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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DORZOLAMIDE HYDROCHLORIDE AND TIMOLOL MALEATE IN OPHTHALMIC DOSAGE FORM

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ABSTRACT

Objective: This new reverse phase high-performance liquid chromatography (RP-HPLC) method demonstrates superiority over the methods found in official monographs, as it is not included in the United States Pharmacopeia, British Pharmacopeia, or European Pharmacopeia (USP, B. P, Ph Eur). It effectively separates all impurities with excellent resolution from the main analyte, and the spectral purity of the main analyte was confirmed through peak purity analysis in specificity studies involving spiked sample solutions and degradation studies. According to the British Pharmacopeia, impurities B and D are associated with dorzolamide hydrochloride (DZH), while timolol maleate (TIM) includes Impurity G. The USP lists maleic acid and DZH-related compounds B and D for DZH, along with G, B, and D for TIM.

Methods: Chromatographic separation was carried out on a Symmetry C8 column using buffer pH 3.0, acetonitrile, and methanol (50:40:10 v/v/v) as mobile phase, at a flow rate of 1.2 ml/min, and a step gradient elution was used. DZH and TIM were detected at 253 nm and 295 nm, respectively, with retention times of 17.6 and 28.4 min.

Results: The method was found to be linear (r^2>0.999) in the concentration ranges of 50-400 µg/ml for DZH and 12-100 µg/ml for TIM. It was validated for precision (both analytes %RSD = 0.3%), accuracy (recoveries 98.7-100.5%), specificity, and robustness. Forced degradation studies also showed the validity of the method with good resolution between the degradation products and the main peaks.

Conclusion: The developed RP-HPLC method is novel, simple, sensitive, precise, accurate, and robust for DZH and TIM in ophthalmic formulations as per the International Council for harmonisation (ICH) for stability studies to perform Quality control (QC) of bulk and finished dosage forms.

Keywords: COSOPT® PF (RLD), Dorzolamide hydrochloride (DZH), Timolol maleate (TIM), RP-HPLC, Stability-indicating method, Forced degradation, Ophthalmic solution, Method validation

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INTRODUCTION

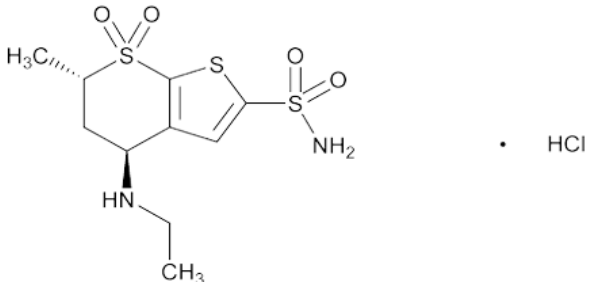
Glaucoma represents a group of eye disorders characterized by progressive optic neuropathy and visual field loss, often associated with elevated intraocular pressure (IOP) [1]. Fixed-dose combination drug products for glaucoma therapy have demonstrated improved efficacy in lowering IOP compared to monotherapy regimens [2]. Dorzolamide hydrochloride (DZH) and timolol maleate (TIM) combination therapy is one such widely used treatment option.

DZH is a carbonic anhydrase inhibitor that decreases aqueous humor secretion, thereby reducing IOP [3]. Chemically, it is (4S,6S)-4-(ethylamino)-6-methyl-5,6-dihydro-4H-thieno[2,3-b]thiopyran-2-sulfonamide 7,7-dioxide hydrochloride. TIM is a non-selective beta-adrenergic receptor blocking agent that reduces the production of aqueous humor [4]. Chemically, it is (S)-1-(tert-butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol maleate.

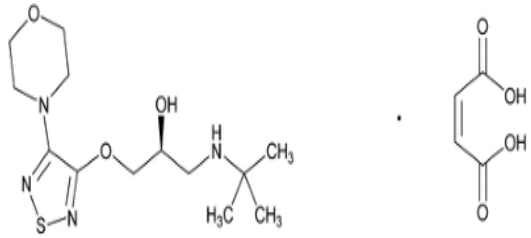
The combination of these agents in ophthalmic solutions, such as COSOPT® PF (RLD) (preservative-free dorzolamide hydrochloride-timolol maleate ophthalmic solution 2% and 0.5%), provides a synergistic IOP-lowering effect [5]. Quality control (QC) of such pharmaceutical formulations requires accurate and reliable analytical methods for quantification of the active ingredients.

Several analytical methods like (ultraviolet) UV spectroscopy [6], RP-HPLC [7], and ultra-performance liquid chromatography (UPLC) [8] have been reported in the literature for the estimation of dorzolamide hydrochloride (DZH) and timolol maleate (TIM) either alone or in combination with other drugs. However, there is limited information available on stability-indicating analytical methods for the simultaneous estimation of DZH and TIM in ophthalmic formulations. Therefore, this study focuses on developing and validating a robust, specific, and sensitive RP-HPLC method for the simultaneous estimation of these active ingredients in their combined dosage form, along with identification using infrared spectroscopy.

The method is Capable of separating all possible degradants and related to process from main analytes and quantifying both analytes in a single method for bulk formulations, finished dosage forms, and even quantifying the reference listed drugs COSOPT® PF (RLD).



Dorzolamide HCL (DZH) structure from USP



Timolol maleate (TIM) structure from USP

MATERIALS AND METHODS

Materials

Drug samples

DZH and TIM ophthalmic solution samples and placebos were received as gift samples from Biogenico Private Limited.

Name of reference list drug

COSOPT® PF (Preservative-free) (dorzolamide hydrochloride-timolol maleate ophthalmic solution 2% and 0.5%) Ophthalmic Solution. (20 mg of dorzolamide and 5 mg of timolol maleate).

Chemicals and solvents

All chemicals and solvents used were of analytical or HPLC grade. Acetonitrile (HPLC grade and AR grade), methanol (HPLC grade and AR grade), orthophosphoric acid (HPLC grade), sodium hydroxide (EMPARTA ACS grade), and HPLC-grade water were procured from S. D. Fine Chemicals Ltd., India; Qualigens Fine Chemicals Ltd., Mumbai, India; Merck Specialities Private Limited, Mumbai; and Ranbaxy Chemicals Ltd., New Delhi, India.

Instruments

Various analytical instruments were used in this study, including a Sartorius MSA6.6S-000DM analytical balance, a Lab India® Thermoscientific Orians tar H211 pH meter, a Life care equipment Pvt ltd fast clean ultra-sonic cleaning system, and a Value 1 stage vacuum pump. For spectroscopic analysis, a Shimadzu model affinity-1 Fourier Transform Infrared spectroscopy (FTIR) Spectrometer and a Shimadzu UV-1800 UV-Visible Spectrophotometer were used. Chromatographic analyses were performed using two HPLC systems: a Waters alliance e 2695 equipped with 2488 UV and Photodiode Array (PDA) Detectors running empower 3 software, and a Shimadzu (Japan) liquid chromatography system featuring an LC–20 AD pump, LC-20A UV/Vis detector, and rheodyne 7725 injection with 100 µl** loop, operated using LC solutions software.

Spectroscopic identification of DZH and TIM

FTIR analysis

The identification of DZH and TIM was performed using FTIR Spectrophotometer (Shimadzu model Affinity-1) with ATR technique. The instrument was configured to operate in % transmittance intensity mode with Happ-General apodization, scanning across a wavenumber range of 650-4000 cm^{-1}. The analysis parameters included 16 scans per sample at a resolution of 4 cm^{-1}. For analysis, approximately 2 mg of each standard (USP) and sample was individually placed under the pressure knob, and their respective spectra were recorded [9]. The identity of the compounds was confirmed by comparing the spectral patterns of the standards with the corresponding samples [10, 11].

