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Simultaneous RP-HPLC determination of dorzolamide hydrochloride and timolol maleate in pharmaceutical preparations

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ABSTRACT

A simple, fast and precise RP-HPLC method is developed for the simultaneous determination of dorzolamide Hydrochloride and timolol maleate using brimonidine tartarate as an internal standard. Chromatographic separation of the two drugs was performed on a Inertsil ODS-3V, C_{18} column (250mm × 4.6 mm, 5µm) as stationary phase with a mobile phase comprising of Buffer* : Acetonitrile (85:15) Buffer* : 0.1 % v/v TriFluoroAcetic acid(1 ml Trifluoroacetic acid to 1000 ml water.), filtered and degassed., at a flow rate of 1.5mL min^{-1} and UV detection at 278nm. The proposed method was validated for linearity, accuracy, precision, LOD, LOQ. Linearity, accuracy and precision were found to be acceptable over the ranges of 140-420µg mL^{-1} for Timolol Maleate, 450-1350µg mL^{-1} for Dorzolamide Hydrochloride. It can be conveniently adopted for routine quality control analysis.

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KEYWORDS

ICH guidelines;
Validation;
Column liquid chromatography;
Pharmaceutical preparations;
Dorzolamide hydrochloride;
Timolol maleate;
Brimonidine tartarate.

INTRODUCTION

Dorzolamide hydrochloride [(4S)-trans-4-ethyl ammonio-6-methyl-5,6-dihydro-4H-thieno[2,3-b] thiopyran-2-sulfonamide 7,7-dioxide chloride], $C_{10}H_{17}N_2O_4S_2^+ \cdot Cl^-$, belongs to a class of drugs called carbonic anhydrase inhibitors.It is us in the treatment of glaucoma, it is an antiglaucoma agent Timolol Maleate ((*S*)-1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol (*Z*)-2-butenedioate (1:1) salt. Timolol maleate is a beta1 and beta2 (nonselective) adrenergic receptor blocking agent, Timolol maleate reduces elevated and normal intraocular pressure, whether or not associated with glaucoma. Timolol maleate is a short-acting, potent, non-selective, beta-adrenergic antagonist. It has no intrinsic sympathomimetic activity and no membrane stabilizing activity.

Timolol maleate eye drops reduce intraocular pressure with little or no effect on accommodation or pupil size[1,2]. The structures of these two drugs are shown in figure 1. One such combination contains 20mg (2.0% w/v) of Dorzolamide Hydrochloride and 5mg (0.5% w/v) of Timolol Maleate. It is widely used as anti-glaucoma. The literature revealed no method was available for simultaneous determination of these two drugs in such pharmaceutical preparations by HPLC. Therefore an HPLC method was developed for determination of Dorzolamide Hydrochloride and Timolol Maleate from their combined dosage form[5-12]. The method described is simple, fast, precise and accurate for simultaneous determination of Dorzolamide Hydrochloride and Timolol Maleate from pharmaceutical preparation.

Chemicals and reagents

Standards were supplied from Hetero Labs Ltd., Mumbai, India. Dorzox T eyed rops manufactured by Cipla, India was procured from the market. Acetonitrile, Trifluoro acetic acid and orthophosphoric acid were from Qualigens. Double distilled water was employed throughout the work. All dilutions were performed in standard volumetric flasks.

