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Method development and validation for simultaneous estimation of brinzolamide and brimonidine in pharmaceutical dosage form by using RP-HPLC

Nagaraju Pappula[1*], M Madhavi[2]
[1] Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India
[2] Department of Botany, Hindu College, Guntur, Andhra Pradesh, India

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Abstract
A high performance liquid chromatographic method was developed and validated for the simultaneous determination of brinzolamide and brimonidine by employing an isocratic RP-HPLC Kromasil C18 (4.6 x 250mm, 5µm) column resulted in an adequate separation for brinzolamide and brimonidine with retention time of 2.121 min and 2.801min respectively. Best resolution was achieved with the Perchloric acid (0.1%) and acetonitrile with ratio of (58:42) as mobile phase pumped at the flow rate of 1.0 ml/min with the detection wavelength of 251 nm. Regression coefficient for both brinzolamide and brimonidine was found to be 0.998 and 0.998 respectively indicating linearity within the concentration range. The validation parameters like linearity, precision, accuracy, limit of detection and limit of quantitation were also found to be suitable. The proposed method can hence be successfully applied to quantify brinzolamide and brimonidine during quality control of formulation.

Keywords: chromatographic, simultaneous determination brinzolamide, brimonidine

Introduction

Brinzolamide is a highly specific, non-competitive, reversible carbonic anhydrase inhibitor [1]. Carbonic anhydrase (CA) is an enzyme found in many tissues of the body including the eye. It catalyzes the reversible reaction involving the hydration of carbon dioxide and the dehydration of carbonic acid. In humans, carbonic anhydrase exists as a number of isoenzymes, the most active being carbonic anhydrase [2] II (CA-II). 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. The result is a reduction in intraocular pressure. Brinzolamide is indicated in the treatment of elevated intraocular pressure[3] in patients with ocular hypertension or open-angle glaucoma. Structurally has (6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-[(Carboxymethoxy) imino]acetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid and molecular weight is 453.45. It is soluble in water and DMSO (figure-1).

Brimonidine is an alpha-adrenergic [4] agonist and 2-imidazoline derivative that was first introduced in 1996. It is considered to be a third generation alpha-2 adrenergic receptor agonist, since it displays preferential binding at alpha-2 Adrenoreceptors over alpha-1 receptors. Chemically 5-Bromo-N-(4, 5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine tartrate and molecular weight 442.22. It displays a higher selectivity toward the alpha-2 adrenergic receptors than clonidine or apraclonidine, which are also alpha-2 adrenergic agonists. Early treatment and management of glaucoma, which predominantly involves the lowering of intraocular pressure, is critical since glaucoma is considered to be a common cause of blindness worldwide. Ophthalmically, brimonidine is used to lower intraocular pressure by reducing aqueous humor production and increasing uveoscleral outflow. Because it is oxidately stable, brimonidine is associated with fewer reports of ocular allergic reactions compared to other alpha-2 adrenergic agonists. Brimonidine mediates vasoconstrictive effects and it was shown to exhibit anti-inflammatory [5] properties in *ex vivo* human skin model and *in vivo* inflammation models. Brimonidine is reported to be metabolized in the cornea. Brimonidine that reaches the systemic circulation upon topical administration undergoes extensive hepatic metabolism mediated by hepatic aldehyde oxidases [6] and the systemic half-life was approximately 3 hours (figure-2).

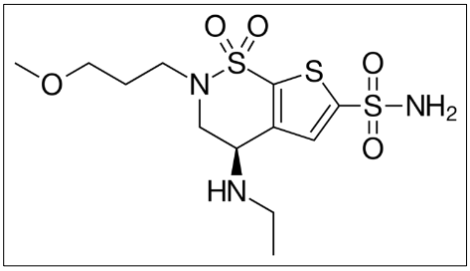

Fig 1: Chemical structure of Brinzolamide

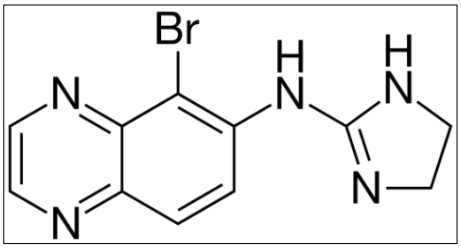

Fig 2: Chemical structure of Brimonidine

Literature survey revealed a few HPLC methods for the estimation of brinzolamide and brimonidine [7, 9]. Also some HPLC methods are available for the determination of brinzolamide and Timolol [10, 12]. Similarly some HPLC methods are reported for the estimation of brimonidine tartrate and timolol maleate [13, 14].Based on results only few works were done on brinzolamide and brimonidine and we plan to develop new simultaneous estimation of both drugs bulk and dosage forms by HPLC method.

