ejpmr, 2019,6(12), 171-175

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT AND VALIDATION OF RP- HPLC METHOD FOR THE ESTIMATION OF TIMOLOL MALEATE IN API AND FAST DISSOLVING TABLETS

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Article Received on 04/10/2019 **Article Revised on 24/10/2019** **Article Accepted on 14/11/2019**

ABSTRACT

A simple, precise and reproducible validation method was develop for Timolol Maleate as per ICH (International Council for Harmonization) guidelines Q2R(1) and subsequently stability indicating method as per ICH guidelines Q1A(R2). Chromatographic evaluation was carried out on C18 column of Agilent (25 cm x 4.6 mm), (i.d. 5 µm.) with a mobile phase consist of Methanol : Water (20:80 v/v). Flow rate was maintained to 0.8ml/min. The stability indicating data revealed that Timolol Maleate was found to be unstable alkaline condition. The validated method was applied various validation parameters like Linearity (calibration curve), Precision, Accuracy, Robustness, LOD and LOQ were found to be 0.9µg/ml and 2µg/ml. The validated method can be used for estimation of Timolol Maleate in prepared Fast Dissolving Tablets (FDTs) by using Croscarmellose Sodium (CCS) as a superdisintegrant a calculated their optical parameters.

KEYWORDS: Timolol Maleate, RP-HPLC, Stability Studies, FDTs.

INTRODUCTION

Timolol Maleate (TM) is a member of a family of drugs called nonselective beta adrenergic blocker. It is used alone or in combination with other antihypertensive agents, especially thiazide type diuretics. Timolol Maleate was available for oral dosing and tablets and for injection and ophthalmic dosing as distinct sterile aqueous solutions. Timolol Maleate chemically described as (S)-1-tert-butylamino-3-(4-morpholino-1,2,5-thiadiazol -3-yloxy)propan-2-ol-hydrogen maleate and 50-60% bioavailability. It blocks both β-1 and β-2 adrenergic receptors to reduce blood pressure decreasing sympathetic outflow. It has a molecular formula $C_{13}H_{24}N_4O_3S$, $C_4H_4O_4$ with molecular weight of 432.50 and pKa 9.21.[1] Timolol Maleate structure is shown in figure 1.

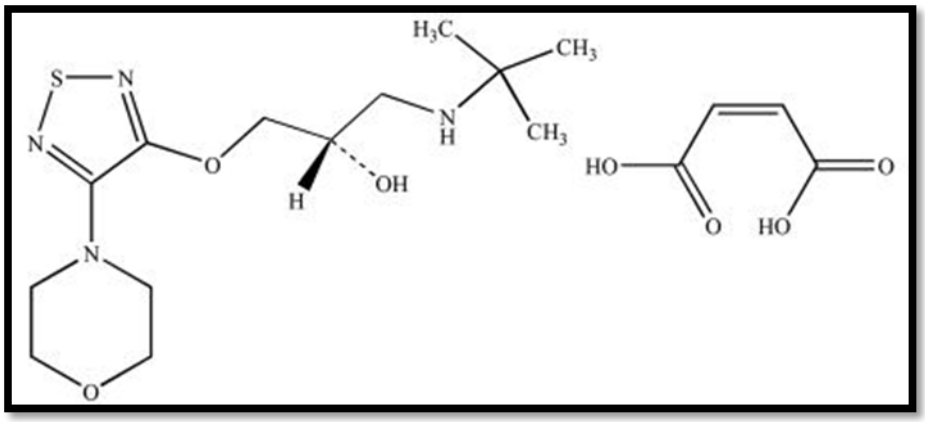

Figure 1: Chemical Structure of Timolol Maleate.

