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RP-HPLC Method Development and Validation for Estimation of Dorzolamide Hydrochloride (Carbonic Anhydrase Inhibitor) In Bulk Dosage Form

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Abstract

A sensitive accurate and fast reverse phase liquid chromatographic method with UV detection at 254nm for Dorzolamide hydrochloride was developed and validated by Isocratic method. Chromatographic separation was achieved on Kromasil C8 column (4.6 x 250mm,10um in particle size) Isocratic elution was carried out with mobile phase which composed of Acetonitrile and phosphate buffer pH.3.5 in the ratio (80:20 v/v) at a flow rate of 1.0ml/min. The method was validated for its linearity, accuracy, precision, specificity, and limit of quantification, limit of detection, robustness and ruggedness based on ICH guideline. The validation studies give satisfactory result. Proposed method has been applied for the quantification of Dorzolamide Hydrochloride in commercial samples. The λ max of Dorzolamide Hydrochloride in water was found to be 253.5nm and in artificial tear fluid was found to be 253.7nm. The retention time of Dorzolamide Hydrochloride were found to be 5.15 ± 0.02. The linearity of the method ranged between 50-250ppm. Correlation coefficient was found to be 0.995 and it is within the acceptance limit. The LOD was found to be 0.393ug/ml and LOQ was found to be 1.193ug/ml. Method was found to be reproducible with relative standard deviation (RSD) for intra and inter day precision less than 2%. Hence rapid and sensitive developed method can be applied for routine quality control analysis of Dorzolamide Hydrochloride in pharmaceutical dosage form.

Key word- *Dorzolamide hydrochloride, Isocratic elution, HPLC, Acetonitrile, phosphate buffer pH.3.5*

1. Introduction

Dorzolamide hydrochloride is a carbonic anhydrase inhibitor; used for the treatment of glaucoma and ocular hypertension [1].

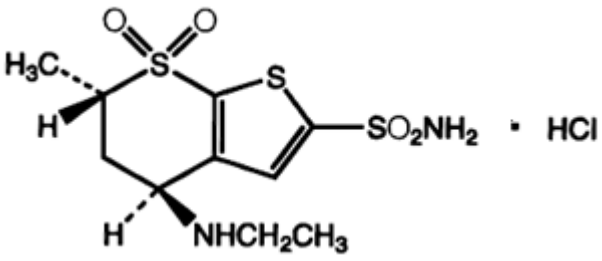

Fig.1 Chemical structure of Dorzolamide

A number of Analytical method like thin layer chromatography, UV/visible spectroscopy , high performance liquid chromatography , liquid chromatography–mass spectroscopy and capillary Electrophoresis have been reported in the literature for the study of Dorzolamide individually or combine with other drug [2]. Describe liquid chromatography methods for the determination of the drug in pharmaceutical dosage forms. The present study is aimed at developing and validating a fast, sensitive, and cost-effective method for the quantification of DZL in ophthalmic dosage form [3, 4,]. A number of Analytical method like thin layer chromatography, UV/visible spectroscopy , high performance liquid chromatography , liquid chromatography–mass spectroscopy and capillary Electrophoresis have been reported in the literature for the study of Dorzolamide individually or combine with other drug [5]. Describe liquid chromatography methods for the determination of the drug in pharmaceutical dosage forms. The present study is aimed at developing and validating a fast, sensitive, and cost-effective method for the quantification of DZL in ophthalmic dosage form [6, 7, and 8].

2. Material and method

2.1 Reagent and sample:

Analytical grade anhydrous disodium hydrogen phosphate (Na_2HPO_4), citric acid monohydrate, HPLC grade water, o-phosphoric acid. HPLC grade Acetonitrile and water. Pure active drug (Dorzolamide) was obtained from Kilipch health care india pvt, (Eye care) Mumbai.

2.2 Instrumental and chromatographic condition:

The analysis of drug was carried out with HPLC (Jasco; 2000 series) system equipped with a kromasil C8 column (4.6 X 250 mm, 10µm in particle size), a PU-2080 Plus isocratic pump, a 20µl injection loop and a UV-2075 Plus absorbance detector (λ=254 nm). Computerized data acquisition and treatment were performed with the Borwin solution Software. Isocratic elution was carried out with mobile phase which composed of Acetonitrile and Phosphate buffer pH 3.5 in the ratio (80:20 v/v) at a flow rate of 1.0 ml/min.

