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Original Article

A NEW RP-UPLC METHOD FOR THE SEPARATION AND SIMULTANEOUS QUANTIFICATION OF DORZOLAMIDE HCl AND TIMOLOL MALEATE

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ABSTRACT

Objective: This investigation demonstrates a stability-indicating and reliable "reverse-phase ultra-performance liquid chromatography" method to simultaneously quantify timolol maleate and dorzolamide HCl in the pharmaceutical dosage form.

Methods: Successful separation was accomplished using Phenyl column (100 mm x 2.1 mm, 1.7µm) with isocratic type of elution using mobile phase containing Acetonitrile+Ammonium Formate buffer (30:70), respectively with 0.2 ml/min flow rate. The wavelength sensor was attuned at 266 nm to quantify timolol maleate and dorzolamide HCl.

Results: Dorzolamide HCl and timolol maleate peaks were eluted with fine resolution at retention times 0.7 min and 1.5 min, respectively. In the 55.75-334.5 µg/ml and 6.25-37.5 µg/ml concentration ranges for dorzolamide HCl and timolol maleate, the calibration graphs were linear, with regression coefficients of 0.99997 and 0.99991, respectively. The suggested ultra-performance liquid chromatography approach has been shown as sensitive, precise, robust, accurate, specific and stability, indicating through the resolution of dorzolamide HCl and timolol maleate from its degradation-based compounds.

Conclusion: The established ultra-performance liquid chromatography technique was effectively extended to the evaluation of dorzolamide HCl and timolol Maleate in the pharmaceutical dosage form and the test results appeared satisfactory.

Keywords: Dorzolamide HCl, Timolol maleate, Development, Validation, RP-UPLC

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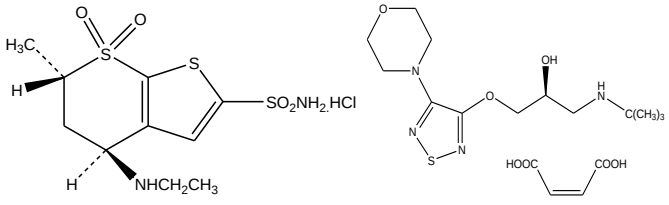
INTRODUCTION

Dorzolamide, sold under the brand name Trusopt , among others, is a medication used to treat high pressure inside the eye, including in cases of glaucoma [1, 2]. It is used as an eye drop. Effects begin within three hours and last for at least eight hours. It is also available as the combination dorzolamide/timolol. Common side effects include eye discomfort, eye redness, taste changes, and blurry vision [3]. Serious side effects include Steven Johnson syndrome [4, 5]. Those allergic to sulfonamides may be allergic to dorzolamide. Use is not recommended in pregnancy or breastfeeding [6, 7]. It is a carbonic anhydrase inhibitor [8] and works by decreasing the production of aqueous humour. It is a second-generation carbonic anhydrase inhibitor. Dorzolamide hydrochloride is used to lower excessive intraocular pressure [9, 10] in open-angle glaucoma and ocular hypertension [11, 12]. This drug is able to cross the cornea, reach the ciliary body of the eye, and produce systemic effects on the carbonic anhydrase enzyme within the eye. Ocular stinging, burning, itching and bitter taste. It causes shallowing of the anterior chamber and leads to transient myopia [13, 14]. As a second-generation carbonic anhydrase inhibitor, dorzolamide avoids systemic effects associated with first-generation carbonic anhydrase inhibitors such as Acetazolamide, Methazolamide, and Dichlorphenamide.

Timolol is a beta blocker medication used either by mouth or as eye drops [15]. As eye drops, it is used to treat increased pressure inside the eye such as in ocular hypertension and glaucoma. By mouth it is used for high blood pressure chest pain [16] due to insufficient blood flow to the heart, to prevent further complications after a heart attack, and to prevent migraines [17, 18]. Common side effects include irritation of the eye mouth include tiredness, slow heartbeat, itchiness, and shortness of breath. Other side effects include masking the symptoms of low blood sugar in those with diabetes [19, 20]. Use is not recommended in those with asthma, uncompensated heart failure, or COPD. It is unclear if use during pregnancy is safe for the baby. Timolol is a non-selective beta blocker [21, 22]. The most serious possible side effects include cardiac arrhythmias [23, 24] and severe bronchospasms. Timolol can also lead to fainting, congestive heart failure, depression, confusion, worsening of Raynaud's syndrome [25, 26] and impotence. Side effects when given in the eye include burning sensation, eye redness, superficial punctate keratopathy, corneal numbness. A Cochrane review compared the effect of timolol versus brimonidine in slowing the progression of open-angle glaucoma in adults but found insufficient evidence to come to conclusions [27]. This paper proposes a novel sensitive stability-indicating RP-UPLC procedure for the assessment of dorzolamide HCl and timolol maleate combination. The process proposed enables the rapid assessment of the dorzolamide HCl and timolol maleate in bulk drugs and formulation preparations without sample pretreatment with high precision and specificity and with no excipient intervention.



