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Validation of Rp-Hplc Method for the Estimation of Timolol Maleate in Fast Dissolving Tablets

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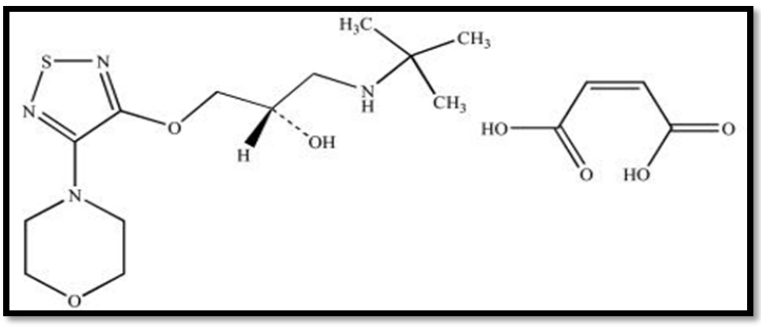
ABSTRACT

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). Flowrate 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(calibrationcurve),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 DissolvingTablets (FDTs) by using Croscar mellose 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 aqueoussolutions.TimololMaleatechemicallydescribed 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.



The literature review showed that there are variousHPLC 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 cRP-HPLCmethod ofanalysis of theTimolol
Maleate and its stability indicating by degradation method and validation parameters are valid according to ICH guidelines.[10-11]

MATERIALSANDMETHODS

Instruments and chemical used

HPLCwithmanualinjectorandUV-Visibledetectorwas employed for investigation. The separation wasachieved 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 dissolvewith 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 nmon methanol solvent. Mobile phase selection was based on peak parameters like symmetry, tailing, peak resolution, cost and runtime. To have an ideal separation of the drug under isocraticconditions,variousmobilephaseswereretriedfor the separation of Timolol Maleate with optimal retention time by using C18 column. A mixture of Methanol: Waterintheratioof20:80v/v wasprovedtobethe 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.Thelinearity,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-dayvariation.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 wavelengthand 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 TimololMaleate,a 20 tablets were taken and calculated their averageweight. 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 thendouble filtered through Whatman filter paper No. 42. Afterward, serial dilutions of the FDTs of Timolol Maleate stock solution wereprepared.Thedilutionswere 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 outunder various conditions likehydrolyti cconditions,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 waterbath. 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.Thepreparedsampleswereheatedat80^0Cfor various time intervals of 2hr-12hr on water bath. Weigh 10mgTimololMaleate wasstoredat80^0Cinhotairoven for required time interval. At the same time another drug containingflaskwaskeptatroomtemperatureascontrol. 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 C18column 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 foundasTimolol -4.56minandMaleic Acid – 4.94 min. The chromatogram of Timolol Maleate was presented in figure B. The Optimized chromatographic conditions are given in Table no.1.

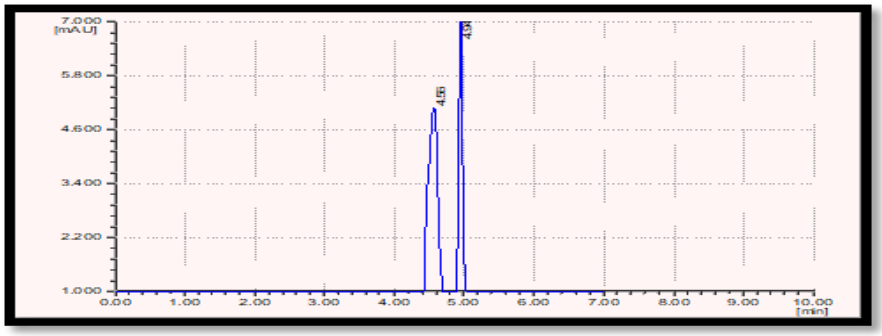
Validation of developed method

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 seriesof 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 deviationoftheslopeandinterceptwerelow.Calibration curve found to be linear with r^2=0.999, Intercept -33942 and Slope (11957) respectively. The calibration curve was plotpeakareav/sconcentration isshown 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 be0.9µg/m land 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 perICH 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.



