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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2011, 3(4): 866-874

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Method Development and Its Validation for Simultaneous Estimation of Timolol Maleate and Dorzolamide Hydrochloride in as API and In Ophthalmic Solution Dosage Form by RPHPLC

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ABSTRACT

Dorzolamide Hydrochloride is used to lower the increased intraocular pressure in open-angle glaucoma and ocular hypertension. Timolol maleate is indicated in the treatment of elevated intraocular pressure in patients with ocular hypertension or open-angle glaucoma. In this article, a high performance liquid chromatography (HPLC) method with UV-Visible detector wavelength (254 nm & 295 nm) for Dorzolamide Hydrochloride & Timolol maleate was developed and validated. The mobile phase consisted of methanol : buffer (60:40), the pH was adjusted to 3 with glacial acetic acid, was run through a Column C-18, (EC 150/4.6 NUCLEOSIL 100-5), reverse phase analytical column. The experiment was carried out at room temperature for Dorzolamide Hydrochloride & Timolol maleate. Analytical run time was less than 10 min. The assay exhibited good linear relationship. Accuracy & Precision were over the concentration range of 10 to 30 µg/ml & 40 to 120 µg/ml for Dorzolamide Hydrochloride and Timolol Maleate respectively. This method was found to be applicable for determination of the Dorzolamide Hydrochloride & Timolol maleate in active pharmaceutical ingredient (API) and pharmaceutical product also.

Key words: Active pharmaceutical ingredient, HPLC, Validation, Dorzolamide Hydrochloride, Timolol maleate.

INTRODUCTION

Dorzolamide is an ophthalmic solution (a liquid that is placed in the eyes) that is for treating glaucoma. It is in a class of drugs called carbonic anhydrase inhibitors which includes brinzolamide. Many parts of the body, including the eye, contain the enzyme carbonic anhydrase

which plays a key role in controlling the pressure within the eye [1]. Carbonic anhydrase controls secretion of fluid within the eye and thereby determines the pressure within the eye (intraocular pressure): the greater the amount of fluid that is secreted, the higher the pressure. Patients with glaucoma have increased intraocular pressure. Dorzolamide blocks carbonic anhydrase thereby decreasing intraocular pressure. This reduces the risk of nerve damage and loss of vision that is caused by increased intraocular pressure in patients with glaucoma.

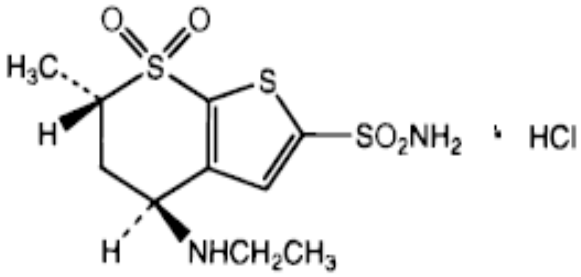

Figure 1: Structure of Dorzolamide hydrochloride

Timolol is a beta-adrenergic blocking drug that is used to treat high blood pressure, angina (heart pain), and heart attacks and to prevent migraine headaches [2]. Timolol is a first generation beta blocker in a class that includes propranolol (Inderal, InnoPran), nadolol (Corgard), penbutolol sulfate (Levatol), sotalol hydrochloride (Betapace), and pindolol (Visken). They differ from other beta blockers because they are non-selective in nature, meaning that they block both beta-1 and beta-2 receptors on nerves and, therefore, will affect not only the heart but also the kidneys, lungs, gastrointestinal tract, liver, uterus, muscles surrounding blood vessels, and skeletal muscle. As a result, they could cause such effects as reduced pumping of blood by the heart and reduced kidney function among other actions. Timolol specifically works by blocking the stimulating actions of the sympathetic nervous system thereby allowing the heart to relax and beat more slowly. This reduces the amount of blood that the heart must pump. Timolol was approved by the FDA in November 1981.