2.3 Preparation of Phosphate buffer pH 3.5:

Weighed quantity of anhydrous disodium hydrogen phosphate (Na_2HPO_4), 0.900 g and citric acid monohydrate 1.298 gm was dissolved in 1000 ml of HPLC grade water, mixed properly and pH was adjusted to 3.5 using o-phosphoric acid. The solution was filtered through 0.45µ membrane filter (USP 28).

2.4 Diluent:

Mixture Acetonitrile and phosphate buffer pH 3.5 in the ratio 20:80 v/v was used as diluent. Mobile phase was prepared freshly, filtered by membrane filter assembly (0.45 µ) and degassed by sonicating for 5 min before use (USP 28).

2.5 Preparation of stock and working standard solutions:

Accurately weighed 10 mg of Dorzolamide was added in 10 ml of HPLC grade methanol and sonicated until it completely dissolved and final volume was made up to suitably. Further 0.5ml of the above stock solution was pipette out into a 10ml volumetric flask and diluted up to the mark with HPLC grade methanol. This was mixed well and filtered through 0.45µm membrane filter assembly and finally dilutions of 100ppm, 150ppm, 200ppm, and 250ppm were prepared with the same diluent.

3. Results and discussion:

3.1 Analytical method development

3.1.1 Estimation of λ max:

Dorzolamide accurately weighed (10mg) and dissolved in 100 ml of methanol to prepare stock solution of 100 ppm. From stock solution 1ml aliquot was removed and further diluted up to 10ml in distilled water. UV spectrum was

recorded from 400 - 200 nm [6]. The λ max of drug in water was found to be 253.5 nm and in artificial tear fluid was found to be 253.7nm (Reported λ max 254nm) shown in Figure 02 and 03.

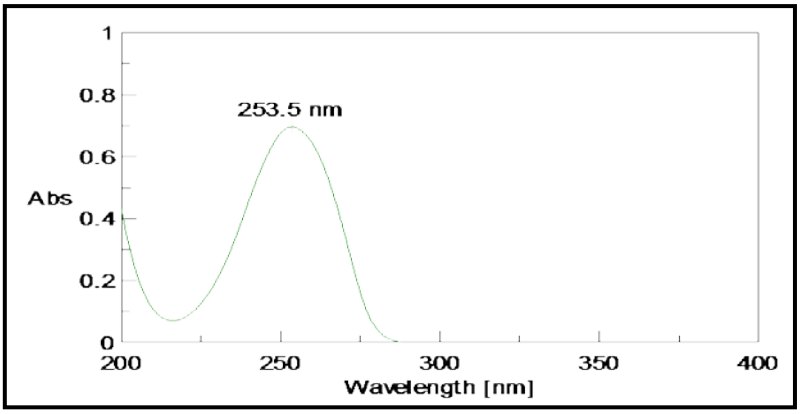

Fig. 2: λ max of Dorzolamide in Water

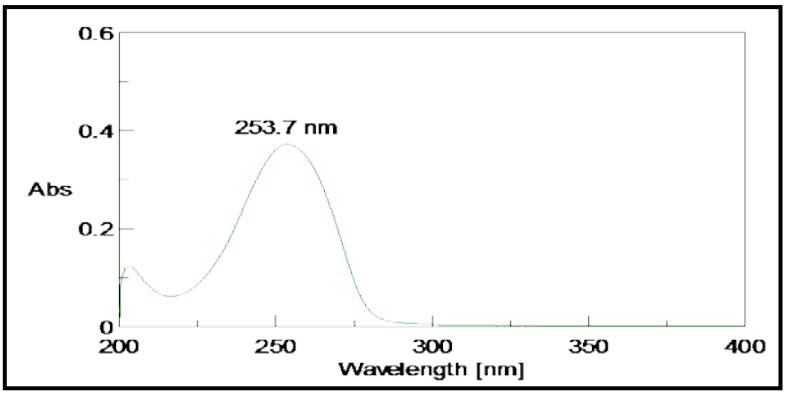

Fig. 3: λ max of Dorzolamide in ATF

3.1.2 Validation of HPLC Method Development: [9-16]

3.1.2.1 Linearity:

The typical chromatogram of Dorzolamide drug of one of the concentration (50 ppm) is shown in Figure 04 along with suitability parameters shown in Table -01