The literature review showed that there are various HPLC methods have been developed for simultaneous estimation of Timolol Maleate in combination of other drugs.[2-8] and few HPLC validation method of Timolol Maleate without combination of other drugs.[9] The aim and objective of the present work was develop and validate the simple, rapid, sensitive, precise and accurate isocratic RP-HPLC method of analysis of the Timolol

Maleate and its stability indicating by degradation method and validation parameters are valid according to ICH guidelines.[10-11]

MATERIALS AND METHODS

Instruments and chemical used

HPLC with manual injector and UV-Visible detector was employed for investigation. The separation was achieved on a Isocratic HPLC Cyberlab LC100, UV detector, Agilent Column (C-18). The analytical balance was used for weighing the materials. The working standard Timolol Maleate was gifted from Jackson Lab.Pvt.Ltd. Amritsar. Methanol HPLC grade was purchased from Merck India Ltd. and water HPLC grade was purchased from Fisher Scientific India Pvt.Ltd.

Preparation of standard solution

Timolol Maleate was weighed 50mg and transferred into 50ml volume metric flask. It was allowed to dissolve with HPLC grade Methanol and volume was made up to 50ml to get the solution concentration 1000µg/ml. Afterward pipette out 10 ml these prepared solution add into 100ml volume metric flask (100µg/ml). Further dilutions were prepared from the above concentrated solution.

Method development

The UV spectrum of the Timolol Maleate was showed the balanced wavelength at 294 nm on methanol solvent. Mobile phase selection was based on peak parameters like symmetry, tailing, peak resolution, cost and run time. To have an ideal separation of the drug under isocratic conditions, various mobile phases were tried for the separation of Timolol Maleate with optimal retention time by using C18 column. A mixture of Methanol: Water in the ratio of 20:80 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

Method Validation

After the method conditions were established as described above, method was validated as per ICH guidelines. The linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantification (LOQ) were determined. The linearity was studied by analyzing five concentrations of Timolol Maleate drug. Precision of the system was evaluated by analyzing five independent standard concentrations and calculated %RSD value to determine intra-day variation. These studies were also repeated on another days to determine inter-day variation. Accuracy studies were carried out for three different concentrations (4 µg/mL, 8 µg/mL and 12 µg/mL) of standard Timolol Maleate solution were carried out using the proposed method and percentage recovery was determined. Robustness studies were carried out by small deliberate changes in wavelength and flow rate and calculated the %RSD value. Limit of detection and Limit of quantification were calculated from linearity plot.

Assay of Prepared Fast Dissolving Tablets of Timolol Maleate

For the analysis of prepare FDTs of Timolol Maleate, a 20 tablets were taken and calculated their average weight. The tablets were crushed and powdered finely with the help of mortar pestle. To prepare assay sample solution, powdered sample equivalent to 50 mg of Timolol Maleate was weighed and transferred to a clean and dry 50 ml volumetric flask. About 20 ml of mobile phase was added as diluting solution and shaken thoroughly to extract the drug from the excipients and then sonicated for 10 min for complete dissolution of drug. After sonicated the solution was allowed to cool at room temperature and make up the volume to the mark with same diluting solution. The solution was then double filtered through Whatman filter paper No. 42. Afterward, serial dilutions of the FDTs of Timolol Maleate stock solution were prepared. The dilutions were prepared in the range of 4, 8, 12, 16 and 20µg/ml for FDTs of Timolol Maleate and were injected in triplicate into the RP-HPLC. The calibration curve was plotted, peak area v/s concentration.

Stress degradation studies

Stress degradation studies were carried out under various conditions like hydrolytic conditions, oxidation condition and thermal condition. The standard working solution was mixed with equivalent volumes with 0.1M hydrochloride acid, 1M sodium hydroxide and heated at 80^0C for various time intervals of 2hr-12hr on water bath. When solution left to reach ambient temperature and neutralized to pH 7 by addition of 1M sodium hydroxide for 1M hydrochloride and by addition of 1M hydrochloride for 1M sodium hydrochloride. Standard working solution mixed with 10%, 15%, and 30% H_2O_2 separately. The prepared samples were heated at 80^0C for various time intervals of 2hr-12hr on water bath. Weigh 10mg Timolol Maleate was stored at 80^0C in hot air oven for required time interval. At the same time another drug containing flask was kept at room temperature as control. After prepared working standard solution for stress degradation studies and injected into system.