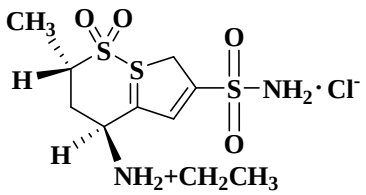
EXPERIMENTAL

Method development and optimization of chromatographic conditions

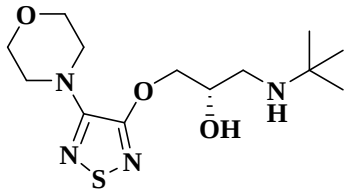
To develop a suitable LC method for the analysis of Timolol Maleate and Dorzolamide Hydrochloride in their combined dosage form, different mobile phases were tried. The criteria employed for selecting the mobile phase for the analyses of the drugs were cost involve, time required for the analysis, better separation of drugs. Chromatographic separation was preformed with Waters Alliance system performance liquid chromatography having HPLC quarternary pump, equipped with auto sampler and a photo-diode array detector. The uv spectrum of all the three drugs were scanned on photo diode array detector for selecting the working wavelength. Peak purity of all the three drugs were checked using photo diode array detector. Chromatograms and data were recorded by means of Empower software. An Inertsil ODS3, C_{18} column (250mm × 4.6 mm, 5 µm particle) was used for the analysis. The mobile phase comprising of 0.1% ortho phosphoric acid: acetonitrile in the ratio (40:60) v/v. The system was run at a flow rate of 1.5mL min^{-1}, 10µL of sample was injected in the chromatographic system and detection wavelength was set at 278nm for simultaneous determination of the two drugs. A typical HPLC chromatogram for simultaneous determination of Dorzolamide Hydrochloride and Timolol Maleate from pharmaceutical formulation is shown in figures 2 and 3.

Preparation of standard stock solutions

The stock solution of timolol maleate(1400µg mL^{-1}) was prepared by dissolving 70.0 mg of Timolol Maleate (99.6 %) in mobile phase in a standard 50mL volumetric flask (solution A). Internal standard (Brimonidine Tartarate) stock solution (800µg mL^{-1}) was prepared by dissolving 40.0 mg of Brimonidine Tartarate in mobile phase in a 50mL standard volumetric flask.



Dorzolamide hydrochloride
($C_{10}H_{17}N_2O_4S_3^+ \cdot Cl^-$)



Timolol maleate ($C_{13}H_{24}N_4O_3S$)

Figure 1: Structures of dorzolamide hydrochloride and timolol maleate

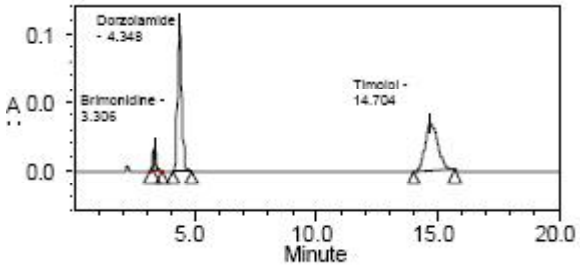


Figure 2: Chromatogram of timolol maleate and dorzolamide hydrochloride with brimonidine tartarate(internal standard) in standard preparation

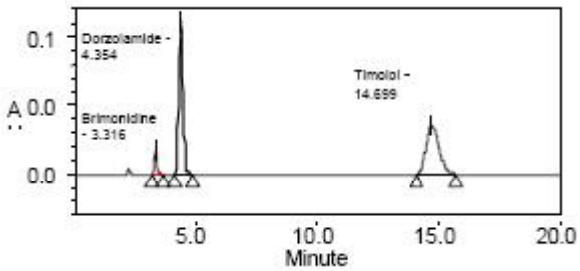


Figure 3: Chromatogram of timolol maleate and dorzolamide hydrochloride with brimonidine tartarate (internal standard) in sample preparation

Working standard solution

Dorzolamide Hydrochloride (900µg mL^{-1}) was prepared by adding 45.0 mg of Dorzolamide Hydrochloride (99.4 %) into a 50ml volumetric flask, into this flask transfer 10.0 mL of stock solutions A and 5ml of

stock solution C and add mobile phase to dissolve Dorzolamide Hydrochloride and diluted up to the mark with mobile phase.

Sample preparation

Transfer about 2.0 g of sample(eye drops) accurately weighed to a 50 ml volumetric flask; add 30 ml of diluent sonicate for 2 minutes and dilute to volume with mobile phase.

RESULTS AND DISCUSSION

System suitability

System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out. System suitability tests were performed as per the USP 31 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 10µL standard solutions of Dorzolamide Hydrochloride and Timolol Maleate of strengths 900µg mL^{-1} and 280µg mL^{-1} respectively using Brimonidine Tartarate as an internal standard in five replicates. The RSD values of Dorzolamide Hydrochloride and Timolol Maleate were 0.40 and 0.32 respectively. The RSD values were found to be satisfactory and meeting the requirements of USP 31 (RSD less than 2.0 %). Theoretical plates, resolution, tailing factor were determined and are presented in TABLE 1.

