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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DORZOLAMIDE HYDROCHLORIDE AND TIMOLOL MALEATE IN OPHTHALMIC SOLUTION BY RP-HPLC

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ABSTRACT

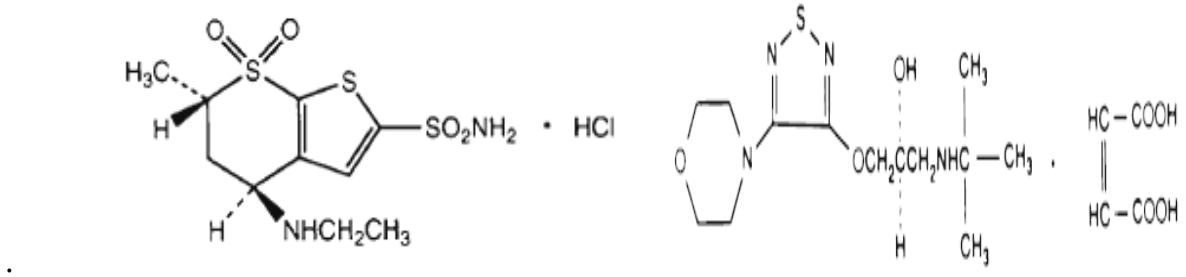
Objective: An accurate and precise HPLC method was developed for the determination of Dorzolamide HCl and Timolol Maleate in ophthalmic solution. **Method:** Separation of the drug was achieved on a INERTSIL ODS 3V 150x4.5 column using a mobile phase consisting of buffer and methanol in the ratio of 50:50v/v adjusted pH of 7.5. The flow rate was 1.0 mL/min and the detection wavelength was 276 nm. **Results and conclusion:** The linearity was observed in the range of 20-100 ppm for Dorzolamide and 5- ppm for Timolol with a correlation coefficient of 1.0 and 1.0 respectively. The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of Dorzolamide and Timolol in Ophthalmic solution.

KEYWORDS: Dorzolamide HCl and Timolol Maleate, HPLC, Validation, Retention time.

INTRODUCTION

[()-(SS)-4 -Ethylamino-5, 6-dihydro-6-methyl-7, 7-dioxide4H-thieno(2,3-b)thio-pyran-2-sulfonamide, dorzolamide (hydrochloride), a potent and selective inhibitor of human carbonic anhydrase, is topically used for reduction of elevated intraocular pressure.[1,2] Timolol (maleate), (S)-3- tert-butylamino -1- [[4 - (4 -morpholinyl) -1, 2, 5-thiadiazol-3- yl]oxy]-2-propanol, is a nonspecific b-adrenergic blocker. It was the first b-blocker used as an antiglaucoma agent, and since then none of the newer b-blockers have been found to be more

effective than timolol (maleate). The two drugs are often combined in eye drops for the therapy of glaucoma.



A **B**

Figure: 1.0: Chemical structures of A) Dorzolamide hydrochloride B) Timolol maleate.

Literature methods for the determination of dorzolamide hydrochloride are mainly based individually on HPLC with UV detection under atmospheric pressure, chemical ionization tandem mass spectrometry in human serum and urine[3–5] and capillary electrophoresis.[6] There are several reports on the determination of timolol maleate, individually or in combination with pilocarpine, including GLC[7] and HPLC of plasma samples,[8,9] HPTLC[10] and, with dorzolamide hydrochloride, spectrophotometry.[11–15] More recently, dorzolamide hydrochloride has been marketed in combination with timolol maleate in eye drops. Dorzolamide hydrochloride is not yet official in any pharmacopoeia. To the best of our knowledge, no HPLC method has been described for the simultaneous determination of both drugs in eye drops. The aim of this work was to develop a rapid, sensitive and specific method for this purpose using HPLC, with simultaneous detection by a diode array detector. This method should be transferrable to quality control laboratories for the determination of both drugs in the presence of each other. The proposed method should require no separation of dorzolamide hydrochloride and timolol maleate before analysis.