RESULT AND DISCUSSION

Development of the Optimized chromatographic conditions

Chromatographic separation studies were carried out on the working standard solutions of Timolol Maleate. Finally, trials were carried out by after various trials, Methanol : water in the ratio 20 : 80 v/v with C18 column at 294 nm wavelength was proved to be the suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing. Retention time were found as Timolol -4.56 min and Maleic Acid – 4.94 min. The chromatogram of Timolol Maleate was presented in figure B. The Optimized chromatographic conditions are given in Table no.1.

Validation of developed method

Validation of the proposed method was carried under ICH guidelines. The results obtained were within acceptable limits. Thus, the system meets suitable criteria. The calibration curve was obtained for a series of concentration in the range of 4-20µg/ml and it was found to be linear. Five points graphs was constructed covering a concentration range 4-20µg/ml. The standard deviation of the slope and intercept were low. Calibration curve found to be linear with r^2=0.999, Intercept -33942 and Slope (11957) respectively. The calibration curve was plot peak area v/s concentration is shown in figure C. The results obtained were within acceptable limits where tailing factor ≤2.0 and theoretical plates >2000. A precision result indicates that the developed method %RSD value for both interday and intraday were less than 2%. Limit of detection and limit of quantitation was found to be 0.9µg/ml and 2µg/ml respectively. The robustness result was found within the acceptance limits. Summary of validation studies are presented in Table no. 2. The prepared FDTs (Fast Dissolving Tablets) of Timolol Maleate by using Croscarmellose Sodium was analyzed with proposed method conditions and good recovery of the drug indicates that the proposed method can be useful for analysis of this formulation and calculate optical parameters. The optical parameters of prepared formulation are presented in Table no. 3.

Stability studies

The forced degradation studies in RP-HPLC method of Timolol Maleate was developed and validated as per ICH guidelines. The degradation nature of Timolol Maleate was studied under various stress conditions of acid, alkali, oxidation and temperature. Drug was found to be unstable in alkali condition.

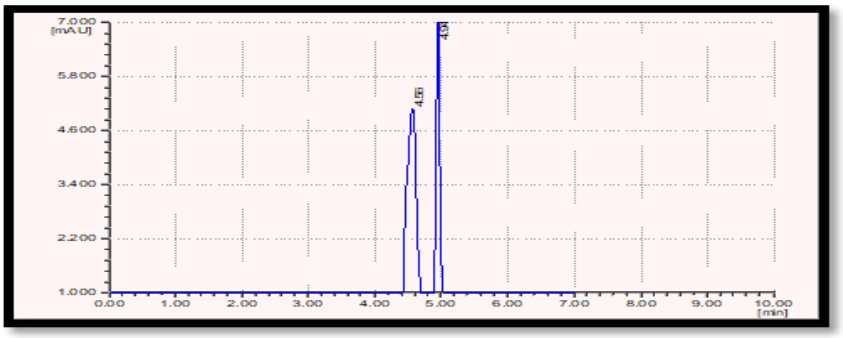

Figure B: Chromatogram of Timolol Maleate.

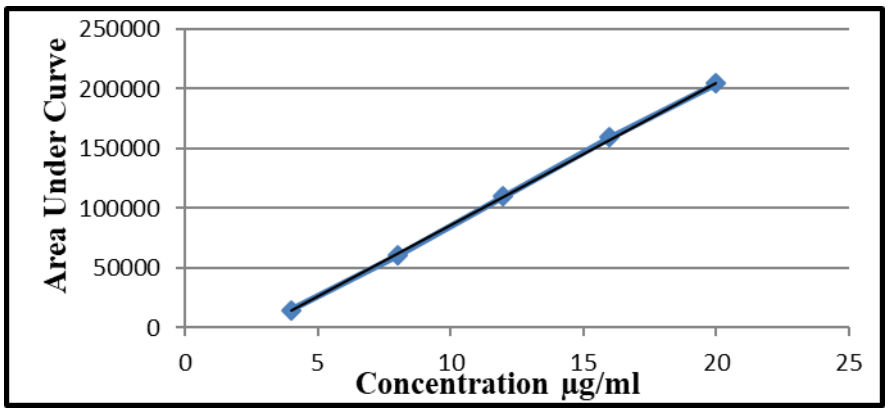

Figure C: Calibration curve of Timolol Maleate.

