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SIMULTANEOUS ESTIMATION OF TIMOLOL MALEATE AND DORZOLAMIDE HYDROCHLORIDE IN AS API AND IN OPHTHALMIC SOLUTION DOSAGE FORM BY RP-HPLC

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Abstract:

A fast, sensitive and accurate reverse phase liquid chromatographic method was developed and validated for the simultaneous determination of Dorzolamide and Timolol maleate in ophthalmic preparations. Chromatographic separation was achieved on Inertsil ODS 3V C18 column (250 X 4.6 mm, 5 µm particle size) with mobile phase consisting of Acetonitrile and 1-Octane Sulphonic acid buffer (0.02M) pH adjusted to 3.5 ± 0.05 with o-phosphoric acid (36:64 V/V) at a flow rate of 1.0 mL/min. The analytes were detected at 254 nm and 295 nm for Dorzolamide and Timolol maleate respectively by PDA detector. Brimonidine was used as internal standard (IS). The retention time of Dorzolamide, Timolol maleate and Brimonidine were found to be at 6.020 ± 0.02, 8.254 ± 0.01 and 4.636 ± 0.01 mins respectively. The linearity of the method ranged between 4-720 and 1-180 µg/mL for Dorzolamide and Timolol maleate respectively with correlation coefficient 0.999 for both the drugs in binary mixture. The LOD was found to be 0.6951 µg/mL and 0.2489 µg/mL for Dorzolamide and Timolol maleate respectively and LOQ was found to be 2.3214 µg/mL and 0.8317 µg/mL for Dorzolamide and Timolol maleate respectively.

Keywords: *Dorzolamide, Timolol maleate, RP-HPLC, Simultaneous estimation.*

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INTRODUCTION:

Dorzolamide is indicated for the management of elevated intraocular pressure in patients with ocular hypertension or open-angle glaucoma. It can also be used in combination with timolol for the same indication in patients who are insufficiently responsive to ophthalmic beta-blockers.[1] Its pre-operative use was also investigated to prevent elevated intraocular pressure after neodynium yttrium aluminum garnet laser posterior capsulotomy.[2] Elevated intraocular pressure is a characteristic manifestation of ocular hypertension or open-angle glaucoma. The level of intraocular pressure (IOP) is governed by the balance between the production of aqueous humour (by ocular ciliary processes) and its outflow from the anterior segment of the eye via trabecular (conventional) or uveoscleral (unconventional) pathways. When there is an increase in the resistance to the trabecular outflow of aqueous humour, the intraocular pressure is elevated. Subsequently, optic nerve damage can occur from blood flow restrictions and mechanical distortion of ocular structures. IUPAC name of (2S,4S)-4-(ethylamino)-2-methyl-1,1-dioxo-2H,3H,4H-1lambda6-thieno[2,3-b]thiopyran-6sulfonamide. Molecular formula is $C_{10}H_{16}N_2O_4S_3$. Molecular Weight is 324.4.

Ophthalmic timolol is indicated for the treatment of increased intraocular pressure in patients with ocular hypertension or open-angle glaucoma. The oral form of this drug is used to treat high blood pressure. In certain cases, timolol is used in the prevention of migraine headaches.[3] Timolol competes with adrenergic neurotransmitters for binding to beta (1)-adrenergic receptors in the heart and the beta(2)-receptors in the vascular and bronchial smooth muscle.[4] This leads to diminished actions of catecholamines, which normally bind to adrenergic receptors and exert sympathetic effects leading to an increase in blood pressure and heart rate. Beta (1)-receptor blockade by timolol leads to a decrease in both heart rate and cardiac output during rest and exercise, and a decrease in both systolic and diastolic blood pressure.[5] IUPAC name of Timolol maleate is (2S)-1-(tert-butylamino)-3-{[4-(morpholin-4-yl)-1,2,5-thiadiazol-3-yl] oxy} propan-2-ol. Molecular formula is $C_{13}H_{24}N_4O_3S$. Molecular Weight is 316.42.

