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Research Article

DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHODS FOR THE ESTIMATION OF METOPROLOL TARTARATE BY RP-HPLC METHOD

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ABSTRACT

A new simple, specific, precise and accurate revere phase liquid chromatography method has been developed for estimation of Metaprolol tartrate single dosage forms. The chromatographic separation was achieved on a 5 – micron C 18 column (250x 4.6mm) using a mobile phase consisting of a mixture of Orthophosphoric acid buffer: Acetonitrile (75:25) was used pH 2.0 The flow rate was maintained at 1.0 ml / min. The detection of the constituents was done using UV detector at 254 nm for Metaprolol tartrate. The retention time of Metaprolol tartrate found is 3.84 min respectively. The developed method was validated for accuracy, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ) and robustness as per the ICH guidelines.

INTRODUCTION

Most of the pharmaceutical industries are manufacturing new drug formulations to meet the market demand based on the literature survey Metaprolol tartrate and their pharmaceutical dosage form. The Metaprolol tartrate is used for the treatment of Anti-hyper tension property. Standard analytical procedure for newer drugs of formulation may not be available in pharmacopoeias; hence it is essential to develop newer analytical methods which are simple, accurate, precise, specific, economic, linear and rapid. The survey reveals that there are only few methods reported for quantitative analysis of, Metaprolol tartrate and their pharmaceutical dosage form by High performance liquid chromatography (RP-HPLC).Estimation of Metaprolol tartrate and their pharmaceutical dosage forms until now made it a worthwhile project .Plan was aimed to presume the present research work by selecting Aspirin and Metaprolol tartrate as drug.

Therefore in the proposed project, a successful attempt has been made to develop simple, accurate, economic and rapid methods for the estimation of Aspirin and Metaprolol tartrate in bulk and various capsule formulations and to validate the methods, as a result for simple, economic, precise and accurate methods were developed and validated as follow Today modern Pharmaceutical analysis has more emphasis to satisfy our query for better understanding of Pharmaceutical compounds, by the use of advanced instrumental methods. It also plays an important tool for quality assurance of Pharmaceutical product throughout the self life. Standard analytical procedure for newer drugs or formulation may not be available in pharmacopoeia; hence, it is essential to develop newer analytical methods, which are accurate, precise, and specific, linear, simple and rapid. Metoprolol, marketed under the tradename Lopressor among others, is a medication of the selective β_1 receptor blocker type. It is used to treat high blood pressure, chest pain due to poor blood flow to the heart, and a number of conditions involving an abnormally fast heart rate. It is also used to prevent further heart problems after myocardial infarction and to prevent headaches in those with migraines. It is sold in formulations that can be taken by mouth or given intravenously. The medication is often taken twice a day. The extended-release formulation is taken once per day. Metoprolol may be combined with hydrochlorothiazide in a single tablet. Common side effects include trouble sleeping, feeling tired, feeling faint, and abdominal discomfort. Large doses may cause serious toxicity. Risk in pregnancy has not been ruled out. It appears to be safe in breastfeeding. Greater care is required with use in those with liver problems or asthma. Stopping this drug should be done slowly to decrease the risk of further health problems. Metoprolol was first made in 1969. It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system. It is available as

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a generic drug. In 2013, metoprolol was the 19th-most prescribed medication in the United States. The methods were developed for the estimation of metaprolol tartrate in pure form and in its Capsule dosage form .the method employed for analysis of metaprolol tartrate are High performance liquid chromatography

RP-HPLC METHOD

An effort has been made to indentify simple, precise, specific and accurate methods for the estimation of aspirin and metaprolol tartrate in bulk and in formulation by using RP-HPLC. The solution of 10µg/ ml and 25 mg of metaprolol tartrate in mobile phase (buffer: acetonitrile) was prepared and the solution was scanned in the range of 200-400 nm. At 254 nm, the drug showed maximum absorbance with 2 hours stability. Hence in this was selected as a detection wavelength. Quantification of metaprolol tartrate was done by external standard calibration method.

MATERIALS AND METHODS

Materials

Drug Samples

Metoprolol Tartarate Was Purchased From Micro Labs –Unit 3, Thiruvandarkovil, And Pudhucherry

Formulation **used**

lopressor (usv pharama pvt. limited) containing metaprolol tartrate equivalent to 10MG AND 75MGmg was purchased from a Local Pharmacy.

