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Article

A Novel and Rapid Validated Stability-Indicating UPLC Method of Related Substances for Dorzolamide Hydrochloride and Timolol Maleate in Ophthalmic Dosage Form

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A novel stability-indicating gradient reversed-phase ultra-performance liquid chromatographic (RP-UPLC) method was developed for the determination of purity of dorzolamide hydrochloride and timalol maleate in presence of their impurities, and forced degradation products and placebo. The method was developed using a Waters UPLC BEH C18, 100 × 2.1mm, 1.7 μm column with mobile phase containing a gradient mixture of solvents A and B. Phosphate buffer (0.04M), pH 2.6 was used as buffer. Buffer pH 2.6 was used as solvent A and Milli-Q water, methanol and acetonitrile in 200:300:600, *v*/*v*/*v* ratios were used as solvent B. The gradient program was set as 0/5, 8/8, 10/15, 16/45, 20/55, 24/80, 25/5 and 30/5. The eluted compound dorzolamide hydrochloride and its impurities were monitored at 254 nm, and timalol maleate and its impurities were monitored at 295 nm. The run time was 30 min, within which dorzolamide hydrochloride and its five impurities as well as timalol maleate and its three impurities were well separated, with resolution more than 2.0. Dorzolamide hydrochloride and timalol maleate were subjected to the stress conditions of oxidative, acid, base, photolytic and thermal degradation. The peak purity of dorzolamide hydrochloride, timalol maleate and their related compounds did not show any flag, thus proved the stability-indicating power of the method. The developed method was validated as per International Conference of Harmonization guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision and robustness.

Introduction

Dorzolamide hydrochloride–timolol maleate ophthalmic solution is the combination of a topical carbonic anhydrase inhibitor and a topical beta-adrenergic receptor blocking agent. Dorzolamide hydrochloride is described chemically as (4S-*trans*)-4-(ethylamino)-5, 6-dihydro-6-methyl-4H-thieno[2,3-b]thiopyran-2-sulfonamide7,7-ioxidemonohydrochloride (Figure 1A–F). Its empirical formula is $C_{10}H_{16}N_2O_4S_3 \bullet HCl$ and molecular weight is 360.91.

Timolol maleate is described chemically as (-)-1-(*tert*-butylamino)-3-[(4-morpholino-1, 2, 5-thiadiazol-3-yl) oxy]-2-propanol maleate (1:1) (salt) (Figure 1G–J). Its molecular formula is $C_{13}H_{24}N_4O_3S \bullet C_4H_4O_4$ and molecular weight is 432.50.

Each milliliter of ophthalmic solution contains 20 mg dorzolamide (22.26 mg of dorzolamide hydrochloride) and 5 mg timolol (6.83 mg timolol maleate).

Inactive ingredients are sodium citrate, hydroxyethyl cellulose, sodium hydroxide, mannitol and water for injection. Benzalkonium chloride (0.0075%) is added as a preservative.

A thin-layer chromatography (TLC)-densitometry, ultraviolet (UV)-spectrophotometry method for the determination of dorzolamide hydrochloride and timolol maleate is available (1). A high-performance thin-layer chromatography (HPTLC) method is also available for the determination of timolol maleate (10). Methods are also available for simultaneous determination of impurities in S-timolol (11, 12). A number of methods are also available for simultaneous determination of dorzolamide HCL and timolol maleate (assay) in eye drops by diode-array, UV detection and capillary electrophoresis assay methods (3–4, 6, 7). A method is also available for quantification of related substances of timolol maleate (13). Two separate methods are available for dorzolamide HCL and timolol maleate in USP (5). To our present knowledge, no validated stability-indicating analytical HPLC or ultra-performance liquid chromatography (UPLC) methods are available in literature for dorzolamide hydrochloride and timalol maleate and its impurities in ophthalmic solution. Attempts were made to develop a stability indicating LC method for the related substance determination of dorzolamide hydrochloride and timalol maleate in the presence of placebo. This paper deals with the forced degradation of dorzolamide hydrochloride and timalol maleate ophthalmic solution under stress conditions like acid hydrolysis, base hydrolysis, water hydrolysis, oxidation and heat. This paper also deals with the validation of the developed method for the accurate quantification of dorzolamide hydrochloride and timalol maleate impurities in ophthalmic solution.

The method was developed on UPLC with low particle size (1.7 micron) and a short 100-mm column. Hence, a rapid, unique, reproducible, stability-indicating UPLC method was developed for the quantitative determination of dorzolamide hydrochloride and timalol maleate and impurities in ophthalmic solution.

Experimental

Chemicals and reagents

Ophthalmic solution, dorzolamide hydrochloride and timalol maleate standard and impurities were supplied by Dr. Reddy's Laboratories (Hyderabad, India). The HPLC-grade acetonitrile analytical grade KH_2PO_4 and ortho-phosphoric acid were purchased from Merck (Darmstadt, Germany). High-purity water was prepared by using a Millipore MilliQ Plus water purification system.