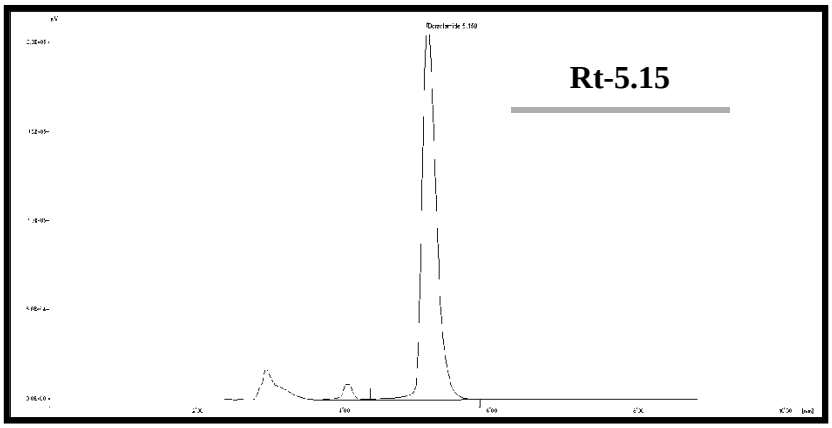

Fig.4: Typical chromatogram of Dorzolamide

Table 1: System suitability parameters of HPLC analysis

Parameters	**Values**
$\lambda_{max\ (nm)}$	254
Concentration range (ppm)	50-250
Retention time (min)	5.15
Theoretical plates	4086.56
Tailing factors	0.998

The linearity was established in the concentration range of 50 to 250µg/ml. Sample of each concentration was injected in triplicate. A standard plot of peak area v/s concentration of drug in µg/ml was plotted. Correlation coefficient and regression equation were obtained from the calibration curve. R^2 (Correlation coefficient) was found to be 0.995 and it is within the acceptable limit. The calibration data is given in Table and graph obtained after plotting absorbance (y) vs. concentration (x) is shown in Figure 05.

Table 2: Calibration data of Dorzolamide by RP-HPLC method

Concentration (µg/ml)	**Peak Area**
0	0
50	1855011
100	3088921
150	4283781
200	5694341
250	74429161

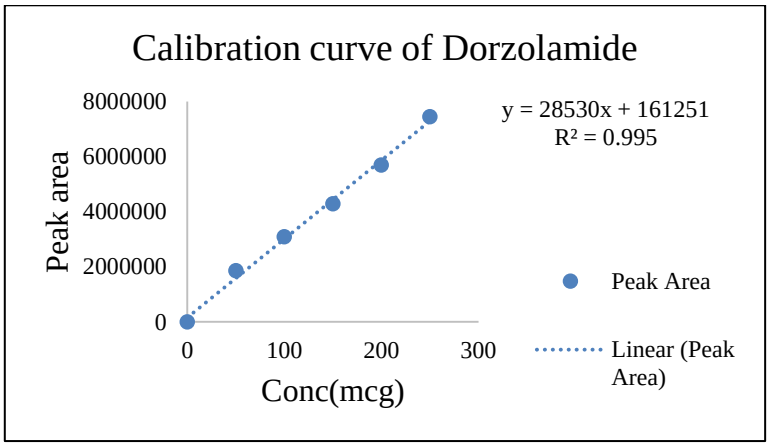

Fig.5: Calibration Data of Dorzolamide by RP-HPLC Method

$$\textbf{LOD} = \frac{3.3 \times \text{Standard Deviation}}{\text{Slope}} = \frac{3.3 \times 1924.207}{16125} = 0.393 \text{ ug/ml}$$

$$\textbf{LOQ} = \frac{10 \times \text{Standard Deviation}}{\text{Slope}} = \frac{10 \times 1924.207}{16125} = 1.193 \text{ ug/ml}$$

3.1.2.2 Accuracy

Accuracy of analytical method was determined by preparing three dilutions of drug viz. 50, 100, and 150µg/ml and injected into HPLC in three times (as per ICH guidelines). Accuracy was established by using regression equation of standard curve. The Accuracy was found to be within the limit of 98-100% as shown in Table 03.