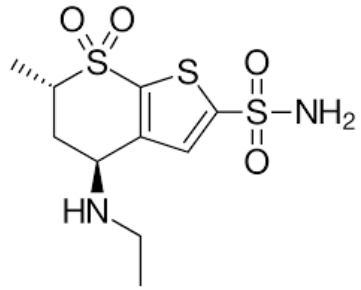

Figure 1: Structure of Dorzolamide

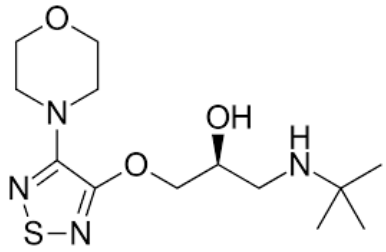

Figure 2: Structure of Timolol maleate

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[6-8] and capillary electrophoresis[9]. There are several reports on the determination of timolol maleate, individually or in combination with pilocarpine, including GLC[10] and HPLC of plasma samples[11-12], HPTLC[13] and, with dorzolamide hydrochloride, spectrophotometry.[14-18] More recently, dorzolamide hydrochloride has been marketed in combination with timolol maleate in eye drops. Dorzolamide hydrochloride is not yet offical in any pharmacopoeia. The aim of the present study was A New Rp-Hplc Method for Simultaneous Estimation of Dorzolamide and Timolol maleate in Its Bulk and Tablet Dosage Form and Its Force Degradation Studies as Per Ich.

MATERIALS AND METHODS:

Chemicals and Reagents: Dorzolamide and Timolol maleate were Purchased from Sun Pharma India Limited. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. The mobile phase consists of a mixture of (0.02M) 1-Octane Sulphonic acid buffer (pH 3.5) and Acetonitrile (64:36 V/V) respectively. The 1-Octane Sulphonic acid buffer was prepared by dissolving 4.35 grams of 1-Octane Sulphonic acid in a 1000ml of volumetric flask with pH adjustment to 3.5 ± 0.05 with o-phosphoric acid. Flow rate of the mobile phase was 1.0 mL/min.

Preparation of solutions:

Mobile phase:

The mobile phase consists of a mixture of (0.02M) 1-Octane Sulphonic acid buffer (pH 3.5) and Acetonitrile (64:36 V/V) respectively. The 1-Octane Sulphonic acid buffer was prepared by dissolving 4.35 grams of 1-Octane Sulphonic acid in a 1000ml of volumetric flask with pH adjustment to 3.5 ± 0.05 with o-phosphoric acid. Flow rate of the mobile phase was 1.0 mL/min.

Preparation of stock solution

Standard stock solution was prepared by dissolving accurately 200 mg Dorzolamide and 50 mg Timolol maleate in 100 ml mobile phase. 5 ml of standard stock solution was diluted to 25 ml with mobile phase to obtain 400 µg/mL Dorzolamide and 100 µg/mL Timolol maleate. The solution was filtered through 0.45µ nylon filter before analysis. 10 mg of Brimonidine was accurately weighed and dissolved in a 100 ml volumetric flask (100 µg/mL) with mobile phase. A series of solutions containing Dorzolamide and Timolol maleate (4:1) were prepared along with Brimonidine (20 µg/mL) as internal standard were prepared with mobile phase and 10 µl of these solutions were injected in to the HPLC system.

Preparation of sample solution

Commercial eye drops MISOPT (2%, 5 ml) and OCUDOR (2%, 5 ml) were purchased from the local market and 2 ml of sample solution was diluted to obtain Dorzolamide and Timolol maleate as 400 and 100 µg/ml with mobile phase. The solution was filtered through 0.45µ nylon filter before analysis. A series of diluted solutions containing the mixture of Dorzolamide and Timolol maleate of the extracted formulation were prepared along with the internal standard (20 µg/mL) were prepared with mobile phase and 10 µl of these solutions were injected in to the HPLC system.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Dorzolamide and Timolol maleate peaks and calculate the %Assay by using the formulae**.**

RESULTS AND DISCUSSION

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved on Inertsil ODS 3V C18 column (250 X 4.6 mm, 5 µm particle size) with mobile phase consisting of Acetonitrile and 1-Octane Sulphonic acid buffer (0.02M) pH adjusted to 3.5 ± 0.05 with o-phosphoric acid (36:64 V/V). The analytes were detected at 254 nm and 295 nm for Dorzolamide and Timolol maleate respectively by PDA detector. Brimonidine was used as internal standard (IS). Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1,2.

