ORIGINAL ARTICLE

A C G publications *J. Chem. Metrol.* XX:X (202X) X-XX

journal of chemical metrology

Development and application of RP–HPLC method for estimation and evaluation of stability of brinzolamide in ophthalmic formulation

Mehul Patel [1*], Ritu Dwivedi [1], Umang Shah [1] and Heta Kachhiya [2]

[1]*Department of Pharmaceutical Chemistry and Analysis, Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology, CHARUSAT Campus, Changa, Anand, Gujarat-388421, India*

[2]*Department of Pharmaceutical Chemistry and Analysis, Indukaka Ipcowala College of Pharmacy, CVM University, Beyond GIDC, P.B. No. 53, Vitthal Udyognagar- 388 121, Gujarat, India*

(Received November 01, 2023; Revised December 22, 2023; Accepted December 28, 2023)

Abstract: This study outlines the development, optimization, and validation of a robust reverse-phase high-performance liquid chromatography (RP-HPLC) technique for the precise quantification of Brinzolamide in ophthalmic products, aligning International Council for Harmonization (ICH) guidelines. The method employed a Phenomenex (C18) (250×4.6mm) column with 5µm particle size as the stationary phase, a 1 mL/min flow rate for the mobile phase (composed of acetonitrile: water 35:65 v/v, pH adjusted to 3 with orthophosphoric acid), and detection at 254 nm. Under these conditions, Brinzolamide displayed Rt of 4.9 minutes. The validation process, following ICH standards, exhibited excellent linearity within the 5–30 µg/mL concentration range, with a limit of detection at 0.22 µg/mL and a limit of quantification at 0.67 µg/mL. Recovery rates from ophthalmic formulations fell between 98.3%-101.08%, indicating high accuracy. Accelerated stability assessments conducted over three months revealed content retention between 98.2%-100.9%, affirming the product's stability. Additionally, Brinzolamide withstood various stress conditions without interference in quantification, as the degradation products had distinct retention times from the pure drug, offering excellent resolution. In conclusion, this RP-HPLC method is suited for routine quality control analysis of Brinzolamide in commercial ophthalmic preparations, due to its specificity, accuracy, precision, and sensitivity, aligning perfectly with ICH guidelines.

Keywords: Brinzolamide; RP-HPLC method; accelerated stability study; force degradation; degradation products.

1. Introduction

Brinzolamide (BZA) is "(4R)-4-(ethylamino)-2-(3-methoxypropyl)-1,1-dioxo-2H,3H,4H-1λ⁶-thieno[3,2-e] thiazine-6-sulfonamide" (represented in Figure S1 in supporting information). It is a colourless solid powder, odourless, slightly water solubility but soluble in methanol, ethanol, and acetonitrile. BZA is sulphonamide and it is used to treat glaucoma [1]. BZA acts as a very specific, reversible blocker of CA-II enzyme, which is mainly responsible in the secretion of aqueous humour [2–10]. BZA is official in IP as well as USP, which recommends HPLC method for its analysis.

Commercially available topical eye drops containing Brinzolamide are widely accessible. Several analytical methodologies have been documented in the previous literature to quantify Brinzolamide [11–

* Corresponding author E-Mail: mehulpatel.ph@charusat.ac.in

The article was published by ACG Publications
http://www.acgpubs.org/journal/journal-of-chemical-metrology Month-Month 202X EISSN:1307-6183
DOI:http://doi.org/10.25135/jcm.2311.2949
Available online: January 11, 2024

17]. However, there is currently no reported method for conducting an accelerated stability study and a stability-indicating study using the Indian Pharmacopeial method. The exact purpose of an accelerated stability study is to determine the shelf life and appropriate packaging for the drug product. Numerous articles have highlighted the instability of Brinzolamide suspension [18–21]. Hence, there is a need to investigate the accelerated stability of Brinzolamide. International Council for Harmonization (ICH) guidelines suggest stress degradation studies for new drug substances and products to understand the stability characteristics of the active ingredient. Methods which can separate the drug from its degradants are considered suitable for drug estimation. Therefore, it is imperative to explore the forced degradation of Brinzolamide using various degradation techniques such as acid hydrolysis, alkaline hydrolysis, oxidative degradation, UV degradation, and photodegradation. In this study, a validated forced degradation method demonstrates the applicability of an RP-HPLC method to quantify Brinzolamide in the presence of its degradants from three different brands. The proposed method offers simplicity, accuracy, repeatability, and stability, making it suitable for regular determination of Brinzolamide content in dosage forms. Furthermore, this procedure has been shown to comply with the ICH regulations. Our study addresses a significant need in the pharmaceutical industry as it provides a reliable analytical method for the estimation and evaluation of Brinzolamide stability in marketed ophthalmic formulations. The findings have direct implications for the quality control and shelf-life determination of Brinzolamide products, ensuring their efficacy and safety for patients with ocular conditions.