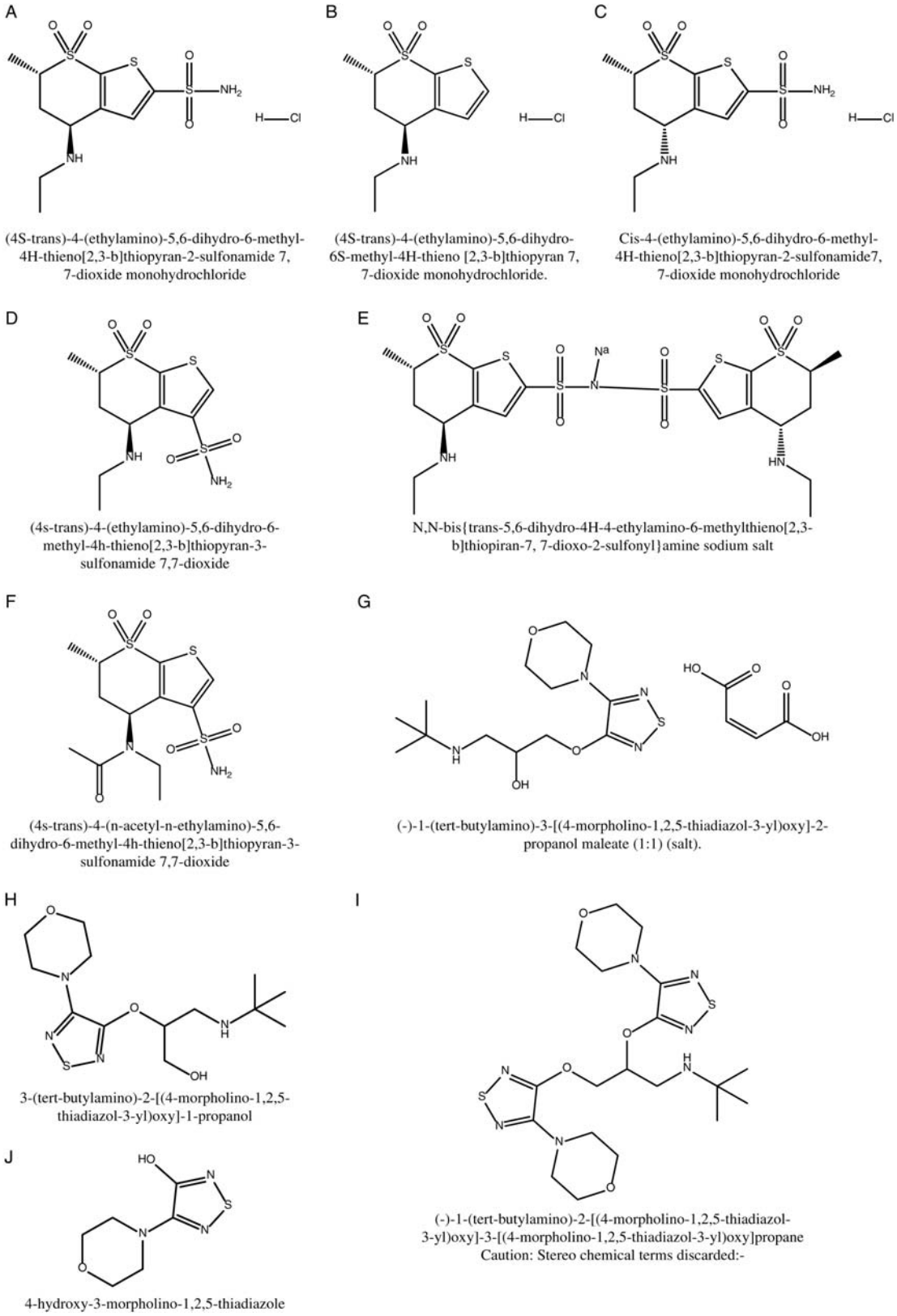

Figure 1. Chemical structures and names of dorzolamide hydrochloride and its impurities and timolol maleate and its impurities: dorzolamide hydrochloride (A); dorzolamide hydrochloride imp-1 (B); dorzolamide hydrochloride imp-2 (C); dorzolamide hydrochloride imp-3 (D); dorzolamide hydrochloride imp-4 (E); dorzolamide hydrochloride imp-5 (F); timolol maleate (G); timolol maleate imp-1 (H); timolol maleate imp-2 (I); timolol maleate imp-3 (J).

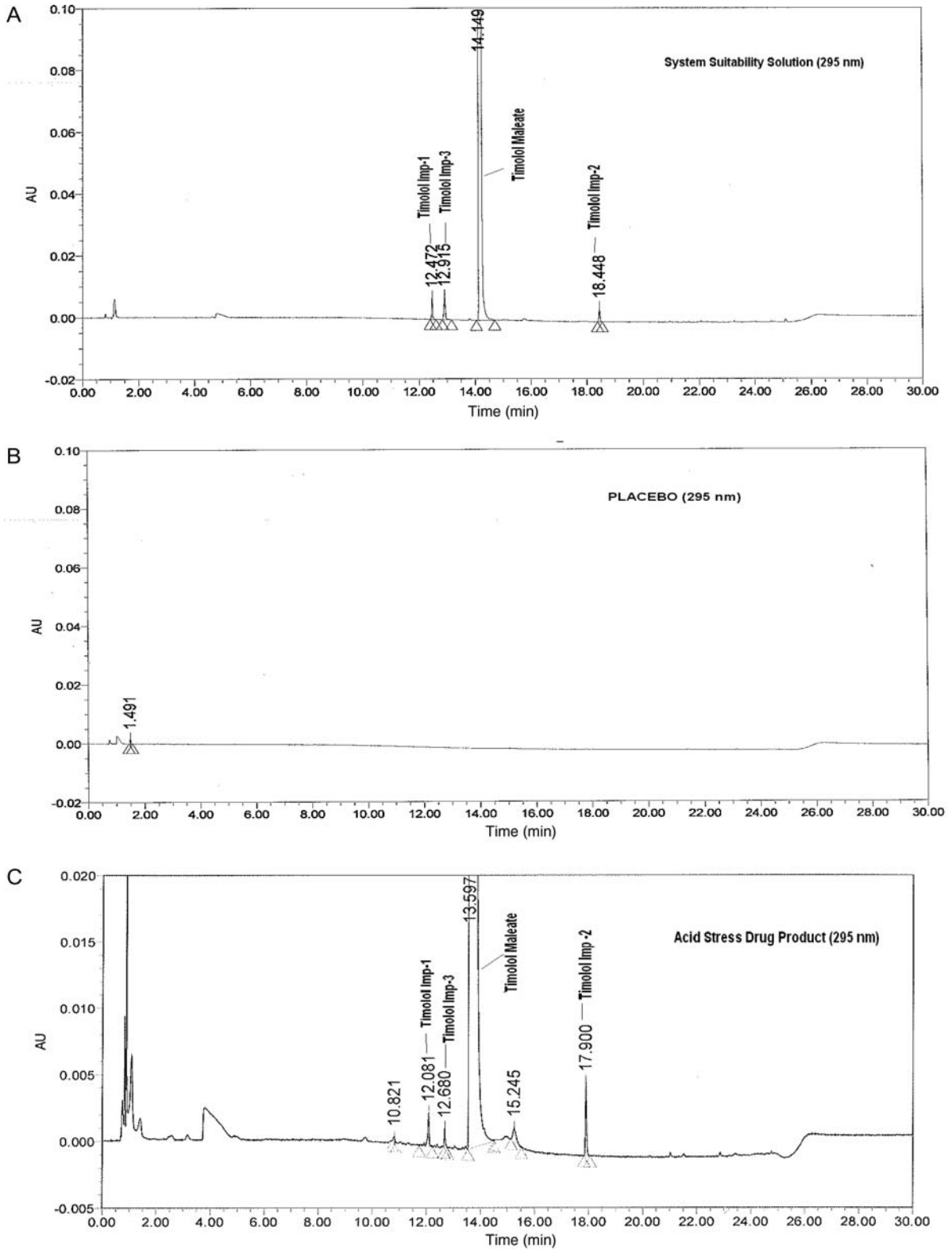

Figure 2. Typical chromatograms of: timolol maleate test spiked with its impurities (A); placebo (B); forced degradation samples at 295 nm wavelength (C–G); dorzolamide hydrochloride test spiked with its impurities (H); placebo (I); forced degradation samples at 254 nm wavelength (J–N).

