Method Development and Validation for Dorzolamide in Ophthalmic Dosage Form using HPLC method

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ABSTRACT

A developed and validated method for the assay of optamalic solution of dorzolamide assay was determined using reversed phase liquid chromatographic method with UV detection at 258 nm. Chromatographic separation was made on a Zorbax SB C_{18} (250 mm 4.6 mm, 5 μm) column kept at 30°C with a mobile phase isocratic mixture of (phosphate buffer, pH 2.5, and acetonitrile, 90 : 10 v/v) at a flow rate of 0.8 mL/min. The method was validated for its accuracy, precision, specificity, linearity, limit of detection, limit of quantification, and robustness based on ICH guidelines,which revealed good results.The developed method is reliable, accurate, specific and sensitive hence can be applied for routine quality control analysis of dorzolamide in pharmaceutical dosage form.

Key points: Method, Validation, Dorzolamide, HPLC

1. INTRODUCTION

Dorzolamide, sold under the brand name trusopt among others, is medications used to treat high pressure inside the eye including glaucoma. It is used as an eye drop is also available as the combination dorzolamide/timolol.[1] Dorzolamide (DZL) hydrochloride, chemically (4*S*,6*S*)-4-(ethylamino)-6-methyl-5,6-dihydro-4*H*-thieno[2,3-b] thiopyran-2 sulfonamide 7, 7-dioxide hydrochloride (**Figure 1),** is a carbonic anhydrase inhibitor, which is used for the treatment of glaucoma and ocular hypertension [1]. A number of analytical methods have been resulted in the literature for the individual assay of dorzolamide. These methods include reversed-phase high-performance liquid chromatography (RP-HPLC) [2-3], spectrophotometry [4] capillary electrophoresis [5], and others [6-8]

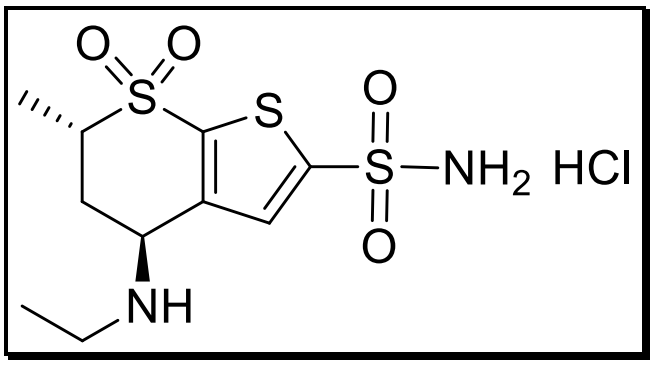

Figure 1: Dorzolamide

Both the British Pharmacopoeia [1] and United States Pharmacopoeia [9] 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.

2. EXPERIMENTAL

Reagents and Samples: Potassium dihydrogen orthophosphate, ortho phosphoric acid of analytical grade, acetonitrile of HPLC grade and water from Merck (Mumbai, India). Pure dorzolamide active substance was from Cipla Pharmaceutical Company, India.

Instrumentation and Chromatographic Conditions. Analysis of HPLC were carried out on Shimadzu (Japan) equipped with LC2010 series pump, Zorbax SB C_{18} column (250 mm 4.6 mm, 5 μm), manual Rheodyne injector (with 20 μL loop size) and SPD-20A UV- visible detector. Spinchrom software was used for data processing and acquisition. Sonicator (Loba, India) and pH meter (Elico, India) were employed to dissolve and/or degas the sample and measure the pH of the buffer. The mobile phase phosphate buffer (50 mM potassium phosphate, adjusted to pH 2.5 with ortho phosphoric acid) and acetonitrile in the ratio of 90 : 10 v/v. It was filtered through a 0.22 μm filter (millipore filter, India), degassed in a sonicator for 10 minutes, and then pumped at a flow rate of 0.8 mL/min. The injection volume was 20 μL, and the UV detection was performed at 258 nm.

Preparation of Reference Solution: DZL stock solution was prepared by dissolving 100 mg of pure DZL in a 100 mL HPLC grade water as solvent to get the solution containing 1000 μg/mL of DZL.

Sample Preparation: From the stock DZL solution, 1 mL was transferred into a 100 mL volumetric flask, sonicated with mobile phase for 10 minutes and made up to the volume. This solution was filtered through a 0.22 μm filter and 0.5 mL of the solution was diluted to 10 μg/mL to get 50 μg/mL with the solvent mobile phase. A 20 μL aliquot was injected into the HPLC instrument for analysis.

