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# Develop an Analytical Procedure for Measure the Quantity Based Quality of Metoprolol Tartrate Drug Content from Marketed Tablets by Reversed Phase-HPLC Method

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**Abstract :** Assay is play an important role for assure the quality of the pharmaceutical product. A large number of techniques have been applied for assure the quality of the pharmaceutical products. The present study has been done by reversed-phase high performance liquid chromatography. Reversed high performance liquid chromatography was equipped with UV detector. The active ingredient metprololtartarate was analysed by reversed phase $C_{18}$ column. The mobile phase (methanol and water) was used in the ratio 70:30. The Wave length (215 nm) of active ingredient metoprolol tartrate was determined by UV spectrophotometer. The retention time of metoprolol tartrate was 5.3 min. The correlation coefficient ($R^2$=0.999) was obtained with correlation range 10-100 ppm. The limit of detection and limit of quantification of the instrument were calculated 0.02 and 0.09 µg/mL, respectively. The accuracy of the method validation was determined by recovery method at three concentration level 5, 10 and 15 µg/mL. The accuracy of the method was obtained 99.80 %, 98.00 % and 102.72 %. The new method was validated according to international conference harmonization guidel.
**Keywords :** Metoprolol tartrate, RP-HPLC, Tablets.

## Introduction

It is a white to off-white crystalline powder. It is soluble in soluble in water. It is Stable under ordinary conditions. Chemically metoprolol tartrate isBis [(2RS)-1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl) amino] 2-propanol](2R,3R)-2,3-dihydroxybutanedioate. Metoprolol tartrate used in the treatment of cardiovascular disorders such as hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction. It is a selective b-adrenergic antagonist. The drug is quite sensitive. Therefore the development of an analytical method for the determination of metoprolol tartrate is of great significance. In addition, several methods have been reported for quantification of metoprolol tartrate using high-performance liquid chromatography HPLC Technique[1-4].

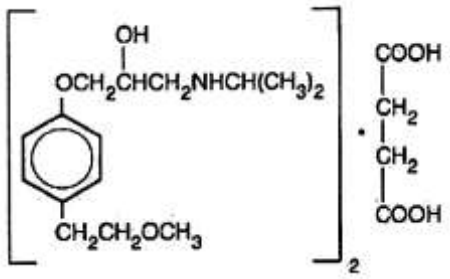


**Figure: 1.** Metprolol Tartrate

## Materials and Methods

The instruments used during the analysis are listed below:

**Table No. 1. Instruments are used during analysis**

| Sl. No. | System Name | Company name |
|---|---|---|
| 1 | HPLC System | Cyberlab$^{TM}$ HPLC |
| 2 | Analytical balance | Vibra |
| 3 | Ultrasonic bath | Toshcon |
| 4 | Syringe 25µL | Eosge, made in Australia |
| 5 | Filter paper 0.45µm | Pall–Life science |
| 6 | Filter assembly | Pall–Corporation |
| 7 | UV-VIS Spectrophotometer | Thermoscientific |

The chromatographic parameters and conditions are listed below:

**Table No. 2. Chromatographic conditions (RP-HPLC)**

| Sl. No. | Parameters | Conditions |
|---|---|---|
| 1 | Stationary Phase | Capcell Pak $C_{18}$(15mmX250mm) |
| 2 | Mobile phase | methanol : water (60:40) |
| 3 | Flow rate | 1.0 mL/min |
| 4 | Injection volume | 25 µL |
| 5 | Wavelength (λ max) | 210 nm |
| 8 | Column temperature | 20 $^{0}$C |

### Sonication

Sonication process is playing an important role for degassing the solvent. The degassing of mobile phase (methanol and water) has been done by ultrasonic sonicator.

### Preparation of mobile phase

Prepare a mobile phase by methanol and water in the ratio of 70:30. Prepared mobile phase was filtered with 0.45µm (pore size) nylon filter membrane.

### Preparation of stock solution

Transfer 10 mg metoprolol tartrate (99.9 %) in 100 mL volumetric flask and dissolved in methanol and water solvent (70:30) and then makeup to meniscus. Prepared stock solution filtered with a nylon filter membrane (0.45 µ).

**Preparation of sample solution**

Crushed 10 tablets after weight variation and transfer the equivalent weight in volumetric flask. The active ingredient metoprolol tartrate was extracted with methanol (thrice) and filtered by whatman filter paper. The aliquot was making up to the meniscus with methanol. The sample solution was prepared by this extracted aliquot.

