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Expert Opinion Article

Stability Indicating HPTLC Method For Estimation Of Timolol Maleate In Bulk & Its PharmaceuticalFormulation

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ABSTRACT
Timolol, the first β-blocker for glaucoma, remains the standard treatment. A simple, accurate, and sensitive HPTLC method was developed to measure Timolol Maleate in bulk and eye drops. Using a mobile phase of chloroform, methanol, and ammonia, the drug was analyzed at 296 nm within a 100–1000 ng/spot range. The method demonstrated high accuracy (R^2 = 0.9949) and an Rf value of 0.53, with low intra-day (0.52–0.99%) and inter-day (0.47–1.85%) variation. Forced degradation studies showed 10–20% degradation under various conditions, but the method remained stable and specific. It is reproducible, selective, and effective for analyzing Timolol Maleate in bulk and ophthalmic formulations, even with additives and impurities.

INTRODUCTION

HPTLC is a key analytical tool for drug analysis, offering low costs and high efficiency, making it a standard technique.[1-2]Pharmaceutical analysis is the process of analyzing both the drug substance (active pharmaceutical ingredient or API) and the drug product (the formulation made by combining the drug substance with excipients).[3]Timolol is a nonselective beta-adrenergic antagonist given in an eye drop solution to reduce intraocular pressure or pressure in the eyes. It is also used in tablet form as a drug to treat hypertension.[4] Timolol blocks beta (1) and beta (2) adrenergic receptors, reducing catecholamine effects. This lowers heart rate, cardiac output, and blood pressure at rest and during exercise.[5]Timolol Maleate chemically known as (S)-1-(tert-Butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol. [6] (Figure No 1) Timolol is metabolized in the liver by the cytochrome P450 2D6 enzyme, with minor contributions from CYP2C19. Timolol and its metabolites are mainly found excreted in the urine.[7]

MATERIAL & METHODS

Materials and Reagents

The drug used for the present investigation was obtained from Astitva Chemicals Valsad Gujarat as a gift sample, and the marketed preparation Timolet 5mg Sun Pharma Ind. Ltd India was purchased from a local drug store at Dhule Maharashtra. All the reagents used for development are analytical grade supplied from Labline India Ltd, Mumbai, hplc grade Methanol Merck Specialties Ltd, Mumbai, Laboratory grade, chloroform, Ammonia from Loba Chemicals India Ltd, Mumbai.

Instrumentation

A Camag HPTLC system containing Camag Linomat IV semiautomatic sample applicator, Hamilton syringe (100 µl), Camag TLC Scanner-3 with win CAT software version 1.3.4, Camag twin- trough chamber (20×10 cm)syringe on pre-coated silica gel alluminium plate 60 F254 [20×10] with 250µm thickness, provide by 79 Anchrom technologists, (Mumbai) through a Camag Linomat V (Switzerland). A UV-visible double-beam spectrometer (Shimadzu-1800) was employed to select the detection wavelength. The calibrated analytical balance (Citizen CY104) was used for the weighing of all the active pharmaceutical ingredients and chemicals, which has a weighing sensi tivity of 0.01 mg.

Preparation of standard stock solution: Timolol Maleate (10 mg) was dissolved in 10 ml methanol to prepare a 1000 µg/ml solution. A 0.1 ml aliquot was further diluted to 10 µg/ml with methanol.

Preparation of sample stock solution:

To prepare the sample stock solution, 1 ml of eye drops (containing 5 mg Timolol Maleate) was dissolved in 5 ml methanol to obtain a 1000 µg/ml solution. After 15 minutes of sonication, the solution

was filtered through Whatman filter paper no. 41 and adjusted to 10 ml with methanol. A 0.1 ml aliquot was further diluted with methanol to achieve a 10 µg/ml concentration of Timolol Maleate.

Preparation of mobile phase:

A suitable solvent system was sought for dense, compact spots with a significant Rf value for Timolol Maleate quantification. Various combinations were tested, and the chloroform: methanol: ammonia (9:1:0.1 v/v) mixture provided the best peak shape. The drug was well-resolved with an Rf value of 0.53 ± 0.027.