Chemicals And Solvents Used

Distilled water (RP-HPLC GRADE), Methanol (RP-HPLC grade), Water for RP-HPLC, Acetonitrile (RP-HPLC grade) was purchased from Qualigens India Pvt. Limited and Loba Chemie India Limited.

Instruments used

A. Shimadzu AUX- 220 Digital balance
B. Shimadzu RP-HPLC system
C. Sonicator – Sonica ultrasonic cleaner
D. Micropipette.

Specifications of instruments

Shimadzu AUX- 220 Digital balance (Shimadzu Instruction Manual)

Specifications	
Weighing capacity	200 gms
Minimum display	0.1 mg
Standard deviation	≤ 0.1 mg
Operation temperature range	5 to 40° C

Methods

LOPRESSOR was selected for analysis, as there was no established method for the same. The identification was done by checking its melting point. The following methods were employed for its analysis..

Rp – Hplc Method

Selection of Chromatographic Method

Proper selection of the method depends upon the nature of the sample, molecular weight, and solubility. The drug selected for the present study was polar. Polar compounds can be separated by reverse phase chromatography, as regularly practiced, reverse phase chromatography utilizes a hydrophobic bonded phase packing, usually processing a C_{18} or C_8 functional group and polar mobile phase, usually a practically or fully aqueous mobile phase. From the above considerations for this Reverse phase chromatographic technique, C_{18} column was chosen as stationary phase, different ratios of mobile phases were performed from that a mixture of ORTHO PHOSPHORIC ACID pH 2.0: Acetonitrile (75:25 % v/v) was selected as mobile phase.

Model: Shimadzu, UV- 1700; Cuvetts: 1 cm quartz cells.

Specifications	
Light source	20 W halogen lamp, Deuterium lamp. Light source position automatic adjustment mechanism
Monochromator	Aberration- correcting concave holographic grating
Detector	Silicon Photodiode
Stray Light	0.04% or less (220 nm: NaI 10g/l) 0.04% or less (340 nm: $NaNO_2$ 50g/l)
Measurement wavelength range	190~ 1100 nm
Spectral Band Width	1 nm or less (190 to 900 nm)
Wavelength Accuracy	± 0.5 nm automatic wavelength calibration mechanism
Recording range	Absorbance: -3.99 ~ 3.99 Abs Transmittance: -399 ~ 399%
Photometric accuracy	± 0.004 Abs (at 1.0 Abs), ± 0.002 Abs (at 0.5 Abs)
Operating Temperature/ Humidity	Temperature range: 15 to 35° C Humidity range: 35 to 80% (15 to below 30° C) 35 to 70% (30 to below 35° C)

Shimadzu HPLC (Shimadzu Instruction Manual)

Detector Specifications	
Light source	Deuterium arc lamp
Wavelength range	190 to 700 nm
Spectral Band Width	5 nm
Wavelength Accuracy	± 1 nm
Cell path length	10 nm
Cell volume	20 µl
Operating temperature range	4 to 40°C (39 to 104°F)
Recording range	0.0001 to 4.000 AUFS
Operating Temperature/ Humidity	4 to 35°C/ 75 %

Pump Specifications	
Pump type	Double reciprocating plunger pump
Pumping methods	Constant flow delivery and constant pressure delivery
Suction filter	45 µm
Line filter	5 µm mesh
Operating temperature	4 to 40°C

Selection of Detection wavelength

A solution of Metaprolol tartrate (10 µg/ml) was scanned in the UV region using ORTHO PHOSPHORIC ACID pH 2.0 (opa): Acetonitrile (75:25 % v/v). The λ_{max} was found at 254nm. Metaprolol tartrates have marked absorbance in all the different ionic strength of phosphate buffer and ratios of mobile phase. There was no significant change in λ_{max}. Hence, 254nm was selected as detection wavelength for the estimation of LOPRESSOR by RP-RP-HPLC method.