A B

Fig. 1: Structure of (A) Dorzolamide HCl and (B) Timolol maleate

MATERIALS AND METHODS

Chemicals

Acetonitrile, HPLC-grade methanol, water were purchased from Merck India Ltd, Mumbai, India. APIs of dorzolamide HCl, timolol maleate standards were procured from Glenmark, Mumbai. Sample formulation (Ophthalmic solution) from Euphoria India Pharma with a lable claim of 22.3 mg/ml dorzolamide HCl and 6.8 mg/ml of timolol maleate was used.

The instrumentation

Waters acquity UPLC with quaternary pump, PDA detector with Empower 2.0 software was employed.

Method optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic and gradient mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally a mixture of acetonitrile and ammonium formate with isocractic elution was selected as mobile phase because it results in a greater response of active pharmacy ingredient. During the optimization of the method, various stationary phases such as C_8, C_{18} and amino phenyl columns were tested. From these trials the peak shapes were relatively good with Phenyl column of 100 x 2.1 mm, 1.7 µ with a PDA detector. The mobile phase flow rate has been done at 266 nm in order to obtain enough sensitivity. By using above conditions, we get retention times of dorzolamide HCl and timolol maleate were about 0.7 min and 1.5 min with a tailing factor of 1.07 and 1.08. The number of theoretical plates for dorzolamide HCl and timolol maleate were 8474, 3810 which indicate the column's successful output the % RSD for six replicate injections was around 0.66% and 0.30%, the proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

There are some HPLC [28-31] methods and no UPLC methods reported in the literature, but these methods are developed only for routine analysis of the selected drugs in bulk and formulation studies. The developed UPLC method was utilized for the estimation of the combined drugs by *in vitro* method.

Validation procedure

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines [32, 33].

Preparation of buffer

Accurately weighed and transfered 6.30 g of Ammonium formate in 1 Lt of HPLC grade water and filter through 0.22 µ filter paper.

Chromatographic conditions

The UPLC analysis was performed on reverse phase UPLC system with isocratic elution mode using a mobile phase of Acetonitrile and Ammonium Formate (30:70) and Phenyl (100x2.1 mm, 1.7 µ) column with a flow rate of 0.2 ml/min.

Diluent

Mobile phase was used as diluent.

Preparation of the standard solution

Standard dorzolamide HCl and timolol maleate solution containing 223 µg/ml and 68 µg/ml was prepared by dissolving 223 mg of dorzolamide HCl and 68 mg of timolol maleate in 100 ml of mobile phase solvent blend. Further, dilute 5 ml to 50 ml with diluents.

Preparation of the sample solution

Sample dorzolamide HCl and timolol maleate sample solution containing 223 µg/ml and 68 µg/ml was prepared by dissolving 0.1 ml of sample (lable claim 22.3 mg of dorzolamide HCl and 6.8 mg of timolol maleate) in 10 ml of mobile phase solvent blend.

RESULTS AND DISCUSSION

In acquiescence with ICH recommendations, the validity parameters were established [34].

System suitability

In System suitability, injecting standard solution and reported USP tailing and plate count values are tabulated in table 1.

Specificity

In this test method placebo, standard and sample solutions were analyzed individually to examine the interference [35]. The below fig. shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence, the method is specific.

Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Drug name	
		Dorzolamide HCl	Timolol maleate
USP Plate Count	NLT 2000	8474	3810
USP Tailing	NMT 2.0	1.07	1.08
USP Resolution	NLT 2.0	-	6.37
% RSD	NMT 2.0	0.66	0.30

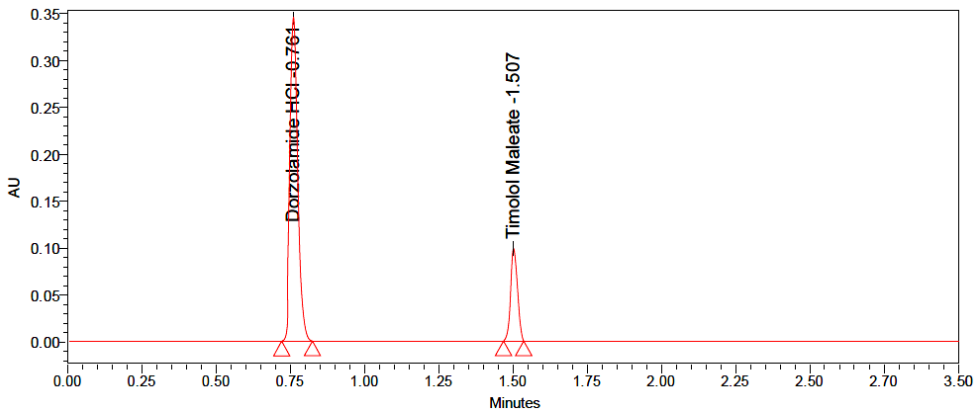


Fig. 2: Chromatogram of standard

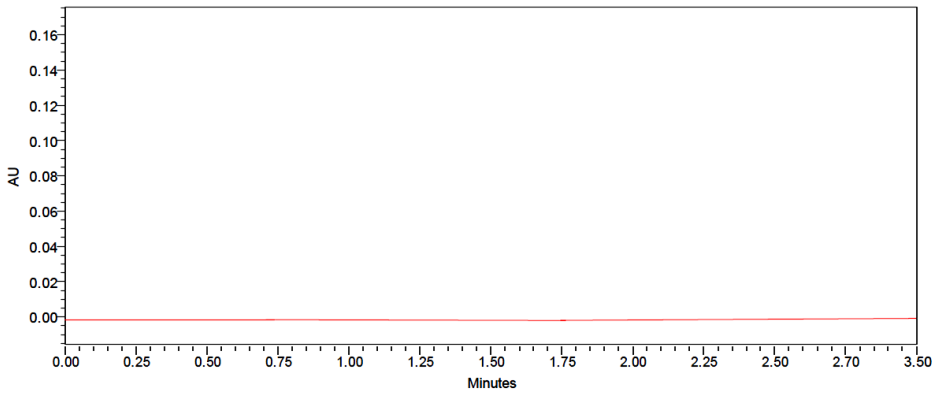


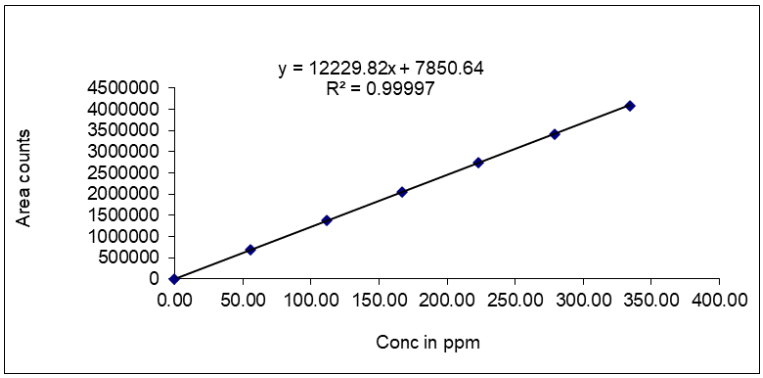
Fig. 3: Chromatogram of blank

Linearity

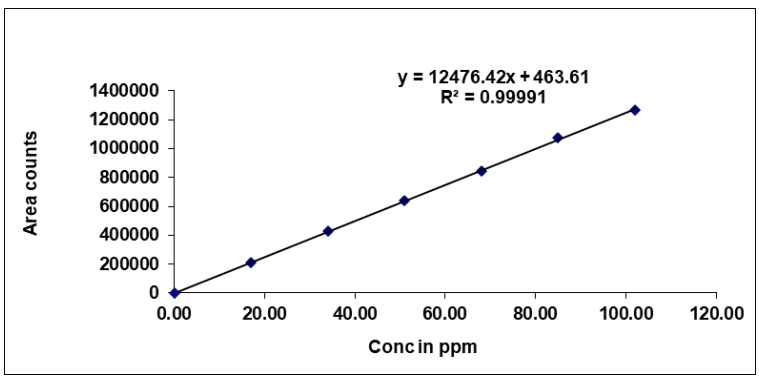
During this work, the linearity of area response was checked for both dorzolamide HCl and timolol maleate. Chromatographed solutions with concentrations of 55.75-334.5 µg/ml for dorzolamide HCl and 6.25-37.5 µg/ml for timolol maleate given linear peak response areas. The regression line equation, regression coefficient and dorzolamide HCl and timolol maleate calibration curves are shown in fig. 4.

Table 2: Linearity of dorzolamide HCl and timolol maleate

S. No.	Conc µg/ml	Dorzolamide HCl area count	Conc. µg/ml	Timolol maleate area count
1	55.75	685412	17.00	210546
2	111.50	1385647	34.00	425781
3	167.25	2044528	51.00	638594
4	223.00	2753261	68.00	844512
5	278.75	3418542	85.00	1072547
6	334.50	4085624	102.00	1265348
Correlation coffiecient		0.99997		0.99991
Slope		12229.82		12476.42
intercept		7850.64		463.61



(A) Dorzolamide HCl



(B) Timolol maleate

Fig. 4: Calibration plots of (A) Dorzolamide HCl (B) Timolol maleate

Accuracy

The accuracy was determined by assay of dorzolamide HCl and timolol maleate in spiked dorzolamide HCl and timolol maleate samples according to proposed method. Three diverse quantities (50% quantity degree, 100% quantity degree and 150% quantity degree) [36, 37] of dorzolamide HCl and timolol maleate standards were put into samples. The results are given in table 3.

Table 3: Results of accuracy

S. No.	% Level	Dorzolamide HCl % recovery	Timolol maleate % recovery