Validation of method

The HPTLC method was validated for specificity, linearity, accuracy, precision, LOD, LOQ, and robustness as per ICH Q2(R1) guidelines.[8]

Precision:

Intra-day and inter-day precision was assessed by analyzing Timolol Maleate at three concentrations (40-120 ng/spot) three times in a day. Each was applied in triplicate, and % RSD was calculated.

Accuracy: Recovery studies were conducted using the standard addition method at 80, 100, and 120% of the label claim. Six determinations were performed at each level, and results were compared with expected values.[9]

Repeatability: Repeatability was evaluated by spotting 4 µl of standard solution six times on a TLC plate, developing it, recording peak areas, and calculating % RSD.

Robustness of the method: Robustness was evaluated at 50, 150, and 200 ng/spot by varying mobile phase composition (±0.1%), volume (±5%), spotting-to-chromatography time (±20 min), and scanning time (±20 min). % RSD of peak area was calculated.

Detection and quantification limit: The Decreasing drug amounts were applied in triplicate, developed, and scanned. The detection limit is the lowest analyte amount detected but not quantified, while the quantitation limit is the lowest amount measured with precision and accuracy.

Forced degradation studies

A stability-indicating method quantitatively analyzes an active ingredient by distinguishing it from its degradation products. It ensures accurate measurement by separating the active pharmaceutical ingredient from decomposition products formed under storage conditions, enabling the detection and quantification of degradation.[10-11]

RESULT & DISCUSSION

Selection of detection wavelength

The ultraviolet absorption spectrum of Timolol Maleate was obtained using Shimadzu 1800- UV visible spectrophotometer and 1cm quartz cells, over a wavelength range of 400 to 200 nm. The wavelength maxima (296nm λmax) were analyzed and showed in Fig. no.2. The mobile phase equilibrated with the stationary phase until a steady baseline was achieved. Timolol Maleate solutions (100–1000 ng/spot) were injected, and peak areas were recorded. A graph of drug concentration vs. peak area was plotted, as depicted in table no.1 and Figure no.3.

HPTLC method development

A Camag HPTLC system with a Linomat IV applicator, Hamilton syringe (100 µl), TLC Scanner-3 (winCAT software 1.3.4), twin-trough chamber (20×10 cm), and Remi centrifuge (C30) was used. Pre-washed silica gel 60 F254 plates (20×20 cm, 250 µm) served as the stationary phase, with a chloroform: methanol: ammonia mobile phase. The chamber was saturated for 20 min, and scanning was performed at 296 nm with a slit size of 5.00×0.45 mm. Spotting parameters included a 6 mm band width and 6 mm spacing. A 4 µl Timolol Maleate (10 µg/ml) solution was applied under nitrogen using a semiautomatic spotter. The plate was developed at constant temperature in a pre-saturated twin-trough chamber, then air-dried. Densitometric measurements were taken at 296 nm in reflectance mode using TLC Scanner-3 with winCAT software, incorporating track optimization and a deuterium lamp as the radiation source shown in Figure No.5.

Validation

Precision

The repeatability study showed a %RSD of 1.68, while intra-day and inter-day precision ranged from 0.73–0.99 and 0.83–0.91, respectively, meeting acceptability criteria**.** The % RSD values of less than 2% demonstrated the robustness of the proposed HPTLC method. (Table No 5). Repeatability was assessed by applying 4 µL of standard drug solution six times on a TLC plate, followed by development and %RSD calculation. (Table No 6)

Specificity

To confirm the specificity of the proposed method, Timolol Maleate was spotted on TLC plate, It was observed that excipients present in formulation did not interfere with peak of Timolol Maleate (Rf, 0.53±0.01).(Figure No.7)

Robustness

Various parameters were changed during method development to study the method's robustness. The % RSD of peak area for each parameter was calculated. The results are depicted in Table No 8.