**Preparation of calibration curve**

A well defined calibration curve was obtained by plotting different peak area versus concentration of the serial dilutions. Theserial dilutions were prepared in ten concentration levels 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/mL.

**Method Validation**

The described method validation was validated according to International Conference Harmonized guidelines[5-7] with respect to linearity, specificity, precision, limit of detection (LOD) and limit of quantification (LOQ).

**Linearity**

As per International Conference Harmonized guidelines the linearity can be calculated by using following equation:

$y = mx + b$

Where 'm' and 'b' are constants (parameters), the constant *m* determines the slope or gradient of that line and the constant term'b' determines the point at which the line crosses they-axis (y-intercept)

**Specificity**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. The term specific generally refers to a method that produces aresponse for a single analyte only. The response of the analyte in the test mixtures containing the analyte and all potential sample components (placebo formulation, synthesis, intermediates, excipients, degradation products, process impurities etc.) is compared with the response of the solution containing only the analyte. Specificity can be calculated by using following equation as per ICH guidelines.

$$\text{Specificity} = \frac{\text{Number of true negatives}}{\text{Number of true negatives+number of false positives}}$$

**Accuracy**

The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value. The accuracy can be calculated by following method:

$$\text{Percentage Recovery} = \frac{\text{Peak Area of the Drug in Standard}}{\text{Peak Area of the Drug in Sample Mix.}} \times 100$$

**System Suitability test**

The Reproducibility of sample was checked of the system to measurement of peak area and was carried out using three replicates of same concentration of standard and sample, respectively.

**Limit of Detection (LOD) and limit of Quantification (LOQ)**

According to ICH guidelines the detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.Limit of detection can be calculated using following equation as per ICH guidelines:

$$\text{Limit of Detection} = 3.3 \times \frac{\text{Standard deviation of the Peak Area of the Drug}}{\text{Slope of the Corresponding Calibration Curve}}$$

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.Limit of quantification can be calculated using following equation as per ICH guidelines:

$$\text{Limit of Quantisation} = 10 \times \frac{\text{Standard deviation of the Peak Area of the Drug}}{\text{Slope of the Corresponding Calibration Curve}}$$

## Results and Discussion

**Selection of mobile phase**

It was a basic need of liquid chromatography.To select the mobile phase,various composition of mobile phase (solvent) were checked with water and methanol (30:70, 40:60, 50:50 and 60:40 by volume) on $c_{18}$ column at wave length 215 nm. The mobile phase methanol and water (70:30, v/v) was selected. The chromatographic condition such as mobile phase, wave length and mobile phase were suitable for retention time and peak area of drug content.

**Chromatographic conditions**

A well defined chromatographic separation was obtained within a run time of 8.0 min.The retention time of Metoprolol Tartrate was 5.3min. The flow rate of mobile phase was 1.0 mL/min.

**System solubility test**

To establish the chromatographic conditions were performed System suitability test (SST) duringthedevelopment and optimization of the method.Thetestwasperformedby injecting the standard mixture in triplicate plates were computed as reported by USP and Internationalconferenceharmonized guidelinesSystemsuitabilityparameterswereshown in table1. The all parameter have been shown that the CV % less than 10. Thus the method is reliable for the analysis.

**Table No. 3. Summary of system suitability parameters**

| Sl. No. | Parameters | Mean | CV % |
|---|---|---|---|
| 1 | Retention Factor | 5.89 | 2.28 |
| 2 | Tailing Factor | 0.95 | 2.00 |
| 3 | Resolution Factor | 1.14 | 1.49 |
| 4 | Theoratical Plates | 1272 | 2.46 |

### Linearity

The detectorresponse for the proposed method was determined to be linear over the range of five concentration levels. The calibration curve was plotted as concentration of the respective drug versus the obtained average peak area.The linearity of the method was evaluated by linear regression analysis.The linear regression equation of proposed method was representing slope and intercept for Metoprolol Tartrate.The statistical data was calculated for Metoprolol Tartrate found tobe accurate, the results of linearity listed in table No 2.

### Limit of detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of method was determined by calculating the signal to noise for metoprolol tartrate is 3 and 10 respectively.The limit of detection (LOD) and limit of quantification (LOQ) values for metoprolol tartrate was found to be 0.02 µg/ mland 0.09 µg/ ml.

