Spectrophotometric method for the determination of Dorzolamide in bulk drug and pharmaceutical dosage form

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ABSTRACT:

In present work a simple, precise, accurate UV spectrophotometric method has been developed for determination of Dorzolamide in bulk drug and its pharmaceutical formulations. Dorzolamide was found to exhibit maximum absorbance at 255nm with methanol as solvent. The present method was validated as per guidelines of the International Conference on Harmonization (ICH) including parameters like linearity, accuracy, precision, limit of detection and limit of quantification. Drug obeyed Beer's law in concentration range of 10-50 µg/mL and the linear regression equation was Y= 0.01008 x+0.0172. The percentage recovery of Dorzolamide was found to be 98.3-100.13. The precision was evaluated and % relative standard deviation (RSD) was less than 2%, LOD was found to be 2.178µg/mL & LOQ was found to be 6.60µg/mL respectively. The results suggest that this method can be employed for routine analysis of Dorzolamide in bulk drug and its pharmaceutical formulations.

KEYWORDS: Dorzolamide, Methanol, UV spectrophotometry, Validation

INTRODUCTION:

Dorzolamide is a Carbonic Anhydrase inhibitor**.** It blocks carbonic anhydrase thereby decreasing secretion of fluid and intraocular pressure Its IUPAC name is (4S,6S)-4-(ethylamino)-6-methyl-7,7-dioxo-5,6-dihydro-4H-thieno(2,3,6)thiopyran-2-sulfonamide.The chemical structure of Dorzolamide was shown in Fig.1. Dorzolamide is used to treat increased pressure in the eye caused by open angle glaucoma or condition called hypertension of the eye. It helps to prevent the gradual loss of vision or eyesight by lowering the increased pressure in the eye[1-2].

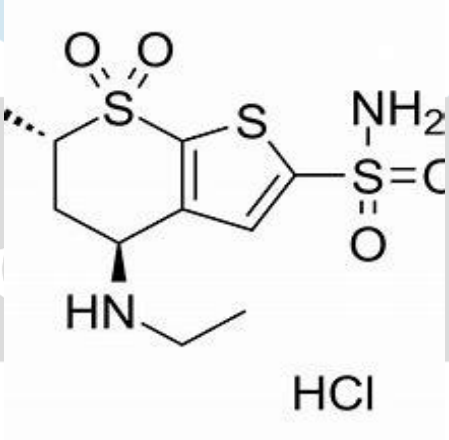

Fig 1 Structure of Dorzolamide

The aim of the present work is to develop a simple accurate, precise and economical spectrophotometric method for the estimation of Dorzolamide in bulk and pharmaceutical formulation and to validate the developed method as per ICH guidelines[3]. Literature review states that few analytical methods[4-9] have been reported for the determination of Dorzolamide.

MATERIALS & METHODS:

Dorzolamide pure drug was obtained from Cipla labs, Hyderabad and formulation (Dorzox eye drop) was purchased from local pharmacy, UV Visible Spectrophotometer (T-60). Methanol was procured from Finar Labs.

Solubility: The drug is soluble in methanol.

Preparation of stock solution:

Standard stock solution was prepared by dissolving 100 mg of Dorzolamide in 100 mL of methanol to get the concentration of 1mg/mL. Pipette out 1mL from the above solution and was further diluted to 10 mL gave 100 µg/ mL.

Absorption Maxima

10µg/mL of the solution was scanned 200 nm to 400 nm and absorption maxima was found to be 255 nm.

Preparation of Dilutions:

Different aliquots of drug solution 0.5mL, 1.0mL, 1.5mL, 2.0mL, 2.5mLwas taken from 100 µg/mL and was transferred into 10mL volumetric flask and the volume was made up to 10 mL with methanol to give 5µg/mL, 10µg/mL, 15µg/mL, 20 µg/mL,25µg/mL absorbance of the solution was measured at 255nm.

Linearity

Calibration curve was plotted by taking absorbance of 5 µg/mL to 25 µg/mL by taking concentration on x -axis and absorbance on y -axis

Precision

Precision of the method was determined by repeatability (intraday precision) and intermediate precision (interday precision) for Dorzolamide standard solution (20µg/mL) by six replicate measurements from the homogenous solution. The results were expressed as % RSD of the measurement.

Limit of Detection and Limit of Quantification

The detection limit of an individual's analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOD =3.3Sa/b

LOQ=10 Sa/b

Sa = the standard deviation of the intercept

b = Slope of the calibration curve

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provide an indication of its reliability during normal usage. It was performed by varying λmax value of ±1 nm for 20µg/ml in triplicate.

Stability

The change in the % assay initially was compared with 24 hours time period. Sample solution of Dorzolamide containing 20µg/ml was taken to test the solution stability.

Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value. It is done by measuring the amount of pure drug recovered at three different concentrations (50%, 100% and 150%) in triplicate.

Assay

5% solution of dorzolamide eye drops was procured and 0.5 mL of eye drops was transferred into a volumetric flask and made up to 10 mL with methanol and was further diluted to give 1mg/mL or 1000µg/mL. 1mL from the above sample solution was made upto 10ml with methanol to give 100µg /mL. Take 0.2 mL solution from the above and made up to 10 mL with methanol gives 20 µg/mL after mixing, filter the solution using Whatmann filter paper to get clear solution. Measure the absorbance of the solution six replicates at 255nm. Calculate the %assay.