MATERIALS AND METHODS

Dorzolamide hydrochloride and Timolol (Figure-1) were obtained as gift sample from MICRO LABS, Bangalore. High purity methanol, water (Milli - Q system, Millipore, Bedford, MA, USA), All chemicals were high purity grade.

Conditions of chromatography

Separation and estimation was performed using HPLC with PDA detector), Inertsil ODS-3V (150mm x 4.6 mm, 5µm) column used in the experiment. The mobile phase was prepared by

mixing of Phosphate buffer and Methanol in the ratio 50:50 (pH is adjusted after the mixing of mobile phase upto 7.5 with diluted OPA) and it was filtered and degassed and detection was at 276 nm. The retention times of Dorzolamide and Timolol were 2.91 min and 4.19 min with the total run time of 10 min.

Preparation of diluent

Prepare required volume of water and methanol in the ratio of 70:30 mix well and sonicated for 30mins for degassing.

Preparation of standard stock solution

Dorzolamide standard stock

Weigh accurately 28.0 mg of Dorzolamide HCl working standard into a 25 ml volumetric flask, dissolve in 10 mL of diluent with the help of sonication after complete dissolving make upto the volume with diluent and mix well.

Timolol maleate standard stock

Weigh accurately 34.0 mg of Timolol Maleate working standard into 100 ml of volumetric flask, dissolve with 30 ml of diluent, sonicate and dilute to volume with diluent and mix well.

Standard preparation

Pipette out 4 ml from each standard stock solution into 20 ml clean, dry volumetric flask and make up to the volume with diluent and mix well.

Sample preparation

Weigh accurately 1.0 gm of sample solution i.e equivalent to 20 mg of Dorzolamide and 5 mg of Timolol from pooled sample solution of 2 to 3 vials into a 100 ml clean, dry volumetric flask and dilute the sample with 30 ml of diluent vortex for few minutes after obtaining clear solution make upto the volume with diluent and mix well.

Method validation

Validation of the method

After the development of RP-HPLC method for the estimation of drug in a dosage form, validation of the method was performed. This section describes the procedure followed for validation of the developed method.

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 (tR), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 60µg/ml, 15µg/ml.

Linearity

Prepare a series of standard solutions (not less than 5 is recommended) in the range of 20µg/ml-100 µg/ml of Dorzolamide standard and 5µg/ml-25µg/ml of Timolol standard injected. A plot of average peak area versus the concentration in µg/ml or mg/ml is made and from this the correlation coefficient, y-intercept (const. of regression) and slope (coefficient of regression) of the regression line were calculated.

Precision

The precision of the test procedure was evaluated for Dorzolamide and Timolol by injecting the six standard solutions. The Relative Standard Deviation of six injections were calculated. The result of Precision studies is given in Table.1.

Specificity

Specificity is the ability of a method to discriminate between the analyte(s) of interest and other components that are present in the sample. A study of placebo interference from excipients was conducted. Equivalent weight of placebo taken as per the test method and placebo interference was conducted in duplicate.

Accuracy

To validate the test method can accurately quantify Dorzolamide and Timolol, prepare samples in three times for higher and lower levels, in triplicate for other levels by spiking Dorzolamide and Timolol active material with equivalent amount of placebo and perform CU as per test procedure. Prepare samples at levels 50%, 100% and 150% of the target assay concentration i.e. 50% of the lowest strength initial concentration to 150% of the highest strength initial concentration level. Table-1.0 shows the results for accuracy of Dorzolamide and Timolol.

Robustness

Robustness of the method is performed by altering the chromatographic conditions such as pH of the buffer, Wavelength, Mobile phase composition and observed the variation of the results which should be within the acceptance criteria.

