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Research Article

STABILITY-INDICATING RP-HPLC METHOD FOR ANALYSIS OF DORZOLAMIDE HCl IN THE BULK DRUG AND IT'S PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A novel stability-indicating RP-HPLC method has been develop and validated for quantitative analysis of Dorzolamide HCl in the bulk drug and in its pharmaceutical dosage form. Using 100 mm × 4.6 mm, 5-µm particle, C18 column with 50:50 (*v*/*v*) Methanol- (0.02M) 1,Octane Sulphonic acid buffer at pH 3.2 as isocratic mobile phase enabled separation of the drug from its degradation products. UV detection was performed at 254 nm. The method was validated for linearity, accuracy (recovery), precision, sensitivity, ruggedness and robustness. The linearity of the method was excellent over the range 20–100 µg/ml (correlation coefficient 0.9997). The limits of detection and quantification were 0.004 and 0.0132 µg/ml, respectively. Recovery of Dorzolamide HCl from the pharmaceutical dosage form ranged from 99.21 to 101.26%.

Dorzolamide HCl was subjected to stress conditions (hydrolysis (acid, base), oxidation, water stress, and thermal degradation) and the stressed samples were analysed by use of the method. Degradation was observed in acid, base, and 30% H_2O_2. The drug was stable under the other stress conditions investigated. The degradation products were well resolved from main peak. The forced degradation studies prove the stability indicating power of the method.

Key words: Dorzolamide HCl, Dosage form, RP-HPLC, Forced degradation, Method validation.

INTRODUCTION

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

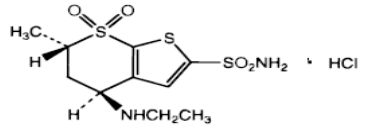

Fig. 1: structural formula of Dorzolamide HCl

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light, which enables storage conditions, retest periods, and shelf life to be recommended.

The two main aspects of study of the stability of a drug product that play an important role in shelf life determinations are assay of the active drug and the degradation products generated during stability studies. Assay of a drug product in a stability test sample must be performed with stability-indicating method, as recommended by the International Conference on Harmonization (ICH).

A literature survey revealed that very few methods [3, 4, 5, 6] were developed and none of the reported procedures enables analysis of the Dorzolamide HCl in pharmaceutical dosage forms in the presence of their degradation products. This manuscript describes the development and validation, in accordance with ICH guidelines, of a rapid, economical, precise, and accurate stability-indicating isocratic RP-HPLC method for analysis of Dorzolamide HCl in the presence of its degradation products.

MATERIALS AND METHODS

Chemicals and solutions

An analytically pure sample of Dorzolamide HCl (purity 99.8%) was procured as gift sample from lake chemicals pvt ltd (Bangalore, India) and Eye drop formulation [DORTAS (Brand name), Intas pharmaceutical Limited, Jaipur, India] was procured from a local pharmacy with labelled amount 2% solution (20mg / ml). Acetic acid and Methanol (HPLC grade) were obtained from Merck Fine Chemicals (Mumbai, India). 1,Octane sulphonic acid, sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) were from Qualigens Fine Chemicals (Glaxo, Mumbai, India). The 0.45-µm Nylon pump filter was obtained from Advanced Microdevices (Ambala Cantt., India). Doubledistilled water was used throughout the experiment. Other chemicals used were of analytical or HPLC grade.

Preparation of standard solutions

Accurately weigh and transfer 10 mg of Dorzolamide HCl Working standard into a 100 ml volumetric flask, add about 50 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (100 µg / ml, Stock solution). Further pipette 2 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent, it was 20 µg / ml. Standard calibration solutions (20–100 µg/ ml) for assessment of linearity were prepared from this stock solution by dilution with suitable diluent.

Preparation of sample solutions

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 diluent and sonicated for 5 min. and then final solution was made with same diluent to get the solution of 1000 µg / ml From this solution, 10 ml was taken in 100 ml standard volumetric flask and diluted to 100 ml with diluent to give a solution of 100 µg / ml From this stock solution, various dilutions of solution were prepared and analysed.