TABLE 1: Result of system suitability

Parameters	Brimonidine tartarate (IS)	Dorzolamide hydrochloride	Timolol maleate
Resolution	-	3.92	15.86
Tailing factor	1.16	1.26	1.22
Theoretical plates	4800	4632	4250

TABLE 2: Results of linearity

Analyte	Slope (mean)	Intercept (mean)	Correlation coefficient (r^2) (n=7)
Dorzolamide hydrochloride	0.0076	0.147	0.9997
Timolol maleate	0.0014	-0.010	0.9998

TABLE 3: Results of assay experiment

	Dorzolamide hydrochloride	Timolol maleate
Drug found in mg/mL (mean)	20.46	5.12
Mean %	99.89	99.68
RSD	0.62	0.38

TABLE 4 : Accuracy of the method

	Initial conc. (mg)	Conc. added (mg)	Total conc. (mg)	Conc. found (mg)	RSD (%) n= 3	Recovery (%)	% Bias
	20	0	20	19.86	0.18	99.30	+0.18
Dorzolamide	20	2	22	21.80	0.24	99.09	+0.67
Hydrochloride	20	4	24	23.84	0.15	99.33	+0.56
	20	6	26	25.78	0.32	99.15	+0.72
	5	0	5.0	4.96	0.21	99.20	+0.24
Timolol	5	0.5	5.5	5.45	0.24	99.09	+0.33
Maleate	5	1.0	6.0	5.95	0.19	99.16	+0.30
	5	1.5	6.5	6.46	0.28	99.38	+0.28

Linearity

Linearity was evaluated by analysis of working standard solutions of Dorzolamide Hydrochloride and Timolol Maleate of seven different concentrations[2,3]. The range of linearity was from 450-1350µg mL^{-1} for Dorzolamide Hydrochloride and 140 - 420µg mL^{-1} for Timolol Maleate. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained for the two pharmaceuticals are represented in TABLE 2. The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration of each drug.

Limit of detection and limits of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively[2,3]. The LOD and LOQ of Dorzolamide Hydrochloride and Timolol Maleate were experimentally determined by six injections of each drug. The LOD of Dorzolamide Hydrochloride and Timolol Maleate were found to be 0.1µg mL^{-1} and 0.2µg mL^{-1} respectively. The LOQ of Dorzolamide Hydrochloride and Timolol Maleate were found to be 0.8µg mL^{-1} and 1.0g mL^{-1} respectively.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions[3]. The relative standard deviations were less than 2% for the two drugs. Method precision was determined from results from ten independent determinations at 100% of the test concentrations of Dorzolamide Hydrochloride and Timolol Maleate in the product. The RSD were 0.62 and 0.38 respectively. Refer TABLE 3.

Accuracy

To study accuracy of the method, recovery experiment was carried out by applying the standard addition method. A known quantity of each drug substance corresponding to 100%, 110%, 120% and 130% of the label claim of each drug was added, to determine if there are positive or negative interferences from excipients present in the formulation[4]. Each set of addition was repeated three times .The accuracy was expressed as the percentage of analytes recovered by the assay. TABLE 4 lists the recoveries of the drugs from a series of spiked concentrations. The results indicate the method is highly accurate for simultaneous determination of the three drugs.

DISCUSSION AND CONCLUSION

Several mobile phases such as water-methanol, water-acetonitrile in different ratios were tried but good peak shape and good resolution between Dorzolamide Hydrochloride, Brimonidine Tartarate and Timolol Maleate was observed using the mobile phase mentioned in chromatographic conditions. The method after being completely validated showed satisfactory data for all the method validation parameters. The method was found to be specific. The low values of %RSD for Method precision suggested that the method is precise. Linearity evaluated for the analyte peak showed a good linear response over a wide range of concentration. The linearity, precision, accuracy of the method proves that the method is specific, accurate,easily reproducible and can be used for simultaneous determination of Timolol Maleate and Dorzolamide Hydrochloride from pharmaceutical preparations.

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