Linearity

As per USP, formulations at 80–120% of the label claim were prepared. Five concentrations of Timolol Maleate from a 1000 µg/mL stock solution were

spotted and analyzed. The linearity equation was y = 12921x - 8.2161 (r^2 = 0.9992). The results are in Table 3 and 4. (Figure No 08)

Limit of detection and limit of quantitation :

The limit of detection is the minimum analyte amount detectable but not quantifiable. The limit of quantitation is the lowest analyte amount measurable with precision and accuracy. (Table No 10)

Forced degradation studies:

Forced degradation studies assessed stability and specificity, including hydrolysis (acidic, alkaline, neutral), oxidative, thermal, and photolytic degradation of Timolol Maleate. Forced degradation studies of Timolol Maleate were carried out under various stress conditions, and resultant chromatograms are depicted in Figure (6-13). Percentage degradation was calculated and recorded. Timolol Maleate undergoes decomposition under acidic, alkaline, oxidative, and thermal conditions with more than one degradation product. Timolol Maleate was moderately degradable in acidic and oxidative conditions. (Table No 11)

Recovery studies

The % mean recovery was found to be 100.64±0.11 for Timolol Maleate which indicates that the proposed method is accurate for the estimation of drugs in formulation. The % recovery of Timolol Maleate at all the levels was found to be satisfactory. The amounts of drug added and determined and the % recovery are listed in Table 9.

Conclusion

A simple, precise, accurate, robust, and specific stability-indicating HPTLC method was developed and validated per ICH Q2(R1) guidelines for determining Timolol Maleate in bulk and pharmaceutical formulations. Stability studies confirmed Timolol Maleate's susceptibility to degradation under various conditions. The developed HPTLC method effectively separates its peak from degradants and excipients in formulations. The developed stability-indicating HPTLC method is suitable for routine quality control analysis of Timolol Maleate in QC laboratories.

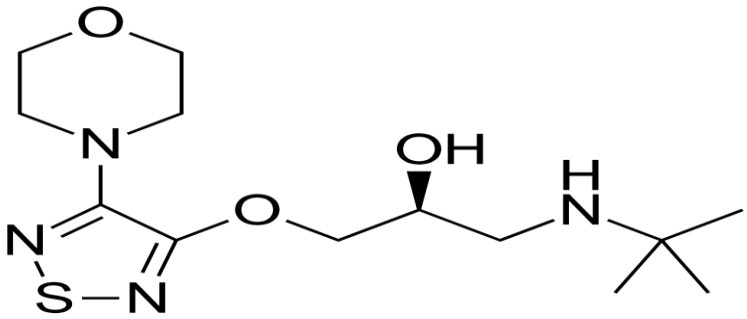

Figure 1 Chemical Structure of Timolol Maleate

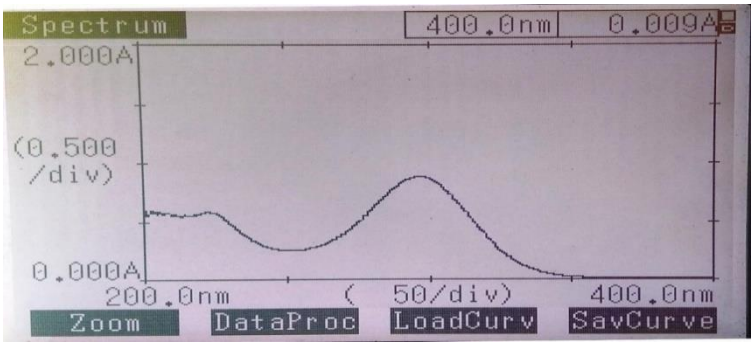

Figure 2 UV Spectra of Timolol Maleate

Sr. No.	Concentration (ng/spot)	Area
1	100	606.5
2	200	1006.6
3	400	1697.3
4	600	2416.1
5	800	3152.9
6	1000	3757.4

Table no.01: Calibration curve data of Timolol Maleate

Sr. No.	Parameter	Timolol Maleate
1	Detection Wavelength (nm)	296
2	Linearity range (ng/band)	100-1000
3	Correlation Coefficient (r)	0.9949
4	Linear Regression Equation (y = mx + c)	y = 3.672x + 179.09
5	Intercept (c)	179.09
6	Slope (m)	3.672

Table no 2: Linear regression data for calibration curve of Timolol Maleate

Sr. No.	Weight of std. (mg)	Weight of sample(mg)	Peak area of std	Peak area of sample	% Drugestimation
	TIMO	TIMO	TIMO	TIMO	TIMO
1.		5		1661.2	100.57
2.		4.9		1637.1	99.15
3.	5	5	1651.5	1649.1	99.88
				Mean	99.86
				±S.D.	0.71
				C.V.	0.007

Table No 3: Results and statistical data for estimation of Timolol Maleate in lab