Table 1: System suitability parameters of Dorzolamide and Timolol maleate

Parameters	Dorzolamide	Timolol maleate
Retention time	6.02	8.24
USP Plate count	8676	10707
USP Tailing	1.05	1.03

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Dorzolamide and Timolol maleate in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-3.

Table 3: Assay results for Dorzolamide and Timolol maleate

Brand Names	Ingredient	Label clai m (%)	Drug Found (%)	% Recover y
MISOPT	Dorzola mide	2.0	1.9965	99.83
	Timo lol maleate	0.5	0.4947	98.94
OCUDOR-T	Dorzola mide	2.0	1.9985	99.93
	Timo lol maleate	0.5	0.4949	98.98

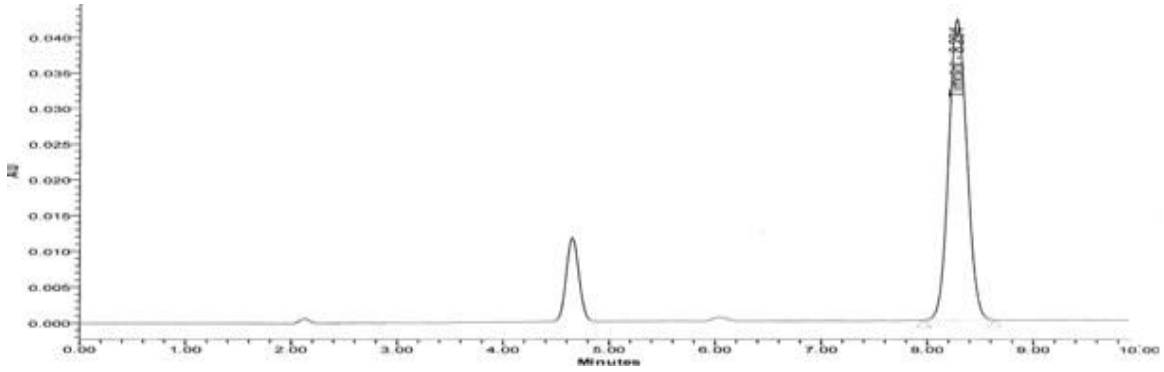

Figure 3: Standard chromatogram

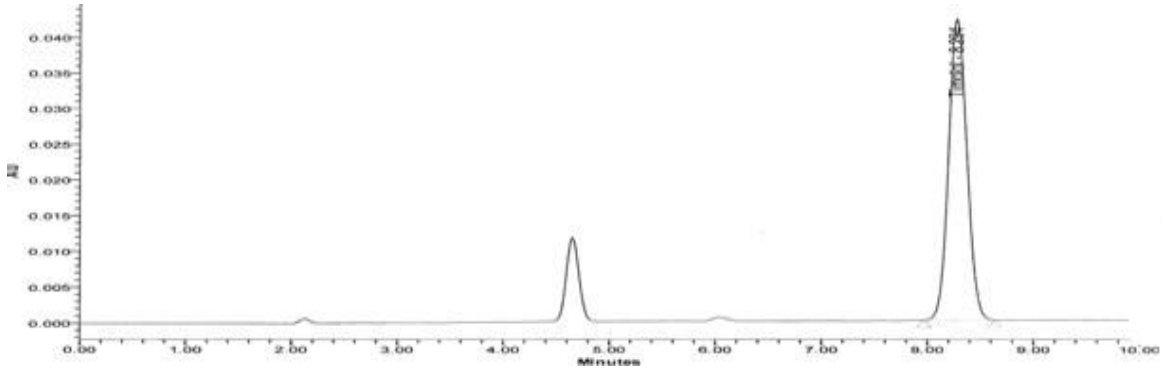

Figure 4: Sample chromatogram

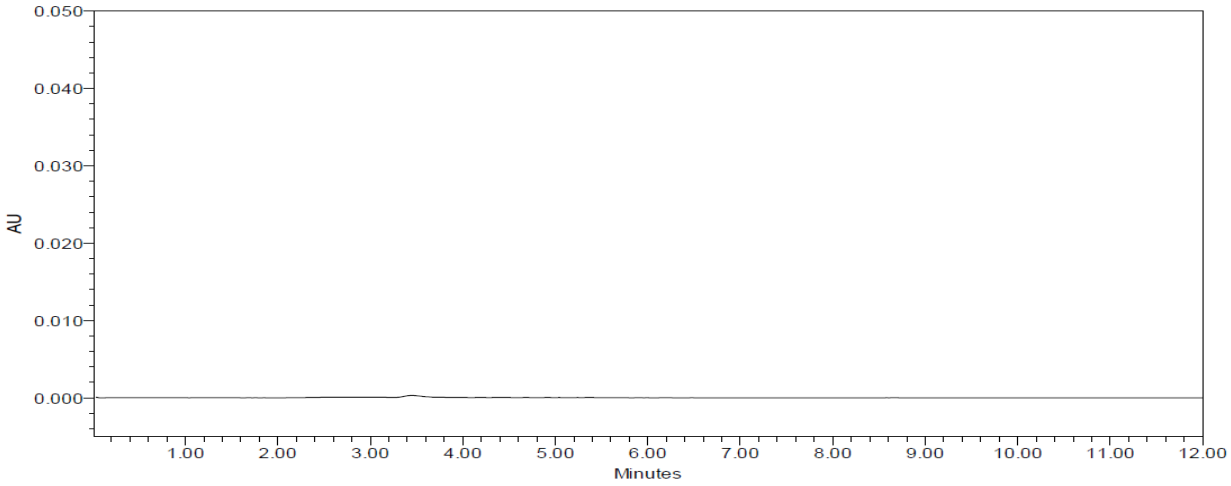

Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: Linearity of the method was evaluated at different concentration levels by diluting the standard Dorzolamide and Timolol maleate (4:1) solutions with IS (20 µg/mL) to give solutions over the range 4-720 µg/mL and 1-180µg/mL respectively. These were injected in triplicate and the peak area ratio value of Dorzolamide to that of IS as well as Timolol maleate to that of IS against their respective concentration were inputted into a Microsoft Excel® spreadsheet program to plot calibration curves. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 4.

Table 4: Linearity results of Dorzolamide and Timolol maleate

Conc. DRZ (µg/mL)	Peak area rati o DRZ / B MD	Conc. TML (µg/mL)	Peak area rati o TML / B MD