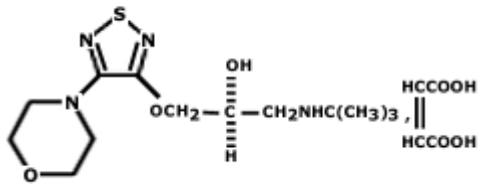

Figure 2: Structure of Timolol maleate

The aim of the present study is to develop a sensitive and rapid RP-HPLC method with UV detection for the quantitative determination of Dorzolamide Hydrochloride and Timolol Maleate in active pharmaceutical ingredient (API). This method offers the advantage of simplicity with adequate sensitivity, selectivity, precision and accuracy. Comparative to other methods this method shows less run time and good sensitivity [3]. This method has been satisfactorily applied to the determination of estimation of Dorzolamide Hydrochloride and Timolol Maleate as API and in ophthalmic solution dosage form [4]. The review of literature reveals that methods including LC/MS, UV, HPTLC, HPLC, Volta metric methods etc. have been reported for the estimation of drugs individually/simultaneously mixture in biological specimen but no method

has been reported so far in literature for estimation of Dorzolamide Hydrochloride and Timolol Maleate as API and in ophthalmic solution dosage form by RP-HPLC method with UV detector.

EXPERIMENTAL SECTION

Materials

Dorzolamide Hydrochloride and Timolol Maleate Jawa Pharmaceuticals (P) Ltd were available from Oasis Laboratories Jaipur, India. HPLC grade acetonitrile, methanol, triethylamine were purchased from Merck. Double distilled water for analytical purpose was obtained from Milli-Q R-O system.

Chromatographic conditions

The HPLC system consisted of a Shimadzu LC-10Advp liquid chromatographic pump, Rheodyne injection port with a 20 µl sample loop and UV-Visible detector (Shimadzu). Data collection, integration and calibration were accomplished using LC Solutions chromatography Data system. The chromatographic separation of Dorzolamide Hydrochloride and Timolol Maleate was accomplished using EC 150/4.6 NUCLEOSIL 100-5 C 18, 5µm reverse phase analytical column [5]. The mobile phase consisted of methanol: buffer (0.02 M Octane-1-sufonic acid buffer) (60:40), the pH was adjusted to 3 with glacial acetic acid. Before use, the mobile phase was filtered by passing it through a 0.45µm filter and the filtrate is degassed by using bath sonicator. The mobile phase was pumped at 1 ml/min at room temperature. All separations were performed at ambient temperature [6].

Preparation of stock and standard solutions

Stock solution of Dorzolamide Hydrochloride and Timolol Maleate was prepared in methanol at (1000 + 250 µg/ml). This stock solution was diluted with methanol to obtain the concentrations required for preparation of standard working solutions [7]. Dorzolamide Hydrochloride. and Timolol Maleate working solutions were in the range of 6.43 to 120 µg/ml & 2.28 to 30 µg/ml. Samples for the determination of recovery, precision and accuracy were prepared by spiking quality control (QC) standard Dorzolamide Hydrochloride (40, 60, 80 ,100, 120 µg/ml) and Timolol Maleate & (10, 15, 20, 25, 30 µg/ml) concentrations.

Validation parameters and procedures

The RP-HPLC assay validation was done as per ICH Q2 (R1) guidelines [8]. These tests included determination of accuracy, precision, linearity, sensitivity and limit of detection and quantification.

Linearity, limit of detection (LOD) and limit of quantification (LOQ)

Standard calibration samples were prepared by making serial dilutions from the stock solution of Dorzolamide Hydrochloride and Timolol Maleate. Calibration curve of concentration versus peak areas was plotted at concentration range of Dorzolamide Hydrochloride and Timolol Maleate working solutions 6.43 to 120 µg/ml & 2.28 to 30 µg/ml 0.5-50 µg/ml simultaneously [9]. The limit of detection (LOD) and the lower limit of quantification (LLOQ) were measured according to the FDA's guidance for bioanalytical method validation in 2001. The limit of detection was defined as the lowest concentration of Dorzolamide Hydrochloride and Timolol Maleate resulting in a peak height greater or equal to three times from background noise (S/N = 3). The LOQ was investigated in extracted samples from five different days [10]. For the determination of LOQ, the percentage deviation and % RSD are to be less than 20%.