Sr. No	Drug	Label Claim(mg)	% of LabelClaim	SD*	% RSD*
1	Timolol Maleate	5	101.15	4.547	0.044

Table No 4: Assay of Timolol Maleate

***Results are mean of three replicates**

Sr No	Precision	% of Label Claim	SD	% RSD
1	Intraday (n=3×3)	101.935	5.360	0.052
2	Interday (n= 3×3)	102.789	4.871	0.047

Table No 5: Intra-day& Inter Day precision of Timolol Maleate

Sr.No	Drug	Amount taken (ng perband)	Amountadded (ng/band)	Total amount (ng/band)	% Recovery*
1	TIMO	200	100	300	100.193
		200	200	400	101.406
		200	300	500	100.082
				Mean	100.56
				SD	0.734
				RSD	0.007

Table No 6: Recovery of Timolol Maleate

Sr.No.	Parameters	Variation	% RSD*
1.	Chamber saturation period	± 10 %	1.09
2.	Time from application to development	0, 10, 20, 30 min	0.069
3.	Time from development to scanning	0, 30, 60, 90 min	0.055

Table No 7: Robustness of Timolol Maleate

Sr. No.	% Label Claim	Area
1	80	996.5
2	90	1150.6
3	100	1297.3
4	110	1416.1
5	120	1550.9

Table no.8: Linearity and range Study for Timolol Maleate

Sr.No	Drug	Amount taken (ng perband)	Amountadded (ng/band)	Total amount (ng/band)	% Recovery*
1	TIMO	200	100	300	100.193
		200	200	400	101.406
		200	300	500	100.082
				Mean	100.56
				SD	0.734
				RSD	0.007

Table No 9: Recovery of Timolol Maleate

Sr. No.	Drug Name	LOD ng/band	LOQ ng/band
1	TIMO	23.54	71.33

Table No 10: LOD and LOQ of Timolol Maleate

Sr. No.	Stress Degradation Condition	Percent recovered ForTIMO (%)
1.	Base (1 N NaOH, kept for 12 Hr.)	76.28
2.	Acid (1 N HCl, kept for 12 Hr.)	87.42
3.	Neutral (kept for 12 Hr.)	93.19
4.	H2O2 30% (kept for 2 Hr)	27.91
5.	Photo stability[UV, 200 watt hrs/square meter Florescence , 1.2 million Lux.]	95.98
6.	Heat dry (60 ^{0}C, 12 hrs.)	99.54
7	Wet Heat, (60 ^{0}C, 12 hrs.)	98.89