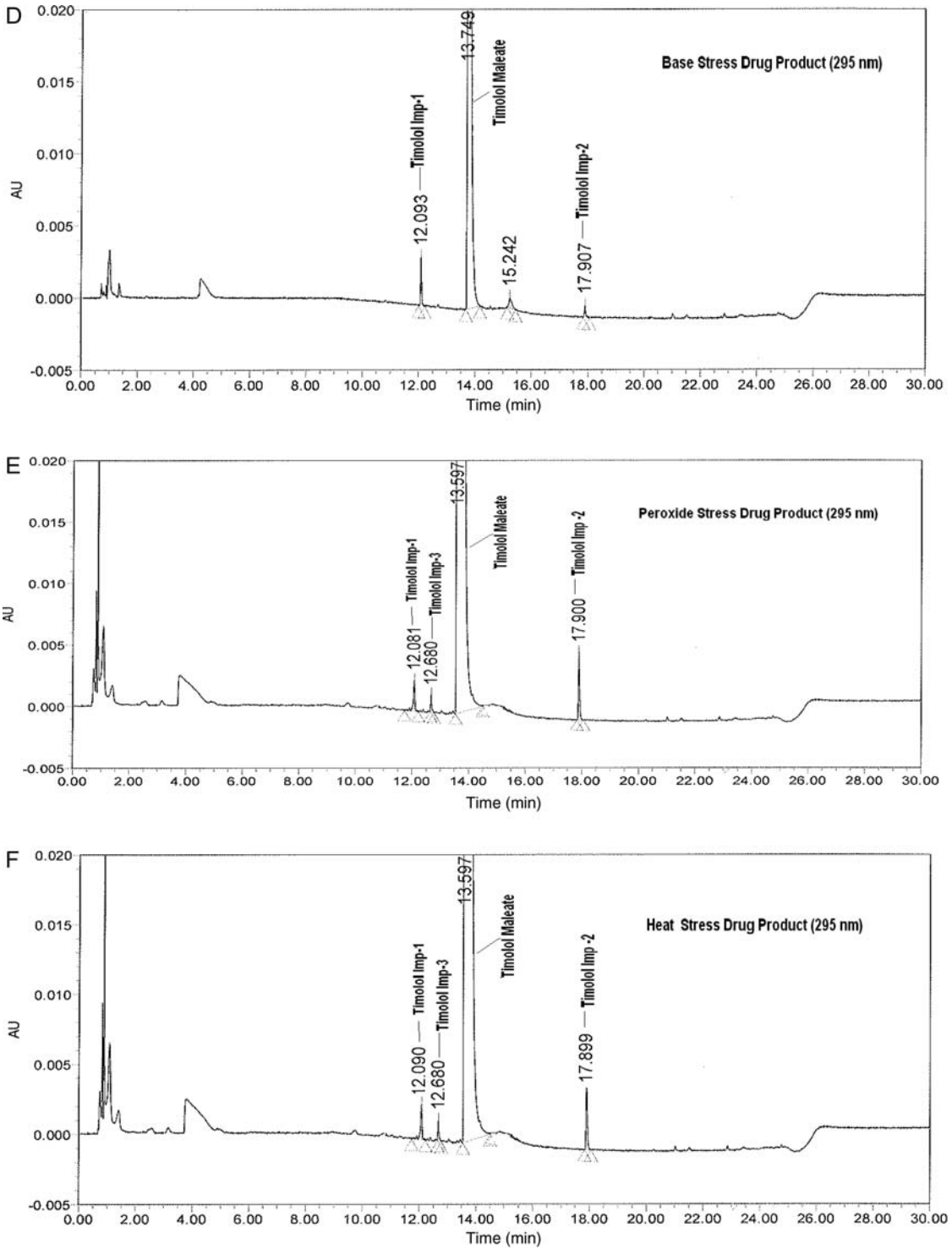

Figure 2. (Continued).

Equipment

UPLC analysis was performed with a Waters (Milford, MA) Acquity UPLC system equipped with a quaternary solvent manager, sample manager, column-heating compartment and photodiode array detector. This system was controlled by Waters Empower software.

An Acquity UPLCTM BEH C18 column, 100 × 2.1 mm, 1.7 μm (Waters) was employed for chromatographic separation. All samples were centrifuged by a Thermo Scientific multifuged machine. The specificity study was conducted by using heating oven (MACK Pharmatech, Hyderabad, India) and photostability chamber, and water baths equipped with a Milli Volt