**Table No.4.The results of linearity, limit of detection (LOD) and limit of quantification (LOQ)**

| Sl.No. | Parameters | HPLC |
|---|---|---|
| 1 | Correlation range | 5-100 µg/mL |
| 2 | Regression equation | y=711.6x-183.1 |
| 3 | Regression coefficient ($R^2$) | 0.999 |
| 4 | Limit of detection (LOD) | 0.02 µg/ ml |
| 5 | Limit of quantification(LOQ) | 0.09µg/ ml |

### Specificity

The specificity ofthemethod was determined by checking the interference with the componentsfrom placebo. There is no interference was observed for any of the components like excipients of the drug content as shown in the following figure:

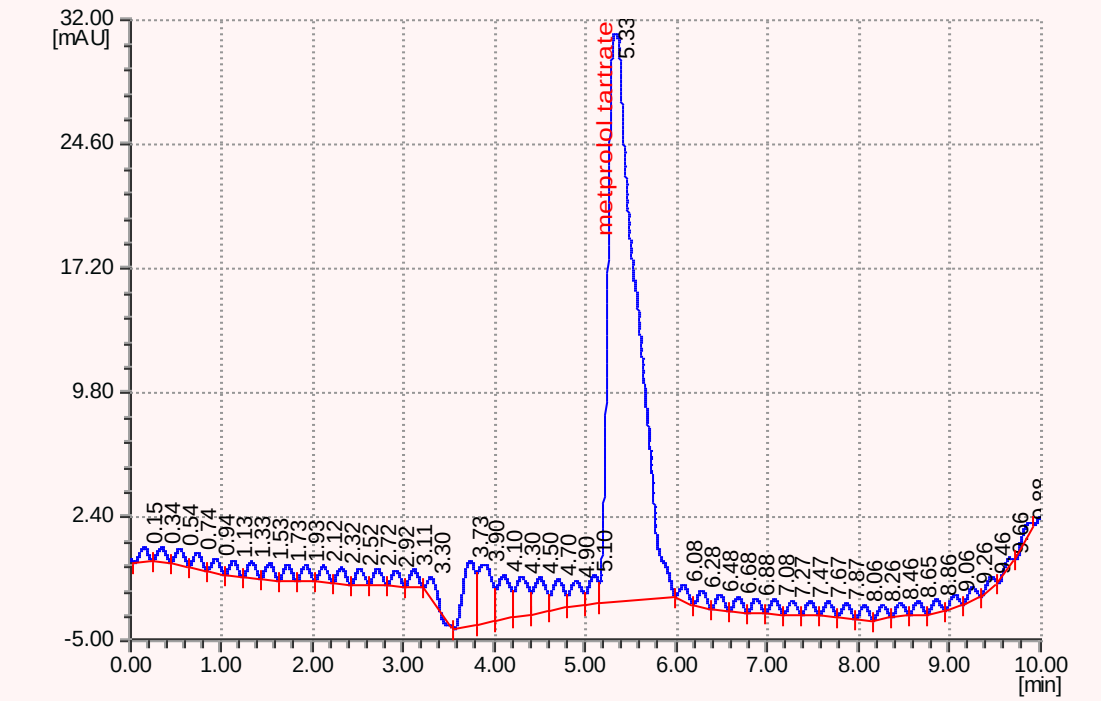


**Figure No. 2. Chromatogram of Metoprolol Tartrate**

**Accuracy**

The accuracy of the method was computed by determination of recovery for at three concentration levels.The amount of metoprolol tartrate was recovered and calculated.The recoveries ofmetoprolol tartrate was analysed by RP-HPLCmethod96.80,98.0 and 102.72 % .

**Table No. 3. Recovery experiment by reversed phase HPLC Method**

| Sl. No. | Added in µg | Recover in µg | Recovery in % |
|---|---|---|---|
| 1 | 5 | 4.84 | 96.8 |
| 2 | 10 | 9.8 | 98 |
| 3 | 25 | 25.68 | 102.72 |

The recovery results were shown that the applied method reliable for the estimation of drug content. Thus the experiment successful for the development and method validation for the simultaneous analysis of metoprolol tartrate from formulated tablets.

## Conclusion

The method validation and development of the method are suitable for assure the quality of Metprolol Tartrate content from formulated marketed tablets. This method can be successfully used for a routine bases analysis.The result also shows that the method could find practical application quality control tool for the simultaneous estimation ofMetprolol Tartrate from their dosage form in a quality control laboratory.

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