Table No 11: Summary of Degradation Study of Timolol Maleate

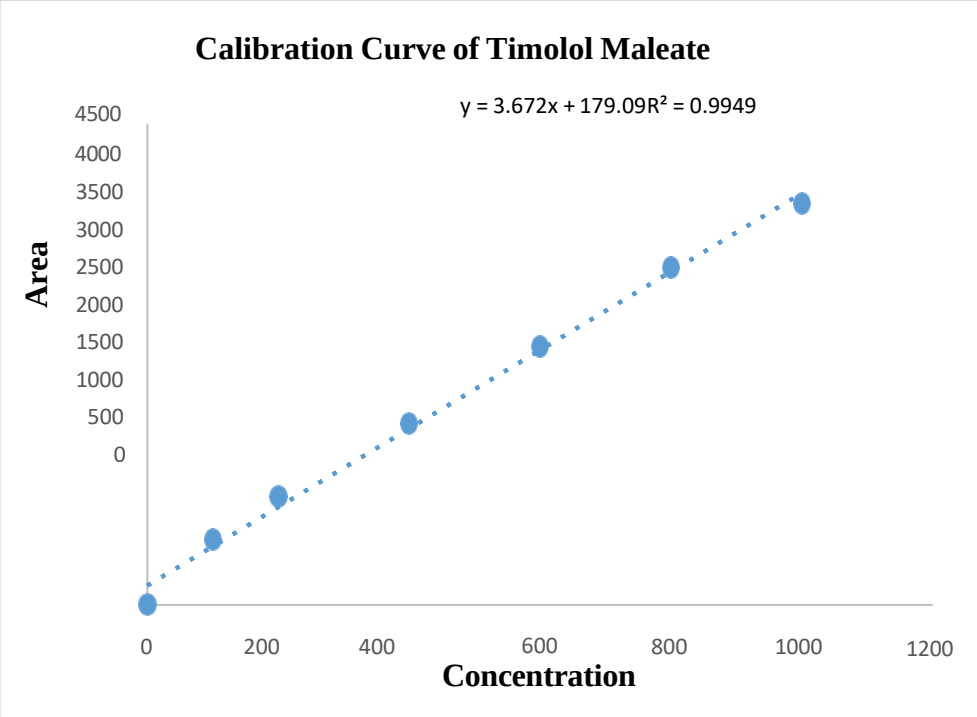

Figure No 03: Calibration curve of Timolol Maleate

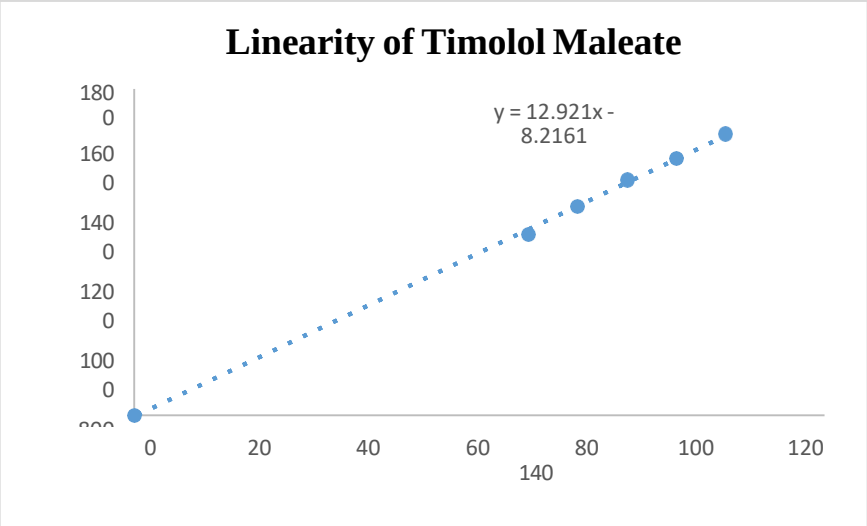

Figure No 04: Linearity of Timolol Maleate

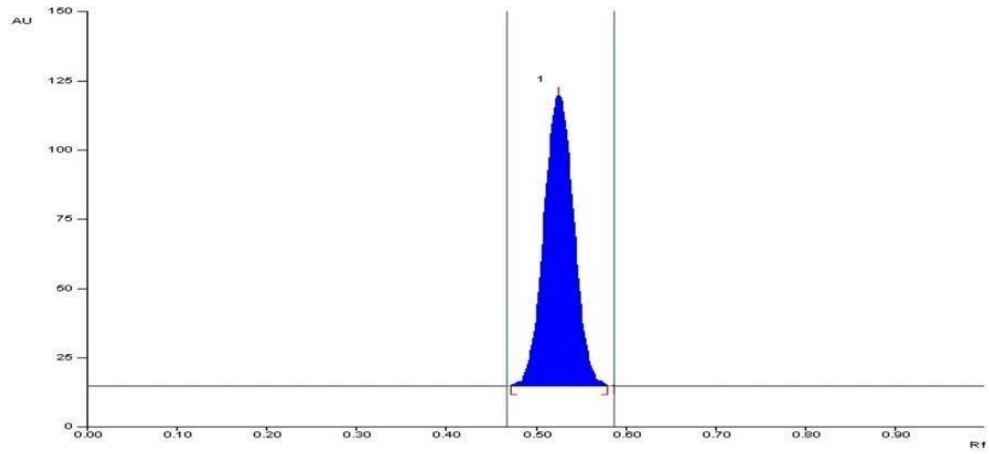

Figure No 05: Densitogram of standard Timolol Maleate; (Rf, 0.53 ± 0.027)

