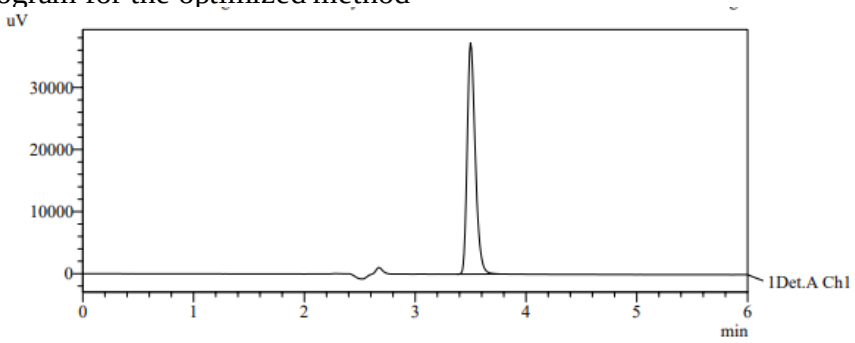
Analytical Method Development for the estimation of Brinzolamide by RP-HPLC.

Method development: Method development was done by changing various, mobile phase ratios, buffers etc.

Chromatographic Conditions:

Mobile phase	**:**	0.1%Formic acid and Methanol (60:40%v/v)
Flow rate	**:**	1ml/min
Column	**:**	PhenomenexC18 (4.6 x 250mm, 5µm)
Detector wave length	**:**	254.0 nm
Column temperature	**:**	30°C
Injection volume	**:**	10µL
Run time	**:**	6min
Diluent	**:**	0.1% formic acid
Results	**:**	In this trail, Brinzolamide was eluted with the solvent front and the plate count was within the limits with a retention time of 3.497min.

The chromatogram for the optimized method



OBSERVATION

Brinzolamide was eluted at 3.497min. Plate count and tailing factor were satisfactory, so this method was optimized and to be validated.

Method Validation: The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH Q2B guidelines, typical analytical performance characteristics that should be considered in the validation of the types of methods are:

1) S pecificity
2) L inearity
3) A ccuracy
4) P recision

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5) System suitability
6) Robustness

1.Specificity

Discussion: Retention time of Brinzolamide was 3.596 min. No interfering peaks were found in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

2. Linearity:

Acceptance criteria:

- Correlation Coefficient should be not less than 0.9990.
- % RSD of peak areas for Solution 2,4,6,8 and 10 should be not more than 2.0 %

Table 1 Linearity table for Brinzolamide

Concentration (µg/ml)	Area
2	29445
4	70429
6	111067
8	148238
10	190669
R^2 Value	0.997

Discussion: Five linear concentrations of Brinzolamide (2-10µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Brinzolamide was y = 20013x -10108. Correlation coefficient obtained was 0.9997 for Brinzolamide. Data presented in Table No.1.

3. Precision:

Table 2. System precision table of Brinzolamide (6ug/ml)

S.NO.	Peak Area	Retention time
1	103067	3.593
2	103273	3.596
3	102268	3.596
4	102928	3.607
5	102478	3.605
Mean	**102802.8**	**3.6014**
STD Deviation	**417.753**	**0.010**
% RSD	**0.406**	**0.272**

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation 0.6% for Brinzolamide. As the limit of Precision was less than "2" the system precision was passed in this method. Repeatability of sample measurement was carried out in six replicates of the

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same sample preparations from same homogenous blend of ,arketed formulation. The data was given table 2 .

4. Accuracy: Table 3. Accuracy table of Brinzolamide

% Level	Amount Spiked (µl)	Amount recovered (µl)	% Recovery	Mean % Recovery
80 %	20	19.92	99.60	99.43
	20	19.89	99.45	
	20	19.82	99.10	
100 %	30	29.89	99.63	
	30	29.33	99.10	
	30	29.99	99.96	
120 %	40	39.69	99.22	
	40	39.70	99.25	
	40	39.85	99.62	

Discussion: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 99.43% for brinzolmaide.

5. Robustness:

Table. 4. Robustness data of Brinzolamide relating to Mobile Phase ratio change

S.No.	Concentration(ug/ml)	Mobile phase	Retention time(min)	Theoretical Plates	Tailing Factor
1	6 (n=3)	60:40	3.058	8057.209	1.436
2	6 (n=3)	65:35	3.622	9389.329	1.289
3	6 (n=3)	70:30	4.723	11243.578	1.351

Table 5. Robustness data of Brinzolamide relating to Flow rate change

S.No.	Concentration (ug/ml)	Flow Rate (ml/min)	Retention time (min)	Theoretical Plates	Tailing Factors
1	6 (n=3)	0.9	3.954	10354.661	1.361
2	6 (n=3)	1.0	3.622	9389.329	1.359
3	6 (n=3)	1.1	3.323	8747.459	1.383

Table 6. Robustness data of Brinzolamide relating to Wave length change

S.No.	Concentration(ug/ml)	Wavelength (nm)	Retention times	Theoretical plates	Tailing Factors
1	6 (n=3)	253	3.59	9266.69	1.375
2	6 (n=3)	254	3.62	9389.33	1.359
3	6 (n=3)	255	3.57	9336.97	1.364

Discussion: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (60:40), mobile phase plus (70:30), wavelength minus (253nm) and wavelength plus (255nm) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

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6. Degradation studies: Standard degraded samples are injected and calculated the percentage of drug degraded in solution by applying different conditions like acid, alkali, and oxidative, photolytic, thermal and neutral analysis.

Table.7 Degradation Data

Type of degradation	Area	% Recovered	% Degraded
Acid	90639	87.9	12.1
Base	91711	88.9	11.1
5% Hydrogen peroxide	103012	99.9	1.3
Thermal	101758	98.7	1.3
Photo	102527	99.4	0.6

CONCLUSION

A new and simple stability indicating RP-HPLC method for the estimation of Brinzolamide in ophthalmic dosage form was successfully developed and validated. The stability indicating method revealed that the Brinzolamide is stable under extreme conditions of acidic, alkaline, thermal etc. From the statistical assessment of the method it was concluded that the developed method was simple, specific, linear, accurate, precise and robust. The high resolution obtained makes this method cost effective and more acceptable. The usage of 0.1% Formic acid buffer which is a volatile in the mobile phase makes this method applicable in the LC-MS instrument.

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