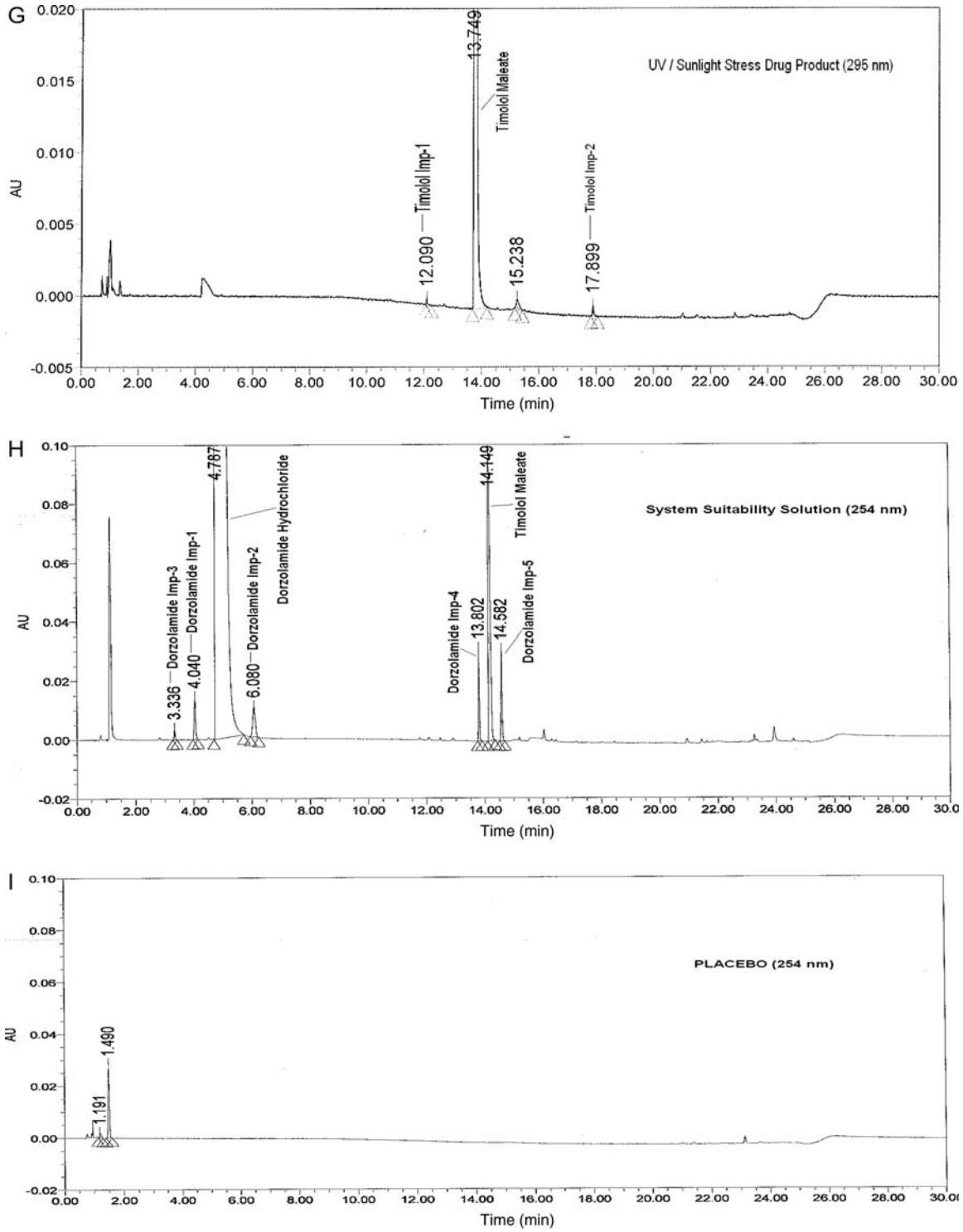

Figure 2. (Continued).

controller (Julabo, Seelbach, Germany) were used for hydrolysis studies.

Chromatographic conditions

The method was developed using a BEH C18, 100 × 2.1mm, 1.7 μm column as stationary phase. The mobile phase used was 0.04M, and phosphate buffer pH 2.6 was used as buffer. Buffer pH 2.6 was used as solvent A and Milli Q water, methanol and acetonitrile in 200:300:600 *v*/*v*/*v* ratios were used as solvent B. A mixture of Milli Q water and acetonitrile in 90:10 *v*/*v* was used for diluent to prepare solutions. The gradient program Time(minutes)/% mobile phase B (T/%B) was set as 0/5, 8/8,