2. Experimental

2.1. Materials and Methods

A generous sample of Brinzolamide (BZA) (Purity: 99.75%) was provided by Sun Pharmaceutical Industries Ltd. located in Vadodara, India. Acetonitrile (HPLC Grade) was procured from Merck chemicals (Purity: 99.9%). Milli-Q water (HPLC grade) was obtained from the Millipore water system at Ramanbhai Patel College of Pharmacy, Charusat, India. Orthophosphoric acid (Purity: 85%) and triethylamine (Purity: 99.5%), both of HPLC grade, were purchased from Loba Chemie Pvt. Ltd. Moreover, Hydrochloric acid (Purity: 37%), Sodium hydroxide (Purity: 98%), and hydrogen peroxide (Purity: 35%) (AR grade) were acquired from the same supplier. The commercially available ophthalmic formulations of BZA, namely Azopt, Brinzox, and Brinolar, containing 1% w/v of BZA, were purchased from a local pharmacy. These formulations were utilized for the analysis of the marketed products and for the accelerated stability study. The chromatographic study was conducted using an automatic Shimadzu Model LC 2010HT liquid chromatography system equipped with UV and PDA detectors, controlled by Class LC Solution software (Supplier: Spinco Biotech). Column with length and width of 250 × 4.6 mm having particle size of 5 µm (Phenomenex (C18)) was employed for the analysis. In addition to the chromatography system, various equipment and instruments were employed, including a Frontier Ultrasonic Cleaner Sonicator, a Mettler-Toledo digital pH meter, a Vijay Scientific hot air oven, and a ThermoLab stability chamber, to facilitate the experimental procedures.

2.2. Mobile Phase and Stock Solutions Preparation

The mobile phase was prepared by adding 2.6 mL of triethylamine to 650 mL of Milli-Q water and adjusting the pH to 3.0 using orthophosphoric acid. Subsequently, 350 mL of acetonitrile was added to the mixture. The resulting mobile phase was subjected to 30 minutes of degassing using a Sonicator and then filtered through a vacuum filtration assembly equipped with a 0.22-micron-pore-size membrane filter.

For the preparation of a 1000 µg/mL Brinzolamide (BZA) solution, precisely 100 mg of BZA was weighed and transferred to a 100 mL volumetric flask. The BZA was dissolved in acetonitrile, and the volume was made 100mL with acetonitrile. To prepare, 100 µg/mL of BZA, 10 mL of the Brinzolamide standard stock solution was taken to a 100 mL volumetric flask and diluted with acetonitrile up to 100mL.

The chromatographic analysis was performed using a Phenomenex (C18) column with a particle size of 5 µm. The flow rate was kept at 1 mL/min, and the UV detector was set at a wavelength of 254 nm. The mobile phase composition consisted of a mixture of acetonitrile (HPLC Grade) and water in a ratio of 35:65 (v/v), and the pH maintained to 3.0 using orthophosphoric acid.

2.3 Method Validation

To meet ICH and USP requirements, the analysis method was tested for robustness, accuracy, linearity, precision, detection limit, and quantitation limit. System suitability testing ensured that the RP-HPLC system could be replicated accurately. A single set of 10 µg/mL standard solution and six injections of standard preparation into liquid chromatography were used to verify the system's repeatability and establish its performance. Table 1 displays the outcomes. The specificity of the analytical technique was validated by testing for analyte detection in the presence of expected interference in the formulation.