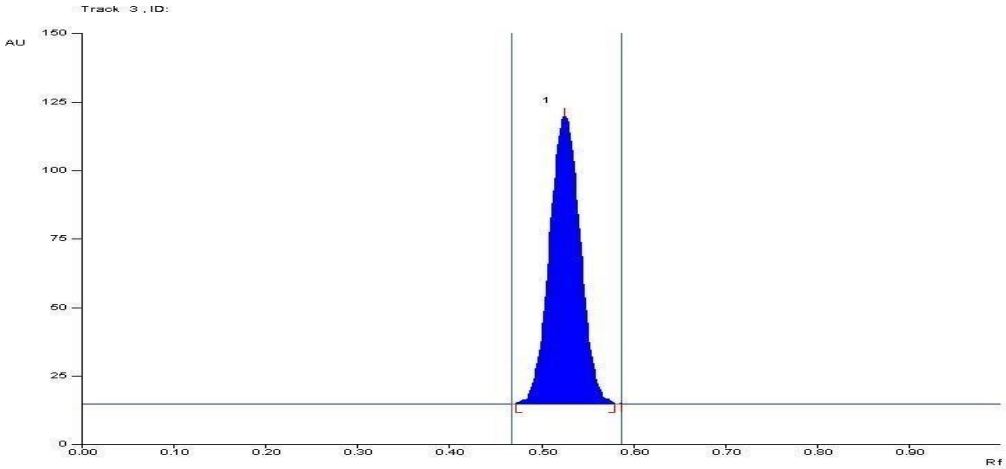

Figure No.6: Densitogram of standard Timolol Maleate; (Rf, 0.53 ± 0.027)

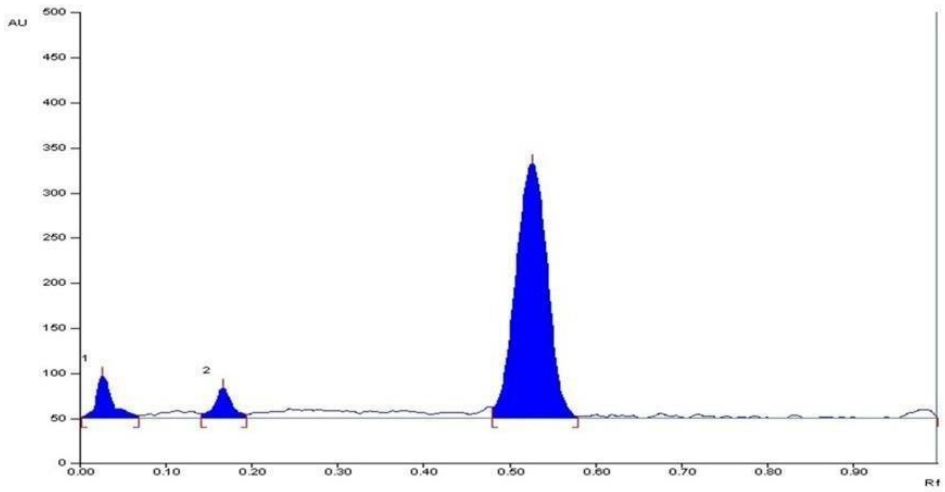

Figure No 07: Densitogram showing the separation of different degradationproducts of TIMO obtained under acidic condition

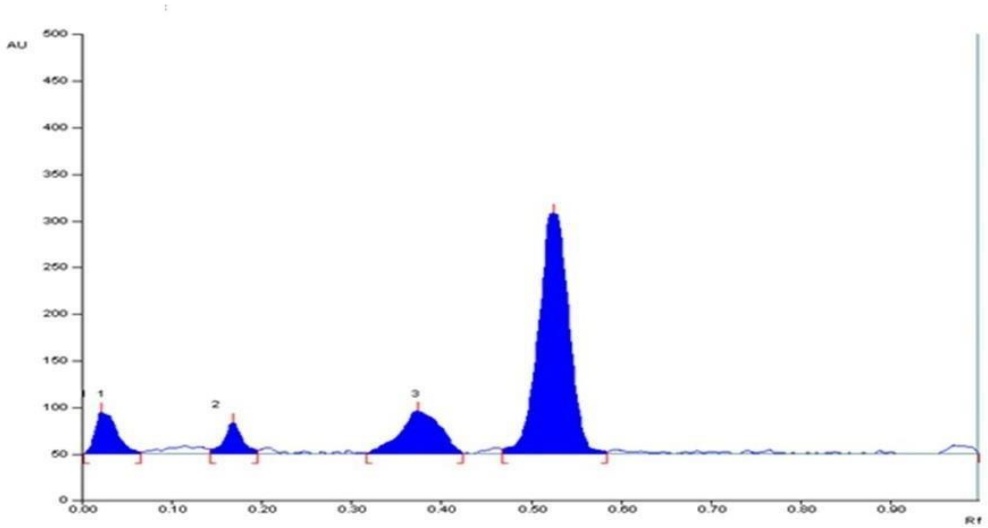

Figure No 8: Densitogram showing the separation of different degradationproducts of TIMO obtained under alkaline condition

