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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF DORZOLAMIDE HCl IN BULK AND PHARMACEUTICAL DOSAGE FORMS

H. M. Sharath*, G babu Jose, K. P. Channabasavaraj, J. S. Modiya

Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathi Nagara, Mandya (District), Karnataka, India

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Correspondence to Author:

H. M. Sharath

Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathi Nagara, Mandya (District), Karnataka, India

ABSTRACT

Three Simple, precise, accurate and economical UV methods have been developed and validated for the quantitative estimation of Dorzolamide HCl in bulk and pharmaceutical dosage form. Dorzolamide HCl has the absorbance maxima at 253 nm in zero order spectra (Method A). In the first order derivative spectra, showed absorbance maxima at 238 nm (Method B) and in the second order derivative spectra, showed peak maxima at 278 nm (Method C). Distilled water was used as solvent for all the methods. Beer's law was found to be obeyed in the concentration range of 3-24 µg/ml. The developed method was validated according to ICH guidelines and was found to be accurate, economic and precise. The proposed method can be successfully applied for the estimation of Dorzolamide HCl in bulk and pharmaceutical dosage forms.

INTRODUCTION: Dorzolamide HCl [1, 2] is an anti-glaucoma agent and topically applied in the form of eye drops (**fig. 1**). Chemically is an [(4*S*, 6S)-4-(Ethylamino)-6-methyl-5, 6-dihydro-4*H*thieno [2, 3*b*] thiopyran-2-sulphonamide 7, 7-dioxide hydrochloride]. Dorzolamide HCl is a carbonic anhydrase inhibitor and used to lower increased intraocular pressure in open-angle glaucoma and ocular hypertension.

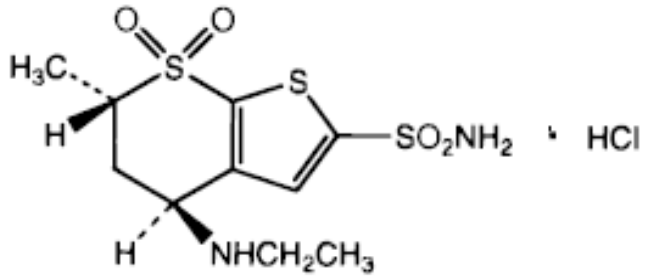

FIG.1: CHEMICAL STRUCTURE OF DORZOLAMIDE HCL

Analytical methods are required to characterize drug substances and drug products composition during all phases of pharmaceutical development. Extensive literature survey reveals that only few methods [3, 4] were reported for determination of Dorzolamide HCl in bulk and in its pharmaceutical dosage form. Hence there is a need to develop new methods for its estimation in bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS:

Instruments and reagents: A Shimadzu-1800 UV/Vis double beam Spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements Distilled water was used as solvent for dilution. Authentic drug sample of Dorzolamide HCl was given as a gift sample by Lake Chemicals Pvt. limited, (Bangalore, India) Eye drop formulation [DORTAS (Brand name), Intas Pharmaceutical Limited, Jaipur, India was procured from a local pharmacy with labelled amount 2% solution (20mg/ml).

Preparation of working standard drug solution: The standard Dorzolamide HCl (100 mg) was weighed accurately and transferred to 100 ml volumetric flask containing few ml of Distilled water and it was sonicated for 5 min to dissolved completely and diluted up to the mark with Distilled water to obtain final concentration of 1000 µg/ml and the resulting solution was used as working standard solution.

Analysis of marketed formulation: The commercially available eye drops contains 2% solution of sterile Dorzolamide HCl (20mg/ml). From this eye drop 1ml Solution was carefully transferred in to volumetric flask of 20 ml capacity containing 10 ml of the Distilled water and sonicated for 5 min. and then final solution was made with Distilled water to get the solution of 1000 µg/ml From this solution, various dilutions of the sample solution were prepared and analysed.

Calibration curve:

Method A:

Zero order spectroscopy: The solutions were scanned in the range from 400-200 nm and the peak was observed at 253 nm. The wavelength selected for the analysis of the drug was 253 nm **(Fig. 2)**. The drug followed the Beer's- law in the range of 3-24 µg/ml. The different concentrations of the sample solution were determined by using the calibration curve **(Fig. 3)**.