Table 1. Summary of system suitability parameters

Parameters	**Result**	
	MEAN ± SD *(n=6)*	**%RSD**
Area	392967.5 ± 886.6	0.22
Theoretical plate	5797.24 ± 92.20	1.59
Tailing factor	1.3 ±0.01	1.35

Calibration standards ranging from 5 to 30 µg/mL of Brinzolamide (BZA) were prepared by accurately transferring appropriate volumes from a 100 µg/mL BZA standard stock solution into 10 mL volumetric flasks. The flasks were then filled to volume with the mobile phase. Prior to analysis, each solution was filtered using a 0.22-micron syringe filter and transferred to respective vials. The vials were then placed in the system, and chromatograms were acquired. The peak areas of the BZA peaks were correlated with their respective concentrations to construct a calibration curve. The linearity range was established in a range of 5-30 µg/mL for BZA. The results of the linearity analysis can be found in Table 2. Additionally, an overlay of the HPLC chromatograms representing the linearity data for BZA is displayed in Figure S2 in supporting information.

Table 2. Calibration curve data for BZA (5 to 30µg/mL) at 254 nm (n=3)

Sr no.	**Brinzolamide**		
	Concentration in µg/mL	**Mean Peak Area (±) SD**	**% RSD**
1	5	195179 ±611.34	0.31
2	10	392803 ±1276.91	0.32
3	15	603273 ±3609.36	0.59
4	20	799412 ±1039.248	0.13
5	25	1092574 ±14150.34	1.29
6	30	1293152 ±19023.02	1.47
Linearity Equation		y = 44488x – 49133	
		R^2 = 0.996	

To assess the precision of the method, Brinzolamide (BZA) was tested at three levels of concentrations (5 µg/mL, 15 µg/mL, and 30 µg/mL) on the same day, allowing for the determination of intraday precision. Similarly, to evaluate interday precision, the same three BZA concentrations were analyzed on three separate days. The limits of detection (LOD) were calculated using LOD = 3.3(SD)/S while Limit of quantitation (LOQ) was determined by LOQ = 10(SD)/S. In these equations, SD is standard deviation and S indicates the slope. Accuracy refers to the degree to which the test findings acquired by an analytical process come close to the actual value. The accuracy was evaluated using the standard spike method at three different levels ranging from 80 to 100 to 120 percent of the sample's working concentration. The preparation of each level was done in triplicate, and the injection of each preparation was also done in triplicate. The findings are presented in Table 3.

Adjusting the detection wavelength (254 nm ± 2 nm) while flow rate of mobile phase (1 mL/min ± 0.2 mL/min), and pH of mobile phase (pH 3 ± 0.2 pH) were used to assess the results' robustness. At one point in time, a single value was used to determine the impact of the parameter. Tabulated in Table 4 are the found robustness values.

Table 3. Accuracy data of BZA (n=3)

Level (%)	Sample conc. (µg/mL)	Std. Conc. (µg/mL)	Total conc. (µg/mL)	Peak Area	Amount recovered	Mean % Recovery ± SD	% RSD
80%	10	8	18	779556 751419 767676	18.6 17.9 18.3	101.7% ± 0.55	0.54
100%	10	10	20	829479 832422 831477	19.7 19.8 19.7	98.3% % ± 0.28	0.2
120%	10	12	22	944529 943700 933170	22.33 22.31 22.08	101.08% ± 0.63	0.6

Table 4. Robustness data of BZA (n=3)

Parameter	Changed condition	Mean ± SD	%RSD
Wavelength(nm) 254 ± 2nm	252 nm	839537.3 ± 4347.3	0.52
	254 nm	840888.7 ± 878.4	0.1
	256 nm	843363 ± 5273.7	0.6
Flow Rate in mL/min (1 ± 0.2)	0.8 mL/min	837247.3 ± 1929.8	0.2
	1 mL/min	840888.7 ± 878.4	0.1
	1.2 mL/min	835881.3 ± 1980.6	0.2
pH of Mobile phase (pH 3 ± 0.2 pH)	2.8 pH	839812.7 ±1023.4	0.12
	3 pH	840888.7 ± 878.4	0.1
	3.2 pH	837211 ± 1739.2	0.2