1	50	100.1	100.1
2	100	99.9	100.1
3	150	98.8	99.6
mean		99.6	99.9
SD		0.7	0.3

(n=3)

Precision

The precision measurements were assessed using measurements of dorzolamide HCl and timolol maleate solution (223 µg/ml and 68 µg/ml) repeated six times within the day. The precision was validated by the RSD measurements of the dorzolamide HCl and timolol maleate peak areas, while the accuracy was validated by the dorzolamide HCl and timolol maleate percentage content assays. These results are given below table 4.

Intraday precision

Six replicates of a standard solution containing dorzolamide HCl (223µg/ml) and timolol maleate (68µg/ml) were analysed on the same day [38, 39]. Peak areas were calculated, which were used to calculate mean, SD and %RSD values.

Intermediate precision

Six replicates of the standard solution were studied by various researchers, and on separate days different instruments were tested. The peak regions used to determine mean percent RSD values have been calculated. The results are given in the following table [40].

Inter-day precision

Six replicates of a sample solution containing dorzolamide HCl (223µg/ml) and timolol maleate (68µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate mean, SD and %RSD values. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100% [41, 42]. The results are given in table 5.

Table 4: Intraday precision results of dorzolamide HCl and timolol maleate

Dorzolamide HCl				Timolol maleate		
S. No.	Conc. (µg/ml)	Area counts	% Assay as is	Conc.(µg/ml)	Area counts	% Assay as is
1		2778459	100.5		844574	99.9
2	223	2763521	99.9	68	841257	99.5
3		2735247	98.9		842365	99.7
4		2756283	99.7		846258	100.1
5		2769854	100.2		842741	99.7
6		2753859	99.6		843659	99.8
% RSD	0.54 0.55			0.21 0.20		
mean	99.8			99.8		
SD		0.551		0.204		

(n=6)

