



## PHYSICO-CHEMICAL CHARACTERIZATION, UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION STUDIES OF TIMOLOL MALEATE

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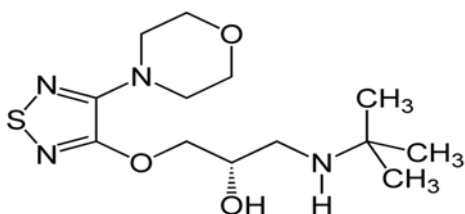
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### ABSTRACT

Timolol maleate (TM) is a nonselective  $\beta$ -adrenergic blocker used in treatment of mild to moderate hypertension, ocular hypertension, chronic open-angle glaucoma, secondary glaucoma, in reduction of mortality after myocardial infarction (MI), and migraine prophylaxis. Physico chemical characterization studies showed that TM has showed a melting point of 197°C. The solubility of drug Timolol followed the order pH 4 > distilled water > pH 7.4 > pH 8 > methanol. The analytical method developed for the estimation of timolol maleate in bulk fluids showed maximum absorbance  $\lambda_{max}$  of 295 nm in distilled water between 200 nm and 400 nm. Linearity studies indicated that estimation of timolol maleate between 2.00  $\mu\text{g/ml}$  to 10.00  $\mu\text{g/ml}$  was found to be linear with regression equation of  $y = 0.0258 \times X - 0.00076$ ; ( $r^2 = 0.9999$ ). The method developed was validated for inter and intraday variation, limit of quantitation studies. The SD values of Inter day and Intraday variation studies indicated that the variation is minimum. Limit of Quantitation of timolol was found to be of 1.00  $\mu\text{g/ml}$ . The above analytical parameters indicated that the developed UV Spectrophotometric method of timolol was simple, accurate and reproducible.

**Keywords:** Timolol maleate (TM), physicochemical characterization, UV Spectrophotometric Method, validation.

### INTRODUCTION



Timolol maleate is nonselective  $\beta$ -adrenergic blocker<sup>1</sup>, it competitively blocks stimulation of  $\beta$ -adrenergic receptors in the heart and decreases renin activity, all of which may play a role in reducing systolic and diastolic blood pressure. Timolol maleate is indicated for treatment of mild to moderate hypertension, reduction of mortality after myocardial infarction (MI), and migraine prophylaxis<sup>2</sup>. Timolol is (S)-1-(tert-butylamino)-3- [(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol. Its molecular formula is  $\text{C}_{13}\text{H}_{24}\text{N}_4\text{O}_3\text{S}$  with molecular weight of 316.42. Timolol maleate has a half life of 2.5-5 h and has a bioavailability of 60% when administered orally<sup>3</sup>.

Review of literature given in sight that very few spectrophotometric and high performance liquid chromatographic method for the analysis of Timolol maleate<sup>4-6</sup>.

Hence the present investigation was undertaken for drug candidate physico chemical characterization, to develop a simple and robust UV Spectrophotometric Method and validation for the quantitation of Timolol maleate in bulk fluids and tablets.

### MATERIALS AND METHODS

Theil's melting point apparatus was used for drug melting point determination. Dhona 200 D electronic balance (Mumbai) was used for weighing of all samples. Shimadzu UV 1700 double beam spectrophotometer (Japan) was used for all the spectrophotometric measurements. The absorption spectra of the reference and test solutions were carried out in a 1 cm quartz cells over the range of 200 - 400 nm. All laboratory glassware like volumetric flasks and pipettes were calibrated and used for experimentation.

Timolol maleate was kindly supplied by Sun pharmaceutical limited, Vapi, Gujarat, India) the drug was used without further purification. All the solvents and chemicals like n-octanol (INR Chemicals, Mumbai). Methanol (Pampasara distillaries) Potassium dihydrogen ortho phosphate and Sodium hydroxide pellets from S.d. Fine chemicals limited, Mumbai used in Spectrophotometric analysis were of LR grade.

#### Methods used for Physico-chemical characterization of the drug:

**Melting point determination:** Melting point of the drugs was determined by taking a small amount of drug in a capillary tube closed at one end and was placed in theil's melting point apparatus and the temperature at which the drug melts was noted. Average of triplicate readings was noted.

**Solubility studies:** The solubility of timolol maleate was determined in distilled water, different buffers, viz., pH 4.0, pH 7.4, pH 8.0 and in methanol according to the

method proposed by Diez et. al. Triplicate readings were taken and average was calculated<sup>7</sup>.

**Partition coefficient:** The partition coefficient of the drugs was determined by taking equal volumes of n-octanol and aqueous phases in a separating funnel. A drug solution was prepared and 1ml of the solution was added to octanol: water (50:50) was taken in a separating funnel and shaken for 10 minutes and allowed to stand for 1 h and is continued for 24 h. Then aqueous phase and octanol phase was separated, centrifuged for 10 min at 2000 rpm. The aqueous phase and octanol phase were assayed before and after partitioning using UV Spectrophotometer at their respective  $\lambda$  max to get partition coefficient. Triplicate readings (n=3) were taken and average was calculated<sup>8</sup>.