Precision and Accuracy

The precision and accuracy were determined by analyzing spiked standard and extracted samples of Dorzolamide Hydrochloride at (40, 60, 80, 100, 120 µg/ml) and Timolol Maleate at (10, 15, 20, 25, 30 µg/ml) different concentrations ranging. The precision of an HPLC method was determined as the coefficient of variation (%RSD) of intra- and inter-day. The intra-day precision was determined by analyzing the spiked standard and extracted samples prepared within a day. The inter-day precision was determined by analyzing the spiked standard and extracted samples analyzed on five different days [11]. After concentrations were calculated by re-fitting peak areas obtained with different standard solutions into a derived regression equation from the set of these standard solutions, %R.S.D. was determined at each concentration of the standard solutions from their average value and S.D. The accuracy of the HPLC method was demonstrated by percentage deviation. The calculated concentrations (or conc. found) were obtained by re-fitting peak areas from standard solutions of known concentrations (or conc. added) into a derived regression equation. The conc. found and conc. added was then used to determine the absolute percentage deviation at each concentration of the standard solutions [12].

Recovery

The absolute recovery was calculated by comparing the peak areas of compounds after liquid-liquid extraction with those obtained on direct injection onto the column of the same amount of Dorzolamide Hydrochloride and Timolol Maleate dissolved in mobile phase [13]. Each measurement was made in triplicates.

$$\text{Recovery (\%)} = \frac{\text{peak area of extracted standard}}{\text{peak area of unextracted standard}} \text{x100}$$

System suitability

The purpose of system suitability to define a set of parameters that are measured prior to each experiment that will tell the analyst if the system is performing adequately or not [14]. The suitability parameters that are evaluated for HPLC method includes peak area reproducibility and retention time.

RESULTS

Chromatography

To facilitate quality control study of Dorzolamide Hydrochloride and Timolol Maleate, a sensitive, specific and reproducible HPLC method has been developed and validated for quantitative determination of Dorzolamide Hydrochloride and Timolol Maleate by reverse phase HPLC with UV detection at 254 nm & 295 nm.

The representative chromatograms of Dorzolamide Hydrochloride and Timolol Maleate spiked in unextracted standard concentration and blank are shown Fig. 2 and Fig. 3. The retention time of Dorzolamide Hydrochloride and Timolol Maleate was 6.08 min & 3.46 min respectively, and the peaks were sharp. There was good baseline separation of Dorzolamide Hydrochloride and Timolol Maleate.

Linearity, limit of detection (LOD) and limit of quantitation (LOQ)

Peak areas of Dorzolamide Hydrochloride and Timolol Maleate were measured. A representative calibration graph of peak area versus concentration in the range Dorzolamide Hydrochloride at

(40, 60, 80, 100, 120 µg/ml) and Timolol Maleate at (10, 15, 20, 25, 30 µg/ml) resulted in regression equation of the calibration curve was calculated as y = 7330.x + 3699 for Dorzolamide Hydrochloride was calculated from calibration curve, where y is peak area and x is the value of various concentrations of standard solutions and correlation coefficient was found to be $r^2 = 0.999$.