Fig. 1: FT-IR stretching frequency data for dorzolamide hydrochloride (DZH)

S. No.	Standard (USP)	Sample
1	3369.65	3367.72
2	3045.60	3045.60
3	2451.53	2451.53
4	2285.65	2337.73
5	2007.90	2073.48
6	1587.42	1587.42
7	1535.34	1535.34
8	1446.62	1446.62
9	1384.89	1384.89
10	1321.24	1321.24

Table 2: FT-IR stretching frequency data for timolol maleate (TIM)

S. No.	Standard (USP)	Sample
1	2964.59	2964.59
2	2889.37	2889.37
3	2852.72	2852.72
4	1703.15	1705.08
5	1618.28	1618.28
6	1585.49	1583.56
7	1406.11	1406.11
8	1379.11	1379.11

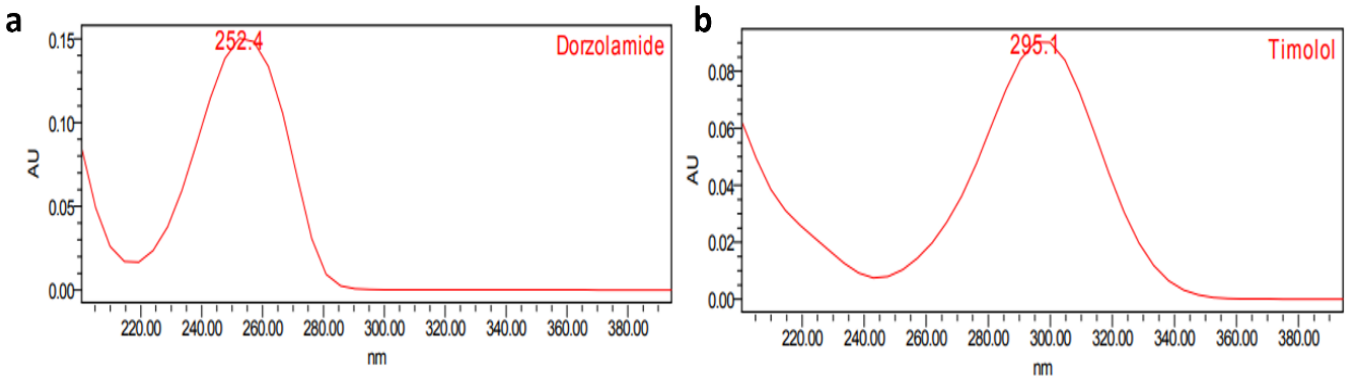


Fig. 1: UV spectra of a. dorzolamide hydrochloride (DZH) and b. timolol maleate (TIM) standards

Method development for RP-HPLC

Selection of wavelength

Based on the UV absorption characteristics, 253 nm was selected as the detection wavelength for dorzolamide hydrochloride (DZH) and 295 nm for timolol maleate (TIM) [12].

Chromatographic conditions

The chromatographic conditions were optimized through multiple experimental trials for efficient separation of DZH and TIM. The final method utilized a Waters Symmetry C8 column (250×4.6 mm, 5 µm) as the stationary phase. The mobile phase consisted of a ternary mixture of buffer pH 3.0 (0.2% orthophosphoric acid adjusted with 5N sodium hydroxide solution), acetonitrile, and methanol in a ratio of 50:40:10% v/v/v. Detection was performed at dual wavelengths: 253 nm for DZH and 295 nm for TIM. The method parameters included a flow rate of 1.2 ml/min, operating pressure of 92 kgf, and column temperature maintained at 37 °C. Sample analysis was conducted using a 10 µl injection volume with a gradient elution mode and a total run time of 55 min [13, 14].

Table 3: Gradient program for HPLC method

Time (min)	Mobile phase-A (%)	Mobile phase-B (%)
0	98	2
18	98	2
25	50	50
45	5	95
46	98	2
55	98	2

Where, mins; minutes, % of means: Ratio of mobile phase form-A and B

Preparation of solutions

Diluent preparation

Buffer of pH 3.0 was mixed with acetonitrile in the ratio 95:5 % (v/v), respectively and sonicated to degas for about 5 min.

Standard solution preparation

Standard solutions of DZH and TIM were prepared by accurately weighing and transferring appropriate amounts to volumetric flasks [15] and diluting with diluent to obtain concentrations of approximately 200 µg/ml for DZH and 50 µg/ml for TIM.

Sample solution preparation

About 1.0 g of DZH and TIM ophthalmic solution was accurately weighed and transferred to a 100 ml volumetric flask, diluted to volume with diluent, and mixed well to obtain concentrations of approximately 200 µg/ml for DZH and 50 µg/ml for TIM.

Method validation

The developed method was validated according to ICH guidelines for specificity, linearity, precision, accuracy, solution stability, and robustness [16, 17].

System suitability

System suitability parameters such as retention time, number of theoretical plates (N), tailing factor, and % RSD of peak areas from five replicate injections of standard solutions were evaluated [18].

Specificity

The specificity of the method was established by analyzing blank, standard, placebo, control sample, and spiked sample solutions. Individual impurity stocks were prepared, and impurities were spiked at 1% level of targeted test concentration. Peak purity was assessed using the PDA detector [19].

Linearity and range

Linearity was evaluated by analyzing standard solutions at concentration levels ranging from 50-400 µg/ml for DZH and 12-100 µg/ml for timolol maleate. Calibration curves were plotted with concentration versus peak area, and the correlation coefficient, slope, and y-intercept were calculated [20].

Precision

Method precision was established by analyzing six replicate sample preparations. The % RSD of assay results was calculated [21].

Accuracy

Recovery studies were performed by spiking known quantities of standard drugs at 50%, 100%, and 150% levels of the target concentration. Each level was analyzed in triplicate, and the percentage recovery was calculated [22].

Solution stability

The stability of standard and sample solutions was evaluated at room temperature and under refrigerated conditions for 24 h [23].

Forced degradation studies

Forced degradation studies were conducted under acid hydrolysis (0.5N HCl at 60 °C), base hydrolysis (0.5N NaOH at 60 °C), and oxidative (3% H_2O_2 at 60 °C) conditions at different time intervals (30 min, 1 hour, and 2 h). The percentage assay and formation of degradation products were monitored [24].

Robustness

The robustness of the method was assessed by deliberately varying the chromatographic conditions such as flow rate (±0.2 ml/min), pH of the buffer (±0.2 units), column temperature (±5 °C), and composition of the mobile phase (±5%) [25].

RESULTS AND DISCUSSION

Identification of dorzolamide hydrochloride (DZH) and timolol maleate (TIM)

FTIR spectroscopy

The FTIR spectra of DZH standard (USP) and sample of active pharmaceutical ingredient (api) showed characteristic peaks at 3369.65/3367.72, 3045.60, 2451.53, 2285.65/2337.73, 2007.90/2073.48, 1587.42, 1535.34, 1446.62, 1384.89, and 1321.24 cm^{-1}, confirming the identity of DZH.

Similarly, the FTIR spectra of TIM standard (USP) and sample of api showed characteristic peaks at 2964.59, 2889.37, 2852.72, 1703.15/1705.08, 1618.28, 1585.49/1583.56, 1406.11, and 1379.11 cm^{-1}, confirming the identity of TIM.

The overlay spectra of standards and samples for both drugs showed good correlation, indicating the authenticity of the drug samples used in the study [26]. The discrepancy was observed in DZM at FTIR Stretching frequency at 2285.65 vs. 2337.73 cm^{-1} in both USP standard and Sample due to sample matrix and has no impact the quality of product.

