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Research Article

Stability indicating RP-HPLC Method for Simultaneous Estimation of Brinzolamide and Brimonidine in Pharmaceutical Dosage Forms

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ABSTRACT

For the simultaneous estimation of BRT and BRZ in their combined dosage form, a RP-HPLC method was developed. Discovery 150 column (Potassium phosphate, pH 3,0) and Buffer: mobile phase acetonitrile (45:55) with a flow rate of 1 ml / min were used to achieve separation. Detection at 225 nm has been performed. BRZ and BRT retention time were respectively 2.444 minutes and 4.518 minutes. The system for linearity, accuracy and accuracy has been validated. Linearity for BRT 0.5-3 µg/ml, and BRZ 3-15 µg/ml have been observed. The method for simultaneous evaluation of BRT and BRZ in the combined dosage form was found to be precise, accurate and rapid. The drug was susceptible to stress conditions such as hydrolysis, oxidation, photolization and thermal degradation. In order to test both drugs simultaneously in a blended commercial form, the proposed approach was successfully used.

1. Introduction

Glaucoma is an eye condition, in which intraocular pressure inside the eye is sufficient to cause optic nerve damage[1]. Brimonidine tartrate (BRT), quinoxaline L- tartrate, chemically is an α2-adrenoreceptor agonist, used to treat glaucoma[2]. This molecule's ocular hypotensive effect will decrease its aqueous humour output[3]. Brinzolamide (BRZ), chemically (R) – 4- (ethyl amino)-3,4-dihydro-2-(3-methoxy propyl)-2H thienol [3,2-e]- 1,2-thiazine-6 sulphonamide 1,1-dioxide, a non-competitive reversible carbonic anhydrase inhibitor is indicated for the treatment of elevated intraocular pressure in patients with glaucoma[4]. A mixture of BRT (0,2% w / w) and BRZ (1% w / w) for the treatment of glaucoma, available as a fixed dose Simbrinza ophthalmic Suppression.

Several analytical methods such as UV [5,6], RP-HPLC[7-9], HPTLC[10], UPLC[11], spectrofluorimetric[12], GC-MS[13], LC/MS/MS[14] and capillary electrophoresis methods[15] are reported for the determination of BRT alone. BRZ is official in IP[16] and USP[17]. Methods such as UV spectrophotometry[18], HPLC and HPTLC[19] are reported for simultaneous estimation of BRZ. Few UV derivative spectrophotometric methods[20-22] have been reported for the determination of BRT and BRZ. As far as we know, up to now no reversed high-performance chromatographic liquid methods for BRT and BRZ have been published. The present study aimed to build, optimize and validate, for the concurrent determination of BRT and BRZ, a simple, quick RP-HPLC process.

2. Materials & Methods

2.1. Chemicals:

Brimonidine tartrate and Brinzolamide, Combination of Brimonidine tartrate and Brinzolamide eye drops dosage forms (Simbrinza), distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, triethyl amine, ortho-phosphoric acid etc.

2.2. HPLC Instrumentation and Chromatographic Condition:

The Waters HPLC was used with an Auto Injector, PDA Detector HPLC 2965 and Empower 2 is the device used. A moving stage consisting of orthophosphoric acid (0.1 per cent) and acetonitrile was added for

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separation. It was screened in an ultrasonic bath for 10 minutes with 0.45 µ membrane filters. The buffer-acetonitrile ratio was 45:55. The column with a column oven (CTO-20AC) was held at 30 °C at a flow rate of 1.0 ml/min. The 10µl volume injected through PDA detector at 254 nm was recorded. The run time was 8 minutes. BRT average retention time is 2.444 minutes and the BRZ optimum is approximately 4.518 minutes. This is illustrated in **Figure 1**.

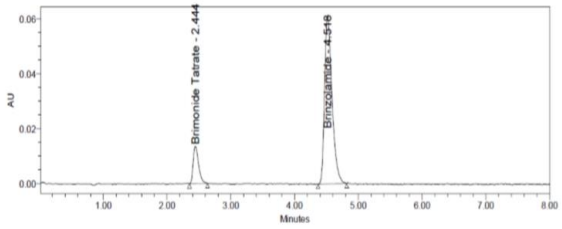

Fig 1. Optimized Chromatogram of BRT and BRZ

2.3. Method Validation

A new, sensitive, convenient method for simultaneous HPLC estimation of BRT and BRZ ophthalmic preparation was developed in order to obtain the present analytical method. The experimental process has been validated for parameters such as precision, device suitability, consistency, linearity, accuracy, robustness, according to the ICH-1996 and USP-30 guidelines.