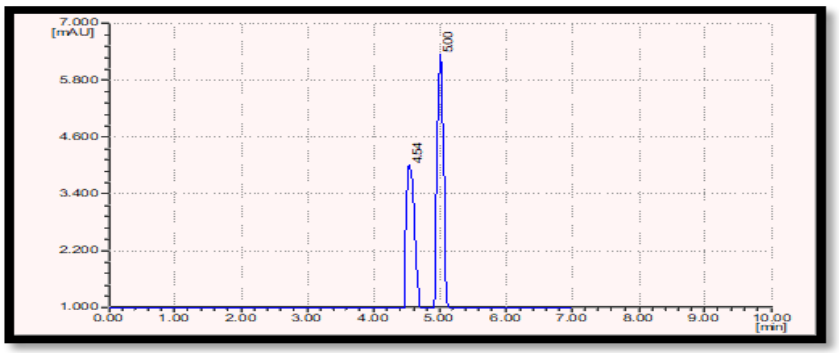

Figure D: Chromatogram of Prepared FDTs of Timolol Maleate.

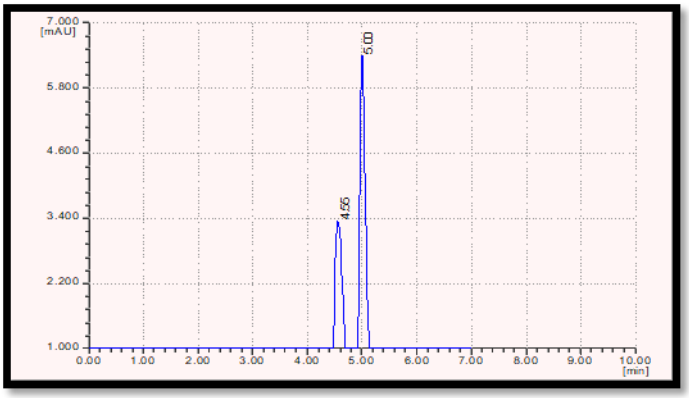

Figure E: Chromatogram of Timolol Maleate Standard.

Table No. 1: Optimized chromatographic conditions for estimation of Timolol Maleate.

Parameter	Results
Mobile phase	Methanol : Water 20 : 80 v/v
Pump mode	Isocratic
Diluent	Mobile phase
Column	C-18 Column (25 cm x 4.6 mm), (i.d. 5 µm.)
Column Temp	Ambient
Wavelength	294 nm
Injection Volume	20 µl
Flow rate	0.8 mL/min
Run time	10 min
Retention Time	Timolol - 4.56 min Maleic Acid - 4.94 min

Table No. 2: Summary of validation results.

S. No.	Parameters	Results
1	Theoretical Plates	6158
2	Tailing Factor	1.30
3	Linearity range	4-20µg/ml
4	Correlation coefficient	0.999
5	Intraday Precision (in %RSD)	0.709
6	Interday Precision (in %RSD)	0.957
7	Recovery range (%)	99.19-100.38
8	Robustness (% Change)	0.644-0.856
9	Limit of Detection	0.9µg/ml
10	Limit of Quantitation	2µg/ml

Table No. 3: Optical parameters of prepared Fast Dissolving Tablets of Timolol Maleate.

S. No.	Parameters	Results
1	Accuracy	99.97±1.9
2	Slope	14744
3	Intercept	-42680
4	Linearity Range	4-20µg/ml
5	Correlation Coefficient	0.999
6	SE of Intercept	3631.831
7	SD of Intercept	79990.029
8	LOD	1.7µg/ml
9	LOQ	5.4 µg/ml

CONCLUSION

The Analytical method of the Timolol maleate raw material and finished product (FDT) is successfully developed and validated using HPLC. All the parameters showed good results. The developed method is accurate, precise with good reproducibility and recovery. The stability studies results reveal that drug unstable in alkali condition.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. R.K. Dhawan, Director-Principal, Khalsa College of Pharmacy, Amritsar (Punjab) for providing all the facilities. The authors duly acknowledge Jackson Lab.Pvt.Ltd. Amritsar, for providing Timolol Maleate as a gift sample.

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