4	0.1533	1	0.0164
8	0.287	2	0.032
20	0.7505	5	0.0859
40	1.4576	10	0.1656
80	2.9295	20	0.3318
160	5.8163	40	0.6641
200	7.2106	50	0.8259
240	8.715	60	1.0005
320	11.592	80	1.3339
400	14.3428	100	1.6548
480	17.0474	120	1.9698
560	19.8324	140	2.3006
600	21.5454	150	2.5033
720	25.8419	180	3.025

Accuracy studies: Accuracy of the method was performed by the standard addition technique. Three levels of solutions (50, 100 and 150%) of the nominal analytical concentrations prepared. Calculate the Amount found and Amount added for Dorzolamide and Timolol maleate and calculate the individual recovery and mean recovery values. The results are shown in table 5.

Table 5: Showing accuracy results for Dorzolamide and Timolol maleate

Drug	Sample level%	Conc. (µg/ ml)			Amount recovered d (µg/ ml)	% Recover y	% RSD
		Pure	Formulation	Total			
DRZ	50	20	40	60	59.91	99.85	0.23
	100	40	40	80	79.45	99.31	0.36
	150	60	40	100	99.76	99.76	0.39
TML	50	5	10	15	14.84	98.93	0.83
	100	10	10	20	19.83	99.15	0.76
	150	15	10	25	24.75	98.99	0.69

Precision Studies: Within-day precision determined by injecting five standard solutions of three different concentrations on the same day (n=5) and between-day precision determined by inject ing the same solutions for consecutive days. Relative standard deviation (RSD %) of the peak area calculated to represent precision. The results are shown in table 6.

Table 6: Intra-Day and Inter-Day Precision for the simu ltaneous analysis of Dorzolamide and Timo lol maleate.