FigureB:Chromatogram of Timolol Maleate.

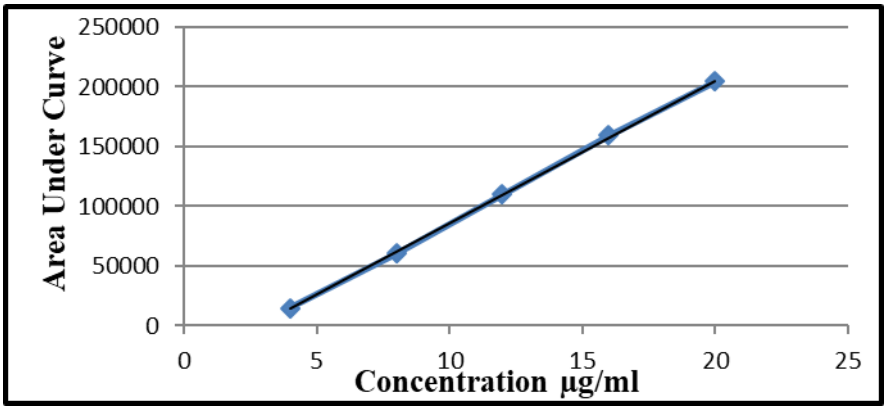
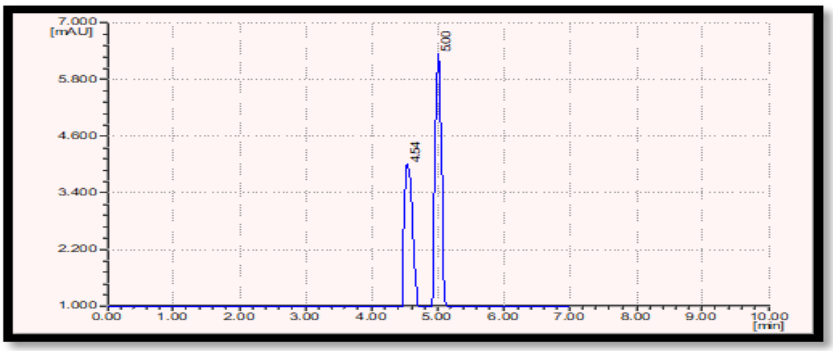
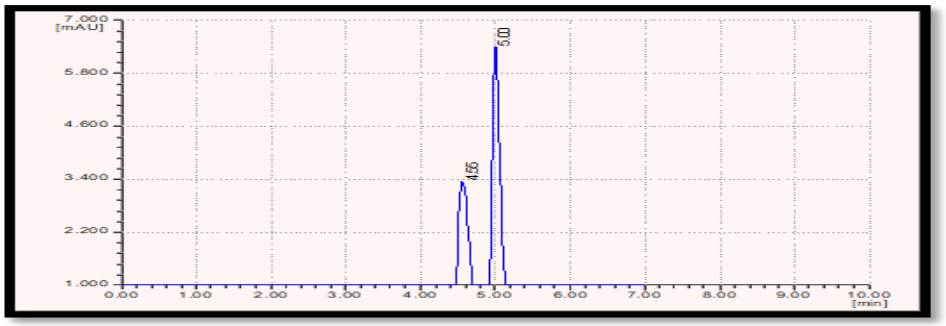


Figure C:Calibration curve of Timolol Maleate.



FigureD:Chromatogram of Prepared FDTs of TimololMaleate



FigureE :Chromatogram of TimololMaleateStandard

TableNo.1: Optimized chromatographic conditions for estimation ofTimolol Maleate.

Parameter	Results
Mobilephase	Methanol:Water20:80v/v
Pump mode	Isocratic
Diluent	Mobilephase
Column	C-18Column(25cmx4.6 mm),(i.d.5µm.)
ColumnTemp	Ambient
Wavelength	294 nm
InjectionVolume	20 µl
Flowrate	0.8mL/min
Runtime	10 min
RetentionTime	Timolol-4.56min MaleicAcid-4.94min

TableNo.2:Summary of validation results.