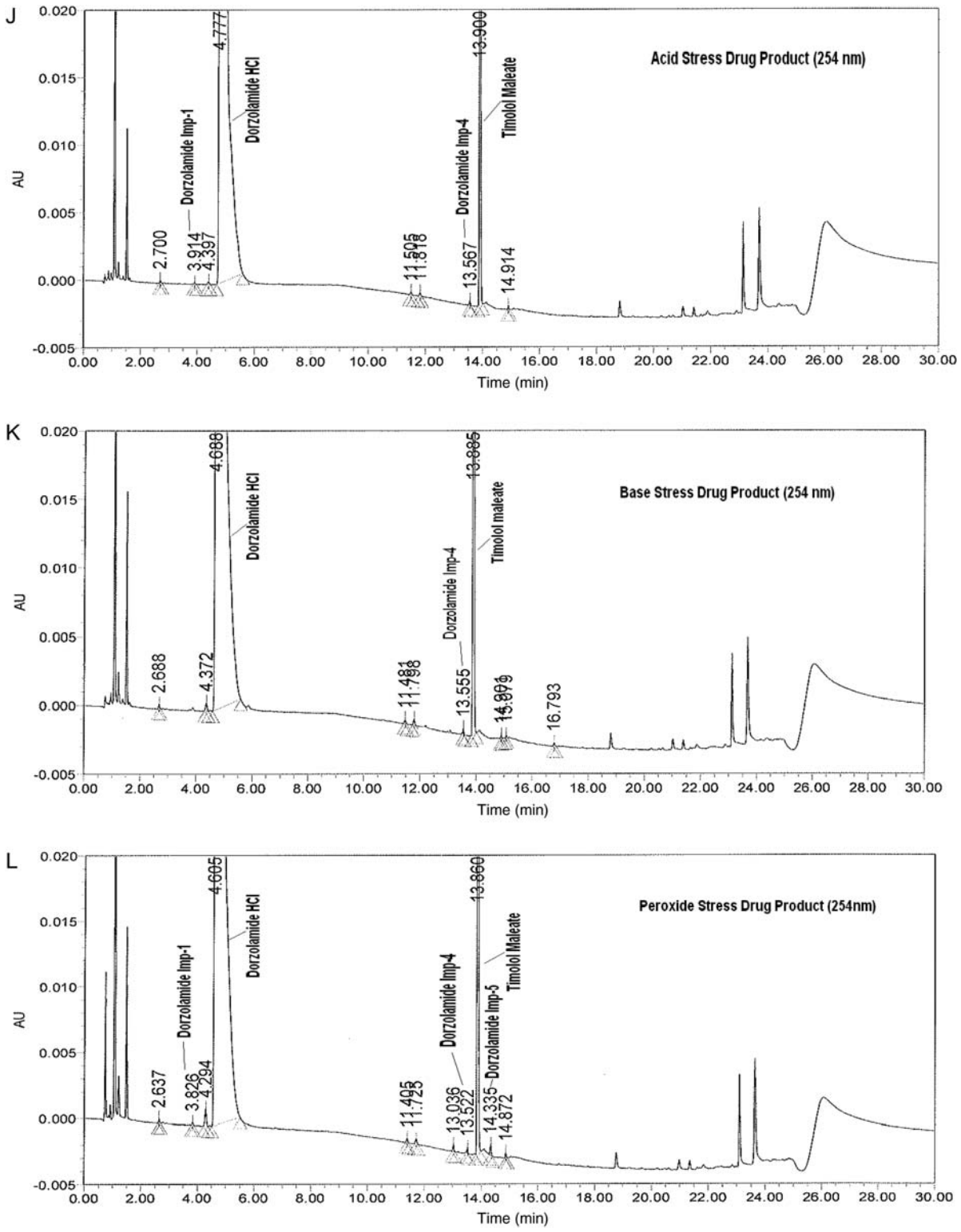

Figure 2. (Continued).

10/15, 16/45, 20/55, 24/80, 25/5 and 30/5, respectively. Before use, the mobile phase was mixed thoroughly and degassed. The mobile phase was pumped at 0.32 mL/min. The eluted compound dorzolamide hydrochloride and its five impurities were monitored at 254 nm and timalol maleate and its three impurities were monitored at 295 nm. The column temperature was maintained at 28°C. The injection volume for samples and standards was 1.5 µL.

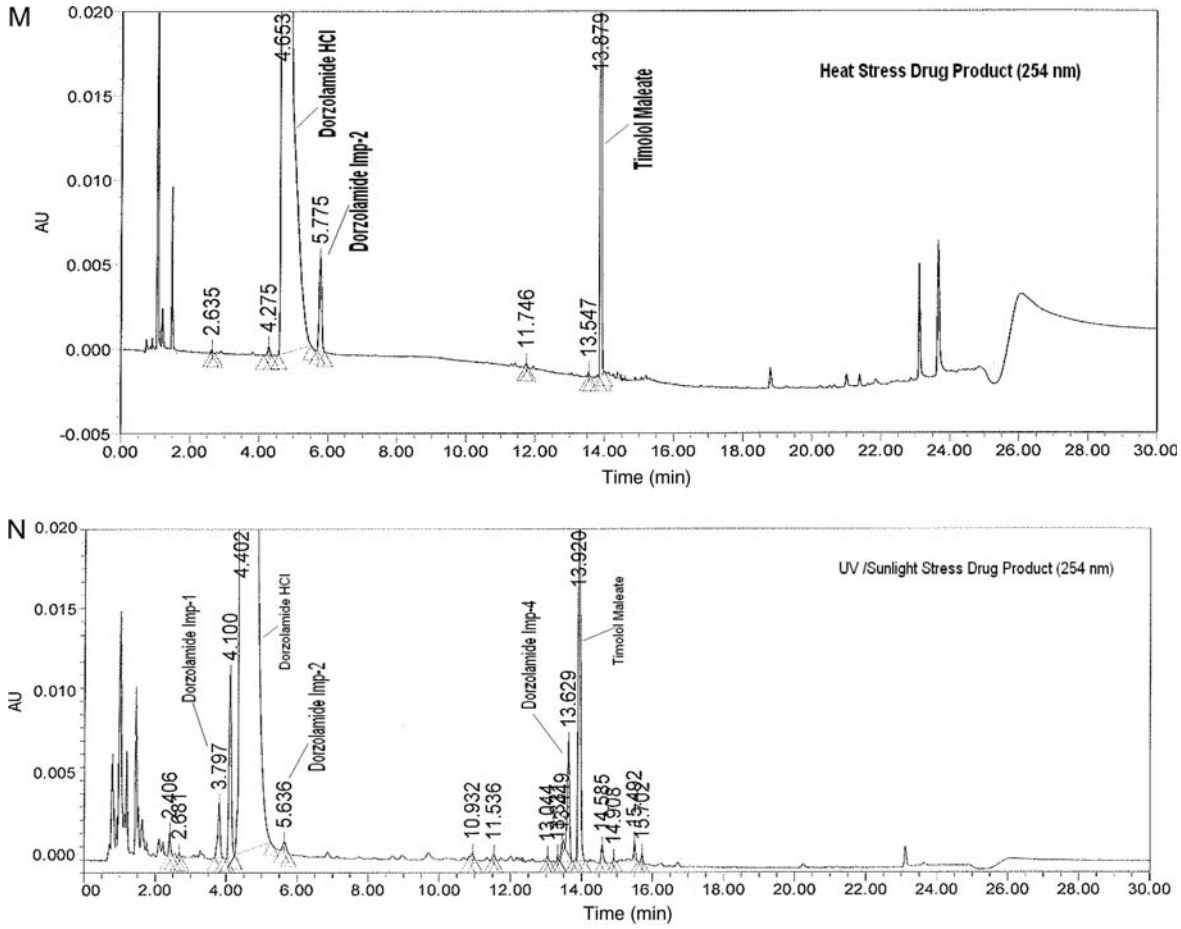

Figure 2. (Continued).

Preparation of stock solutions

A stock solution of dorzolamide (1.224 mg/mL) and timolol (0.61 mg/mL) was prepared by dissolving an appropriate amount of the drug in diluent (Milli-Q water and acetonitrile in 90:10, *v*/*v*). Working solutions of 12.24 and 3.06 μg/mL were prepared from this stock solution for the related substance determination. A stock solution of impurity (a mixture of imp-1, imp-2, imp-3, imp-4 and imp-5 for dorzolamide at 0.0608 mg/mL was also prepared in the diluent and imp-1, imp-2 and imp-3 for timolol) at 0.08 mg/mL was also prepared in the diluent. A system suitability solution containing (2.4 mg/mL) dorzolamide and 7.2 μg/mL of the previously described impurity stock and (0.6 mg/mL) timolol and 1.8 μg/mL of the previously described impurity stock.

