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

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ABSTRACT

Aim of the study is to develop and validate RP-HPLC method for the determination of Dorzolamide HCl and Timolol Maleate in ophthalmic solution. 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. The linearity was observed in the range of 20-100 ppm for Dorzolamide and 5- ppm for Timolol with a correlation coefficient of 0.999 and 0.999 respectively. The proposed method was validated for its linearity, accuracy, precision and robustness. 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 %.

Key words: Dorzolamide HCl, Timolol Maleate, RP-HPLC method, ophthalmic solution.

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INTRODUCTION

Dorzolamide Hydrochloride **(**(4S, 6S)-2-ethylamino-4-methyl-5,5-dioxo-5-6,7-dithiabicyclo [4.3.0] nona-8, 10-diene-8-sulfonamide, hydrochloride) is an inhibitor of human carbonic anhydrase II. Inhibition of carbonic anhydrase in the ciliary processes of the eye decreases aqueous humor secretion, presumably by slowing the formation of bicarbonate ions with subsequent reduction in sodium and fluid transport. Timolol Maleate ((S)-1-(tert-butylamino)-3-[(4-morpholin-4-yl-

1,2,5-thiadiazol-3-yl)oxy]propan-2-ol,maleate) is a beta1 and beta2 (non-selective) adrenergic receptor blocking agent. The combined effect of these two agents administered as Dorzolamide HCI/Timolol Maleate Ophthalmic Solution results in additional intraocular pressure reduction compared to either component administered alone (1, 2).Literature review reveals only few methods has been reported for analysis of Dorzolamide and Timolol in eye drops (3-9). An attempt was made in the present investigation to develop new RP-HPLC method for the estimation of Dorzolamide and Timolol in eye drops .Dorzolamide and Timolol have been reported to possess Diuretic, non-selective β receptor antagonist. Used in the treatment of glaucoma. The newly developed HPLC method would be then validated as per ICH Guidelines to indicate that the analytical procedure employed is recommended to be suitable for its routine analysis in terms of various parameters like specificity, LOD, LOQ, linearity, precision, accuracy, system suitability.

MATERIALS AND METHODS

Materials

Sample was collected from Aurobindo Pharma Pvt Ltd, Hyd. Orthophosphoric Acid, Water, Methanol, Di Sodium Hydrogen Posphate, Di Potassium Hydrogen Posphate from Merck Spl Ltd., Mumbai.

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 upto 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 dilutethe sample with 30 ml of diluent vortex for few minutes after obtaining clear solution make upto the volume with diluent and mix well.After the development of RP-HPLC method for the estimation of drug in a dosage form, validation of the method was performed for parameters such as System Suitability, Linearity, Precision, Specificity, Accuracy, Limit of Detection, Limit of Quantitation according to ICH guidelines (10, 11)

RESULTS AND DISCUSSION

Good resolution was produced in mobile phase Phosphate buffer and Methanol in the ratio 50:50 (pH is adjusted after the mixing of mobile phase upto 7.5 with diluted OPA). Assay was carried out for marketed formulation and the results are given in table-1 and fig-1. % Assay of Dorzolamide and Timolol in ophthalmic formulation is found to be 98.9 %and 99.8% are within acceptance criteria 95-105%.

Table-1 Results of %Assay of Dorzolamide and Timolol

Name	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

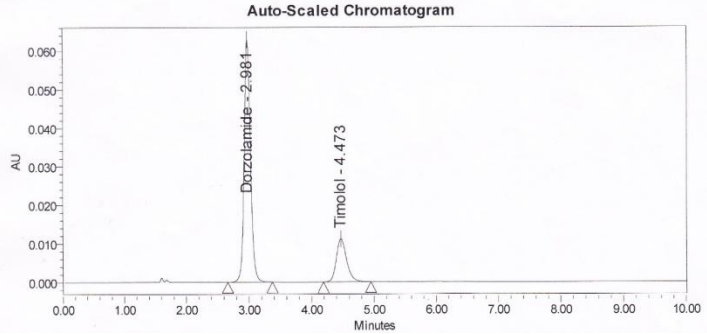

Fig-1 chromatogram for Dorzolamide and Timolol in ophthalmic formulation

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. The results were within acceptable limits.

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. The calibration data of Dorzolamide and Timolol is given in Table-2 and the calibration curve of linearity is shown in Fig 2 and 3.

Table-2 Results of Linearity of Detector Response

Standard concentration (µg/ml)		Area		Mean Area	
Dorzolamide	**Timolol**	**Dorzolamide**	**Timolol**	**Dorzolamide**	**Timolol**
20	5	108974	31001	109802.5	30793.7
		109731	30586		
40	10	218013	62699	218245	62844
		218478	62990		
60	15	325221	93092	325366	93302
		325512	93512		
80	20	436224	126886	436461	126898
		436699	126912		
100	25	541710	158075	542172	158074
		542634	158074		
Regression Dorzolamide= 0.999 Timolol= 0.999					