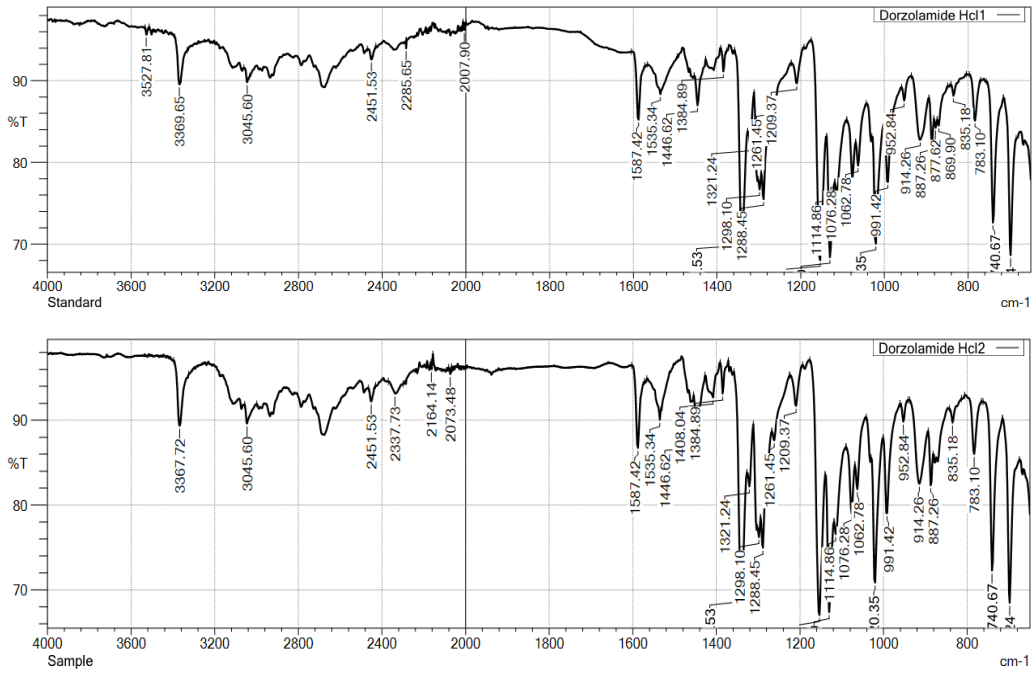


Fig. 2: FTIR-spectra of standard (USP) VS sample for dorzolamide hydrochloride (DZH)

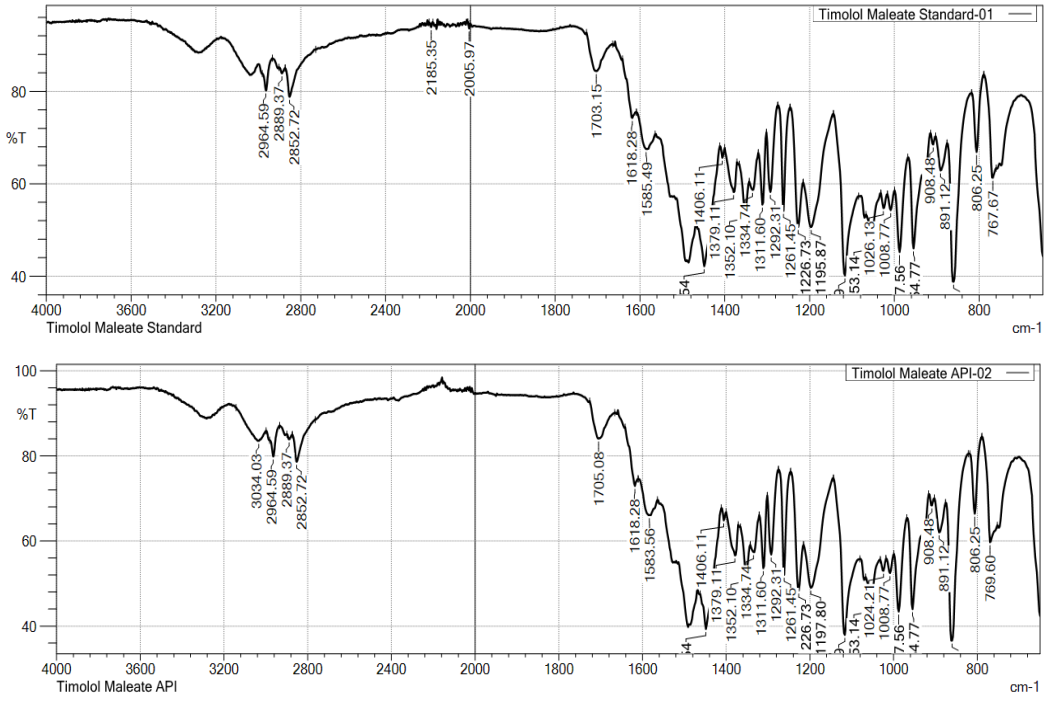


Fig. 3: FTIR-spectra of standard (USP) VS sample for timolol maleate (TIM)

Method development

The method development process involved a systematic approach to achieve optimal separation of DZH and TIM. Various mobile phase compositions, flow rates, column temperatures, and pH conditions were evaluated. The selection of appropriate wavelengths (253 nm for DZM and 295 nm for TIM) was based on the UV absorption maxima of the compounds.

Initial trials showed that a mobile phase composed of buffer pH 3.0, acetonitrile, and methanol (50:40:10% v/v/v) with gradient elution on a Symmetry C8 column provided good symmetric peaks with adequate resolution. The optimized method resulted in retention times of 17.6 min for DZH and 28.4 min for TIM.

Method validation

System suitability

The system suitability parameters met the acceptance criteria, indicating the suitability of the chromatographic system for analysis. The tailing factors for DZH and TIM were 1.2 and 1.1, respectively, which are well within the acceptance limit of NMT 2.0. The theoretical plate counts were 5543 for DZH and 7850 for

TIM, exceeding the minimum requirement of 2,000. The % RSD values for peak areas from five replicate injections were 0.3% for both compounds, indicating good precision of the injection system [27].

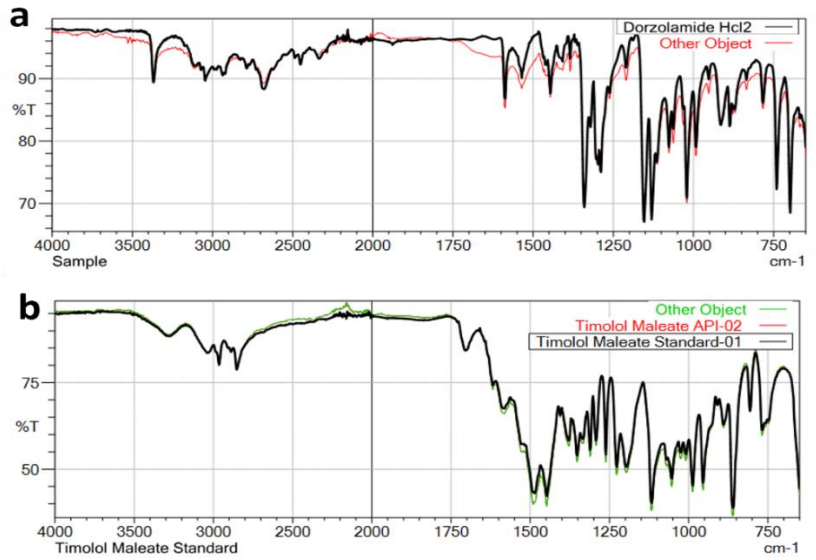


Fig. 4: Overlay FTIR-spectra comparison of standard vs sample for a. dorzolamide hydrochloride (DZH) and b. timolol maleate (TIM)