Table 3: Accuracy studies

Sr. No	Concentration (µg/ml)	Average Peak area	Concentration Found	% Accuracy
1	50	260422.8	49.99	99.98
2	100	303849.7	98.91	98.91
3	150	405360.1	148.29	98.86

3.1.2.3 Precision

Precision of analytical method was determined by injecting three concentration of drug viz. 100, 150, 200µg/ml in triplicate. From the data obtained standard deviation (SD) and % RSD were calculated. %RSD values for intra and inter day variability were found to be less than 2%, hence given method passes the test for precision (Table 04 & 05). There was good reproducibility of the results.

Repeatability data (Intraday variability)

Repeatability (Intraday variability) was studied by injecting 3 replicates in morning, afternoon and evening. Reproducibility (Inter-day variability) was studied by injecting 3 replicates of 100, 150, 200µg/ml on two consecutive days.

Table 4: Repeatability data (Intra-day variability)

	Conc. µg/ml	Peak area				SD	% RSD
		I	II	III	Average		
Day 1	100	277768	280248.7	278569	278956.1	1563.2	0.75
	150	406983	416983.1	412568	278861.7	1265.6	0.45
	200	606971.1	608835.9	605925.7	412178.1	5011.3	1.21
Day 2	100	247798	250246	251569	607244.2	1474.20	0.24
	150	396083	389685	396868	249871	1913.26	0.76
	200	586951.6	598856.9	586325.7	394212	3940.09	0.99

Table 5: Reproducibility data (Inter-day variability)

	Conc. µg/ml	Peak area				SD	% RSD
		I	II	III	Average		
9.30 am	100	245698	243159	250698	246518.3	3835.86	1.55
	150	436983	436983	442568	438844.7	3224.5	0.73
	200	586971.1	578835.9	575925.7	580577.6	5724.6	0.98
12.30 pm	100	245698	250159	241698	245851.7	2876.32	1.19
	150	435983	442983	452568	443844.7	4414.3	1.12
	200	586971.4	576835.1	575425.7	579744.1	6298.6	1.08
4 pm	100	2404256	242569	236851	239892	2876.32	1.19
	150	435968	445698	442568	441411.3	4967.05	1.12
	200	586971.2	576835.1	575425.7	583834.9	4376.25	0.74

3.1.2.4 Robustness

Robustness was performed to check reliability of an analysis with respect to deliberate variations in method parameters. Results of which are shown in table 06, 07, and 08

Table 6: Robustness data (change in mobile phase ratio)

Mobile phase ratio	Conc.(µg/ml)	Peak area	Retention time (min)
75:15	100	277685	6.10
80:20	100	280224	5.15
85:25	100	275698	3.78
Avg.	277869		
SD	2268.60		
% RSD	0.8164		

Table 7: Robustness data (change in flow rate)

Flow rate(ml/min)	Conc.(µg/ml)	Peak area	Retention time (min)
0.9	100	280252	6.56
1	100	281756	5.15
1.1	100	275897	3.72

Avg.	279301.7
SD	3042.91
% RSD	1.089

Table 8: Robustness data (change in wavelength)

Wavelength	**Conc.(µg/ml)**	**Peak area**	**Retention time (min)**
252	100	290235.4	6.58
254	100	294168.3	5.15
256	100	290569.1	3.78
Avg.	291657.6		
SD	2180.72		
% RSD	0.7476		

4. Conclusion

Although several studies have provided different methods for determination of Dorzolamide hydrochloride, this study provide another alternative method, which is rapid, specific, and sensitive for Dorzolamide hydrochloride assay in ophthalmic pharmaceutical dosage form. The method run time is short with excellent sensitivity: a limit of detection and quantification values of 0.393µg/mL and 1.193µg/mL, respectively. The developed method has been applied to ophthalmic samples.

5. Acknowledgments

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