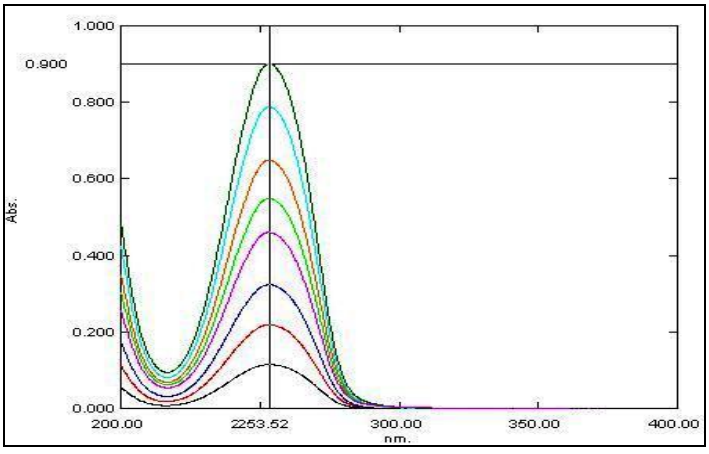

FIG. 2: ZERO ORDER SPECTRA OF DORZOLAMIDE HCl AT 253nm

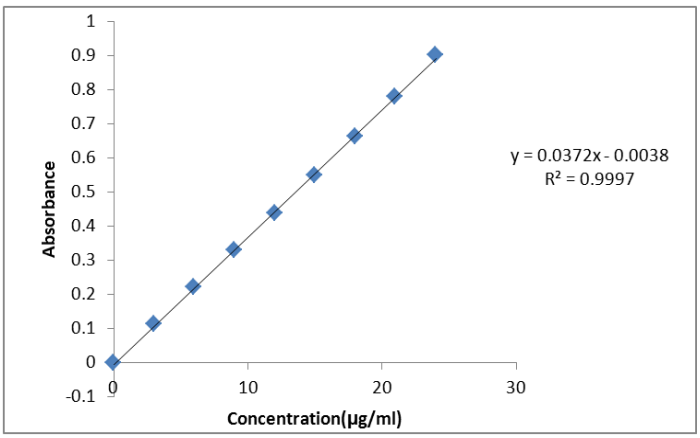

FIG. 3: CALIBRATION CURVE OF DORZOLAMIDE HCl

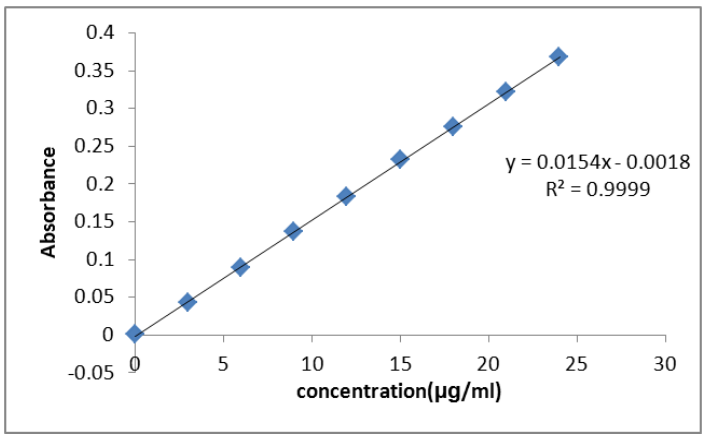

FIG. 5: CALIBRATION CURVE OF DORZOLAMIDE HCl

Method B:

First Order Derivative Spectroscopic method: The first order derivative spectra at n=1, showed a sharp peak at 238nm as shown in **(Fig. 4)**. The absorbance difference at n=1 (dA/dλ) is calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solution. The standard drug solution was diluted, so as to get the final concentration in the range of 3-24 µg/ml and derivatized in to first order derivative spectra mode. The calibration curve of dA/dλ against concentration of the drug showed linearity. Similarly absorbances of samples solution were measured and the amount of Dorzolamide HCl in the sample was determined from standard calibration curve (**Fig. 5**).

Method C:

Second Order Derivative Spectroscopic method: The Second order derivative spectra at n=2, showed a sharp peak at 278nm **(Fig. 6)**. The absorbance difference at n=2 ($d^2A/d\lambda^2$) is calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solution. The standard drug solution was diluted so as to get the final concentration in the range of 3-24 µg/ml and derivatized in to second order derivative spectra mode. The calibration curve of $d^2A/d\lambda^2$ against concentration of the drug showed linearity. Similarly absorbances of samples solution were measured and amount of Dorzolamide HCl was determined from standard calibration curve (**Fig. 7**).