Initial Separation Conditions

The following chromatographic conditions were fixed initially to improve the separation of LOPRESSOR

Mode of operation : GRADIENT
Stationary phase : C_{18} Column (150 mm × 4.6 mm i.d., 5 µ)
Mobile phase : Buffer: Acetonitrile

Ratio	:	75:25:% V/v
Detection wavelength	:	254nm
Flow rate	:	1 micro litre/ min
Temperature	:	Ambient
Sample volume	:	50 µl
Operating pressure	:	205 kgf
Quantification method	:	External Standard Calibration Method

The mobile phase was allowed to run for 60 minutes to record a steady baseline. LOPRESSOR drug solution was injected and chromatogram was recorded. It was observed that the drug was eluted metaprolol tartrate at 3.84 minutes. Hence the different ratios of mobile phase were tried to get the good peak shape, short retention time and acceptable system suitability parameters.

Effect of Ratio of mobile phase

The mobile phase concentration of buffer was changed in different proportions like 65:35% v/v, 55:45% v/v and 75:25 % v/v of ORTHO PHOSPHORIC ACID pH 2.0: Acetonitrile (75:25 % v/v). The chromatograms were recorded for the above ratios. In this ORTHO PHOSPHORIC ACID pH 4: Acetonitrile (75:25 % v/v), the drug was eluted aspirin and atravastatin at 3.84 minutes. In the ratio of (ORTHO PHOSPHORIC ACID pH 2.0 (opa): Acetonitrile (75:25 % v/v), the peak shape and system suitability parameters were good. Hence, the ratio was selected for further analysis.

Effect of ratio of mobile phase

- In the ratio of (75:25) of buffer: Acetonitrile; the peak shape and system suitability parameters were good. Hence, the ratio was selected for further analysis.
- Buffer; Water previously adjusted to ph 2.0 with orthophosphoric acid filter and degas before use.

Preparation of Standard Solution

- Weigh accurately about 100 mg of metaprolol tartrate working standard into a 100 ml volumetric flask. Add about 100 ml of methanol, sonicate to dissolve, and make up to the mark with mobile phase.
- Take 10ml of standard solution to 100 ml with mobile phase.

Sample Preparation

Weigh the tablets and crush the content of 20 capsules, weigh accurately powder sample (50 mg equivalent of metaprolol tartrate) into a 100 ml volumetric flask, add 100 ml of methanol. Sonicate for 10 minutes and dilute to the volume with mobile phase sonicate to dissolve too completely. Filter with o.45µ membrane filter. Dilute 10ml of the above solution to 50 ml with mobile phase.

Preparation of Calibration Curve

In this method, the aliquots of stock solution of metaprolol tartrate (1-5ml) were transferred into a 25 ml of volumetric flask and made up to the mark with mobile phase. A solution contains 10, 20, 30, 40, 50 µg /ml of metaprolol tartrate in mobile phase were injected and the chromatograms were recorded at 254nm. It was found that the above concentration range was linear. The procedure was repeated for three times. The peak areas were plotted against concentration and the calibration curve was constructed.

Estimation of Metaprolol Tartrate In Tablet Formulation

Weigh the tablet and crush the content of 20 tablets, the average weight was found and powdered (50 mg equivalent of metaprolol tartrate) and into a 100 ml volumetric flask, add 20 ml of methanol. Sonicate for 10 minutes and dilute to the volume with mobile phase sonicate to dissolve completely. Filter with o.45 µ membrane filter. Dilute 5 ml of the above solution to 50 ml with mobile phase .Inject the solution and recorded the chromatogram. The concentration of each test solution was determined by using slope and intercept values from calibration graph.

Recovery Studies

To ensure the reliability of the methods, recovery studies were carried out by mixing a known quantity of standard drug solution with the pre – analyzed sample formulation and the content were mixed and made to the volume with mobile phase and re- analyzed by the proposed method, the percentage recovery was calculated.

Limit of Detection (Lod) And Limit of Quantification (Loq)

Calibration of standard was repeated for three times. The limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation of intercept.

System Suitability Studies

The system suitability studies carried out as specified in ICH guidelines and USP. The parameters like tailing factor, asymmetry factor, number of theoretical plates, capacity factor were calculated.

RESULTS AND DISCUSSION

The methods were developed for the estimation of metaprolol tartrate in pure form and in its tablet dosage form .the method employed for analysis of metaprolol tartrate are
High performance liquid chromatography

Rp-Hplc Method

An effort has been made to indentify simple, precise, specific and accurate methods for the estimation of aspirin and metaprolol tartrate in bulk and in formulation by using RP-RP-HPLC.