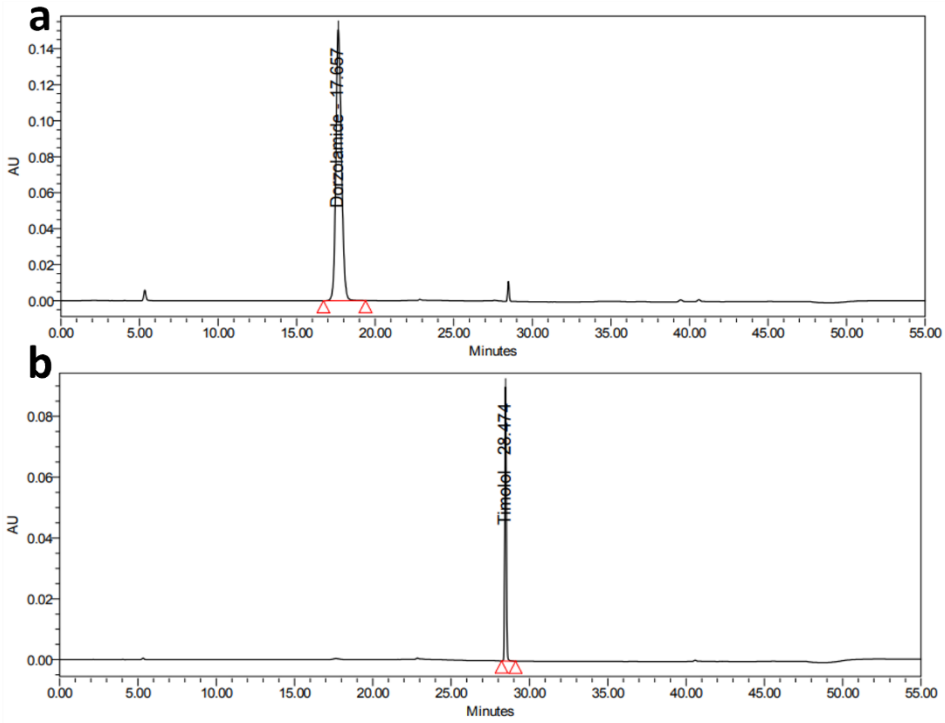


Fig. 5: Representative chromatograms of a. dorzolamide hydrochloride (DZH) at 253 nm and b. timolol maleate (TIM) at 295 nm

Table 4: System suitability parameters for dorzolamide hydrochloride (DZH) and timolol maleate (TIM)

S. No.	System suitability parameter	Acceptance criteria	Dorzolamide	Timolol maleate
1	For Interference check	No interference should be observed	No interference	No interference
2	The USP Tailing factor	NMT 2.0	1.2	1.1
3	The USP plate count	NLT 2000	5543	7850
4	The %RSD of peak	NMT 2.0	0.3	0.3
5	% Recovery of Standard solution against check standard	98-102%	99.9	100.0
6	% RSD of peak area standard against standard preparation	NMT 2.0	0.3	0.3
7	Bracketing standard	NMT 2.0%	0.2	0.2

Specificity

The specificity of the method was demonstrated by the absence of interference from placebo components, degradation products, and impurities at the retention times of dorzolamide hydrochloride (DZH) and timolol maleate (TIM). Peak purity analysis using the PDA detector showed purity angles less than purity thresholds for both compounds in standard solutions, control samples, and spiked samples, confirming the specificity of the method.

The retention times for DZH and its related impurities (DZH RC-D, DZH RC-B (In-house-C), Maleic Acid Adducts-A, Maleic Acid Adducts-B, and DZH In-house-B) were well separated from the main peak. Similarly, timolol maleate (TIM) and its related impurities (TIM Imp-G, TIM Imp-B, TIM Imp-D, TIM Imp-F, TIM Imp-C, TIM imp-J, TIM Imp-H, and TIM Imp-I) showed adequate separation.

Linearity and range

The calibration curves for DZH and timolol maleate TIM showed excellent linearity in the concentration ranges of 50-200 µg/ml and 12-100 µg/ml, respectively. The correlation coefficients (r^2) were 0.9999 for both compounds, indicating a strong linear relationship between concentration and peak area. The slopes and intercepts of the calibration curves were 13,160.89275 and 10,101.18953 for TIM, and 19,859.50102 and 48,164.58577 for DZH, respectively. (The Linearity was performed as per the specification limits is related to specification limits for both components i.e.) 2.0% of DZH is 20 mg/ml and 0.5% of TIM is 5 mg/ml).

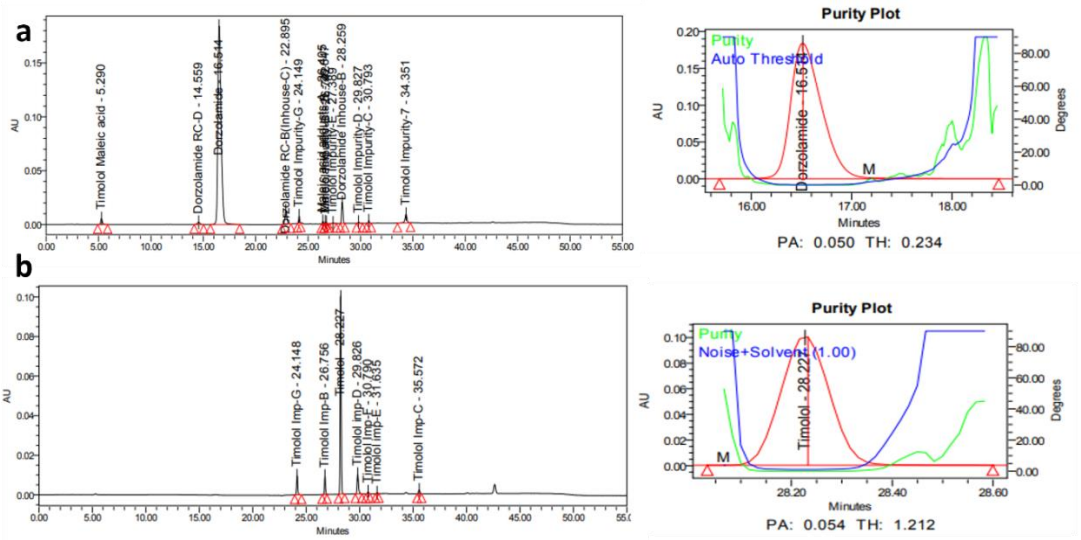


Fig. 6: Specimen chromatogram for purity plot of spiked sample of a. dorzolamide hydrochloride (DZH) and b. timolol maleate (TIM)

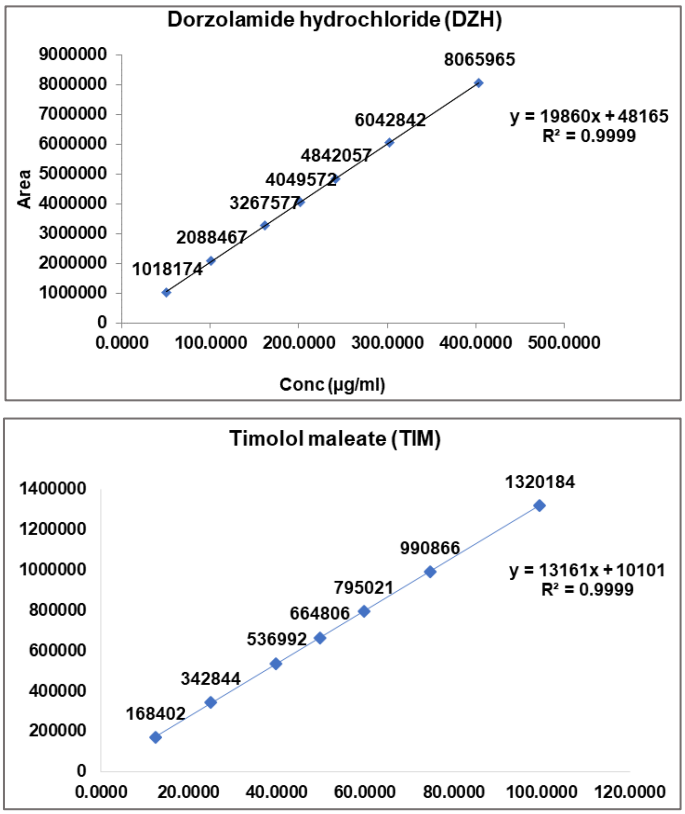


Fig. 7: Linearity plot of dorzolamide hydrochloride (DZH) and timolol maleate (TIM)