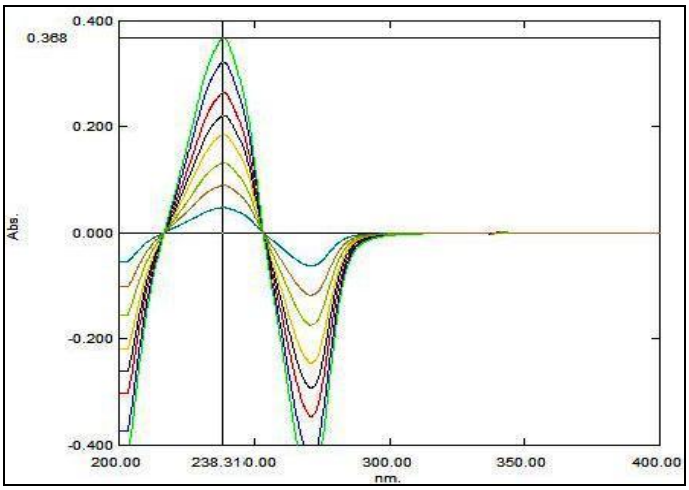

FIG. 4: FIRST ORDER DERIVATIVE SPECTRA OF DORZOLAMIDE HCl AT 238 nm

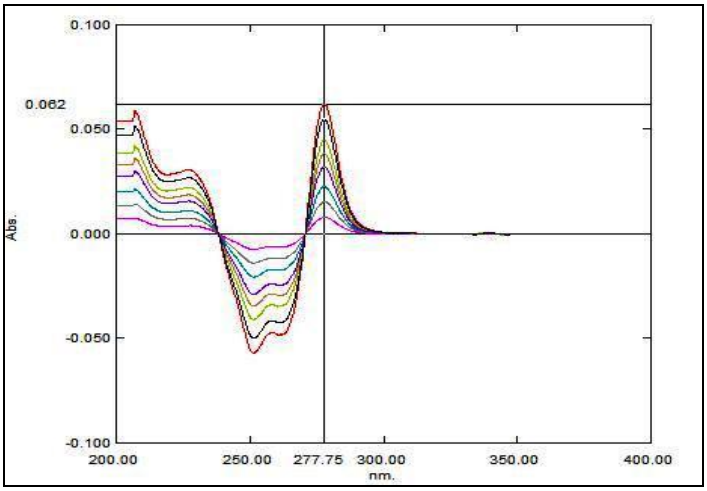

FIGURE 6: SECOND ORDER DERIVATIVE SPECTRA OF DORZOLAMIDE HCl AT 278nm

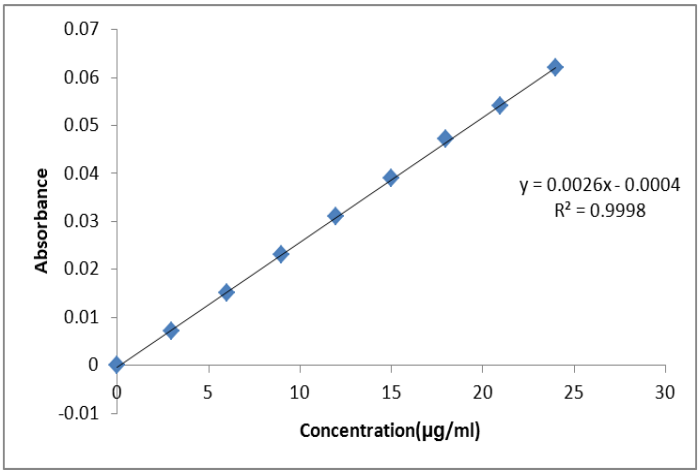

FIG. 7: CALIBRATION CURVE OF DORZOLAMIDE HCl

Validation of the method: All these methods were validated according to ICH guidelines [5, 6] in terms of linearity, precision, accuracy and ruggedness parameters.

Linearity: The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method **(Table 1)**. The linearity was found to be 3-24 µg/ml for zero, first and second order derivative spectrophotometeric methods. Optimum conditions, Optical characteristics and Statistical data of the Regression equation in UV method are given in **table 2**.