Limit of detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

1. Based on Signal-to-Noise for LOD (3:1), LOQ (10:1)
2. Based on the Standard Deviation of the Response and the Slope

Limit of quantitation (LOQ)

The quantitation 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. From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\,\sigma}{S}, \quad LOQ = \frac{10\,\sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

LOD and LOQ of Dorzolamide and Timolol are performed by spiking of known concentrations of the sample into the placebo of formulation and inject the sample the results are estimated by signal to noise ratio.

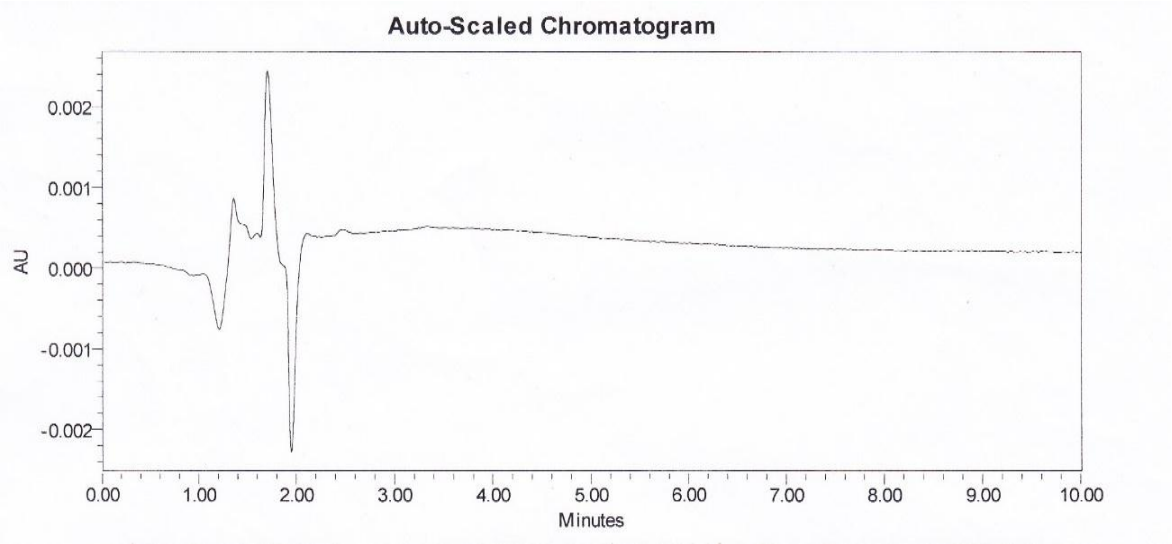


Figure 1.0: Blank chromatogram.

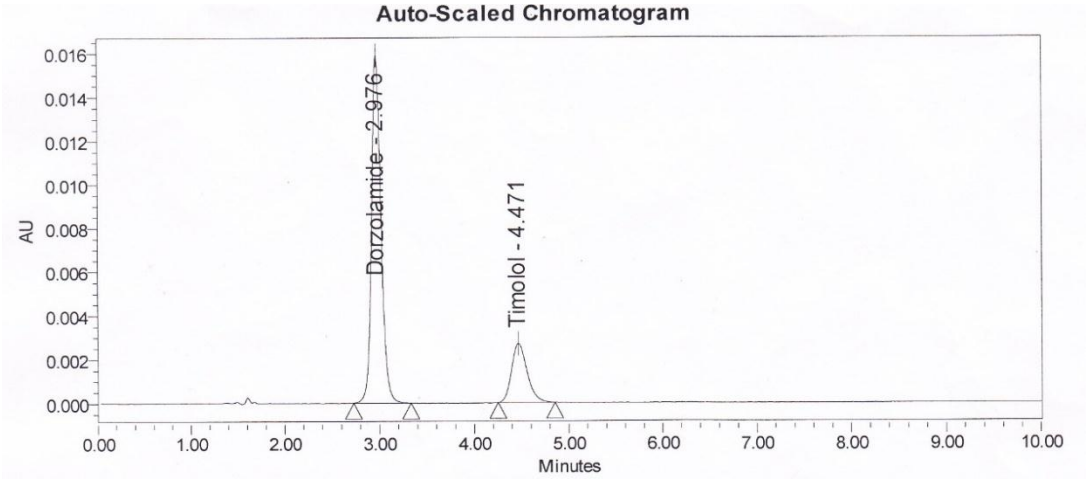


Fig. 5.0: Chromatogram of Dorzolamide & Timolol.