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD is calculated using the formula 3.3 times σ/s, where "σ" is the standard deviation of the intercept obtained for calibration curve and "s" is the slope of the calibration curve. Similarly, LOQ is calculated using the formula 10 times σ/s. The calculated LOD and LOQ is for DZH is 5.3 µg/ml and 16.2µg/ml and for TIM is 1.6 µg/ml and 4.9µg/ml, which is 0.5% of DZH and 0.1% for TIM for specification level.

Precision

The method precision was evaluated by analyzing six replicate sample preparations. The % RSD values for the assay results were 0.001% for both DZH and TIM, which are well below the acceptance criterion of NMT 2.0%, indicating the precision of the method [28].

Accuracy

The accuracy of the method was assessed through recovery studies at three concentration levels (50%, 100%, and 150% of the target concentration). The mean recoveries for dorzolamide hydrochloride (DZH) were 99.2%, 99.2%, and 98.7% at the 50%, 100%, and 150% levels, respectively, with overall %RSD values ranging from 0.1% to 0.2%. For timolol maleate, the mean recoveries were 100.5%, 99.0%, and 99.1% at the 50%, 100%, and 150% levels, respectively, with overall %RSD values ranging from 0.1% to 0.3%. These results demonstrate the accuracy of the method within the specified range [29]. We acknowledge the observation of slightly low recovery for dorzolamide (DZH) at the 100% accuracy level. Upon further investigation, we considered potential contributing factors such as matrix effects, which is having the acceptable limit is 98.0 to 102.0 and the samples shows precise in replicate injections in table 7.

Table 6: Method precision results

Name of the preparation	Dorzolamide area	Timolol maleate area	Dorzolamide (% assay)	Timolol maleate (% assay)
Sample Preparation-1	3690022	630064	101.7	99.3
Sample Preparation-2	3679044	627620	101.4	99.0
Sample Preparation-3	3678772	627927	101.4	99.0
Sample Preparation-4	3683306	628801	101.5	99.2
Sample Preparation-5	3672121	627186	101.2	98.9
Sample Preparation-6	3677702	628052	101.3	99.0
AVG			101.4	99.1
SD			0.1722	0.1506
%RSD			0.2	0.2

Where, AVG; average, SD; standard deviation, RSD; Related standard deviation, All the values are presented as mean±SD, n=6

Table 7: Accuracy (Recovery) results for dorzolamide hydrochloride (DZH)

% Recovery for dorzolamide							
Recovery level	**Sample no**	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Average mean**	**SD**	**%RSD**
50%	1	101.15	100.32	99.2	99.2	0.1	0.1
	2	101.12	100.25	99.1			
	3	101.23	100.57	99.3			
100%	1	200.65	198.83	99.1	99.2	0.2	0.2
	2	200.56	199.52	99.5			
	3	200.38	198.79	99.2			
	4	200.47	198.50	98.9			
	5	200.38	198.27	98.9			
	6	200.56	198.93	99.3			
	1	310.15	306.47	98.8	98.7	0.2	0.2
150%	2	310.24	306.16	98.7			
	3	310.42	305.84	98.5			

Where, AVG; average, SD; standard deviation, RSD; Related standard deviation, All the values are presented as mean±SD, n=3 and 6

Table 8: % Accuracy (recovery) results for timolol maleate (TIM)

% Recovery for timolol maleate							
Recovery level	**Sample no (n)**	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Average mean**	**SD**	**%RSD**
50%	1	25.25	25.41	100.6	100.5	0.3	0.3
	2	25.25	25.31	100.2			
	3	25.25	25.45	100.8			
100%	1	50.50	49.97	98.95	99.0	0.3	0.3
	2	50.50	50.15	99.31			
	3	50.50	50.02	99.05			
	4	50.50	50.04	99.09			
	5	50.50	49.73	98.48			
	6	50.50	49.94	98.89			
	1	75.75	75.18	99.25	99.1	0.1	0.1
150%	2	75.75	75.06	99.09			
	3	75.75	75.07	99.10			

Where, AVG; average, SD; standard deviation, RSD; Related standard deviation, All the values are presented as mean±SD, n= 3 and 6

Solution stability

The stability of standard and sample solutions was evaluated at room temperature and under refrigerated conditions for 24 h. The % difference in peak areas between initial and 24 h measurements was less than 1% for both compounds under both storage conditions, indicating that the solutions are stable for at least 24 h.

Table 9: Solution stability data for check standard vs calibration standard

Solution stability for dorzolamide check standard				
Condition	**Standard at room temperature**		**Standard at Refrigerator**	
	Area	**% Difference**	**Area % recovery**	**% Difference**
Initial	4241158	NA	4241158	NA
24Hrs	4243218	-0.05	4227893	0.31
Solution stability for calibration standard				
Initial	3956116	NA	3956116	NA
24Hrs	3959482	-0.09	3926178	0.76
Solution stability for timolol check standard				
Initial	694375	NA	694375	NA
24Hrs	695149	-0.11	692017	0.34
Solution stability for timolol calibration standard				
Initial	674038	NA	674038	NA
24 H	677773	-0.55	671713	0.34

Forced degradation studies

Forced degradation studies were conducted to evaluate the stability-indicating nature of the method. DZH showed minimal degradation under acid hydrolysis (approximately 1-3% degradation) and moderate degradation under basic and oxidative conditions (approximately 2-8% degradation). TIM was more susceptible to degradation, particularly under basic and oxidative conditions, with degradation ranging from approximately 10% to 92% depending on the stress conditions and duration.

The chromatographic method was capable of resolving the degradation products from the main peaks, confirming its stability-indicating nature. Peak purity analysis showed that the peaks of interest remained pure even in the presence of degradation products [30].