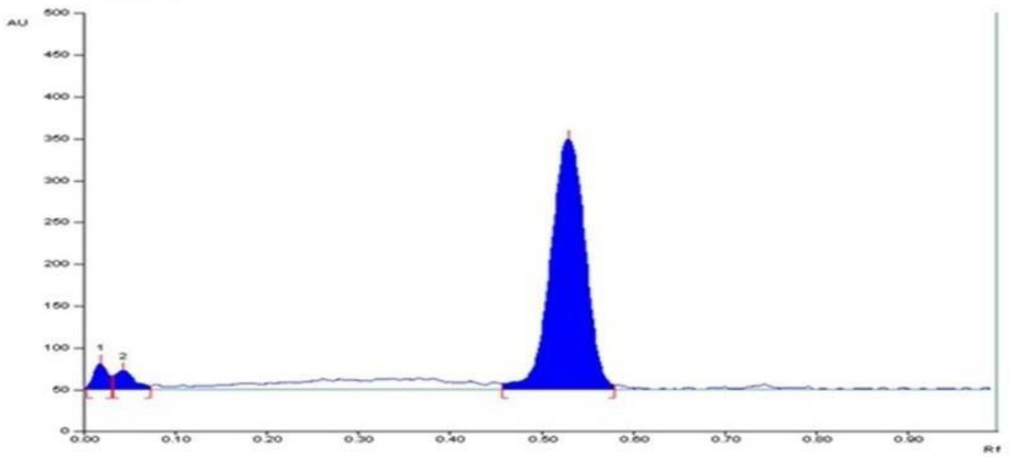

Figure No9: Densitogram showing the separation of different degradation products of TIMO obtained under neutral condition.

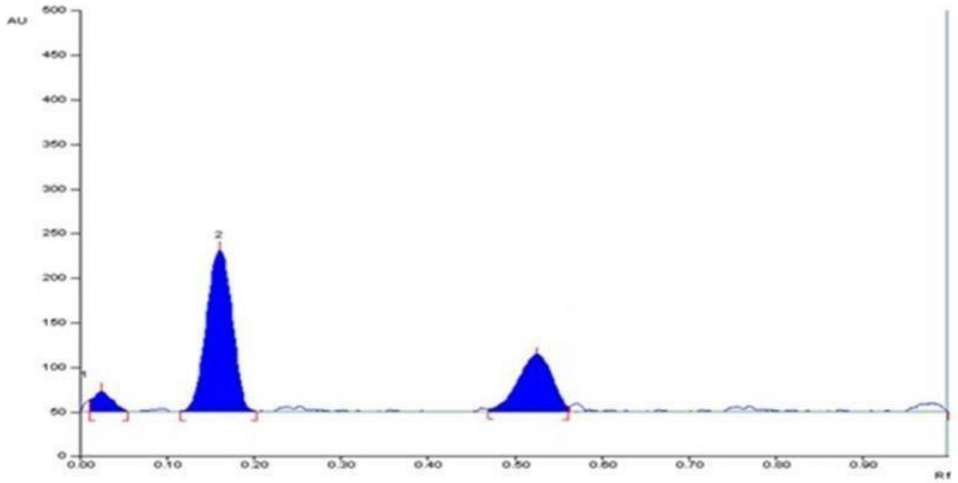

Figure No 10: Densitogram showing the separation of different degradation products of TIMO obtained under oxidative condition