Table 1.0: Summary of validation parameters.

Validation	Parameters	Dorzolamide	Timolol
System suitability	Tailing factor	1.1	1.2
	%RSD	0.1	0.1
	Theoretical plates	3245	3127
	Resolution	5.2	N.A
Linearity	Correlation coefficient	0.999	0.999
	Slope	5450	6309
Precision	%RSD	0.1	o.2
Accuracy	Mean % recovery for 50, 100, 150% respectively	99.06 97.03 97.84	103.61 100.60 101.716
Specificity		No interference	No interference
Robustness	1. Flow (± 10%), wavelength (± 5 nm), organic variation in mobile phase (±2%), Column Oven Temperature (±5^{0}C), and P^H (± 0.2 units)] at which robustness studies were performed. 2. The system suitability criteria is meeting the requirement for all variable conditions. 3. The test method is robust for all the variable conditions except flow variation (-10%).		

Assay of Dorzolamide and Timolol is performed by weighing method. Wt/mL for the formulation is performed in a clean and dried specific gravity bottle (pycnometer) and its value implemented in the calculation of assay.

$$\frac{Wt}{mL} = \frac{W3-W1}{W2-W1} \times \frac{Weight}{Volume}$$

W1= Weight of empty specific gravity bottle (10mL)

W2 = Weight of bottle with water

W3 = Weight of bottle with ophthalmic solution.

Assay formula

$$= \frac{At}{As} \times \frac{Ws}{Ds} \times \frac{Dt}{Wt} \times \frac{PA}{100} \times \frac{Mol\ wt.1}{Mol\ wt.2} \times \frac{Wt}{mL} \times \frac{Labelled\ claim}{Amount\ obtained}$$

At = Area of sample, As = Area of standard

Ws = Weight of standard, Wt = Weight of sample (1.013gms)

Dt = Dilution of sample, Ds = Dilution of standard

PA = Potency of standard (Dorzolamide =99.5%, timolol=96.5%)

Weight/mL =1.0154gm/ mL

Table 2: Results of %Assay of Dorzolamide and Timolol.

	As	At	Wt. taken mg	Mol.wt$_1$ gm/mol	Mol.wt$_2$ gm/mol	%Assay
Dorzolamide	1190424	1216813	26.99	324.4	360.9	98.9
Timolol	421432	442451	33.6	316.43	432.49	99.8

RESULTS AND DISCUSSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost RP-HPLC method. It is successfully applied for the determination of Dorzolamide and Timolol in pharmaceutical preparations without the interferences of other constituent in the formulations.

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Methanol: Posphate Buffer (50:50v/v) with 1.0 ml/min flow rate is quite robust.

The optimum wavelength for detection was 276 nm at which better detector response for drug was obtained. The average retention time for Dorzolamide and Timolol were found to be 2.89and4.20.The linearity was observed in the range of 20-100 ppm for Dorzolamide and 5-25 ppm for Timolol with a correlation coefficient of 0.999 and 0.999 respectively. The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of 99.0 – 99.9 %.

Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different

days, over a period of three days. These results show the accuracy and reproducibility of the assay. %Assay of Dorzolamide and Timolol in ophthalmic formulation is found to be 98.9 %and 99.8% are with in acceptance criteria 95-105%.

CONCLUSION

The chromatographic method developed for Dorzolamide and Timolol is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

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