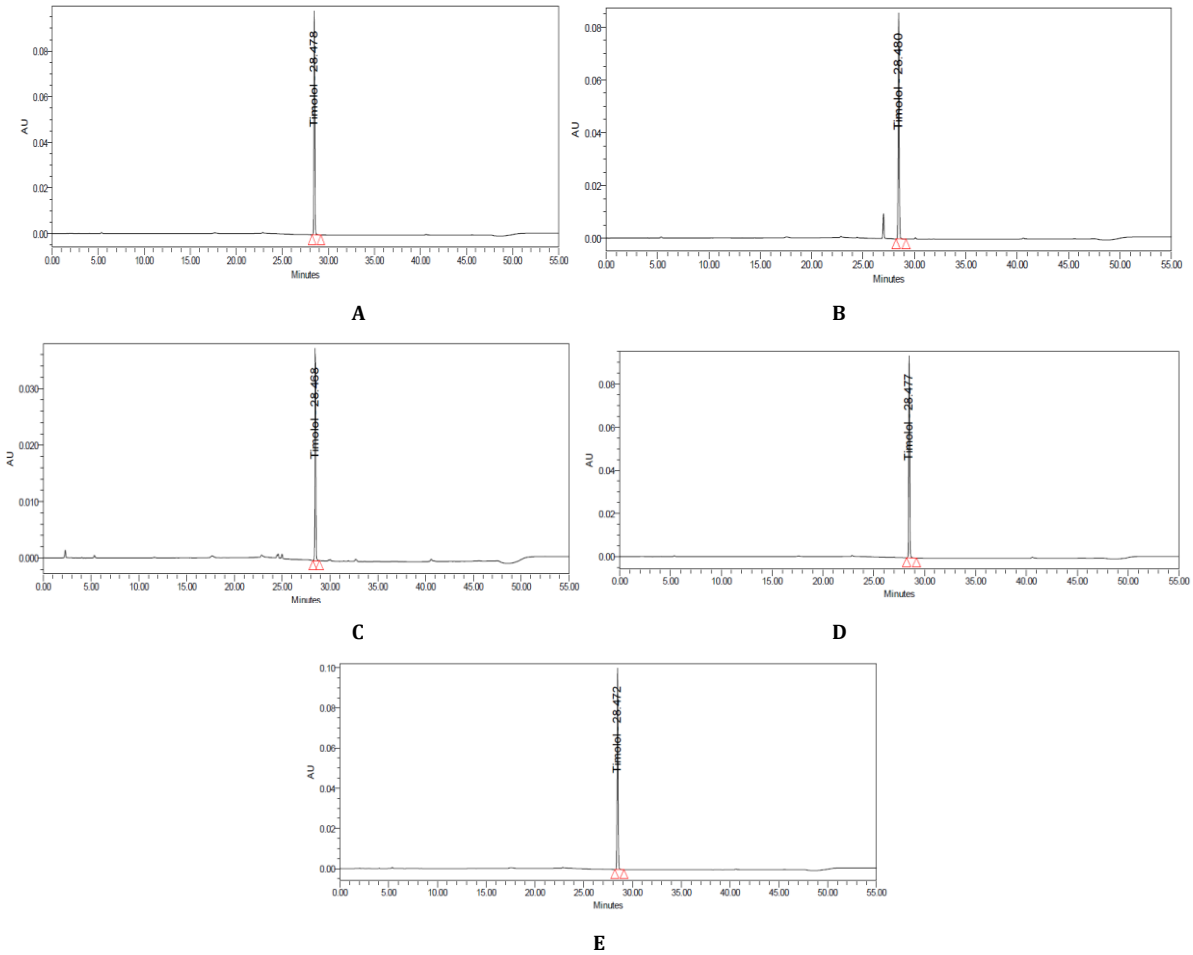


Fig. 8: Degradation chromatograms of timolol maleate (TIM) at @ 293 nm A Acid, B Base, C Peroxide, D Thermal, E Photolytic

Table 10: Forced degradation study results

S. No.	Degradation mechanism/Condition	% Assay dorzolamide	% Assay timolol maleate
1	Control Sample	101.7	99.3
2	Acid_60 °C_0.5N HCl _2H	103.2	100.6
3	Base_60 °C_0.5N NaOH_2H	99.3	83.0
4	Peroxide_60 °C_0.5N HCl_2H	93.2	40.0
5	Acid_60 °C_0.5N HCl _1H	101.3	98.9
6	Base_60 °C_0.5N NaOH_1H	103.	90.1
7	Peroxide_60 °C_3% H_2O_2_1H	97.1	65.0
8	Acid_60 °C_0.5N HCl _30 min	101.4	97.1
9	Base_60 °C_0.5N NaOH_30 min	95.3	7.7
10	Peroxide_60 °C_0.5N HCl_30 min	99.9	82.0
11	Base_2 ml spl_60 °C_0.5N precisionNaOH_30 min	100.6	82.5
12	Thermal sample 60 °C_10 d	97.5	96.8
13	Humidity_75% RH _10 d	97.6	97.1
14	Photolytic UV_10 d	97.5	97.0

Where, H; hours, min; minutes

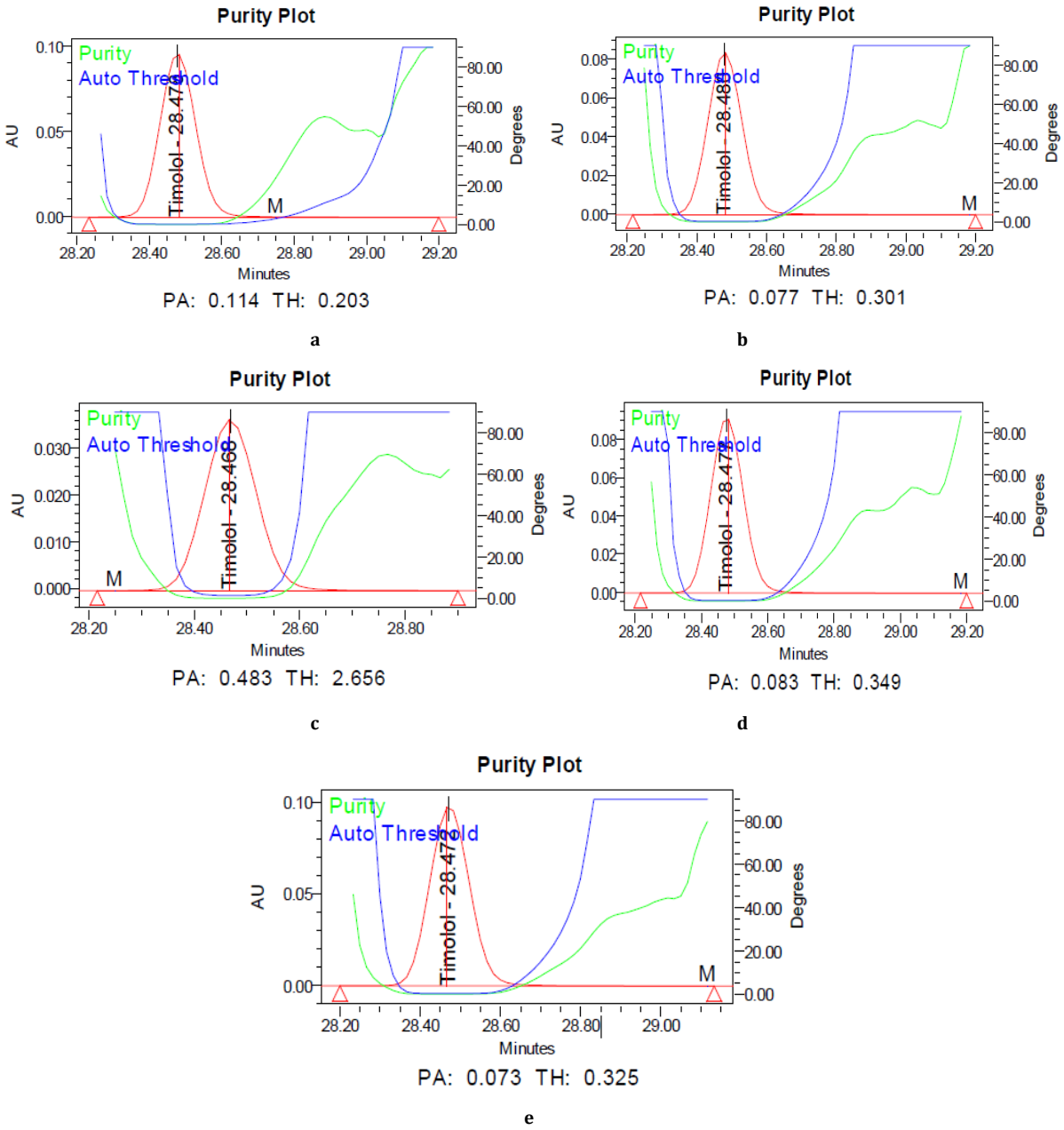


Fig. 9: Purity plots of timolol maleate (TIM) at @ 295 nm a Acid, b Base, c Peroxide, d Thermal, e Photolytic