RESULTS AND DISCUSSION:

Linearity

From the graph it was found that Dorzolamide obeys Beers law and the linearity concentration lies between 10-50 µg/mL. The linearity data and calibration curve were shown in Table 1 and Fig.2. The correlation coefficient, intercept and slope were calculated for Dorzolamide and the results were shown in Table 2.

Table1: Linearity data of Dorzolamide

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.115
2	20	0.227
3	30	0.316
4	40	0.415
5	50	0.525

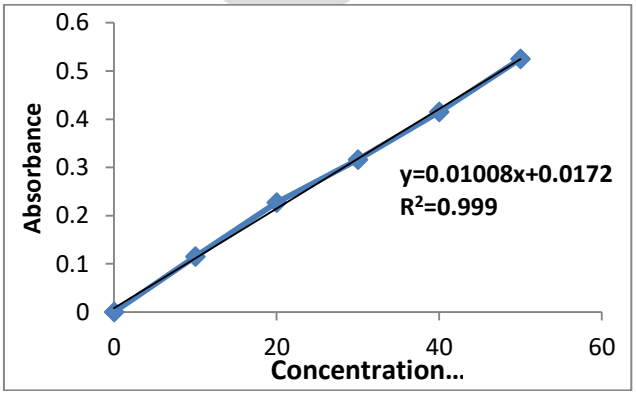

Fig 2 Calibration curve of Dorzolamide at 255 nm

Table 2 : Optical characteristics of dorzolamide

Parameters	Dorzolamide
λmax	255 nm
Slope	0.01008
Intercept	0.0172
Linearity	10-50µg/mL
Correlation coefficient	0.999

Discussion: Calibration curve was plotted and correlation coefficient was found to be 0.999. So, there was a good relation between absorbance and concentration.

Precision

Intraday and Interday precision data was shown in Table 3 and 4 respectively.

Table3 Intraday Precision data of dorzolamide

Concentration (µg/ml)	Absorbance
20	0.228
20	0.230
20	0.234
20	0.230
20	0.229
20	0.226
Mean	0.229
Standard Deviation	0.0026
%RSD	1.16

Table 4 Interday precision data of dorzolamide

S. No.	Concentration (µg/ml)	Absorbance DAY 1	Absorbance DAY 2	Absorbance DAY 3
1	20	0.228	0.237	0.230
2	20	0.230	0.239	0.232
3	20	0.234	0.234	0.234
4	20	0.230	0.230	0.236
5	20	0.229	0.231	0.237
6	20	0.226	0.233	0.236
MEAN		0.229	0.234	0.234
STD DEV		0.0026	0.0034	0.0027
%RSD		1.16	1.47	1.15

Discussion: The % RSD for Intraday and Interday precision was found to be < 2%. It indicates that the method was precise.

Accuracy

Recovery studies: Recovery studies were carried out by spiking the samples solution with standard solution at 50%, 100%, and 150% at 3 replicates and data was shown in Table 5.

Table 5 Accuracy data of dorzolamide

Sample (%level)	Amount taken(µg/mL)	Amount added(µg/mL)	Amount recovered (µg/ml)	% Recovery	Average
50	20	10	10	100	98.33
50	20	10	9.80	98	
50	20	10	9.70	97	
100	20	20	19.9	99.5	100.13

100 100	20 20	20 20	20.01 20.18	100 100.9	
150 150 150	20 20 20	30 30 30	29.84 30.03 29.64	99.4 100 98.8	99.23

DISCUSSION: The average % recovery of Dorzolamide was found to be in between 98.33-100.13.

Limit of Detection and Limit of Quantification

LOD and LOQ values for dorzolamide was shown in Table 6.

Table 6 Sensitivity data of dorzolamide

Parameter	Dorzolamide(µg/mL)
LOD	2.178
LOQ	6.601

Discussion: LOD and LOQ values for dorzolamide was found to be 2.178 µg/mL and 6.601µg/mL. It indicates that the method was sensitive.

Robustness

Robustness data was shown in Table 7.

Table 7 Robustness data of dorzolamide

S. No	Wavelength	Absorbance
1	254nm	0.228
2	255nm	0.231
3	256nm	0.230

Discussion: There was no much variation in the absorbance with change in wavelength.

Stability: Sample solution of Dorzolamide containing 20µg/ml was taken to test the solution stability. The data of stability was shown in Table 8.

Table 8 Stability data of dorzolamide

Time	% Assay
Initial	98.43
24 hours	100.15

Discussion: It was observed that the difference in the result was NMT 2% for formulation, indicating stability of dorzolamide.

Assay:

Table 9 Assay of dorzolamide (n=6)

Label claim	Amount found	Assay%±SD
100 mg	100.40	100.40±0.15

Discussion: Dorzolamide was 100.4±0.15 which was comparable with the label claim amount. It shows that UV Visible method developed was successful in determining dorzolamide from ophthalmic formulation

CONCLUSION

The developed UV spectroscopic method was simple, sensitive, cost effective with good precision. The parameters were validated as per ICH guidelines. The findings of work suggest that the method may be applied for quantitative estimation of Dorzolamide from bulk and pharmaceutical dosage forms. Hence this method can be used in the routine work of quality control aspects.

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