Drug	Conc. (µg/ mL)	Intra-day Precision		Inter-day precision	
		Mean pe ak are a r ati o ± SD	RSD (%)	Mean pe ak are a r ati o ± SD	RSD (%)
DRZ	200	7.2193 ± 0.0317	0.439	7.91227 ± 0.0497	0.628
	400	14.3793 ± 0.0549	0.382	15.9748 ± 0.0809	0.507
	600	21.4301 ± 0.1787	0.834	21.5478 ± 0.2413	1.12
TML	50	0.8269 ± 0.00373	0.449	0.9256 ± 0.00631	0.682
	100	1.6594 ± 0.00581	0.349	1.9934 ± 0.00993	0.498
	150	2.4896 ± 0.02163	0.868	2.5034 ± 0.0313	1.25

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 7.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Table 7: LOD, LOQ of Dorzolamide and Timolol maleate

Drug	LOD	LOQ
Dorzolamide	0.695	2.32
Timolol maleate	0.248	0.83

CONCLUSION:

The advantages of the proposed method involve a simple procedure for sample preparate ion and relatively short time of analysis. Apart from th is, it can be used for assays of Dorzolamide and Timolol maleate in biological fluids or in pharmacokinetic investigations. The proposed method was validated by testing its linearity, accuracy, precision, limits of detection and quantitation. The results of the analysis of pharmaceutical dosage forms by the proposed methods are highly reproducible, reliable, and are in good agreement with the label claims of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with Dorzolamide and Timolol maleate. It may be said that the proposed methods are precise, sensitive, and accurate, so that these can be used as standard pharmacopeial methods for the simultaneous determination of Dorzolamide and Timolol maleate using the HPLC systems with PDA detector.

REFERENCES:

1. FDA Approved Drug Products: TRUSOPT (dorzolamide hydrochloride) ophthalmic solution
2. Arieta C, Amaral M, Matuda E, Crosta C, de Carvalho Moreira Filho D, Jose N: Dorzolamide X apraclonidine in the prevention of the intraocular pressure spike after Nd : YAG laser posterior capsulotomy. Curr Eye Res. 2002 Oct;25(4):237-41. doi: 10.1076/ceyr.25.4.237.13484. (PubMed ID 12658557)
3. Migliazzo CV, Hagan JC 3rd: Beta blocker eye drops for treatment of acute migraine. Mo Med. 2014 Jul-Aug;111(4):283-8. (PubMed ID 25211851)
4. Leier CV, Baker ND, Weber PA: Cardiovascular effects of ophthalmic timolol. Ann Intern Med. 1986 Feb;104(2):197-9. doi: 10.7326/0003-4819-104-2-197. (PubMed ID 3946944)
5. Valvo E, Gammaro L, Tessitore N, Fabris A, Ortalda V, Bedogna V, Maschio G: Effects of timolol on blood pressure, systemic hemodynamics, plasma renin activity, and glomerular filtration rate in patients with essential hypertension. Int J Clin Pharmacol Ther Toxicol. 1984 Mar;22(3):156-61. (PubMed ID 6370874)
6. Matuszewski, B. K.; Constanzer, M. L.; Woolf, E. J.; Au, T.; Haddix, H.: J. Chromatogr. B. 653, 77 (1994)
7. Constanzer, M. L.; Chavez, C. M.; Matuszewski, B. K.: J. Pharm. Biomed. Anal. 15, 1001 (1997)
8. Satuf, M. L.; Robles, J. C.; Goicoechea, H. C.; Oliveri, A. C.: Anal. Lett. 32, 2019 (1999)
9. Tim, R. C.; Kautz, R. A.; Karger, B. L.; Electrophoresis 21, 220 (2000)
10. Carlin, J. R.; Walkar, R. W.; Davies, R. O.; Ferguson, R. K.; Vandenheuvel, W. J. A.: J. Pharm. Sci. 69, 1111 (1980)
11. Lennard M. S.; Parkin, S.: J. Chromatogr. B. 338, 249 (1985)
12. Kubota, K.; Nakamura, H.; Koyama, E.; Yamada, T.; Kikuchi, K.; Ishizaki, T.: J. Chromatogr. B. 533, 255 (1990)
13. Kulkarni, S. P.; Amin, P. D.: J. Pharm. Biomed. Anal. 23, 983 (2000)
14. Erk, N.: J. Pharm. Biomed. Anal. 28, 391 (2002)
15. Bebawy, L. I.: J. Pharm. Biomed. Anal. 27, 737 (2002)
16. Patel, B. R.; Krischbaum J. J.; Poet, R. B.: J. Pharm. Sci. 70, 336 (1981)
17. Mazzo D. J.; Snyder, P. A.: J. Chromatogr. 438, 85 (1988)
18. Mazzo, D. J.: J. Chromatogr. 299, 503 (1984)