#### Analytical method developed for the estimation of timolol maleate in bulk fluids

**Preparation of stock solution of timolol maleate:** Stock solution of Timolol maleate was prepared by dissolving 100 mg of accurately weighed amount of Timolol maleate in 10 ml of distilled water and then the volume was adjusted to 100 ml with the same solution to get 1 mg / ml solution.

**Procedure:** The above stock solution of drug was subsequently diluted with distilled water to get 2  $\mu$ g, 4  $\mu$ g, 6  $\mu$ g, 8  $\mu$ g and 10  $\mu$ g, of drug per ml. Later, 5 ml of 10  $\mu$ g / ml solution was pipetted out into quartz cuvettes of UV spectrophotometer (against blank of methanol), and scanned for maximum absorbance between 200 nm and 400 nm in a UV-VIS 2000 Spectrophotometer, (double beam) Hitachi. Japan. Average of triplicate readings was taken. The peaks (n=3) with their respective absorbance's were noted and from the maximum absorbance, a  $\lambda$ max of 295 nm was obtained. This value corroborates to literature value 294 nm.<sup>9</sup>

Further calibration curve of timolol maleate was plotted by measuring absorbances of 2  $\mu$ g /ml, 4  $\mu$ g/ml, 6  $\mu$ g/ml, 8  $\mu$ g/ml and 10  $\mu$ g /ml solutions at a  $\lambda$ max of 295 nm. Average of triplicate readings was taken and tabulated. Regression equation was derived from the slope of the curve ( $y = 0.025 \times X - 0.00076$ ;  $r^2 = 0.999$ ). Also the analytical method so developed was validated for linearity. Further the analytical method will be validated for other parameters like accuracy, precision and etc in prospective work.

**Limit Of Quantitation studies (LOQ) of Timolol maleate:** LOQ studies were conducted by taking concentrations of 0.25  $\mu$ g /ml, 0.50  $\mu$ g/ml, 0.75  $\mu$ g/ml, 1.00  $\mu$ g/ml and 1.50  $\mu$ g /ml for lower limit and 15.00  $\mu$ g /ml, 18.00  $\mu$ g/ml, 20.00  $\mu$ g/ml, 25.00  $\mu$ g/ml and 30.00  $\mu$ g /ml for upper limit to find out the deviation from beers lamberts law.

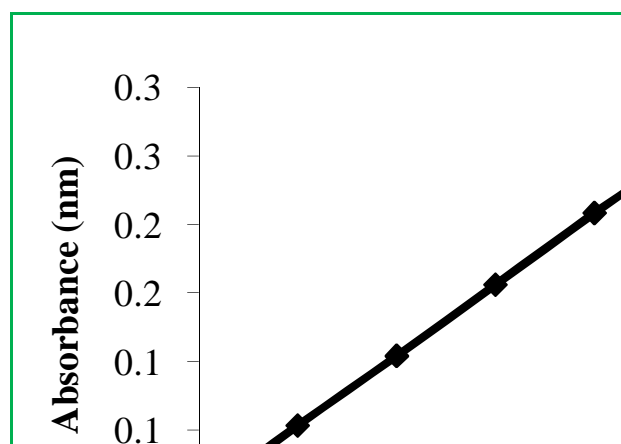
## RESULTS AND DISCUSSION

The melting point of timolol maleate was 197<sup>o</sup> C which corroborates with the literature value 199<sup>o</sup> C<sup>10</sup>. The  $\lambda$ max of timolol maleate was 295nm which corroborates to the reported literature value 294 nm. Results of studies on melting point and UV absorption maxima of drug suggested the values to be corroborating with previously reported literature values.

**Table 1:** Spectrophotometric data for the estimation of timolol maleate at 295 nm

Sl. No	Concentration ( $\mu$ g/ml)	Absorbance
1	0.00	0
2	2.00	0.053
3	4.00	0.104
4	6.00	0.156
5	8.00	0.208
6	10.00	0.258

**Figure 1:** Calibration curve of timolol maleate.



$$y = 0.025 \times X - 0.00076; (r^2 = 0.999)$$

The slope (m) of calibration curve of timolol maleate was 0.025.

**Table 2:** Linearity studies of timolol maleate

Concentration ( $\mu$ g/ml)	Absorbance (nm)	Regression Data		
2	0.053	$m^* = 0.0258$	$c^* = -0.00076$	$r^* = 0.9999$
4	0.104			
6	0.156			
8	0.208			
10	0.258			

\*m = slope; c = intercept; r = regression.

Linearity studies indicated that estimation of timolol maleate between 2.00  $\mu$ g / ml to 10.00  $\mu$ g /ml was found to be linear with slope (m) 0.025, intercept (c) is -0.00076 ( $r^2 = 0.999$ ).

### Validation of analytical method of timolol maleate developed in the laboratory

The analytical method so developed in the laboratory was validated for accuracy, precision and linearity. Inter day and intraday variation was also studied.