Method Development: This method was validated according to the ICH guidelines [10] for its accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity, linearity and robustness.

3. RESULTS AND DISCUSSION

Method Development and Optimization: For the determination of DZL, various parameters such as detection wavelength, effect of composition of mobile phase, pH of mobile phase, flow rate, concentration of buffer solution, column temperature and injection volume were studied and optimized are considered . The wavelength was standardized at 258 nm to achieve good resolution and symmetric peak shape for the drug many analysis were performed using various composition and pH of mobile phase, flow rate, concentration of buffer solution, column temperature and injection volume . The optimized mobile phase consisted of phosphate buffer (50 mM potassium phosphate, adjusted to pH 2.5 with ortho phosphoric acid) and acetonitrile in the ratio of 90 : 10 v/v. Similarly the best signal was obtained at a column temperature of 30°C, an injection volume of 20 μL and a flow rate of 0.8 mL/min , Zorbax SB C_{18} (250 mm × 4.6 mm, 5 μm) column. System suitability studies were conducted by injecting DZL standard solutions in six replicates and system suitability parameters such as USP plate number, 2910; tailing factor is 1.08, retention time is 2.653±0.0461 min.

Method Development

Specificity : The specificity of the developed method was examined by injecting solutions of standard, sample, and placebo separately. The absence of interfering peaks of additives in a pharmaceutical formulation at the retention time of DZL proved the specificity of the method.

Linearity: Linear graph were obtained between the concentrations of the analyte and the peak areas which calculated correlation coefficient (r^2 = 0.9999) Linearity was evaluated by analyzing a series of various concentrations of DZL. Six concentrations (10 , 25 , 50, 100, 125, and 150 μg/mL) of DZL were injected in triplicate.

Accuracy: The reliability and validity of the were examined by the standard addition technique. Known amounts of standard drug at 50%, 100%, and 150% of the test concentration were added and analyzed in triplicate. Percent recoveries ranged from 99.86-100.57% to 100.32%, which indicate the excipients in ophthalmic preparations do not interfere with DZL assay (**Table 1).**

Table 1: Recovery study of dorzolamide from pharmaceutical formulation.

	Amount (µg/mL)	Amount (µg/mL)	Amount (µg/mL)	% recovery ±SD
Eye Drop	50	50	50.11	100.21±0.3431
	50	75	75.18	100.30±0.2781

Precision : The intra- and inter-day precisions were determined from the prepared samples on the same and three consecutive days, respectively, while the results of day 3 were obtained by a second analyst. The low % RSD values of the peak areas illustrate acceptable precision of the proposed methods. The precision determinations are summarized in **Table 2.**

Sensitivity : The LOD and LOQ for DZL were determined based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. An LOD value of 0.0403 μg/mL and an LOQ value of 0.1228 μg/mL were found.

Robustness : To verify the robustness of the proposed method, the effect of small changes of relevant

chromatographic parameters such as flow rate and mobile phase composition on the results was investigated. One factor at a time (OFAT) was examined sequentially and peak areas were evaluated as a response variable.

Table 2: Results of the precision study

%RSD (n=6)	Day1	0.08
	Day2	0.11
	Day3	0.12
%RSD (n=8)	Day1-2	0.08
%RSD (n=12)	Day1-3	0.10

The influence of flow rate at 0.7 mL/min and 0.8 mL/min and the effect of different amounts of acetonitrile (8%, 10%, and 12%) in the mobilewere examined.The results of analysis of variance demonstrated that the peak areas were not significantly (p.>0.5) affected by changing these variables. Therefore, the value was not influenced by these small variations in there

Application of the Method:

Analysis of Real Samples:

The validated method has been successfully applied to determine DZL concentrations in eye drop products. Average content of 99.92% label claim was observed. Which was in good agreement with the label claim for the formulation. The developed method is more sensitive and faster than the reported analytical methods in the literature. The pro- posed method had lower limit of detection and quantification [11-15] and analytical run time [16]. The shorter run time leads to the low volume of mobile phase consumption, which makes the method cost effective. Furthermore, this method is more precise than the previous method [16-18].

4. CONCLUSION

This study understood the importance of this method, which is rapid, specific, and sensitive for DZL assay in pharmaceutical dosage form. The method run time is very less with excellent sensitivity: a limit of detection and quantification values of 0.0403 μg/mL and 0.1228 μg/mL, respectively. The developed method has been applied to ophthalmic samples.

5. CONFLICTS OF INTEREST

The authors declare that there are no conflict of interest regarding the publication of this article.

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