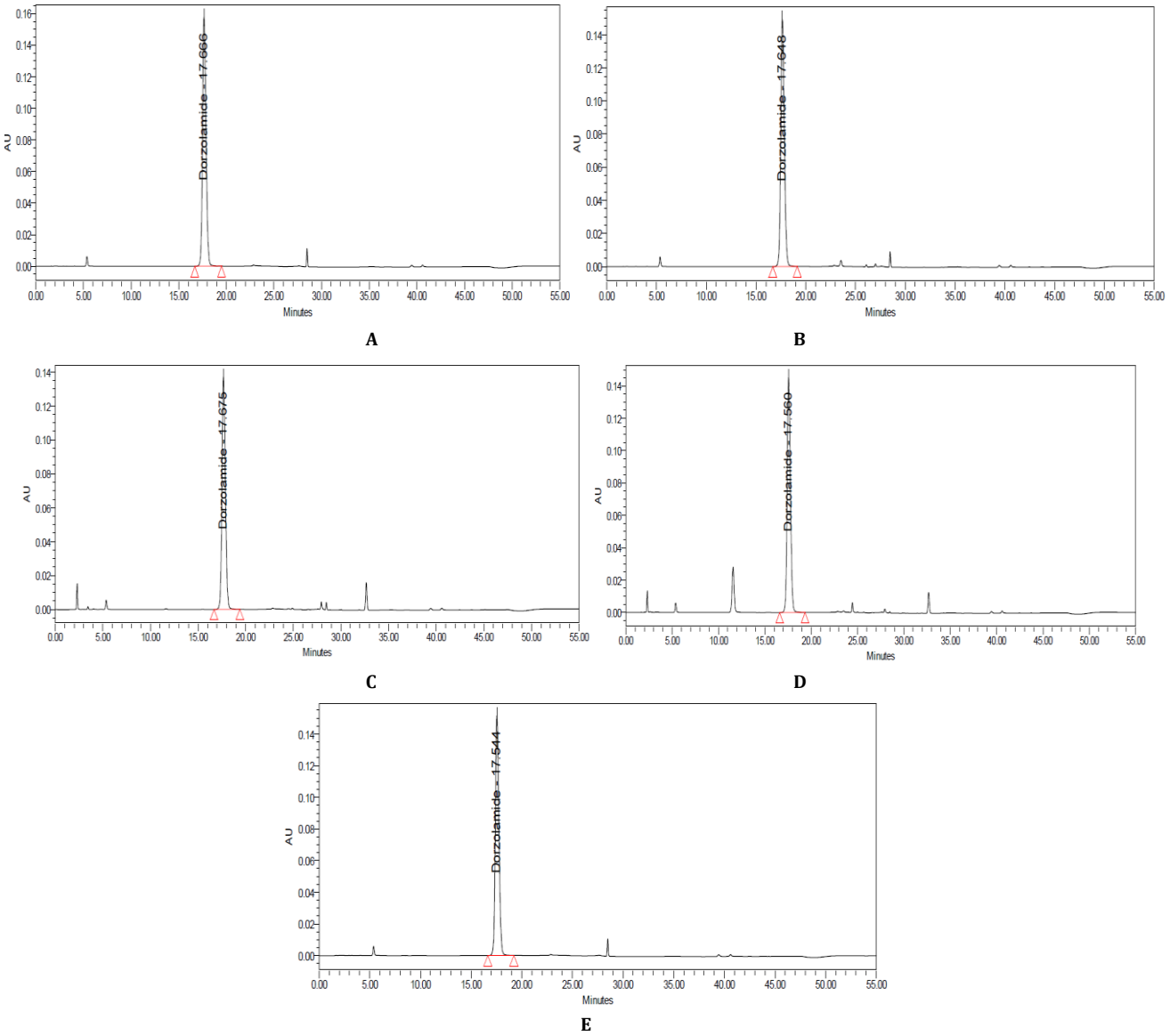


Fig. 10: Degradation chromatograms of dorzolamide hydrochloride (DZH) at @ 253 nm A Acid, B Base, C Peroxide, D Thermal, E Photolytic

Table 11: Robustness data for critical parameters

Parameters	Retention time (min)	
Mobile phase composition	**Dorzolamide (DZH)**	**Timolol maleate (TIM)**
85:15%v/v	16.3	27.7
95:5%v/v	16.9	28.9
pH of buffer		
pH2.8	16.2	28.2
pH3.2	17.3	28.4
Flow Rate Variation		
1.0 ml/min	20.0	29.3
1.4 ml/min	15.2	27.7
Column Temperature Variation		
32 °C	17.6	27.8
42 °C	13.2	27.5

Robustness

The robustness of the method was assessed by deliberately varying the chromatographic conditions. Small changes in flow rate (±0.2 ml/min), pH of the buffer (±0.2 units), column temperature (±5 °C), and composition of the mobile phase (±5%) did not significantly affect the system suitability parameters such as retention time, tailing factor, and theoretical plate count. This demonstrates the robustness of the method within the studied range of variations.

The most significant changes were observed with variations in flow rate and column temperature and mobile phase pH, which affected the retention times of both compounds. However, the method remained suitable for analysis as the system suitability criteria were still met.