Material and Methods

Quantitative simultaneous estimation of brinzolamide and brimonidine was done by using an isocratic Shimadzu HPLC instrument on Kromasil C18 (4.6 x 250mm, 5µm) column. The Instrument is equipped with binary pump and variable wavelength PDA detector. A 20µL Hamilton syringe was used for injecting the samples. Shimadzu UV-Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials. Brinzolamide and brimonidine pure drugs were provided by Rankem pharmaceuticals, Hyderabad. Optimised conditions: the mobile phase with 0.1% perchloric acid and Acetonitrile (58:42 %v/v) was employed in isocratic mode at a flow rate of 1.0 mL/min. The run time was 6 min and 20µL of the sample was injected for every run into the column at wavelength of 251nm.

Chromatographic Conditions

A mixture of 0.1% perchloric acid and Acetonitrile in the ratio of 58:42 %v/v was employed to be the most suitable mobile phase for identical chromatographic separation of Mucomelt Forte (Combination of Brinzolamide and Brimonidine). The solvent mixture was filtered through 0.45 µ membrane filter and sonicated to dissolve it completely. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20µL and the column was maintained at ambient temperature and run time was set to be 6 min and detected of the drug was monitored at wavelength of 251nm and retention time was to be 2.121 and 2.801 min.

Preparation of standard stock solution

Accurately weighed 16mg of Brinzolamide, 3.2mg of Brimonidine and transferred to 25ml volumetric flask and3/4 thof diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labelled as Standard stock solution. (640µg/ml of Brinzolamide and 128µg/ml of Brimonidine). 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (64µg/mlBrinzolamide of and 12.8µg/ml of Brimonidine)

Preparation of sample solution

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10)ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (640µg/ml of Brinzolamide and 128µg/ml of Brimonidine). 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (64µg/mlBrinzolamide of and 12.8µg/ml of Brimonidine)

Linearity

The calibration graphs shows that the linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrate that the methods have adequate sensitivity to the concentrations of the analyte. Several aliquots of standard solution of Ticagrelor was taken in different 10 mL volumetric flasks and diluted up to the mark with diluents such that the final concentration of Mucomelt Fortewas in the range 16-96 µg/mL for brinzolamide and 3.2-22.4 µg/mL for brimonidine. Evaluation of the drug was performed with UV detector at 251 nm; peak area was recorded for all the peaks. The correlation coefficient value of both brinzolamide and brimonidine was 0.9989. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated. The data is tabulated in table 1 and Figure 3 & 4.

System suitability

System suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

Accuracy

The recovery studies for the method were carried out by standard addition method. It was evaluated at three concentration levels (32, 64 and 96 µg/mL) and the percentage recoveries were calculated. The data is tabulated in table 2& 3 and figure 5, 6 &7.

Precision

The precision of the method was determined by intra and inter precision studies. This was evaluated by injecting three independent sample preparations of ticagrelor from a single formulation at three different concentration levels on the same day (Intraday) and on three different days (Inter day). The % RSD was then calculated. The data is represented in table 4 & 5 and figure 8 & 9.

Limit of detection and limit of quantification

LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve. The sensitivity of the method was established by the LOD and the LOQ values. Data is represented in table 6.

Robustness

Robustness was established by introducing small changes in the HPLC optimised conditions which include mobile phase, flow rate and temperature. Data is represented in table 7.

Results and Discussion

The proposed method was found to be simple. Six linear concentrations of Brinzolamide (16-96µg/ml) and Brimonidine (3.2-22.4µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for brinzolamide was y =53463x + 61322 and of brimonidine was y = 62525x + 21764 and correlation coefficient obtained was 0.9989 for the two drugs. System suitability parameters indicates high column efficiency with large number of theoretical plates (>2000).The tailing factors were found to be 1.04 and 1.34 which does not exceed the critical value 2.The average retention time of brinzolamide and brimonidine was found to be 2.121 and 2.801

min. No interference was seen from any of the components of the pharmaceutical dosage form indicating the specificity of the method. The % RSD was found to be 0.3 and 0.3 for intraday and 0.2 and 0.7 for inter day precision studies. Thus the method was found to be accurate and precise as the %RSD was not more than 2%. The limit of detection of brinzolamide and brimonidine were 0.05 & 0.04, limit of quantification for brinzolamide and brimonidine were found to be 0.15 and 0.11µg/mL respectively. The RSD for the % assay of sample was calculated for each parameter in robustness and was found to be less than 2% confirming the robustness of the method.

Conclusion

A validated RP-HPLC method was developed for the determination of brinzolamide and brimonidine in tablet dosage form and bulk forms. As the proposed method is simple, rapid, accurate, precise and specific it can be employed for the routine analysis of brinzolamide and brimonidine in pharmaceutical dosage forms.