In order to determine the assay of the pharmaceutical dosage form containing Brinzolamide (BZA), a suspension containing an equivalent amount of 10 mg of the drug was transferred to a 10 mL volumetric flask. Acetonitrile was added to achieve the desired volume, and the resulting mixture was subjected to sonication for a duration of 5 minutes. Subsequently, the solution was filtered using a syringe filter with a pore size of 0.22 microns (1000 µg/mL). From the prepared solution, 1 mL was accurately measured and transferred to a 10 mL volumetric flask, followed by adjustment of the volume with acetonitrile. This step resulted in the final solution with a concentration of 100 µg/mL of BZA. Further, 1 mL of this solution was transferred to a 10 mL volumetric flask, and the volume was adjusted with mobile phase. For the subsequent chromatographic analysis, 20 µL of the solution was injected into the system, and the percentage assay was determined by calculating the response using a linear equation. Detailed results of the analysis can be found in Table 5.

Table 5. Assay data of BZA

Drug (Brand name)	Conc. (µg/mL)	Area	Assay	%Assay ± * SD	% RSD
Brinzolamide (Brinzox)	10	401259	10.12	100.2% ± 1.10	1.09 %
	10	394889	9.9		
	10	399504	10.08		

2.4. Accelerated Stability Study

This study was performed on three different brands of ophthalmic suspension which contain BZA (1%w/v). Accelerated stability studies should be conducted on the eye drop packed in closed container. Three different brands (Brinzox, Brinolar and Azopt) formulations were kept at 40^0 C ± 2^0 C/75% RH ± 5% RH in stability chamber for storage and tested every week up to 12 weeks (3 Months). The time and concentration of the active ingredient studies at 40^0C temperature for 3 months.

2.5. Forced Degradation Studies

For the forced degradation studies of Brinzolamide (BZA), various degradation conditions including hydrolysis, oxidation, photolysis (UV light), and thermal degradation were employed. To initiate the experiments, 10 mg of BZA was precisely weighed and added to 100 mL volumetric flasks. In these flasks acetonitrile was added, to make final concentration of 100 µg/mL. This solution served as stock solution as the starting point for investigating the effects of forced degradation.

To evaluate acid degradation, 1 mL of a 100 µg/mL BZA standard stock solution was added to a 10 mL volumetric flask, later addition of 1 mL of 0.1N HCl. This flask was kept at room temperature for three hours to allow for acid degradation. Subsequently, 1 mL of freshly prepared 0.1N NaOH was introduced to the mixture to neutralize it and terminate the process. The resulting solution was then diluted with mobile phase upto 10mL and subjected to analysis using a liquid chromatography system.

In a comparable manner, the base degradation experiment was carried out by transferring 1 mL of a 100 µg/mL BZA standard stock solution to a 10 mL volumetric flask. followed by the addition of 1 mL of 0.1N NaOH. The flask was left at room temperature for one hour to allow for base degradation. After the designated time, for neutralization, 1 mL of 0.1N HCl was added to complete the procedure. The acid-degraded sample was subsequently diluted with mobile phase to make a final volume of 10 mL and analyzed using a liquid chromatographic device.

For oxidation degradation, a 10 mL volumetric flask was utilized, and 1 mL of a 100 mg/mL BZA standard stock solution was added. Then the flask was kept at room temperature for five hours. Following acid degradation, solution was diluted to 10 mL with the mobile phase and injected into a liquid chromatographic apparatus.

To assess photolytic breakdown, 100 mg of Brinzolamide powder was placed in a petri dish and exposed to light for six hours using a UV light source. The resulting solution, with a concentration of 10 µg/mL, was utilized for the subsequent experiments. After photodegradation, the solution was injected into a liquid chromatography system for analysis.

Thermal degradation involved weighing 100 mg of Brinzolamide powder and placing it in a petri dish, which was then subjected to a hot air oven at 70°C for six hours. The resulting solution, which underwent thermal stress for the specified duration, was used to prepare a 10 µg/mL concentration for the experiments. The thermal degradation solution was subsequently injected into the liquid chromatography apparatus.

To analyze the BZA solution that had not undergone degradation, a sample containing 10 µg/mL of BZA was injected into an optimized high-performance liquid chromatography (HPLC) system. The chromatographic analysis was performed using the appropriate parameters and conditions.