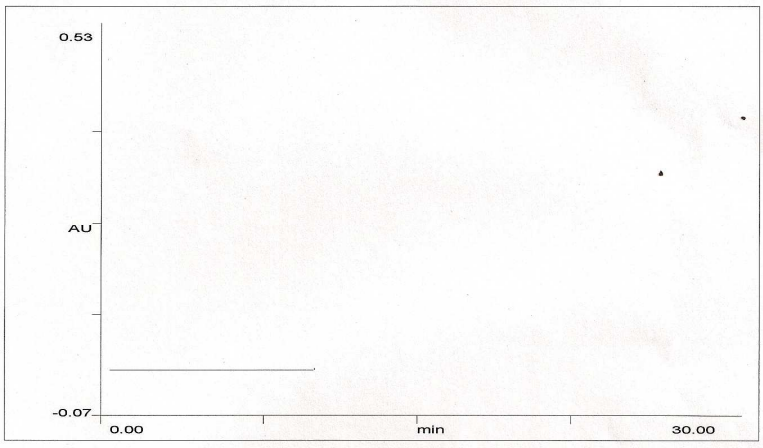

Figure 3: Chromatogram of Baseline

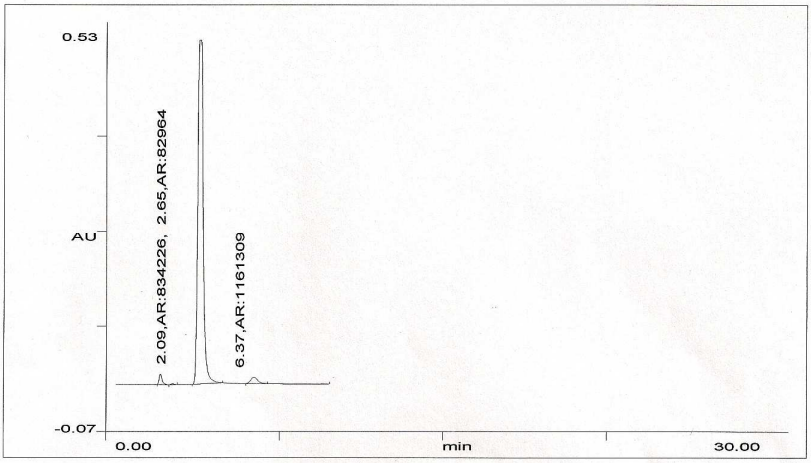

Figure 4: Chromatogram of standard Timolol Maleate and Dorzolamide Hydrochloride

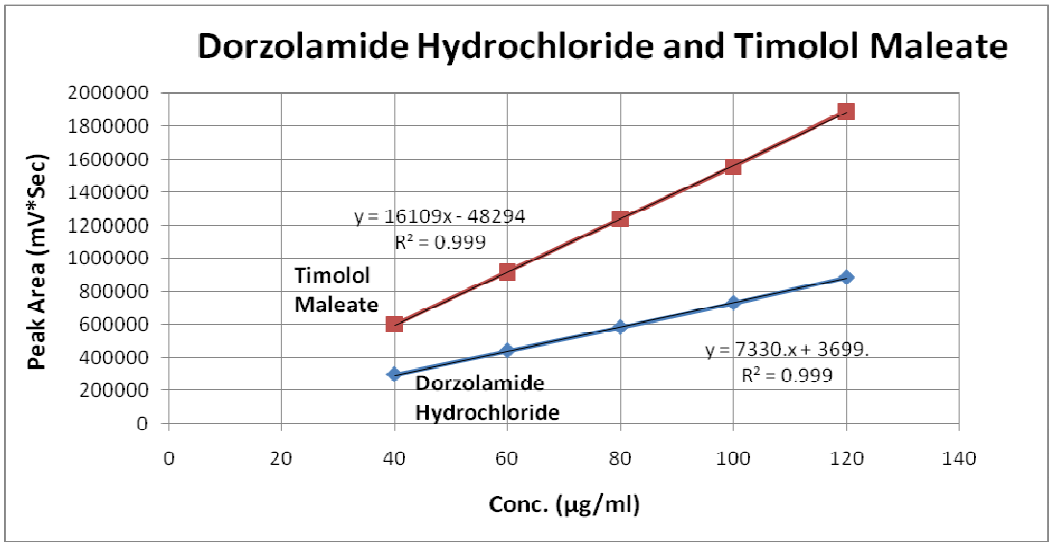

Figure 5: Calibration curve of standard Timolol Maleate and Dorzolamide Hydrochloride

The linear regression equation the linear regression equation y = 16109x – 48294 for Timolol maleate was calculated from calibration curve, where y is peak area and x is the value of various concentrations of standard solutions and correlation coefficient was found to be $r^2 = 0.999$. These results demonstrated a good linearity between the peak areas versus concentrations. The LOD was found to be 0.755 µg/ml and 2.122 µg/ml and LOQ was found to be 2.289 µg/ml and 6.432 µg/ml for Timolol Maleate and Dorzolamide Hydrochloride respectively.

Precision

The precision of the assay method was validated by the determination of the intra- and inter-day coefficient of variation (%R.S.D.) and percentage deviation. The intra-day precision data over the concentration range of 40, 60, 80, 100, 120 µg/ml for Dorzolamide Hydrochloride and 10, 15, 20, 25, 30 µg/ml for Timolol Maleate and all mean RSD (%) was found to be within acceptance limit (≤2%), for Dorzolamide Hydrochloride and Timolol Maleate.