Tables and Figures

Table1: Linearity values of both brinzolamide and brimonidine

Brinzolamide		Brimonidine	
Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area
16	917929	3.2	234004
32	1766764	6.4	413515
48	2676790	9.6	632851
64	3391238	12.8	798128
80	4389813	16	1020948
96	5189115	22.4	1432899

Table 2: Accuracy data of brinzolamide

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	32	31.752	99.22	99.16%
	32	31.581	98.69	
	32	31.844	99.51	
100%	64	64.088	100.14	
	64	64.028	100.04	
	64	63.822	99.72	
150%	96	94.220	98.15	
	96	94.365	98.30	
	96	94.701	98.65	

Table-3: Accuracy data of brimonidine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	6.4	6.453	100.82	99.43%
	6.4	6.419	100.30	
	6.4	6.350	99.22	
100%	12.8	12.58	98.25	
	12.8	12.75	99.59	
	12.8	12.55	98.05	
150%	22.4	22.21	99.14	
	22.4	22.23	99.24	
	22.4	22.46	100.26	

Table 4: Intraday precision (Repeatability) of brinzolamide

S. No	Area of Brinzolamide	Area of Brimonidine
1.	3286339	792187
2.	3294580	797745
3.	3265437	796380
4.	3280334	795969
5.	3274953	791564
6.	3290337	792160
Mean	3281997	794334
S.D	10708.5	2664.7
%RSD	0.3	0.3

Table 5: Intermediate precision of brimonidine

S. No	Area of Brinzolamide	Area of Brimonidine
1.	3309839	820216
2.	3308316	811974
3.	3299277	808454
4.	3313000	809483
5.	3322118	810069
6.	3301706	804178
Mean	3309043	810729
S.D	8209.5	5322.3
%RSD	0.2	0.7

Table 6: LOD & LOQ Values of Brinzolamide and Brimonidine

Molecule	LOD	LOQ
Brinzolamide	0.05	0.15
Brimonidine	0.04	0.11

Table 7: Robustness of Both Brinzolamide and Brimonidine

S. No.	Condition	%RSD of Brinzolamide	% RSD of Brimonidine
1	Flow rate (-) 1.1ml/min	0.0	1.0
2	Flow rate (+) 1.3ml/min	0.1	0.1
3	Mobile phase (-) 35B:65A	0.0	1.9
4	Mobile phase (+) 45B:55A	0.7	0.9
5	Temperature (-) 25°C	0.3	0.2
6	Temperature (+) 35°C	0.6	0.9