The solution of 10µg/ ml and 25 mg of metaprolol tartrate in mobile phase (buffer: acetonitrile) was prepared and the solution was scanned in the range of 200-400 nm . At 254 nm, the drug showed maximum absorbance with 2 hours stability. Hence in this was selected as a detection wavelength. Quantification of metaprolol tartrate was done by external standard calibration method. The optimization was done by various mobile phase such as acetonitrile;water ,acetonitilwe;methanol and only acetonitrile is used were employed and the chromatogram was recorded for aspirin and metaprolol tartrate.These are shown in figure 1-3 after considering suitability test parameters [acetonitrile :buffer] of 75:25 ratio was selected for analysis .The retention time of metaprolol tartrate is 9.4 minutes. The system suitability test parameters were calculated for optimized chromatograms and are shown in the table-2.The linearity was done by using external standard calibration method with the optimized chromatographic conditions; stock solutions of aspirin were prepared by 20 ml of methanol and make upto mobile phase with 100ml. In that prepare various concentration in the range of metaprolol tartrate in mobile phase.20 µl of each solution

were injected individually. The chromatogram was shown in figures. The calibration curve was plotted using concentration against peak area .The procedure was repeated for three times. The co-relation coefficient value was found to be 0.9999 indicates that the concentration of aspirin and metaprolol tartrate has good linearity. The calibration graph is shown in figures. The optical characteristics of aspirin and metaprolol tartrate are shown in tables. The limit of detection and the limit of quantification were determined by using slope and standard deviation .The LOD AND LOQ was calculated. The table formulation LOPRESSOR was selected for analysis from the calibration curve, the nominal concentration was prepared 20 µl of formulation was injected and the chromatograms were recorded. The percentage of aspirin and metaprolol tartrate present in formulation was found to be the table. The precision of the method was confirmed by repeatable injection of the formulation for six times and there chromatograms are shown in the figures. The percentage RSD value was found to be tables. This indicates that the method has good precision .The values are shown in tables

Accuracy was confirmed by recovery studies by adding known amount of pure drug to the previously analysed formulation and the mixture was re analysed by the proposed method and there chromatograms were recorded as shown in the figures. The percentage recovery of aspirin and metaprolol tartrate present in formulation was found to be the table. It was extremely low when compared to the normal value the high percentage recovery indicates that there is know interface produced due to the excipients used in formulation. Hence, the developed method was found to be accurate. All the above parameters combined with simplicity and ease of operation ensures that the application of proposed method for the assay of drug in pharmaceutical dosage forms.

ANALYTICAL WORK SHEET

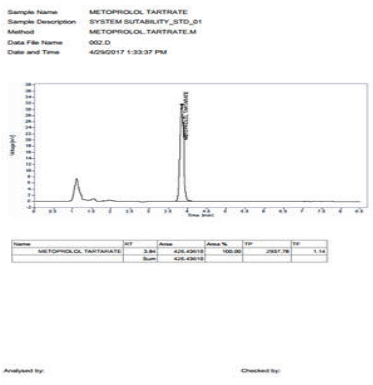
SYSTEM SUITABILITY

STANDARD DILUTION:

102.59	mg---->	100	ml	10	ml---->	100	ml

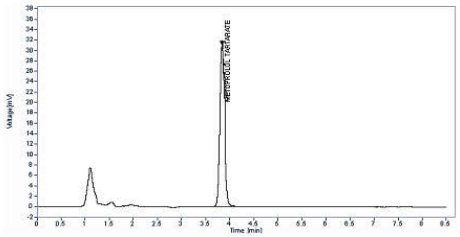
S.No.	Area
1	426.43610
2	431.57492
3	434.12817
4	434.55750
5	429.58740429
Average	58740
SD	431.25682
% RSD	3.36
	0.78

HPLC AREA PERCENTAGE REPORT



HPLC AREA PERCENTAGE REPORT

Sample Name	METOPROLOL TARTRATE
Sample Description	PRECISION SOLUTION_SAMPLE_01
Method	METOPROLOL.TARTRATE.M
Data File Name	019.D
Date and Time	4/29/2017 4:39:14 PM