System suitability

The suitability of the device was achieved through the injection of six regular replicates and two sample preparedness replicates at 100 % to check the chromatographic system's accuracy and precision. The study of retention time, tailoring factor, and theoretical plates in the column was carried out in this method and shown in **Table 1**.

Table 1: System suitability of Brimonidine tartrate& Brinzolamide

Parameter	BRT	BRZ
Retention time (RT)	2.444 ± 0.3 min	4.518 ±0.3min
Theoretical plates (N)	4398 ± 163.48	7301 ± 163.48
Tailing factor (T)	1.42± 0.117	1.43± 0.117

Specificity

The method's specificities were tested to make sure that excipients, BRT and BRZ diluent solution, do not intervene. The specificity of the BRT and BRZ was studied by injecting placebo, diluting and regular solution.

Linearity

By grafting the BRT and BRZ standard concentration vs. peak area and evaluating the correlation coefficients (R^2) of the two compounds, the linearity of the chromatographic procedure was established. BRT and BRZ standard solution were injectable to the HPLC system in a concentration level of 40, 60, 80, 100, 120 and 140 %. For both standard solutions, the response from the detector was linear from 40 to 140 % of the test concentration. The column was balanced with the mobile process at least 60 minutes before the solutions were injected. **Table 2** and **Figure 2 & 3** demonstrate the linearity curves of BRT and BRZ.

Table 2: Calibration data of Brimonidine and Brinzolamide

Concentration BRT (µg/ml)	Response	Concentration BRZ (µg/ml)	Response
0	0	0	0
0.5	17320	2.5	106300
1	32652	5	214999
1.5	48552	7.5	319015
2	62691	10	414098
2.5	79666	12.5	524927
3	97231	15	621417

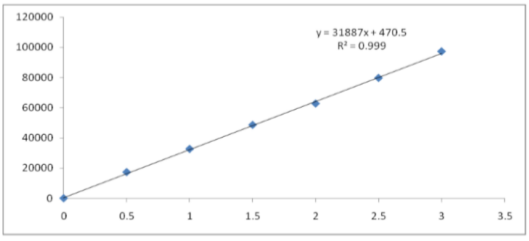

Fig 2. Linearity curve of Brimonidine

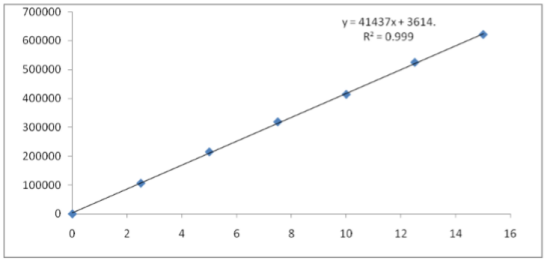

Fig 3. Linearity curve of Brinzolamide

Precision

The consistency of an analytical procedure is the extent to which the procedure is repeatedly applied to several samples between different testing outcomes. The accuracy of the test was tested for repetitiveness, reproducibility and intermediate accuracy through an estimate of the test in six separate sample preparations of the same batch. **Table 3** provide the statistical analysis for repeatability, intermediate accuracy and reproductivity of BRT and BRZ.

Table 3: Repeatability and Inter day precision results for Brimonidine and Brinzolamide

S.No	Repeatability		Inter day precision	
	BRT	BRZ	BRT	BRZ
1	62400	415956	61111	403813
2	62381	410651	61012	405886
3	62470	413431	61841	408739
4	62212	414650	61792	402934
5	62621	418334	61715	407917
6	62525	411666	61470	405145
Mean	62435	414115	61490	8782254
S.D	139.9	2826.3	357.1	2267.0
% RSD	0.2	0.7	0.6	0.6

Accuracy

The precision of the procedure is the proximity of the result to the true value. Recovery studies have identified the accuracy of the system. The restoration was performed by the addition of BRT and BRZ to the placebo (excipient blend) of test concentrations (40, 60, 80, 100, 120 and 140 %) and expressed in percent. For each recovery stage, three samples have been prepared. Recovery ranged from 99.46 to 100.38

percent and 99.34 to 100.35 percent for BRT and BRZ. **Table 4** shows the data.

Table 4: Accuracy results of Brimonidine and Brinzolamide

Sample	Amount added (µg/ml)	Recovery (%)	% RSD
BRT	1	101.17	0.31
	2	98.64	0.70
	3	100.58	0.43
BRZ	5	99.39	0.63
	10	98.28	0.13
	15	99.41	1.01

Robustness

The strength of an analytical method is a measure of its capacity to not be compromised by minor but deliberate variations in the parameters of the analytical procedure. For individual parameters, the standard deviation of the peak is measured, and the percent RSD is less than 2 percent. **Table 5** shows results.