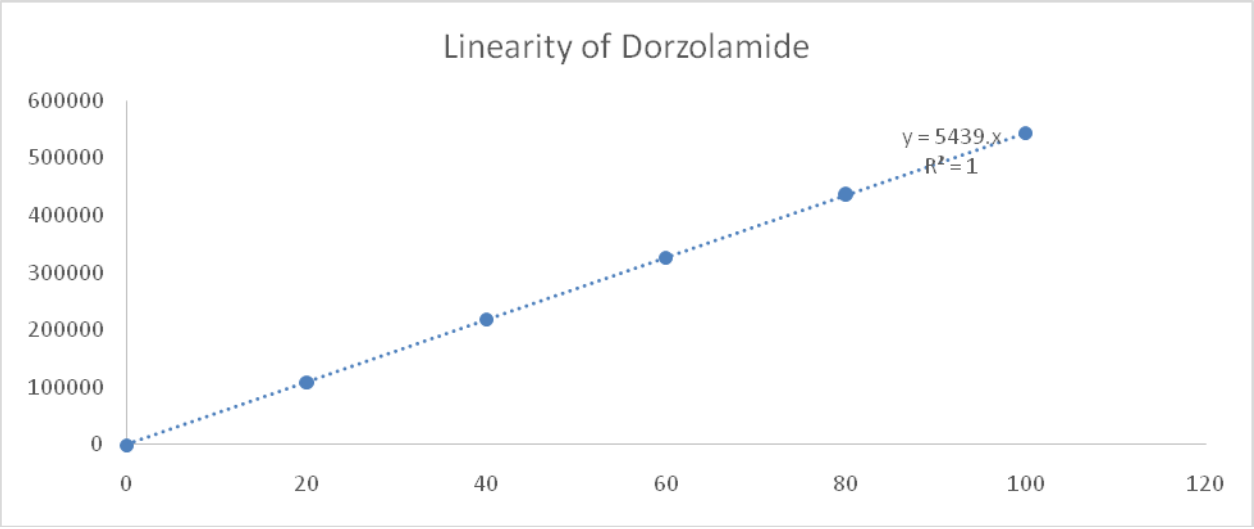

Fig-2 Graph of Linearity of Dorzolamide

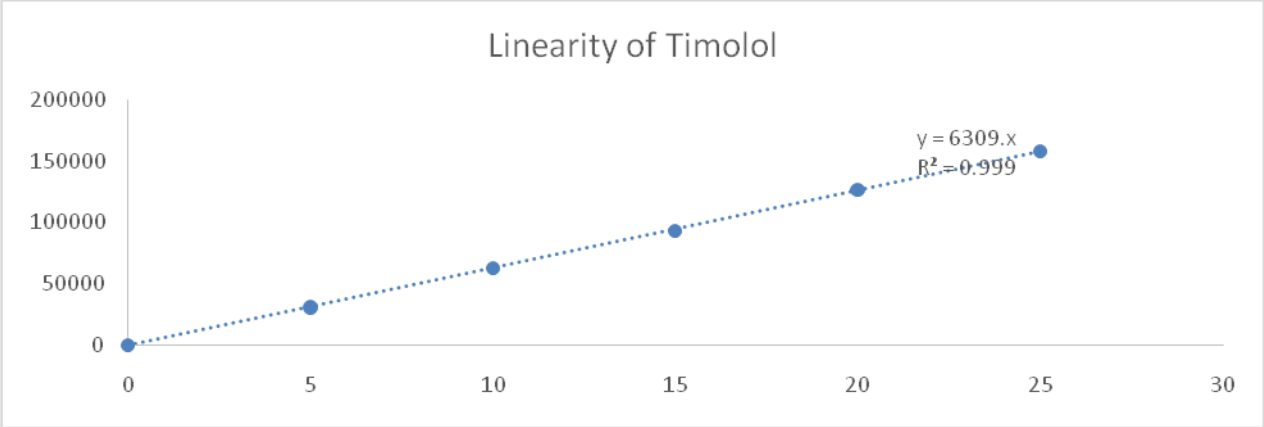

Fig-3 Graph of Linearity of Timolol

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 was calculated. The result of Precision studies is given in Table-3.

Table-3 Precision for Dorzolamide and Timolol

S.No	Injection Number	Peak area for Dorzolamide	Peak area for Timolol
1	Standard 1	1192745	420820
2	Standard 2	1192045	420776
3	Standard 3	1191767	420784
4	Standard 4	1190839	420198
5	Standard 5	1190674	420660
6	Standard 6	1191866	420628
Mean		1191656	420644
%RSD		0.1	0.1

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-4 shows the results for accuracy of Dorzolamide and Timolol

Table-4 Accuracy for Dorzolamide and Timolol

% Spiked	Weight added (mg)		Weight recovered (mg)		% Recovery	
	Dorzolamide	Timolol	Dorzolamide	Timolol	Dorzolamide	Timolol
50	10.2	2.5	9.912	2.59	99.10	103.90
	10.2	2.5	9.910	2.55	99.08	103.60
	10.2	2.5	9.908	2.51	99.02	103.30
100	20.0	5.0	19.45	5.4	97.20	100.69
	20.1	5.0	19.42	5.40	97.07	100.56
	20.0	5.0	19.40	5.38	96.82	100.55
150	30.0	7.5	29.37	7.30	97.91	101.79
	30.0	7.5	29.33	7.26	97.82	101.71
	30.0	7.5	29.32	7.24	97.78	101.65

LOD and LOQ was calculated and results is given in table-5.

Table-5 Results of LOD and LOQ

Sample	LOD	LOQ
Dorzolamide	0.4710µg/ml	1.4275µg/ml
Timolol	0.1208 µg/ml	0.3661µg/ml

CONCLUSION

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. 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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