Chromatography

The liquid chromatographic system consisted of following components: Shimadzu HPLC model containing LC-20AT (VP series) pump, variable wavelength programmable UV/ VIS detector SPD-20A (VP series) and Hamilton syringe (705 NR, 50 µl). Chromatographic analysis was performed using Chromtech N-2000 software on a Sigma Aldrich Supelco C18 column with 100 x 4.6 mm i.d. and 5 µm particle size.The optimized mobile phase was consisted

of Methanol and pH 3.2 buffer (50 : 50 v/v). The flow rate was 1.2 ml / min. Detection wavelength 254 nm was selected by scanning drug over a wide range of wavelength 200 nm to 400 nm in spectrophotometer. The 20 µl sample was injected and the total run time was 15 min. Chromatogram showed peak of Dorzolamide HCl at retention time of 6.865 ± 0.02 min.

Forced degradation study

To study the effect of acid, approximately 100 mg Dorzolamide HCl was accurately weighed and dissolved in 25 ml of 1M hydrochloric acid (HCl) and refluxed for 70°C for approximately 2 hr in water bath. The solution was then left to reach ambient temperature,and neutralized to pH 7 by addition of 1M sodium hydroxide(NaOH) then diluted to 50 ml with diluent (Mobile Phase) from this solution target concentration was prepared and injected.

To study the effect of alkali, approximately 100 mg Dorzolamide HCl was accurately weighed and dissolved in 25 ml of 1M sodium hydroxide (NaOH) and refluxed for 70°C for approximately 2 hr in water bath. The solution was then left to reach ambient temperature,and neutralized to pH 7 by addition of 1M hydrochloric acid (HCl) then diluted to 50 ml with diluent (Mobile Phase) from this solution target concentration was prepared and injected.

To study the effect of oxidising conditions, approximately 50 mg Dorzolamide HCl was accurately weighed and dissolved in 25 ml of 30% H_2O_2 and refluxed for 40°C for approximately 2 hr in water bath. The solution was then left to reach ambient temperature, and diluted to 50 ml with diluent (Mobile Phase) from this solution target concentration was prepared and injected.

To study the effect of water stress, approximately 50 mg Dorzolamide HCl was accurately weighed and dissolved in 25 ml of water (HPLC grade) and refluxed for 70°C for approximately 2 hr in water bath. The solution was then left to reach ambient temperature, and diluted to 50 ml with diluent (Mobile Phase) from this solution target concentration was prepared and injected.

To study the effect of temperature, approximately 50 mg Dorzolamide HCl was stored at 80°C for 48 hr, then dissolved in few ml of diluent and sonicate for 5min then diluted to 50 ml with diluent (Mobile Phase) from this solution target concentration was prepared and injected.

Method validation

The method was validated according to International Conference on Harmonization [7, 8] guidelines for validation of analytical procedures.

RESULTS AND DISCUSSION

System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (t_R), number of theoretical plates (N), tailing factor (T), and peak asymmetry (A_f) were evaluated for five replicate injections of the drug at a concentration of 60 µg / ml. The results given in **Table 1** were within acceptable limits. A typical chromatogram of Dorzolamide HCl is presented in fig 2.

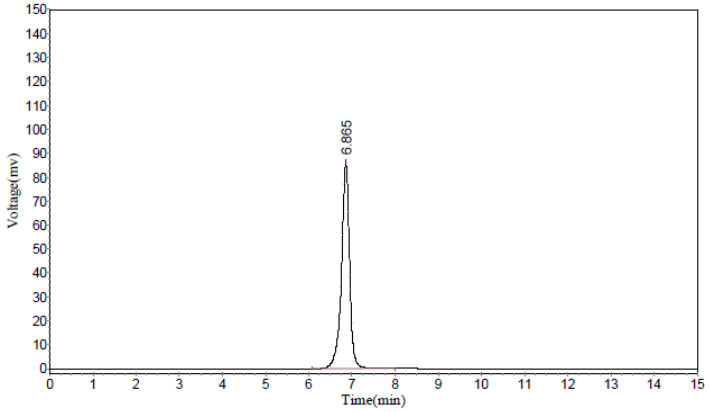

Fig. 2: Chromatogram of Dorzolamide HCl formulation at 254 nm

Table 1: Results from system suitability studies

Property	Values*	Required limits
Retention time (t_R)	6.86	RSD ≤ 1%
Theoretical plates (N)	7988.17	$N > 2000$
Tailing factor (T)	0.88	$T \leq 2$
Asymmetric factor (A_f)	0.795	$A_f \leq 1.5$