Table 5: Robustness data of Brimonidine Tartrate & Brinzolamide

Condition	% RSD of BRT	%RSD of BRZ
Flow minus	1.0	1.1
Flow Plus	0.5	1.1
Mobile phase minus	0.5	0.9
Mobile phase Plus	0.6	0.1
Temperature minus	1.4	1.0
Temperature Plus	0.4	1.1

2.4. Degradation Studies

Acid Degradation Studies

1 ml of 2N hydrochloric acid was applied and refluxed for 30 minutes at 60 °C for 1 ml of the stock solution of BRT and BRZ. The resulting solution was diluted to achieve the solution of 2 µg/ml and 10 µl was injected into the device.

Alkali Degradation Studies

1 ml of 2N sodium hydroxide was applied and refluxed for 30 minutes with 60 °C in 1 ml of BRT and BRZ solutions. The resulting solution was injected into the system with 2 &10 µg/ml solution, and the chromatograms were injected into the system for sample stability evaluation.

Oxidation

One ml of hydrogen peroxide was added separately to 1 ml of BRT and BRZ stock solution. The solutions were held at 60 °C for 30 minutes. In the HPLC analysis, the resulting solution was diluted and the chromatograms were injecting 10 µl into the device to test the stability and obtain 2 &10 µg/ml solution.

Dry Heat Degradation Studies

Standard solution for the treatment of dry heat degradation was put in the oven at 105 °C for 6 h. In HPLC, a 2 & 10 µg/ml solution has been diluted and 10 µl into the device, and chromatograms have been reported to determine samples stability.

Photo Stability studies

The drug's photochemical stability was also measured in the exposure of 20 + 100 µg/ml to UV light by keeping the beaker in the UV Chamber in a photo stable chamber for 7 days or 200-watt hours/m^2. The resulting solution for an HPLC analysis was diluted to achieve 2 µg/mL and 10 µl was injected into the system and sample stability chromatograms were reported.

Neutral Degradation Studies

Stress checks were studied by refluxing the medication into water at a temperature of 60 °C for 6 hours in neutral conditions. The resulting solution for the HPLC analysis was diluted to 2 & 10 µg/ml solution and a 10 µl chromatogram was injected in the device to determine sample stability. The values were shown in **Table 6**.

Table 6: Degradation data

Type of Deg	Brimonidine			Brinzolamide		
	Area	%Rec	%Deg	Area	%Rec	%Deg
Acid	59788	95.30	4.70	395900	95.23	4.77
Base	61024	97.27	2.73	403947	97.17	2.83
Peroxide	61489	98.01	1.99	408006	98.14	1.86
Thermal	62281	99.27	0.73	412366	99.19	0.81
UV	62388	99.44	0.56	412900	99.32	0.68
Water	62250	99.44	0.56	412382	99.20	0.80

3. Results and Discussion

The procedure was validated in compliance with the directives of the ICH. The process was unique since the chromatogram of BRT and BRZ was not interfered with by excipients, diluting solution and impurity. The method demonstrates detector response linearity and generates the linears of 0.5–3 µg/ml for BRT (**Figure 1, Table 1**) and BRZ 2–15 µg/ml (**Figure 2, Table 1**). This method is the most popular and proved the results of their accuracy, and both compounds were less than 2 percent RSD percentage points, which is within a reasonable cap (**Table 3**). The findings for BRT and BRZ (**Tables 4, 5 & 6**) were also satisfactory for their accurate and robust assessment. This approach is therefore applicable simultaneously from its preparation for evaluating BRT and BRZ.

4. Conclusion

To simultaneously estimate the BRT and BRZ in ophthalmic solution form, a simple, accurate, precise method was developed. The retention time was 2.444 & 4.518 min and RSD for BRT & BRZ respectively and was found to be of 0.2 and 0.7. A 100.13 and 99.03 % recovery were achieved for BRT and BRZ. Percent recovery is achieved. LOD, LOQ values of 0.02, 0.07 and 0.05, 0.15 ppm, respectively, were derived from regression equation BRT and BRZ. Brimonidine's regression equal shall be y = 31877x+470.5 and y = 41437x + 3614 of BRZ. The retention periods are reduced, and the retention time has been reduced, making it easy and inexpensive for the process to be implemented in the industry in a standard quality management test.

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Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

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