Preparation of sample solution

Five sample solution vials were well-mixed into a dry cleaned test tube to get a uniform solution. Three milliliters of sample solution containing 60 mg dorzolamide and 15 mg of timolol were pipetted and transferred to a 25-mL volumetric flask. Finally, the drugs were dissolved in 15 mL of diluent and the pipette was rinsed 3–4 times with diluent. The mixture was then sonicated for approximately 10 min and made up to volume with diluent. The solution was filtered through a 0.2-μm Millipore PVDF filter. Then, 1.5 μL of these solutions were injected into the liquid chromatograph and a chromatogram was recorded, which is shown in Figure 2. The retention times of dorzolamide and timolol were found to be approximately 4.7 and 14.1 min, respectively.

Method validation

The proposed method was validated as per ICH guidelines (2).

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential degradants. Stress studies were performed for ophthalmic solution to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted to determine the stress conditions of heat (60°C), photolytic sunlight for approximately 1.2 million lux hours and UV light, both at shorter and longer wavelengths for approximately 200 Wh/m^3, acid (0.1 N HCl), base (0.1 N NaOH) and oxidation (1% H2O2) to evaluate the ability of the proposed method to separate dorzolamide and timolol from their degradation products. For the acid, base, water, hydrolysis and oxidation, the

study period was 3 h, and for heat it was 6 h. Peak purity test was carried out for the dorzolamide and timolol peaks by using photodiode array (PDA) detector in stress samples.

Precision

The precision of the related substances method was checked by injecting six individual preparations of dorzolamide hydrochloride (2.4 mg/mL) and timalol maleate (0.6 mg/mL) and its impurities in ophthalmic solution and spiking with 0.30% of imp-1, imp-2, imp-3, imp-4 and imp-5 for dorzolamide and imp-1, imp-2 and imp-3 for timalol with respect to analyte concentration. The relative standard deviation (RSD) of the area for each impurity was calculated.

The intermediate precision of the method was also evaluated using different analysts and different instruments in the same laboratory

Limits of detection and quantification The limit of detection (LOD) and limit of quantification (LOQ) for impurities and analyte (with respect to unknown impurities) were determined at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. A precision study was also carried out at the LOQ level by injecting six individual preparations of timalol maleate and its known impurities (imp-1, imp-2, imp-3, imp-4 and imp-5) and dorzolamide hydrochloride and its known impurities (imp-1, imp-2 and imp-3) and calculating the RSD of the area.

Linearity

Linearity test solutions for the related substance method were prepared by diluting stock solutions (described previously) to the required concentrations. The solutions were prepared at five concentration levels from LOQ to 250% of the specification level: 0.3% of the respective analyte concentrations of dorzolamide hydrochloride (2,400 μg/mL) and timalol maleate (600 μg/mL). Correlation coefficient, value for the slope, *Y*-intercept and percent bias of the calibration curve was calculated.

Accuracy

The accuracy study of all impurities was carried out in triplicate at LOQ, 50%, 100%, 150% and 250% of the target concentration level: 0.3% of respective analyte concentrations of dorzolamide hydrochloride (2,400 μg/mL) and timalol maleate (600 μg/mL). The percentages of recoveries for impurities were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolutions between all peaks were recorded. The flow rate of the mobile phase was 0.32 mL/min. To study the effect of flow rate on the resolution, flow was changed by 0.02 units from 0.30 to 0.34 mL/min. The effect of the column temperature on resolution was studied at 25 and 30°C instead of 28°C. The effect of the percent organic strength on resolution was studied by varying acetonitrile by ±10% with constant ratio of methanol and water. The effect of the percent organic strength on resolution was studied by varying methanol by ±10% with a constant ratio of acetonitrile and water. While other mobile phase components was held constant as stated previously.

Solution stability and mobile phase stability

The solution stability of dorzolamide and timalol and their impurities in the related substance method was carried out by leaving spiked sample solutions in tightly capped volumetric flasks at room temperature for 24 h. The contents of dorzolamide imp-1, imp-2, imp-3, imp-4 and imp-5 and timalol imp-1, imp-2 and imp-3 were determined for 24-h intervals up to the study period. The mobile phase stability was also carried out for 24 h by injecting the freshly prepared sample solutions for each 24-h interval. The content of impurities was checked in the test.

Results and Discussion

Method development and optimization

No single analytical HPLC or UPLC method is available in the literature for determination of dorzolamide hydrochloride and timalol maleate and its impurities in ophthalmic solution formulation. Hence, it is necessary to develop a rapid, accurate and validated method for the simultaneous determination of related substances and degradants from the combined dosage form.

The primary objective of the UPLC method is a reduction in run-time to 30 min, without compromising the efficiency, compared with a run-time of approximately 60 min on traditional LC analysis of combined dosage form. The UPLC method will reduce acetonitrile and methanol consumption (at least 80%) without compromising productivity and performance.

The effect of organic modifier and stationary phase was studied for the separation of critical, closely-eluting dorzolamide hydrochloride imp-1 and imp-2 peaks from the dorzolamide hydrochloride peak and dorzolamide imp-4 and imp-5 from the timolol maleate peak. Initial trials were made on a BEH C18 50 × 4.6 mm, 1.7-μm column for method development with gradient elution with solvents A and B (described previously). T/%B was set as 0/4, 8/8, 10/15, 16/45, 21/70, 23/4 and 26/4, respectively but in the previously described conditions, resolution between dorzolamide hydrochloride peak and its imp-1 and imp-2 peaks was less than 2. Attempts were made to achieve resolution between these pairs of impurities with the gradient program (T/%B) set as 0/2, 8/8, 10/15, 16/45, 21/70, 23/2 and 26/2, respectively, but the dorzolamide hydrochloride peak merged with the imp-1 peak. Setting a fast gradient with (T/%B) as 0/8, 10/15, 16/45, 21/70, 23/8 and 26/8 resulted in less resolution between dorzolamide imp-4 imp-5 and the timolol maleate peak and between the dorzolamide hydrochloride peak and the imp-2 peak. Mobile phase buffer pH trials were made at pH 2.1, 2.6 and 2.9 on a BEH C18 50 × 4.6 mm 1.7-μm column. The chromatographic conditions were set as described previously. Different types of columns, a BEH C8 100 × 4.6 mm 1.7 μm column and a BEH Shield C18 100 × 4.6 mm 1.7 μm column, were used for method development, but good resolution was achieved by using the BEH C18 100 × 4.6 mm 1.7 μm column. Using a buffer of 0.04M potassium dihydrogen phosphate prepared by adjusting the pH of 2.6 with orthophosphoric acid (solvent A) and Milli-Q water–methanol–acetonitrile in the ratio 200:300:600 (*v*/*v*/*v*) (solvent B), with column temperature maintained at 28°C and gradient elution