* Mean ± S.D. from six determinations

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

Sr. No.	Concentration (µg / ml)	Retention time (min)	Peak area (mv)
1	20	6.857	358623.5
2	40	6.855	740898.1
3	60	6.863	1148993
4	80	6.852	1516295
5	100	6.859	1938022

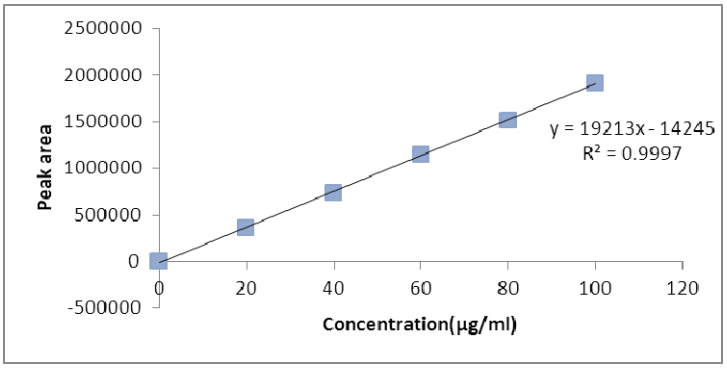

Fig. 3: Calibration curve of Dorzolamide HCl at 254 nm

Linearity and range

Appropriate aliquots of standard Dorzolamide HCl stock solutions (100 µg / ml) were taken in different 10 ml volumetric flask and resultant solution was diluted up to the mark with diluent to obtain final concentration of 20-100 µg / ml. The solutions were prepared in triplicate. Calibration curve were constructed by plotting the concentration of Dorzolamide HCl versus corresponding mean peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range and the results given in **Table 2** and **Fig: 3.**

Precision

The intra-day precision was determined by analyzing standard solution of concentration 60 µg / ml for 6 times on the same day while inter-day precision was determined by analysing corresponding standards daily for 6 day over a period of one week. The values of percentage relative standard deviation (% RSD) for intra-and inter-day variation are given in **Table 3.**

Accuracy

Accuracy of the method was checked by recovery study using standard addition method known amount of standard Dorzolamide HCl was added into pre analysed sample and subjected it to the proposed high performance liquid chromatographic method. These studies were carried out at three levels i.e,(50, 100 and 150%). The recovery studies were carried out and the % recovery and standard deviation of the % recovery were calculated and presented in **Table 4.**

Sensitivity

The sensitivity of measurement of Dorzolamide HCl by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). The LOQ and LOD were calculated by the use of the equations LOD = 3 x N / B and LOQ = 10 x N / B where N is the standard deviation of intercept of calibration plot and B is the average of the slope of the corresponding calibration plot. The limit of detection (LOD) was 0.004 µg / ml and the limit of quantitation (LOQ) was 0.0132 µg / ml.

Table 3: Precision results for Dorzolamide HCl

Sr. No.	Concentration (µg / ml)	Intraday precision (Area)	Interday precision (Area)
1	60	1151774	1152673
2	60	1156494	1155492
3	60	1152263	1156012
4	60	1150362	1168826
5	60	1149837	1156261
6	60	1143898	1149842
Mean		1150771	1156518
Std.Dev		4104.927	6514.96
%RSD		0.356	0.563

Table 4: Results from recovery studies

Brand used	Label claim (mg/ml)	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery ± SD* (%)	% RSD
Dortas	20	60	30	30.38	101.26 ± 0.23	0.253
		60	60	59.53	99.21 ± 0.31	0.412
		60	90	90.82	100.93 ± 0.18	0.129

*Average of six determinations

Table 5: Ruggedness studies of Dorzolamide HCl by RP-HPLC method

Brand used	Label claim (mg/ml)	Analyst I		Analyst II	
		Amount found (mg/ml)	Recovery ± SD* (%)	Amount found (mg/ml)	Recovery ± SD* (%)
Dortas	20	19.84	99.20 ± 0.27	19.94	99.70 ± 0.39

*Average of six determinations

Ruggedness and robustness

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 5.**

Robustness is a measure of capacity of a method to remain unaffected by small but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and chromatographic response was evaluated. The organic composition in the mobile phase was varied from 45% to 55%, and the variation in mobile phase flow rate by 1.1 ml / min (0.5 and 0.8 ml / min) had no significant effect on the retention time and chromatographic response of the 20 µg / ml solution, indicating that the method was robust. The results are shown in **Table 6.**