**Table 3:** Inter day and intraday variation studies of timolol maleate Concentration

Morning	Day 1 Avg	Day 2 Avg	Day 3 Avg	Avg±SD
Concentration (µg /ml)	Absorbance			
0	0	0	0	0.000
2	0.049	0.049	0.054	0.051±0.003
4	0.099	0.104	0.104	0.102±0.003
6	0.149	0.15	0.147	0.149±0.002
8	0.201	0.189	0.196	0.195±0.006
10	0.252	0.249	0.251	0.251±0.002
Afternoon	Day 1 Avg	Day 2 Avg	Day 3 Avg	Avg±SD
0	0	0	0	0.000
2	0.045	0.053	0.043	0.047±0.005
4	0.093	0.11	0.105	0.103±0.009
6	0.145	0.149	0.147	0.147±0.002
8	0.195	0.202	0.199	0.199±0.004
10	0.246	0.248	0.248	0.247±0.001
Evening	Day 1 Avg	Day 2 Avg	Day 3 Avg	Avg±SD
0	0	0	0	0.000
2	0.045	0.046	0.048	0.046±0.002
4	0.097	0.103	0.095	0.098±0.004
6	0.144	0.145	0.148	0.146±0.002
8	0.193	0.199	0.196	0.196±0.003
10	0.244	0.245	0.246	0.245±0.001

**Table 4:** Average readings of Inter day and intraday variation studies of timolol maleate

Concentration (µg /ml)	Absorbance			Avg±SD
	3 Days M* Avg	3 Days A* Avg	3 Days E* Avg	
0	0	0	0	0.000
2	0.051	0.047	0.046	0.048±0.003
4	0.102	0.103	0.098	0.101±0.003
6	0.149	0.147	0.146	0.147±0.002
8	0.195	0.199	0.196	0.197±0.002
10	0.251	0.247	0.245	0.248±0.003

\* M = Morning; A = Afternoon E = Evening

The SD values of Inter day and intraday variation studies indicated that the variation is minimum.

**Table 5:** LOQ studies of Timolol maleate

Sl.no.	Concentration (µg /ml)	I	II	III	Avg ± SD
		Absorbance			
1	0.00	0	0	0	0.000
2	0.25	0.003	0.004	0.003	0.003±0.001
3	0.50	0.011	0.013	0.009	0.011±0.002
4	0.75	0.019	0.021	0.017	0.019±0.002
5	1.00	0.023	0.024	0.023	0.023±0.001
6	1.50	0.038	0.039	0.036	0.038±0.002
7	2.00	0.051	0.050	0.051	0.052±0.001
8	4.00	0.102	0.102	0.103	0.101±0.002
9	6.00	0.151	0.150	0.152	0.150±0.002
10	8.00	0.201	0.201	0.202	0.200±0.001
11	10.00	0.251	0.252	0.252	0.250±0.002
12	15.00	0.333	0.337	0.332	0.334±0.003
13	18.00	0.370	0.375	0.369	0.371±0.003
14	20.00	0.411	0.407	0.411	0.410±0.002
15	25.00	0.420	0.418	0.423	0.420±0.004
16	30.00	0.431	0.434	0.430	0.432±0.002

Results of studies on melting point and UV absorption maxima of drug suggested the values to be corroborating with previously reported literature values. The slope (m) of calibration curve of timolol maleate was 0.025 (Table-1, Fig-1). Linearity studies indicated that estimation of timolol maleate between 2.00 µg / ml to 10.00 µg /ml was found to be linear with slope (m) 0.025, intercept (c) is - 0.00076, regression coefficient r<sup>2</sup> is 0.999 (Table-2). The SD values of Inter day and Intraday variation studies indicated that the variation is minimum (Table-3, Table-4). Limit of Quantitation of timolol was found to be of 1.00 µg /ml (Table-5) in UV spectrophotometer (UV 1700, Shimadzu, Japan). The solubility of drug timolol follows the order buffer pH 4.0 > distilled water > buffer pH 7.4 > buffer pH 8.0. The log p value of timolol maleate was found to be 1.48, which corroborates to the reported literature value 1.44 [11]. The physico-chemical characterization studies showed that the bulk sample obtained was pure and analytical work for the estimation of timolol was found to obey Beer's limit and the curve was found to be linear. Using the proposed analytical technique, further quantitation work of prospective *in vitro* studies of timolol could be carried out.

### CONCLUSION

The literature review encompasses the literature reports on various analytical methods of Timolol maleate estimation useful in the study. The corroborating experimental values suggest the bulk sample drug obtained is pure. The solubility study of timolol maleate suggests that the drug is highly soluble in alkaline buffer pH 8.0, and distilled water, it is least soluble in acidic buffer pH 4.0. The analytical method developed using UV spectrophotometer is linear and LOQ is 1 µg /ml and the inter-day and intra-day variation is minimum. Although there are reports and publications of either colorimetric and HPLC methods for timolol maleate estimation, but



there is no report or publication corresponding to the intended investigation, development and validation of UV Spectrophotometric method of timolol maleate. Therefore it could conclude that the proposed investigation is a novel work and the investigation would help in estimation of drug candidate spectrophotometrically in the bulk fluids.

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