Table I
System Suitability Parameters*

Compound name	RT	RRT†	Tailing factor	Similarity factor
Dorzolamide (A)				
Imp-1	4.040	0.84	1.0	0.99
Imp-2	6.080	1.27	1.0	1.00
Imp-3	3.336	0.70	1.0	0.98
Imp-4	13.802	2.88	1.0	1.01
Imp-5	14.582	3.04	1.0	0.99
Dorzolamide	4.787	1.00	1.0	1.00
Timolol (B)				
Compound name	RT	RRT‡	Tailing factor	Similarity factor
Imp-1	12.472	0.88	1.0	0.99
Imp-2	18.448	1.30	1.0	0.99
Imp-3	12.915	0.91	1.0	1.01
Timolol	14.149	1.00	1.0	1.00
Resolution Parameters (C)				
Peak name	Resolution			
Resolution between dorzolamide and imp-2 peaks at 254 nm	4.4			
Resolution between dorzolamide imp-4 and timolol peaks at 254 nm	3.8			
Resolution between dorzolamide imp-5 and timolol peaks at 254 nm	4.2			
Resolution between timolol imp-1 and timolol imp-3 peaks at 295 nm	4.2			

*Specifications for the system suitability parameters: (1) similarity factor was calculated as the ratio of the peak area of two injections (limit $1.02 < 0.98$); (2) resolution between each pair of peaks should be $\leq$2.5%; (3) tailing factor of impurity peak and standard peak should be $\geq$2%.
†Relative retention times (RRT) were calculated against the retention time (RT) of dorzolamide.
‡RRTs were calculated against the RT of timolol.

(T/%B) set as 0/5, 8/8, 10/15, 16/45, 20/55, 24/80, 25/5 and 30/5, respectively, enabled the separation of all pairs of related compounds and eluted dorzolamide hydrochloride, timolol maleate and their impurities as a symmetrical peak (Figure 2 and Table I). Interference from the excipients was also checked by injecting the common excipient without drug, excipient with dorzloamide hydrochloride and excipient with timolol maleate, and no interference was observed (Figure 2).

Validation of the method

System suitability

System suitability parameters were measured to verify the system, method and column performance. Results of other system suitability parameters such as relative retention time of each impurity, tailing factor and similarity factor (between two preparations) are presented in Tables IA and IB. As the data show, the acceptable system suitability parameters are: relative retention time of each impurity should be comparable, tailing factor for dorzolamide hydrochloride and timalol maleate in standard solution should not be more than 2.0 and similarity factor (between two standard preparations) should not be less than 0.98 and not more than 1.02. Resolution between all peaks should be more than 2.0, as presented in Table IC. A spiked chromatogram of impurity and degradation products with dorzolamide hydrochloride and timalol maleate is presented in Figure 2.

Specificity

All forced degradation samples were analyzed at an initial concentration of dorzolamide hydrochloride and timalol maleate with previously described UPLC conditions, using PDA detector to ensure the homogeneity and purity of the dorzolamide and timalol peaks. Significant degradation of dorzolamide and timalol was observed in the following conditions: heat (60°C for 3 h), photolytic UV light (200 Wh/m^3), sun light (1.2 million lux hours), oxidation (1.0% H_2O_2 at 50°C for 3 h), acid (0.1 N HCl at 50°C for 3 h) and base (0.1 N NaOH at 50°C for 3 h), leading to the formation of impurities percent degradation should be $<1\% > 20\%$). Degradation was performed on the drug product, placebo (without active ingredient), placebo for dorzolamide hydrochloride (containing timolol maleate with excipient) and placebo for timolol maleate (containing dorzolamide hydrochloride with excipient). The percent degradation values are presented in Tables IVA and IVB.

Precision

The RSD for the area of dorzolamide imp-1, imp-2, imp-3, imp-4 and imp-5 and timalol imp-1, imp-2 and imp-3 in related substances are presented in Table II. The method precision and intermediate precision study found to be less than 2% (which should be less than 10.0%), conforming good precision of the method. *LOD and LOQ*

The determination of LOD and LOQ of all the impurities; namely, dorzolamide hydrochloride and its impurities imp-1, imp-2, imp-3, imp-4 and imp-5, and timalol maleate and its impurities imp-1, imp-2 and imp-3, are reported in Table II. The precision at the LOQ concentrations for dorzolamide hydrochloride and its impurities imp-1, imp-2, imp-3, imp-4 and imp-5, and timalol maleate and its impurities imp-1, imp-2 and imp-3 were found to be below 5% (which should be less than 10.0%). LOD and LOQ of all the impurities values presented in Table II.

Linearity

The result shows that an excellent correlation existed between the peak area and concentration of the analyte. A linear calibration plot for the related substance method was obtained over the calibration ranges tested, i.e., LOQ to 250% for dorzolamide and its impurities (imp-1, imp-2, imp-3, imp-4 and imp-5) and timalol and its impurities (imp-1, imp-2 and imp-3). The correlation coefficient obtained was greater than 0.997 (Table II). These results show that an excellent correlation existed between the peak area and the concentration. The percent bias was also calculated for all related compounds and main analytes and found to be less than 4.2% (Table II).

Accuracy

The percentage recovery of dorzolamide hydrochloride and timolol maleate impurities in ophthalmic solution varied from 85 to 115% and RSD of three samples at each level was found to be less than 15 % at LOQ, 50%, 100%, 150% and 250% levels of the target 0.3% level of the target concentrations, respectively. An LC chromatogram of the spiked sample at the 0.3% level of all eight impurities in the ophthalmic sample solution is shown in Figure 2. Percent recovery values for impurities are presented in Table III (Percent recovery should be between 90 and 110%).