Name	RT	Area	Area %	TP	TF
METOPROLOL TARTARATE	3.85	431.87982	100.00	3026.95	1.11
	Sum	431.87982			

Analysed by: Checked by:

ANALYTICAL WORK SHEET

ACCURACY

Label Claim: Metoprolol Tartarate 50 mg Average Weight. (mg):

STANDARD DILUTION: Standard Potency(as such) %: Conversion Factor:

102.59 mg----> 100 ml, further 10 ml dilute to 100 ml

Area:

Metoprolol Tartarate	429.74122	430.98562	435.98441	440.78457	435.01420
Average :	434.502	S.D.:	4.386	% R.S.D.:	1.01

SAMPLE DILUTIONS & CALCULATIONS:

ID	DILUTIONS					Area	ASSAY (mg/tab.)
LOW LEVEL CONCENTRATION (80%)							
100Spl.- 01 106.58 mg---->	ml----->	10	ml----->	50	ml	343.74114	50.1581
MIDDLE LEVEL CONCENTRATION (100%)							
100Spl.- 01 133.76 mg---->	ml----->	10	ml----->	50	ml	433.25874	50.3740
HIGH LEVEL CONCENTRATION (120%)							
100Spl.- 01 159.60 mg---->	ml----->	10	ml----->	50	ml	517.69312	50.4457

ANALYTICAL WORK SHEET

LINEARITY AND RANGE

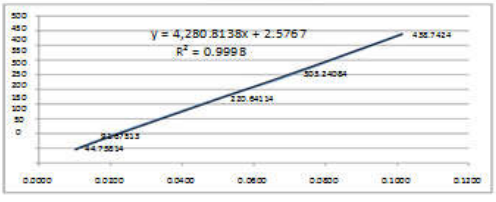
Standard purity : 98.93
Conversion Factor 1

STANDARD DILUTIONS							FINAL CONCENTRATION
102.59 mg---->	100	ml; further	1.0	ml diluted to	100	mL	0.0101 mg/mL
102.59 mg---->	100	ml; further	2.0	ml diluted to	100	mL	0.0203 mg/mL
102.59 mg---->	100	ml; further	5.0	ml diluted to	100	mL	0.0507 mg/mL
102.59 mg---->	100	ml; further	7	ml diluted to	100	mL	0.0710 mg/mL
102.59 mg---->	100	ml; further	10	ml diluted to	100	mL	0.1015 mg/mL

STANDARD CONCENTRATIONS AND AREA RATIO:

Conc.(mg/mL) (X) :	0.0101	0.0203	0.0507	0.0710	0.1015
Area Ratio(Y) :	44.75814	91.67513	220.64114	303.24084	435.7424

LINEARITY GRAPH



Analysed by Checked by

ANALYTICAL WORK SHEET

METHOD PRECISION

Metoprolol tartarate	50 mg	Average Weight. (mg):	133.160
STANDARD DILUTION:			Standard Potency(as such) %: Conversion Factor:

102.59 mg----> 100 ml, further 10 ml dilute to 100 ml

Area:

Metoprolo Succinate	438.88563	442.30972	441.56827	444.55222	432.01406
Average :	439.866	S.D.:	4.833	% R.S.D.:	0.011

SAMPLE DILUTIONS & CALCULATIONS:

ID		DILUTIONS							Area:	ASSAY (mg/tab.)	ASSAY (%)
Spl.-	01	133.86	mg-->	100	ml----->	10	ml----->	50 ml	431.87982		49.5643
Spl.-	02	132.47	mg-->	100	ml----->	10	ml----->	50 ml	432.85414		50.1973
Spl.-	03	133.92	mg-->	100	ml----->	10	ml----->	50 ml	431.84512		49.5381
Spl.-	04	133.02	mg-->	100	ml----->	10	ml----->	50 ml	435.20020		50.2607
Spl.-	05	133.51	mg-->	100	ml----->	10	ml----->	50 ml	436.15401		50.1860
Spl.-	06	133.04	mg-->	100	ml----->	10	ml----->	50 ml	432.98569		49.9974
									Average :		49.9573
									S.D.SD :		0.33
									% R.S.D. :		

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