3. Results and discussion

3.1. Chromatographic Separation

After optimization, the ideal conditions for the analysis of Brinzolamide (BZA) were determined. The mobile phase consisting of acetonitrile and water in a ratio of 65:35, v/v, with a pH of 3.0 adjusted using orthophosphoric acid, exhibited excellent performance. It was found that 1 mL/min flow rate was suitable for achieving well-defined peaks with minimal tailing of BZA which shown in Figure 1.

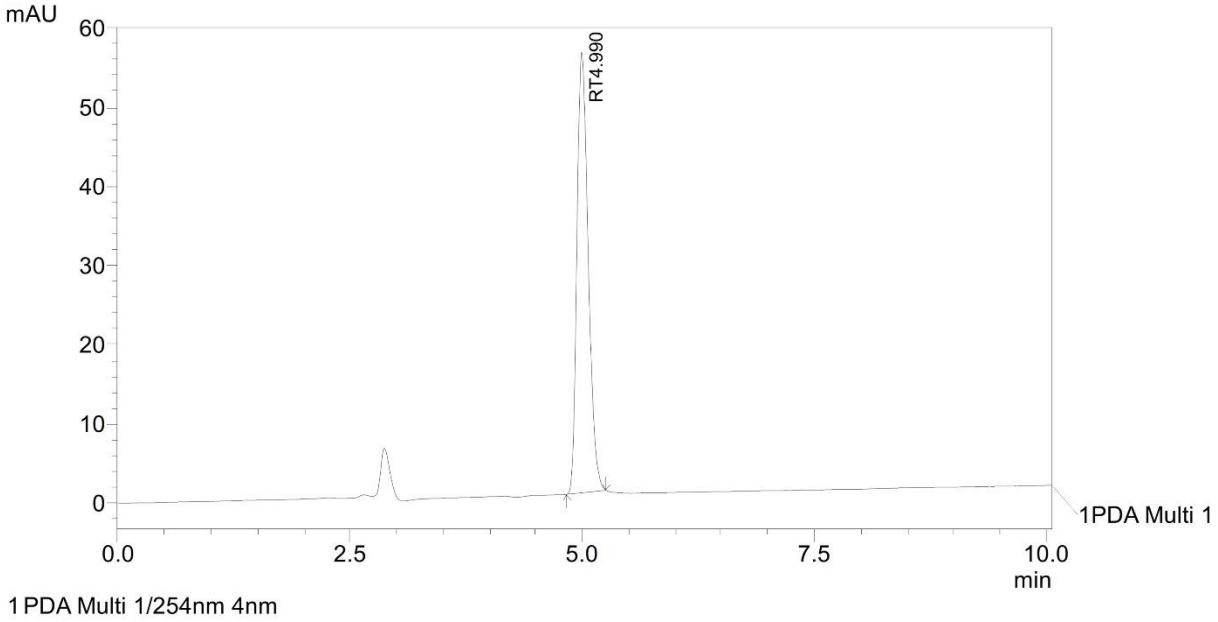

Figure 1. Chromatogram of Brinzolamide (10 µg/mL)

It was determined that the retention time for BZA was 4.9 minutes. The UV overlay spectra of BZA revealed good absorbance at 254 nm, thus it was chosen as the detection wavelength. Additionally, it was found that the developed procedure was very specific. Within the range of 5-30 g/mL, it was found that the BZA calibration curve follows a linear pattern. Peak purity analysis displayed in Figure S3 in supporting information. Table 2 summarizes the results of a regression analysis performed on the data from the calibration curves.

The limit of detection (LOD) for Brinzolamide (BZA) was determined to be 0.22 µg/mL, while the limit of quantitation (LOQ) was 0.67 µg/mL. The robustness data, demonstrating the system's ability to withstand small variations in method parameters, are presented in Table 4. Additionally, a comprehensive summary of the validation results for the parameters mentioned can be found in Table 6.