RESULT AND DISCUSSION:

TABLE 1: RESULTS OF CALIBRATION CURVE FOR DORZOLAMIDE HCl

Sr. No.	Concentration (µg/ml)	Method A	Method B	Method C
		Absorbance at 253 nm	Absorbance at 238 nm	Absorbance at 278 nm
1	3	0.112	0.043	0.007
2	6	0.221	0.089	0.015
3	9	0.328	0.137	0.023
4	12	0.438	0.188	0.031
5	15	0.458	0.232	0.039
6	18	0.662	0.275	0.047
7	21	0.779	0.322	0.047
8	24	0.900	0.368	0.054

TABLE 2: OPTIMUM CONDITIONS, OPTICAL CHARACTERISTICS AND STATISTICAL DATA OF THE REGRESSION EQUATION IN UV METHOD

PARAMETERS	RESULTS		
	METHOD A	METHOD B	METHOD C
Absorption Maxima (nm)	253	238	278
Beer's-Lambert's range (µg/ml)	3-24	3-24	3-24
Regression equation (y)*			
Slope (b)	0.0372	0.0154	0.00026
Intercept (a)	-0.0038	-0.0018	-0.0004
Correlation coefficient	0.9997	0.9999	0.9998
Sandell's sensitivity (mcg / cm^2-0.001 absorbance units)	0.02737	0.0646	0.3846
Molar extinction coefficient ($L\ mol^{-1}\ cm^{-1}$)	0.03653 $X10^4$	0.0154 $X10^4$	0.0026 $X\ 10^4$
Intraday precision (% RSD)	0.342	0.842	1.422
Interday precision (% RSD)	0.395	0.885	1.460
Accuracy	100.41±0.61	99.86±0.43	100.60±0.73
Limit of detection (µg / ml)	0.107	0.146	0.575
Limit of quantification (µg / ml)	0.325	0.442	1.743

Accuracy: The accuracy of the method was assessed by recovery studies at three different levels i.e. 50%, 100%, 150%. The values of standard deviation were satisfactory and the recovery studies were close to 100% **(Table-3)**. Hence these methods can be useful in routine analysis of Dorzolamide HCl in bulk drug and formulations.

Ruggedness: Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst. The results of ruggedness testing are reported in the **Table 4**.

Precision: The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation (CV). The results of inter-day and intra-day were reported in **Table 5**.

TABLE: 3 ACCURACY RESULTS FOR DORZOLAMIDE HCl

Brand	Methods	Initial amount (µg/ml)	Amount of pure drug added (µg/ml)	Amount Recovered (µg/ml)	% Recovery ± SD**
Dortas	Method A	15	7.5	7.47	99.73 ± 0.592
		15	15	15.06	100.4 ± 0.841
		15	22.5	22.76	101.1 ± 0.632
Dortas	Method B	10	7.5	7.53	100.40±0.431
		10	15	14.97	99.80±0.462
		10	22.5	22.48	99.91±0.534
Dortas	Method C	20	7.5	7.48	99.73±0.642
		20	15	15.12	100.80±0.789
		20	22.5	22.51	100.04±0.828

**Average of six determinations

TABLE: 4 RUGGEDNESS RESULTS FOR DORZOLAMIDE HCl

Brand	Methods	Label claim (mg/ml)	Analyst I		Analyst II	
			Amount found (mg/ml)	Recovery ± SD** (%)	Amount found (mg/ml)	Recovery ± SD** (%)
Dortas	Method A	20	20.02	100.1 ± 0.17	20.11	100.5 ± 0.12
	Method B	20	19.98	99.9 ± 0.21	20.05	100.2 ± 0.29
	Method C	20	20.21	101.5±0.32	19.99	99.95±0.34

** Average of six determinations

TABLE: 5 PRECISION RESULTS FOR DORZOLAMIDE HCl

Methods	Conc. (µg /ml)	Inter-day Absorbance Mean ± SD**	% CV	Intra-day Absorbance Mean ± SD**	% CV
Method A	12	0.4371 ± 0.001472	0.33	0.4366 ± 0.001633	0.42
	15	0.5463 ± 0.00216	0.39	0.5451 ± 0.001472	0.34
	18	0.6625 ± 0.001871	0.28	0.6643 ± 0.001751	0.28
Method B	12	0.1835 ± 0.001871	1.01	0.1836 ± 0.00216	1.17
	15	0.2333 ± 0.002066	0.88	0.2333 ± 0.001966	0.84
	18	0.2741 ± 0.002366	0.86	0.2738 ± 0.002408	0.87
Method C	12	0.0321 ± 0.000753	2.34	0.0315 ± 0.000548	1.53
	15	0.0375 ± 0.000548	1.46	0.0385 ± 0.000548	1.42
	18	0.0451 ± 0.000753	1.66	0.0455 ± 0.000548	1.20

** Average of three determinations

CONCLUSION: It can be concluded that the proposed methods were validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and relatively inexpensive. So, the developed methods can be easily applied for the routine Quality Control analysis of Dorzolamide HCl in pharmaceutical preparations.

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