Table II
Degradation*

Stress condition	Drug product							
	Degradation (%)	Imp-1	Imp-2	Imp-3	Imp-4	Imp-5	Unknown	Unknown
Dorzolamide HCl (A)								
Refluxed with 0.1N HCl solution for approximately 3 h at 50°C.	1.6%	0.2%	0.0%	0.0%	0.2%	0.0%	0.4%	0.3%
Refluxed with 0.1N NaOH solution for approximately 3 h at 50°C.	1.4%	0.0%	0.0%	0.0%	0.2%	0.0%	0.4%	0.4%
Refluxed with 1.0% hydrogen peroxide for approximately 3 h at room temperature.	1.7%	0.2%	0.0%	0.0%	0.2%	0.2%	0.4%	0.5%
Exposed to sunlight for approximately 1.2 million lux h; UV light both at shorter and longer wavelengths for approximately 200 Wh/m^3.	8.2%	1.5%	0.2%	0.0%	2.2%	0.0%	3.0%	0.2%
Dry heating done at 60°C for approximately 3 h.	4.4%	0.0%	3.5%	0.0%	0.2%	0.0%	0.3%	0.2%

Timolol Maleate (B)

Stress condition	Drug product					
	Degradation (%)	Imp-1	Imp-2	Imp-3	Unknown	Unknown
Refluxed with 0.1N HCl solution for approximately 3 h at 50°C.	2.0%	0.5%	1.2%	0.2%	0.5%	0.1%
Refluxed with 0.1N NaOH solution for approximately 3 h at 50°C.	2.6%	1.1%	1.0%	0.0%	0.5%	0.0%
Refluxed with 1.0% hydrogen peroxide for approximately 3 h at room temperature.	3.1%	0.8%	0.8%	1.5%	0.0%	0.0%
Exposed to sunlight for approximately 1.2 million lux h; UV light both at shorter and longer wavelengths for approximately 200 Wh/m^3.	1.8%	0.5%	0.5%	0.0%	0.8%	0.0%
Dry heating done at 60°C for approximately 3 h.	2.1%	0.4%	1.2%	0.5%	0.0%	0.0%

*Impurities designated "unknown" showed a maximum of two unknown impurities.

Table III
Regression and Precision Data

Parameter	Imp-1	Imp-2	Imp-3	Imp-4	Imp-5	Dorzolamide
Dorzolamide (A)						
LOD (μg/mL)	0.1	0.09	0.17	0.05	0.05	0.07
LOQ (μg/mL)	0.26	0.17	0.46	0.17	0.14	0.24
Regression equation (*y*)	$Y = MX + C$					
Slope (m)	6298.91	9197.17	2951.98	8444.053	9740.29	9095.60
Intercept (c)	−1166.01	−1782.65	−138.29	−768.67	−475.85	1433.97
Correlation coefficient	0.9997	0.9999	0.9999	0.9991	0.9999	0.9999
Percent bias	−2.71	−2.84	−0.66	−1.38	−0.70	2.12
Precision (RSD)	0.5%	0.8%	1.1%	0.3%	0.2%	NA
Intermediate precision (RSD)	1.5%	1.2%	1.5%	0.2%	0.5%	NA
Precision at LOQ (RSD)	4.8%	4.9%	3.9%	1.0%	1.0%	4.0%

Timolol (B)				
Parameter	Imp-1	Imp-2	Imp-3	Timolol
LOD (μg/mL)	0.08	0.05	0.07	0.06
LOQ (μg/mL)	0.22	0.18	0.20	0.18
Regression equation (*y*)	$Y = MX + C$			
Slope (m)	6632.53	6676.04	11712.63	5000.57
Intercept (c)	355.94	−582.27	531.5157	387.48
Correlation coefficient	0.9997	0.9990	0.9998	0.9996
Percent bias	2.89	−4.20	2.42	4.20
Precision (RSD)	0.9%	0.5%	1.3%	NA
Intermediate precision (RSD)	1.0%	1.0%	2.0%	NA
Precision at LOQ (RSD)	2.4%	1.7%	5.0%	3.5%

Robustness

In all of the deliberate varied chromatographic conditions (flow rate, column temperature and composition of organic solvent), the resolution between critical pairs was greater than 2.5, illustrating the robustness of the method.

Stability in solution and in the mobile phase

During solution stability and mobile phase stability experiments performed using the related substances method, no significant changes were observed in the content of impurities; namely, imp-1, imp-2, imp-3, imp-4 and imp-5 of dorzolamide, and imp-1, imp-2 and imp-3 of timolol. The solution stability and mobile phase stability experiment data confirm that the sample solutions and mobile phases used during the related substances determination were stable for 24 h.

Conclusions

The gradient UPLC method developed for dorzolamide hydrochloride and timolol maleate related substances in liquid pharmaceutical dosage forms is found to be precise, accurate, linear, robust, rugged and specific. Satisfactory results were obtained from

Table IV
Evaluation of Accuracy*

Amount spiked		Imp-1	Imp-2	Imp-3	Imp-4	Imp-5
Dorzolamide (A)						
LOQ	Recovery (%)	100.3	95.2	93.9	101.7	106.1
	RSD (%)	4.5	6.9	6.3	10.2	0.0
50%	Recovery (%)	96.5	98.1	93.9	90.7	94.0
	RSD (%)	0.4	1.5	1.2	0.4	0.4
100%	Recovery (%)	93.0	102.8	97.0	91.1	93.8
	RSD (%)	0.0	0.4	0.4	0.4	0.2
150%	Recovery (%)	94.7	100.4	91.5	94.4	92.7
	RSD (%)	0.2	0.5	0.3	0.5	0.5
250%	Recovery (%)	99.4	97.1	93.9	90.0	91.5
	RSD (%)	0.3	0.4	0.6	0.1	0.2
Timolol (B)						
Amount spiked						
		IMP-1	IMP-2	IMP-3		
LOQ	Recovery (%)	101.2	98.4	100.3		
	RSD (%)	10.0	5.3	10.0		
50%	Recovery (%)	100.9	102.5	99.9		
	RSD (%)	1.2	1.9	3.4		
100%	Recovery (%)	106.2	106.7	103.3		
	RSD (%)	1.2	1.0	0.0		
150%	Recovery (%)	101.2	101.7	101.4		
	RSD (%)	1.3	0.6	1.1		
250%	Recovery (%)	101.6	92.7	101.4		
	RSD (%)	0.6	0.5	0.2		

*Percent RSD values calculated with three sample recoveries at each level.

validation of the method. Hence, the method is stability-indicating and can be used for routine analysis of production samples and to check the stability of samples of ophthalmic solution.

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