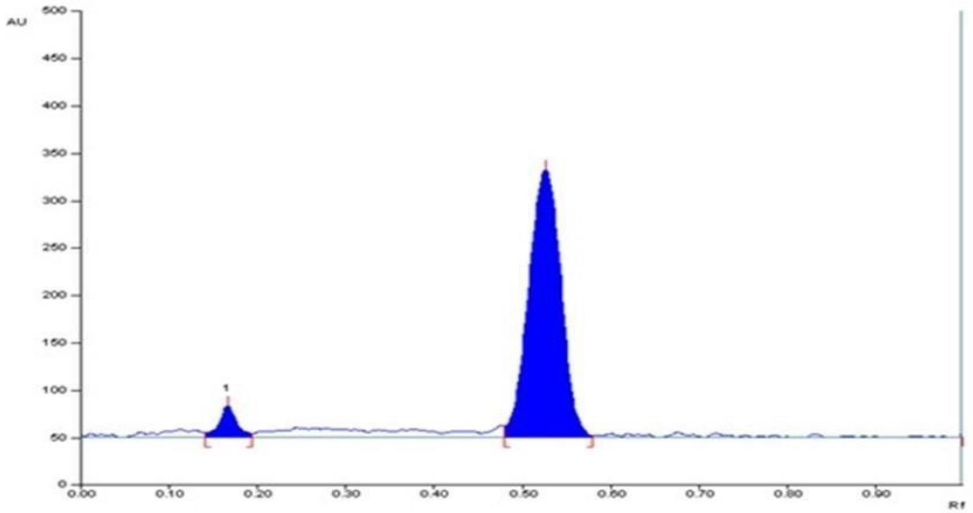

Figure No 11: Densitogram showing the separation of different degradation productsof TIMO obtained under Photolytic condition

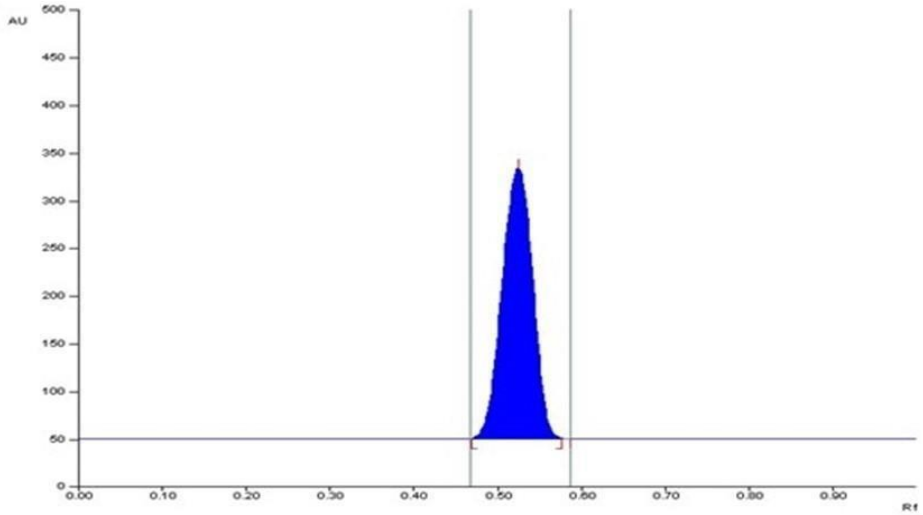

Figure No 12: Chromatogram showing the separation of different degradationproducts of TIMO obtained under Dry heat condition

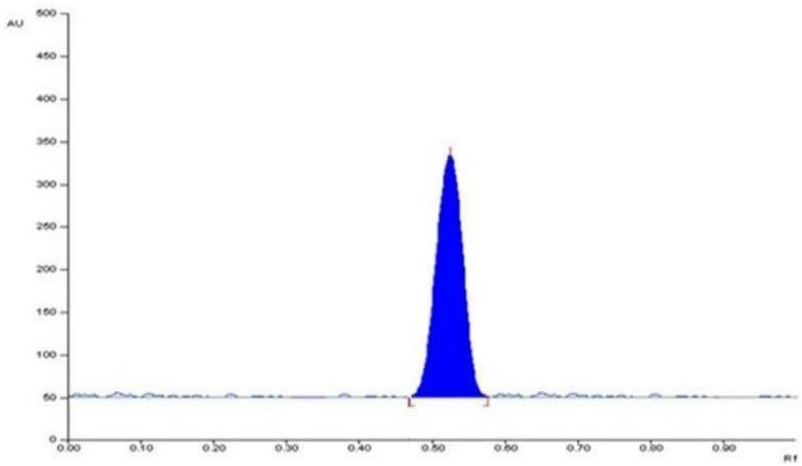

Figure No 13: Chromatogram showing the separation of different degradationproducts of TIMO obtained under Wet heat condition

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