S.No.	Parameters	Results
1	TheoreticalPlates	6158
2	TailingFactor	1.30
3	Linearityrange	4-20µg/ml
4	Correlationcoefficient	0.999
5	IntradayPrecision(in%RSD)	0.709
6	InterdayPrecision(in%RSD)	0.957
7	Recoveryrange(%)	99.19-100.38
8	Robustness(%Change)	0.644-0.856
9	LimitofDetection	0.9µg/ml
10	LimitofQuantitation	2µg/ml

TableNo.3:Optical parameters of prepared Fast Dissolving Tablets of TimololMaleate.

S.No.	Parameters	Results
1	Accuracy	99.97±1.9
2	Slope	14744
3	Intercept	-42680
4	LinearityRange	4-20µg/ml

5	CorrelationCoefficient	0.999
6	SEofIntercept	3631.831
7	SDofIntercept	79990.029
8	LOD	1.7µg/ml
9	LOQ	5.4µg/ml

CONCLUSION

The Analytical method of the Timolol maleate raw material and finished product (FDT) is successfully developedand validatedusing 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.

REFERENCES

1. K.S. Rathore, Dr. R. K. Nema, Dr. S. S. Sisodia, "Timolol maleate a gold standard drug in glaucoma used as ocular films and inserts: An overview"*Int.J.Pharm.Sci.Rev.Res.,2010;3:23-29.*
2. A. S. Geetha, B Anusha and G Rajitha, "Analytical method development and validation of new RP-HPLC method for simultaneous estimation of brinzolamide and Timolol Maleate in ophthalmic solutions" *Res.J.Pharm. Bio.Chem. Sci., 2016;7: 1290-1298.*
3. R. Khatun and S.M. A. Islam, "Development and validation of analytical method for simultaneous estimation of brinzolamide and timolol by HPLC from ophthalmic preparation" Int. J. Pharma. Sci. Res., 2014; 5: 1001-1007.
4. W.A.Shadoul,E.A.GadKariem,M.E.Adamand K.E.E. Ibrahim, "Simultaneous determination of dorzolamide hydrochloride and Timolol Maleate in ophthalmic solutions using HPLC" Elixir Pharm. J., 2011; 4060-4063.
5. S. P. Bansode, R. Kamble and C. S. Chauhan, "HPLC method development and its validation for simultaneous estimation of Timolol Maleate and hydrochlorothiazide in their combined tablet dosage form" Int. J. Inst. Pharm.Life Sci., 2015; 5: 1-6.
6. A. A. Elshanawane, L. M. Abdelaziz, M. S Mohram and H. M. Hafez, (2014). "Development and validation of HPLC method for simultaneous estimation of brimonidine tartrate and Timolol Maleate in bulk and pharmaceutical dosage form" J. Chromatogr. Sep. Tech., 2014; 5: 1-5.
7. R.U.Kanchan, S.M.N. Roy and R.B. Rane, "Simultaneous RP-HPLC determination of dorzolamide hydrochloride and Timolol Maleate in pharmaceuticalpreparations" Aanl. Chem. Indian J., 2008; 7: 638-641.
8. R.V Rele, V.V. Mhatre, J. M. Parab and C. B. Warkar, "Simultaneous RP-HPLC determination of latanoprost and Timolol Maleate in combined pharmaceutical dosage form" J. Chem. Pharm. Res., 2011; 3: 138-144.
9. D.U.Laddha,R.K.Barse,R.V.ZilpelwarandA. A. Tagalpallewar, "Development and validation of stability indicating reverse phase high performance liquid chromatography method forTimololMaleate" Int. J. Pharma. Tech. Res., 2014; 6: 1429-1435.
10. ICH Q1A(R2), Stability Testing Guidelines:Stability Testing of new Drug Substances and Products, 2003.
11. ICHQ2R(1),Validationofanalyticalprocedure,test and methodology, 1994.