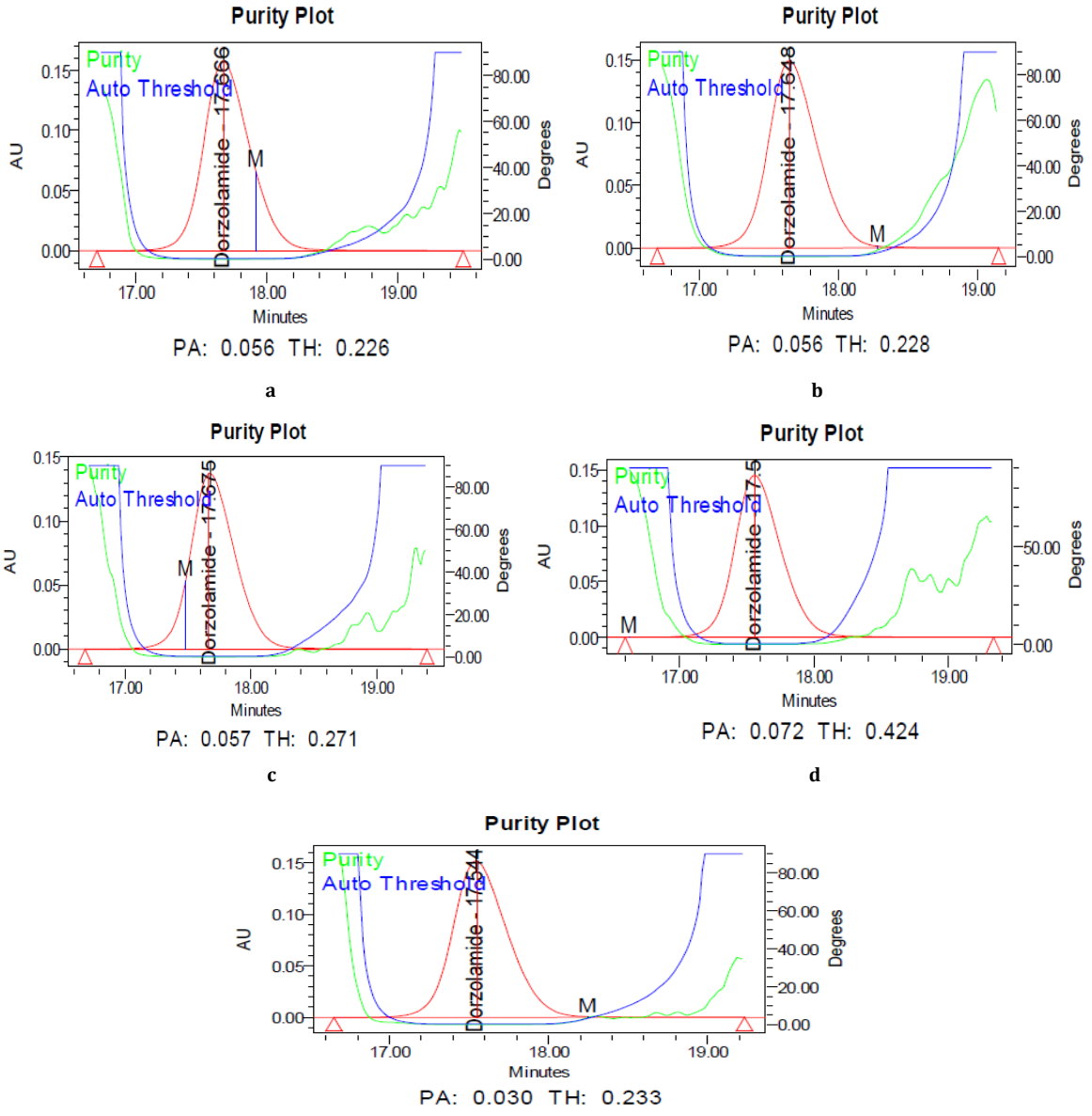


Fig. 11: Purity plots of dorzolamide hydrochloride (DZH) at @ 253 nm a Acid, b Base, c Peroxide, d Thermal, e Photolytic

Table 12: Robustness data for DZH As such method

Name	Area	RT	USP Tailing	USP plate count
Average of Six Standards	4018132	17.406	1.3	10709
Control sample	3863672	17.308	1.2	12611
BKT Standard	3996407	17.313	1.3	12602

Table 13: Robustness data for DZH low flow method_1.0 ml

Name	Area	RT	USP tailing	USP plate count
Average of Six Standards	4832292	20.037	1.3	9332
Control sample	4668216	19.984	1.2	7681
BKT Standard	4816460	18.437	1.3	8758

Table 14: Robustness data for DZH high flow method_1.4 ml

Name	Area	RT	USP Tailing	USP Plate count
Average of Six Standards	3434829	15.243	1.5	5792
Control sample	3333998	15.228	1.6	6100
BKT Standard	3441024	15.277	1.6	6383

Table 15: Robustness data for DZH low temperature method_32 °C

Name	Area	RT	USP Tailing	USP Plate count
Average of Six Standards	3450509	17.655	1.8	5616
Control sample	3339747	17.640	1.8	5656
BKT Standard	1709857	17.730	1.7	6044

Table 16: Robustness data for DZH high temperature method_42 °C

Name	Area	RT	USP Tailing	USP Plate count
Average of Six Standards	3462632	13.241	1.7	5592
Control sample	3338902	13.213	1.7	5433
BKT Standard	3463786	13.169	1.8	5325

Table 17: Robustness data for DZH low pH-2.8

Name	Area	RT	USP tailing	USP plate count
Average of Six Standards	4016582	16.207	1.5	9291
Control sample	3917791	16.163	1.5	9059
BKT Standard	4016274	16.212	1.5	9422

Table 18: Robustness data for DZH high pH-3.2

Name	Area	RT	USP Tailing	USP Plate count
Average of Six Standards	4018798	17.344	1.5	7186
Control sample	3911586	17.325	1.6	6880
BKT Standard	4013318	17.307	1.6	7084

Table 19: Robustness data for DZH low organic

Name	Area	RT	USP tailing	USP plate count
Average of Six Standards	4825354	16.352	1.5	11040
Control sample	4516177	16.420	1.4	11570
BKT Standard	4749970	16.508	1.5	11362

Table 20: Robustness data for DZH organic

Name	Area	RT	USP tailing	USP plate count
Average of Six Standards	4393697	16.939	1.4	10324
Control sample	3948760	16.945	1.3	9923
BKT Standard	4107294	16.944	1.3	8578

Table 21: Robustness data for TIM as such method

Name	Area	RT	USP tailing	USP plate count
Average of Six Standards	658730	28.380	1.0	7521
Control sample	655387	28.380	1.0	7514
BKT Standard	658650	28.386	1.0	7554

Table 22: Robustness data for TIM Flow method_1.0 ml

Name	Area	RT	USP Tailing	USP Plate count
Average of Six Standards	794461	29.302	1.0	4548
Control sample	790318	29.302	1.0	4498
BKT Standard	794346	29.282	1.0	4588

Table 23: Robustness data for TIM flow method_1.4 ml

Name	Area	RT	USP Tailing	USP Plate count
Average of Six Standards	565869	27.710	1.1	4588
Control sample	565495	27.705	1.1	4598
BKT Standard	568649	27.721	1.1	4578

Table 24: Robustness data for TIM temperature method_32 °C

Name	Area	RT	USP Tailing	USP Plate count
Average of Six Standards	568272	27.833	1.2	4896
Control sample	568508	27.831	1.2	4856
BKT Standard	290311	27.845	1.2	4825

Table 25: Robustness data for TIM temperature method_42 ℃

Name	Area	RT	USP Tailing	USP Plate count
Average of Six Standards	570896	27.586	1.1	4514
Control sample	566612	27.574	1.2	4514
BKT Standard	570923	27.600	1.2	4572

Table 26: Robustness data for TIM pH-2.8

Name	Area	RT	USP Tailing	USP Plate count
Average of Six Standards	676376	28.295	1.1	4112
Control sample	678216	28.301	1.1	4115
BKT Standard	675360	28.283	1.1	4105

Table 27: Robustness data for TIM pH-3.2

Name	Area	RT	USP tailing	USP plate count
Average of Six Standards	663475	28.467	1.1	4512
Control sample	667968	28.457	1.1	4514
BKT Standard	663275	28.469	1.1	4517

Table 28: Robustness data for TIM low organic

Name	Area	RT	USP tailing	USP plate count
Average of Six Standards	794177	27.780	1.0	4507
Control sample	767106	27.779	1.0	4498
BKT Standard	782625	27.768	1.0	4857

Table 29: Robustness data for TIM high organic

Name	Area	RT	USP tailing	USP plate count
Average of Six Standards	725438	28.971	1.0	4758
Control sample	669571	28.964	1.0	4728
BKT Standard	669571	28.964	1.0	4799

CONCLUSION

A Novel, simple, sensitive, precise, accurate, and robust RP-HPLC method has been developed and validated for the simultaneous estimation of DZH and TIM in ophthalmic formulations. The method is superior compared with others and provides good resolution between the two active ingredients and their degradation products as per the USP, BP, and Ph. Eur. The validation studies demonstrate that the method is specific, linear, precise, accurate, and stability-indicating as per the ICH guidelines. The developed method can be effectively applied for routine quality control analysis of DZH and TIM in their combined ophthalmic formulations and bulk dosages. The stability-indicating nature of the method makes it particularly suitable for stability studies and for the analysis of samples that may contain degradation products. FTIR (Fourier Transform Infrared) spectroscopy is indeed used to compare dorzolamide hydrochloride (DZH) and timolol maleate (TIM), both in their standard forms and in a sample to identify similarities and differences in their molecular structures. This technique helps confirm the identity of the api and assess the purity and stability of the pharmaceutical formulation.

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AUTHORS CONTRIBUTIONS

Ramakrishna K Conceptualized the study, methodology, formal analysis, investigation, writing-original draft and Validation, data curation, writing-review and editing and Software, resources, visualization, Srujan Kumar M: Supervised the project.

CONFLICT OF INTERESTS

The authors report that there is no conflict of interest

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