Table 6. Summarize data of method validation

Sr No.	Parameters	Results
1	Purity index	0.9998
2	Linearity range (µg/mL)	5 µg/mL – 30 µg/mL
3	Co-relation coefficient (R2)	$R^2 = 0.996$
4	Regression Equation	y = 44488x - 49133
5	LoD (µg/mL)	0.22
6	LoQ (µg/mL)	0.67
7	Intraday Precision (% RSD NMT 2)	0.3 – 0.5
8	Interday Precision (%RSD) (n = 3)	0.6 - 0.8
9	Accuracy (%Recovery) (n=3)	98.3 % – 101.08 %
10	Robustness	Robust
11	% Assay	100.2 %

Following a rigorous accelerated stability study conducted at a temperature of 40 ℃ ± 20 ℃ and relative humidity (RH) of 75% ± 5% in a stability chamber for a duration of three months, no observable alterations were observed in the physical characteristics or pH values of the three formulations under investigation. Based on these findings, it can be concluded that all three formulations exhibit stability throughout their anticipated shelf life. A comprehensive summary of the results obtained from the accelerated stability testing can be found in Table 7.

Table 7. Summary of accelerated stability study for azopt, brinolar and brinzox

Test	Result		
	Azopt	**Azopt**	**Azopt**
Color	White color	White color	White color
Clearance	Free from Particle/ Impurities	Free from Particle/ Impurities	Free from Particle/ Impurities
pH	6.9	6.9	6.9
Initial content	99.4 %	99.4 %	99.4 %
After 3 months content	98.2 %	98.2 %	98.2 %

Brinzolamide (BZA) demonstrated sensitivity to various stress conditions, including acids, bases, hydrogen peroxide, heat, and UV light, with the most significant deterioration observed in acidic and photolytic environments. Under the influences of acid hydrolysis, base hydrolysis, and oxidation, BZA underwent degradation and transformed into degradation products. During thermal degradation, two additional peaks corresponding to degradants were observed in the chromatogram. The chromatogram of acid degraded BZA exhibited an extra signal at Rt 7.4 (displayed in Figure S4 in supporting information). Similarly, the base degraded BZA chromatogram displayed an additional peak at Rt 5.8 (Shown in Figure S5 in supporting information). The oxidatively degraded BZA chromatogram exhibited an extra signal at Rt 7.4 (refer to Figure S6 in supporting information). In the case of photolytic degradation, a decrease in peak areas was observed compared to non-degraded samples, although no additional degradation peaks were observed (represented in Figure S7 in supporting information). Thermal degradation resulted in the appearance of two more peaks at Rt 3.8 and 5.9 minutes, respectively (represented in Figure S8 in supporting information). By comparing the peak areas of the drug in non-degraded conditions with those of the degraded peaks in all degradation conditions, the percentage rate of degradation was calculated. The comprehensive findings of the degradation experiments are summarized in Table 8.

Table 8. Summary of force degradation study for BZA

Type of Degradation	Stress Condition	Brinzolamide			
		Time (hours)	**Area**	**Rt (min) of degradation products**	**% Degradation**
Acid Degradation	0.1 N HCl (Room Temperature)	3 h	363901	7.4 min	11.3 %
Base Degradation	0.1 N NaOH (Room Temperature)	1 h	384889	5.8 min	6.19 %
Oxidation Degradation	3 % H_2O_2 (Room Temperature)	5 h	389218	7.4 min	5.1 %
Photolytic Degradation	UV Light	6 h	339920	-	17.1 %
Thermal Degradation	70^0 C (Hot Air Oven)	6 h	370190	3.8 min and 5.9 min	9.7 %

4. Conclusions

The primary objective of this research project is to establish and validate a stability-indicating high-performance liquid chromatography (HPLC) method that ensures accurate quantification of Brinzolamide (BZA), adhering to the guidelines given by the International Council for Harmonisation for Pharmaceuticals for Human Use (ICH). Through comprehensive statistical analysis, the method has been assessed for its accuracy, precision, robustness, and repeatability. Moreover, the developed method has demonstrated its simplicity, specificity, sensitivity, and selectivity in the analysis of BZA in ocular preparations. The obtained assay results reveal a BZA concentration of 100.2% ± 1.10% in the ocular dosage form.