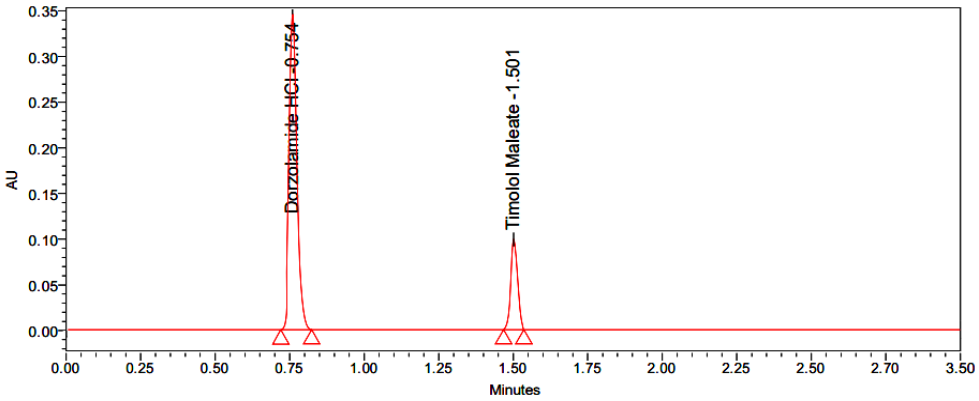


Fig. 5: Chromatogram of method precision

LOD and LOQ

Both LOD and LOQ were measured utilizing a signal-to-noise methodology. LOQ and LOD were defined as the dorzolamide HCl and timolol maleate concentration levels that ensuing a peak height of 10 times and 3 times, respectively the baseline noise [43].

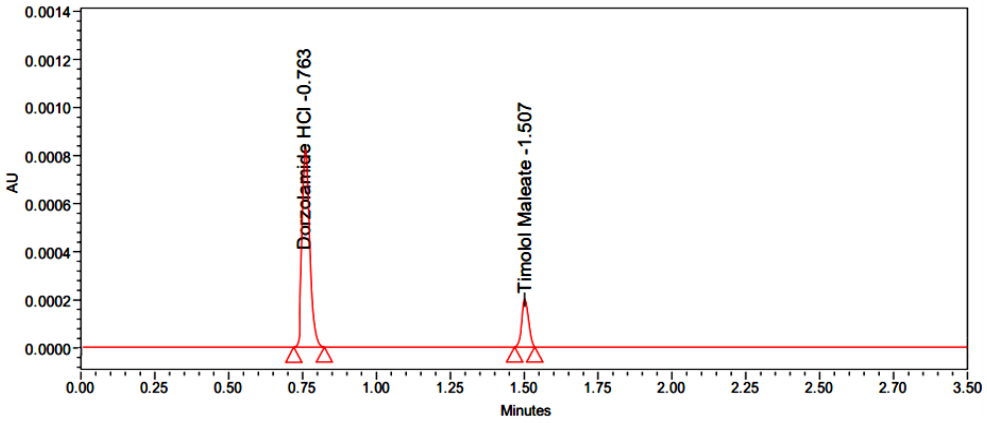
Table 5: Inter-day outcomes of accuracy of dorzolamide HCl and timolol maleate

Dorzolamide HCl				Timolol maleate		
S. No.	**Conc.(µg/ml)**	**Area counts**	**% assay as is**	**Conc.(µg/ml)**	**Area count**	**% assay as is**
1		2769123	100.1	68	847254	100.3
2	223	2748154	99.3		843516	99.8
3		2759854	99.7		846041	100.1
4		2743215	99.1		847148	100.2
5		2768974	100.1		848752	100.4
6		2745820	99.2		844643	99.9
%RSD	0.42 0.45			0.23 0.23		
Mean	99.6			100.1		
SD	0.449			0.232		

(n=6)

Table 6: LOD and LOQ for dorzolamide HCl and timolol maleate

Dorzolamide HCl				Timolol maleate			
LOD		**LOQ**		**LOD**		**LOQ**	
Concentration	s/n	Concentration	s/n	concentration	s/n	Concentration	s/n
0.54µg/ml	3	1.78µg/ml	10	0.16µg/ml	3	0.54µg/ml	10