Repeatability

The repeatability study which was conducted on the solution having the concentration of about 20 µg/ml for Timolol Maleate and 80 µg/ml for Dorzolamide Hydrochloride showed a RSD of 0.573% for Timolol Maleate and 0.429% for Dorzolamide Hydrochloride. Thus it was concluded that the analytical technique showed a good repeatability.

Accuracy

The accuracy of the method was verified by comparing the concentrations measured from market sample with actual added concentrations. The recovery of Dorzolamide Hydrochloride and Timolol Maleate was evaluated at concentrations of 80, 100 and 120 µg/ml prepared by using standard & spiked solutions of Dorzolamide Hydrochloride and Timolol Maleate. % recovery was calculated and was found to be 99.31 % for Timolol Maleate and 99.89% for Dorzolamide Hydrochloride. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be concluded that the proposed method is accurate.

Table 1: Recovery of Dorzolamide Hydrochloride and Timolol Maleate

S. No.	Conc. before spiking C_1 (µg/ml)	Reference Std. added C_2 (µg/ml)*	Conc. after spiking C_3 (µg/ml)*	% recovery
1.	40	40	81.34	101.67
	10	10	20.03	100.15
2.	40	60	98.87	98.87
	10	15	24.80	99.21
3.	40	80	118.95	99.13
	10	20	29.56	98.53
Mean ± SD	DORZO	99.89 ±1.55		
	TIMO	99.31 ±0.83		

System suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table 2:

Table 2: Data of System Suitability Parameter

S. No.	Parameter	Limit	Result
1.	Resolution	Rs > 2	3.09
2.	Theoretical plate	N > 2000	Timolol Maleate 2100 Dorzolamide Hydrochloride 2300
3.	Retention time	-------	Timolol Maleate 6.08 min Dorzolamide Hydrochloride 3.7 min
4.	Tailing factor	</= 1.5	Timolol Maleate 1.09 Dorzolamide Hydrochloride 1.15
5.	Asymmetric Factor	</=2.0	Timolol Maleate 1.11 Dorzolamide Hydrochloride 1.17

DISCUSSION

The present method for the determination of Dorzolamide Hydrochloride and Timolol Maleate is sensitive, specific accurate, and reproducible. The excellent separation is demonstrated in the chromatograms and no interfering peaks were observed. The calibration curve was linear and the method was suitable for the analysis of samples. The accuracy of the method was in compliance with the proposed limits and the precision of the method was satisfactory. The system suitability of the method shows that the performance of the chromatographic system is not significantly influenced by variations of the operational parameters inside an accepted domain. This method shows the system suitability parameters are within the limits only.

The validation parameters according to I.C.H Q2 (R1) guidelines were studied. The accuracy of the methods was proved by performing recovery studies in the available formulations. Values obtained were found to be greater than 98%, which indicated that the proposed method is accurate for the analysis of drug. Summary of various validation parameters performed for RP-HPLC method are shown in Table 3.

Table 3: Summary of Validation Parameters by RP-HPLC Method

Validation parameters		DORZOLAMIDE HYDROCHLORIDE	TIMOLOL MALEATE
Specificity		% interference <0.5 %	
Range (µg/ml)	Linear range	40-120	10-30
	Working range	30-140	2.28-40
	Target range	64, 80, 100	16, 20, 24
	Target concentration	80	20
Precision (%RSD)	Repeatability	0.429	0.573
	Inter day	0.16	0.366
	Intra day	0.422	0.788
Accuracy (% recovery)		99.89	99.31
LOD		2.122	0.755
LOQ		6.432	2.289

Industrial Application

It is evident from the study that the developed methods are simple, specific, precise, accurate and cost effective. The newly developed method can be used for routine analysis as method for the estimation of Timolol Maleate and Dorzolamide Hydrochloride as API and in ophthalmic dosage forms.

Future Aspects

From the current work it was finally concluded that the developed RP-HPLC method is found to very simple, reliable and selective providing satisfactory accuracy and precision. The method is suitable for routine quantitative analysis in pharmaceutical dosage forms. The recoveries achieved were highly significant in the developed method.

Hence, the chromatographic method developed for Timolol Maleate and Dorzolamide Hydrochloride can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories and in pharmacokinetic studies

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