Table 9. Comparison of method validation with reported method

Sr No.	Parameters	Results	Reported method [12]
1	Purity index	0.9998	-
2	Linearity range (µg/mL)	5 µg/mL – 30 µg/mL	50-1600
3	Co-relation coefficient (R2)	$R^2 = 0.996$	0.993
4	Regression Equation	y = 44488x - 49133	y=51.234x+978.3
5	LoD (µg/mL)	0.22	6.06
6	LoQ (µg/mL)	0.67	18.38
7	Intraday Precision (% RSD NMT 2)	0.3 – 0.5	0.26
8	Interday Precision (%RSD) (n = 3)	0.6 - 0.8	0.45
9	Accuracy (%Recovery) (n=3)	98.3 % – 101.08 %	99.84%-100.62%
10	Robustness	Robust	Robust
11	% Assay	100.2 %	99.7%
12	Mobile Phase	Acetonitrile and water	Phosphate buffer and methanol

The presented methodology proves to be more apt for the routine analysis of brinzolamide in isolation, as well as in the presence of its degradants, in comparison to previously documented approaches. Table 9 provides a comprehensive comparison of diverse parameters between the developed method and the previously reported technique. This study extends its application to stability assessment, while the reported method focuses on bulk drug determination with an emphasis on validation[22]. The results indicate that the developed method exhibits greater sensitivity, enabling detection and quantification at the lowest levels. Additionally, the proposed method is characterized by its simplicity and cost-effectiveness, achieved through the utilization of a mobile phase consisting of acetonitrile and water for analyte separation.

In terms of stability assessment, this study provides further evidence supporting the hypothesis that Azopt, Brinolar, and Brinzox, which are all BZA products, remain stable under accelerated storage conditions for a period of up to 120 days. The developed method proved to be suitable for analyzing the stability of BZA under various forced degradation conditions, including acidic, basic, photolytic, oxidative, and thermal conditions. However, it is important to note that no characterization of the degradation products was performed in this study.

Acknowledgements

The authors express their gratitude to Sun Pharmaceuticals Industries Ltd, Vadodara, for generously providing the gift samples of Brinzolamide (BZA) for the purpose of conducting this research. Additionally, the authors extend their sincere appreciation to Dr. Manan Raval, Principal of Ramanbhai Patel College of Pharmacy, CHARUSAT Campus, Anand (India), for facilitating the necessary resources and support to successfully carry out the research work on BZA.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ journal-of-chemical-metrology

ORCID
Mehul Patel: 0000-0002-2405-0243
Ritu Dwivedi: 0009-0004-2088-0022
Umang Shah: 0000-0001-6748-2137
Heta Kachhiya:0000-0003-4534-6243

References

[1] R. Hall, G. Havner, J. Baker, G. Stafford, W. Schneider, W.-Y. Lin, J. May, M. Curtis, C. Struble, B. McCue, D. Jasheway and D. McGee (1999). Brinzolamide, in: H.G. Brittain (Ed.), Analytical profiles of drug substances and excipients, *Academic Press* **26,** 47–96.

[2] M. Iester (2008). Brinzolamide ophthalmic suspension: a review of its pharmacology and use in the treatment of open angle glaucoma and ocular hypertension, *Clin Ophthalmol.* **2**, 517–523.

[3] Y. Shen, J. Sun and X. Sun (2023). Intraocular nano-microscale drug delivery systems for glaucoma treatment: design strategies and recent progress, *J. Nanobiotechnol.* **21**,1-27.

[4] L.M. C De Guzman, G.Q. De Guzman and E.C. Borromeo (2022). Brinzolamide-loaded soft contact lens for ophthalmic delivery, *Ther Deliv*. **13**, 233–247.

[5] X. Chen and T. Shen (2023). Advances in innovative delivery systems for antiglaucoma drugs, *Curr Opin Ophthalmol.* **34**, 123-128.

[6] J. Välimäki, P. Nukareddy, H. Uusitalo and U. Aapola (2022). Intraluminal deposits: A rare cause of glaucoma drainage implant total obstruction, *Eur. J. Ophthalmol.* **33**, 307–311.

[7] S. Kumar, S. Rulhania, S. Jaswal and V. Monga (2021). Recent advances in the medicinal chemistry of carbonic anhydrase inhibitors, *Eur. J. Med. Chem.* **209**, 112923.

[8] A. Erras, B. Shahrvini, R.N. Weinreb and S.L. Baxter (2023). Review of glaucoma medication adherence monitoring in the digital health era, *Br. J. Ophthalmol.* **107**, 153-159.