Table 6: Robustness studies of Dorzolamide HCl by HPLC method

Condition	Modification	Mean area ± SD *	RSD (%)	Mean t_R ± SD (min)
Mobile phase composition (v / v)	45 : 55	1164212 ± 11180.68	0.961	6.990 ± 0.019
	50 : 50	1116299 ± 7180.693	0.643	6.849 ± 0.015
	55 : 45	1134925 ± 10627.68	0.936	6.723 ± 0.022
Mobile phase pH	3.0	1157102 ± 10045.71	0.868	6.895 ± 0.014
	3.2	1160444 ± 7263.466	0.625	6.831 ± 0.015
	3.4	1146383 ± 9011.458	0.786	6.789 ± 0.022
Flow Rate Of Mobile Phase	1.1	1221462 ± 11490.41	0.930	7.515 ± 0.02
	1.2	1163091 ± 10240.34	0.880	6.87 ± 0.025
	1.3	1136552 ± 9384.887	0.825	6.382 ± 0.02

*Average of six determinations

Table 7: Characteristic parameters of Dorzolamide HCl for the RP-HPLC method

Parameters	RP-HPLC
Calibration range (µg / ml)	20-100
Detection wavelength	254 nm
Mobile phase	50 : 50
(Methanol : Buffer)	(v / v, pH 3.2)
Retention time	6.865 ± 0.02
Regression equation (Y*)	Y = 19213x-14245
Slope (b)	19213
Intercept (a)	-14245
Correlation coefficient(r^2)	0.9997
Intraday Precision (% RSD*)	0.396
Interday Precision (% RSD*)	0.596
Limit of detection (µg / ml)	0.004
Limit of quantitation (µg / ml)	0.0132

*Y = bx + a, where x is the concentration of compound in µg / ml and ; Y is the peak area ; *Average of six determinations

Forced degradation study

When establishing the stability-indicating properties of analytical methods, the intermediate degradation products should not interfere with any stage of drug analysis. The System suitability parameters of forced degradation studies are summarised in **Table 8.** The results from forced degradation studies are given **Table 9.** Chromatograms obtained from after degradation under different stress conditions are shown in **Fig: 4 – 8,** respectively. No peaks co-eluted with the drug peak, suggesting the method enabled specific analysis of Dorzolamide HCl in the presence of its degradation products.

Table 8: System suitability parameters of Dorzolamide HCl by degradation studies

Sr. No.	System suitability parameters	Dorzolamide HCl	Degradation products	
			A	B
1	Retention time (minutes)	6.832	2.965	3.515
2	Theoretical plates	5396	4623.99	5784.12
3	Resolution	9.747	0.000	2.638
4	Asymmetry	1.378	1.123	0.969
5	Tailing Factor	1.287	1.032	0.997

Table 9: Results from analysis of samples by the forced degradation study, showing percentage degradation of Dorzolamide HCl

Sr. No.	Parameters (Stress condition/duration)	Degradation products (t_R)		Total % degradation
		A	B	
1	Acidic/1 M HCl/70°C/2 hr	2.95	3.532	29.707
2	alkali/1 M NaOH/70°C/2 hr	2.965	3.515	8.873
3	Oxidising/30% H2O2/40°C/2 hr	2.907	-	13.741
4	Neutral/H2Oat pH7/70°C /2 hr	3.530	-	4.664
5	Thermal/80°C/48 hr	-	-	No degradation

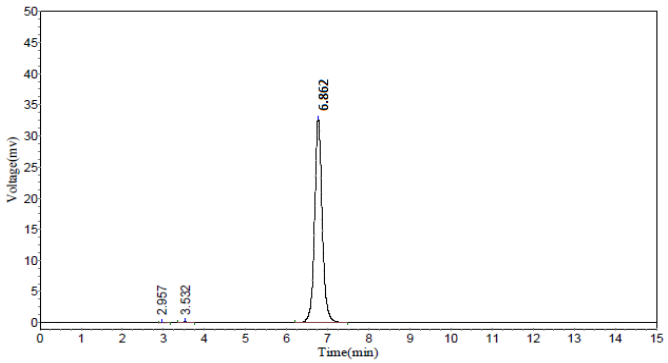

Fig. 4: Typical chromatogram obtained after degradation of Dorzolamide HCl under acidic conditions