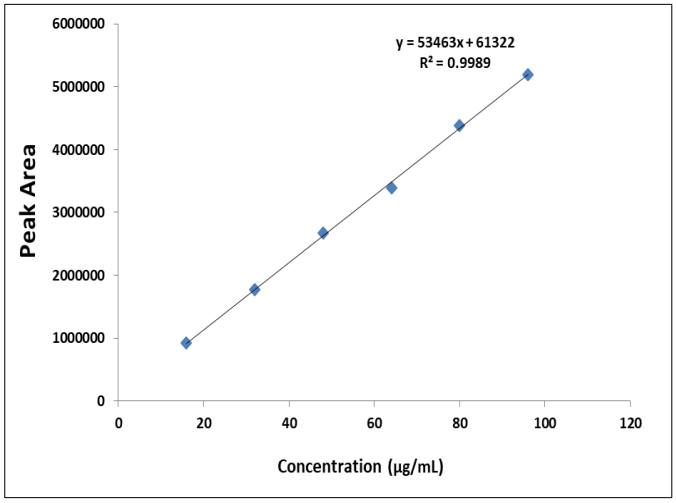

Fig 3: Calibration curve of Brinzolamide

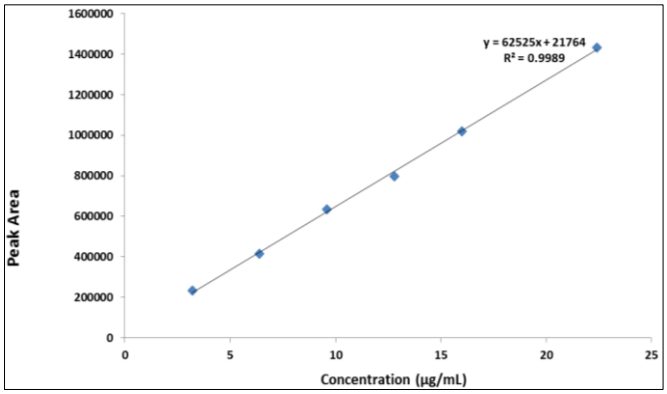

Fig 4: Calibration curve of Brimonidine

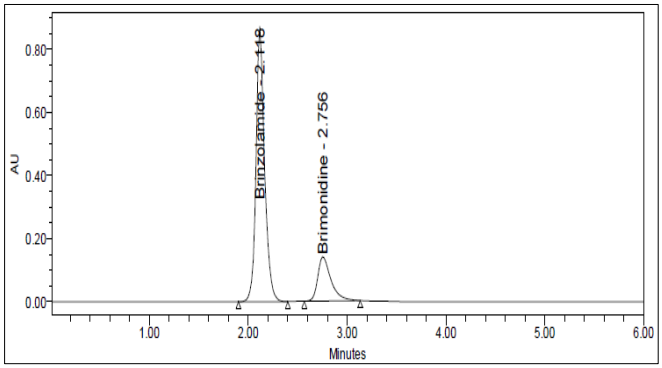

Fig 5: Accuracy 50% chromatogram of both brinzolamide and brimonidine

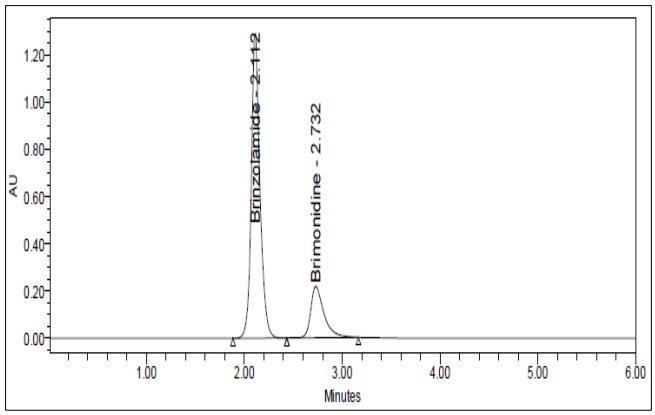

Fig 6: Accuracy 100% chromatogram of both brinzolamide and brimonidine

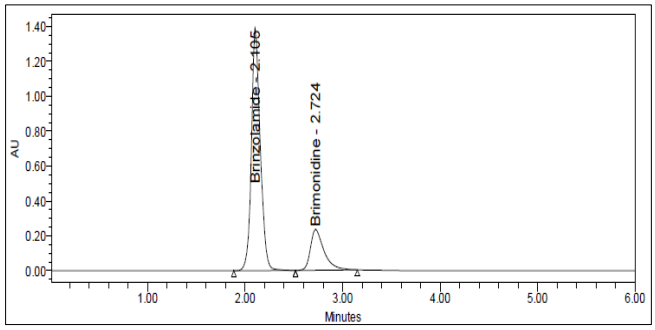

Fig 7: Accuracy 150% chromatogram of both brinzolamide and brimonidine

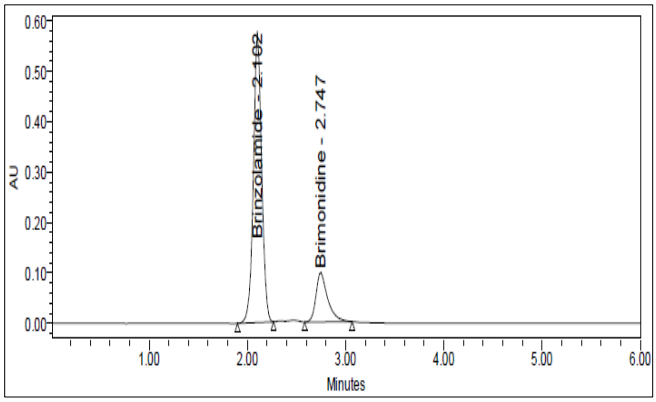

Fig 8: Repeatability of precision of both brinzolamide and brimonidine

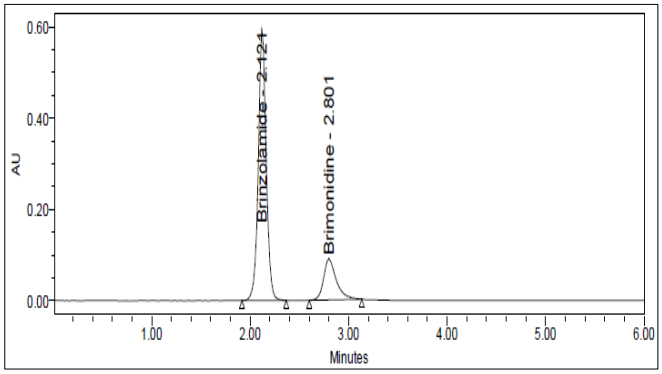

Fig 9: Intermediate Precision of Both Brinzolamide and Brimonidine

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