[9] H. Onoe, K. Hirooka, M. Nagayama, H. Mochizuki, A. Hirota, K. Suzuki, T. Sagara and Y. Kiuchi (2022). The efficacy, safety, and satisfaction associated with switching from brinzolamide or brimonidine to brinzolamide/brimonidine in open-angle glaucoma patients, *J. Pers. Med.* **12**, 1-7.

[10] Y. Sharma, K. Chahar, L. Mishra, L. Kumari, A. Singla, P. Patel, D. Singh and B. Das Kurmi (2023). Recent overviews on the drug delivery aspects and applications of brinzolamide for the management of glaucoma, *Health Sci. Rev.* **6**, 100083. 1-9.

[11] A.F. Lo Faro, A. Tini, M. Gottardi, F. Pirani, A. Sirignano, R. Giorgetti and F.P. Busardò (2021). Development and validation of a fast ultra-high-performance liquid chromatography tandem mass spectrometry method for determining carbonic anhydrase inhibitors and their metabolites in urine and hair, *Drug Test. Anal.* **13**, 1552–1560.

[12] J.R. Christian, K. Patel and T.R. Gandhi (2016). Validation and experimental design assisted robustness testing of RPLC method for the simultaneous analysis of brinzolamide and brimonidine tartrate in an ophthalmic dosage form, *Indian J. Pharm. Sci.* **78** (**5**), 631-640.

[13] M.S. Eissa, I.M. Nour, M.R. Elghobashy and M.A. Shehata (2017). Validated TLC-densitometry method for simultaneous determination of brinzolamide and timolol in their ophthalmic preparation, *Anal. Chem. Lett.* **7**, 805–812.

[14] M. Walash and R. El-Shaheny (2016). Fast separation and quantification of three anti-glaucoma drugs by high-performance liquid chromatography UV detection, *J. Food Drug Anal.* **24**, 441–449.

[15] A.A. Mandour, N. Nabil, H.E. Zaazaa and M. Abdelkawy (2020). Review on analytical studies of some pharmaceutical compounds containing heterocyclic rings: brinzolamide, timolol maleate, flumethasone pivalate, and clioquinol, *Future J. Pharm .Sci.* **6** (**52**), 1-10. Doi:10.1186/s43094-020-00068-4.

[16] M. Damle, K. Bothara, A. Khodke and L. Potale (2010). A validated stability indicating HPTLC method for simultaneous estimation of irbesartan and hydrochlorothiazide, *Pharm Method.* **1**, 39-43.

[17] B. Tiwari, M.K. Shirsat and A. Kulkarni (2020). Analytical method development and validation for the determination of Brinzolamide by RP-HPLC, *J. Drug Deliv. Therapeut.* **10**, 92–96.

[18] A.A. Mandour, N. Nabil, H.E. Zaazaa, M.M. Ibrahim and M.A. Ibrahim (2023). Two stability indicating chromatographic methods: TLC densitometric versus HPLC method for the simultaneous determination of brinzolamide and timolol maleate in ophthalmic formulation in the presence of probable carcinogenic oxidative degradation product of timolol maleate, *Separations* **10** (**37**), 1-14.

[19] C. Vishnuvardhan, D. Swain, R. Borkar, S. Gananadhamu, R. Srinivas and N. Satheeshkumar (2016). Study of forced degradation behaviour of brinzolamide using LC–ESI–Q-TOF and *In silico* toxicity prediction, *Chromatographia* **79**, 1293–1308.

[20] P.G. Firat, E. Samdanci, S. Doganay, M. Cavdar, N. Sahin and A. Gunduz (2012). Short-term effect of topical brinzolamide-timolol fixed combination on ocular surface of glaucoma patients, *Int. J. Ophthalmol.* **5**, 714–718.

[21] M.M. Mahboobian, A. Seyfoddin, I.D. Rupenthal, R. Aboofazeli and S.M. Foroutan (2017). Formulation development and evaluation of the therapeutic efficacy of brinzolamide containing nanoemulsions, *Iran J. Pharm. Res.* **16**, 847–857.

[22] B. Tiwari, M.K. Shirsat and A. Kulkarni (2020). Analytical method development and validation for the determination of Brinzolamide by RP-HPLC, *J. Drug Deliv. Ther.***10** (**1**),92-96.