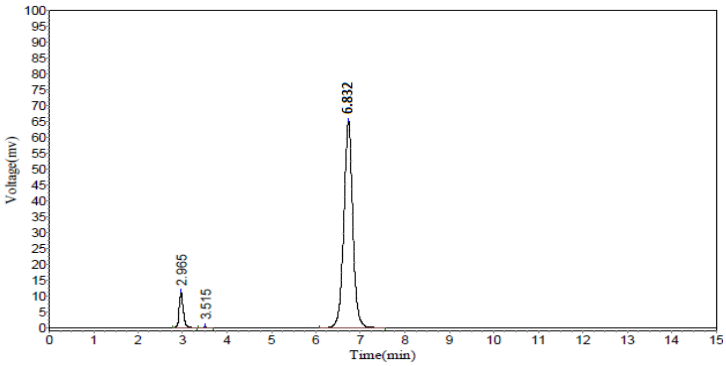

Fig. 5: Typical chromatogram obtained after degradation of Dorzolamide HCl under alkali conditions

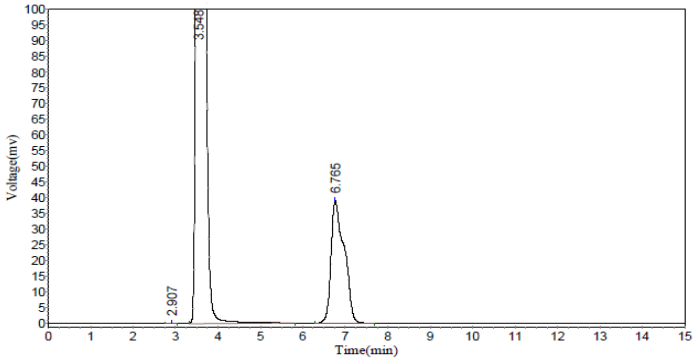

Fig. 6: Typical chromatogram obtained after degradation of Dorzolamide HCl under oxidising conditions

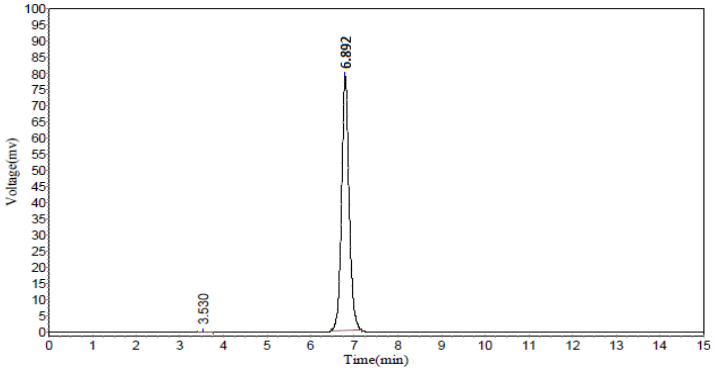

Fig. 7: Typical chromatogram obtained after degradation of Dorzolamide HCl under water stress conditions

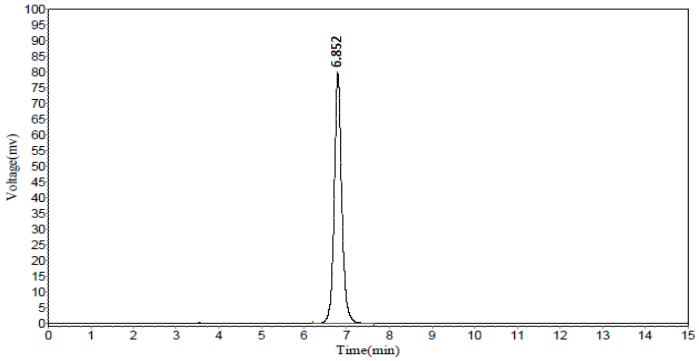

Fig: 8. Typical chromatogram obtained after thermal degradation of Dorzolamide HCl

CONCLUSION

The method developed for quantitative analysis of Dorzolamide HCl is rapid, precise, accurate, and selective. The method was completely validated as per ICH guidelines and satisfactory results were obtained for all the characteristics tested. The method is stability-indicating and can be used to assess the stability of Dorzolamide HCl in bulk and pharmaceutical dosage forms. The method can be conveniently used for assay of Dorzolamide HCl in the bulk drug and in pharmaceutical dosage forms. The method can be conveniently used in quality control laboratory.

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