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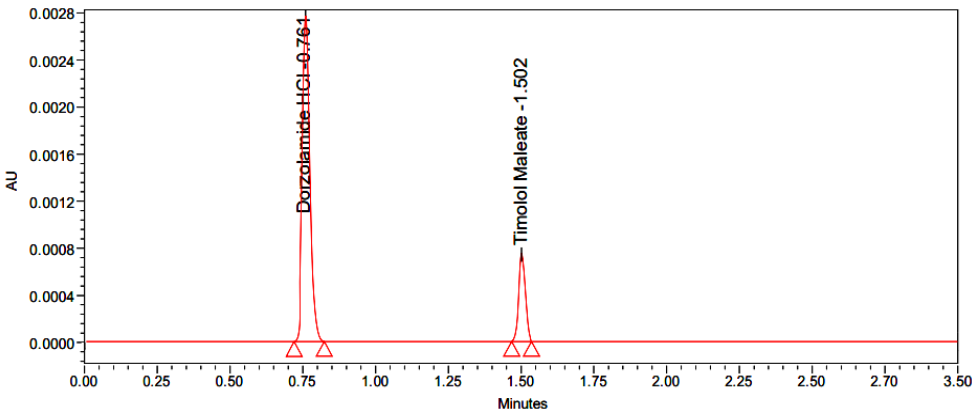

B

Fig. 6: Chromatogram of (A) LOD and (B) LOQ

Table 7: Robustness data of dorzolamide HCl and timolol maleate

Parameter name	% RSD	
	Dorzolamide HCl	**Timolol maleate**
Flow minus (0.9 ml/min)	0.60	0.30
Flow plus (1.1 ml/min)	0.58	0.55
Organic minus (-10%)	0.66	0.17
Organic plus (+10%)	0.51	0.15

Robustness

The robustness was measured using peak area measurements of dorzolamide HCl and timolol maleate solution (223µg/ml and 68µg/ml) with considerably changed parameters in UPLC assay operating conditions. The changed parameters and peak areas obtained were presented in table 7 [44].

Degradation studies

The dorzolamide HCl and timolol maleate sample was subjected into various forced degradation conditions to effect partial degradation of the drug. Studies of forced degradation have carried out to find out that the method is suitable for products of degradation [45, 46]. In addition, the studies provide details about the conditions during which the drug is unstable in order that measures are often taken during formulation to avoid potential instabilities [47, 48].

Acid degradation was done by using 1N HCl and 12.3% of dorzolamide HCl and 11.1% of timolol maleate degradation was observed. Alkali degradation was done at 1N NaOH and 13.9% of dorzolamide HCl and 10.6% of timolol maleate degradation was observed. Peroxide degradation was performed with 30% hydrogen peroxide and 15.6% dorzolamide HCl, 13.4% of timolol maleate degradation was observed. Reduction degradation was performed with 10% sodium bi sulphite solution, 1.5% dorzolamide HCl and 3.9% timolol maleate degradation was observed. In thermal degradation the sample was degraded to 10.1% of dorzolamide HCl and 10.2% of timolol maleate. In Photolytic degradation the sample was degraded to 2.5% of dorzolamide HCl and 2.8% of timolol maleate. In hydrolysis degradation the sample was degraded to 1.1% of dorzolamide HCl and 2.0% of timolol maleate. All degradation results are tabulated in table 9.

Table 9: Forced degradation results of dorzolamide HCl and timolol maleate

Degradation condition	Dorzolamide HCl		Timolol Maleate	
	% Assay	% Degradation	% Assay	% Degradation
Acid degradation	87.7	12.3	88.9	11.1
Alkali degradation	86.1	13.9	89.4	10.6
Peroxide degradation	84.4	15.6	86.6	13.4
Reduction degradation	98.5	1.5	96.1	3.9
Thermal degradation	89.9	10.1	89.8	10.2
Photolytic degradation	97.5	2.5	97.2	2.8
Hydrolysis degradation	98.9	1.1	98.0	2.0

CONCLUSION

An Ultra-performance liquid chromatography process for determining the combination of dorzolamide HCl and timolol maleate in combined formulation form and pure form has been described in the established method. The present Ultra-performance liquid chromatography process is exemplified by its speed, ease and relatively inexpensive. The successful validity criteria of the proposed approach permit its use in laboratories for quality control.

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AUTHORS CONTRIBUTIONS

Contributions from all authors were substantial in the following areas: ideation, design, data acquisition, analysis, and interpretation; writing the article or critical revision for important intellectual content; acceptance of the work for submission to the journal; final approval of the published version; and acceptance of full responsibility for all parts of the work. All of the writers meet the criteria set forth by the International Committee of Medical Journal Editors (ICMJE) for inclusion as authors in medical journals.

CONFLICTS OF INTERESTS

Declared none

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