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Development and Validation of Spectrophotometric and RP-HPLC Method for Determination of Metoprolol Succinate

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Abstract : In this study, a simple, sensitive and highly accurate ultraviolet spectrophotometric and RP-HPLC method has been developed and validated for determination of metoprolol succinate in bulk and pharmaceutical formulations. The method is based on the measurement of the absorbance of metoprolol succinate solution in 0.1N HCL at 222 nm. Beer's law was obeyed in the concentration range of 2-10µg/ml. The slope, intercept and correlation coefficient were also calculated. For HPLC method validation the analyte was resolved by using a mobile phase [Phosphate buffer: acetonitrile in the ratio of (80:20, v/v)] at a flow rate 1 ml/minute, with a column of Agilent, eclipse XDB-C18, 150 mm× 4.6 mm, 5µm at a wavelength of 223 nm. The linear dynamic range for Metoprolol Succinate was 5ng/ml-100ng/ml. The limit of detection [LOD] and Limit of quantification [LOQ] for Metoprolol Succinate was found to be 25.5 ng.mL-1 and 96.22 ng.mL-1 respectively.
Keywords : Metoprolol succinate, Spectrophotometry, HPLC, linearity, validation.

Introduction

Metoprolol succinate (MS) is a β1-selective (cardioselective) adrenoreceptor blocking agent[1] used extensively in the treatment of hypertension, angina pectoris and coronary heart diseases has oral bioavailability of <50% perhaps because of its rapid first pass metabolism and degradation in colon.[2,3] MS is highly soluble throughout physiological pH. Drug solubility was 157 mg mL–1 in water (pH = 5.5) and 183 mg mL–1in 0.1 mol L–1 HCl solution (pH =1.0).[4]

Literature survey revealed that chromatographic[5] and spectrophotometric methods[6] were reported for estimation of metoprolol succinate individually or in combination with other drugs. Here we have made an attempt to validate both UV and HPLC methods for determination of metoprolol succinate.

Experimental

Chemicals and materials

Metoprolol succinate was obtained as a gift sample from Wockhardt Limited, Aurangabad. All

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chemicals and solvents were purchased from Thermofischer scientific Pvt. Ltd, Mumbai and were of analytical grade. Distilled water was used to prepare all solutions and HPLC grade water was used for HPLC method validation.Freshly prepared solutions were always employed.

Instrumentation

The UV-Visible Spectrophotometer Shimadzu UV-1800 was used. The sample solutions were recorded over the range of 200-400nm. Digital Weighing Balance of Shimadzu AX200 was used for weighing the samples. An Ultra Sonicator of Toshniwal process instruments, pvt. Ltd Ajmer was used for sonication of the drug solution.

The development and validation of the RP-HPLC method was performed on Agilent, eclipse XDB-C18, 150 mm× 4.6 mm, 5µm column. The work was carried out in an air-conditioned room maintained at temperature 20 °C. The flow rate was 1 ml/minute. The analytes were monitored at 223 nm.

Determination of λmax

The uv spectrum of metoprolol succinate was obtained by using shimadzu UV-1800, Japan. Accurately weighed 100mg of the drug and was dissolved in sufficient quantity of 0.1N HCL and volume was made upto 100ml .The stock solution was diluted to obtain a concentration of 100(µg/ml).This resultant solution was scanned from 200-400 nm and the spectrum was recorded to obtain the wavelength of maximum absorbance.

Preparation of calibration curve

The above prepared stock solution of 100 (µg/ml) was used to prepare further dilutions of 2, 4, 6, 8, 10(µg/ml).The absorbance of prepared dilutions were measured at 222nm using 0.1N HCL as blank by UV – visible spectrophotometer.

Mobile phase preparation for HPLC

Preparation of a buffer: weigh accurately about 6.8g of potassium dihydrogen phosphate (KH2PO4) and transfer it into 1000ml volumetric flask. Dissolve and dilute with HPLC grade water.

Transfer 800ml solution of buffer and 200ml of acetonitrile (80:20, v/v) into a 1000ml volumetric flask, dissolve and filter through 0.45 micron filter paper.

Preparation of stock solution: weigh accurately 10mg of MS into a 100ml of volumetric flask, dissolve and dilute with mobile phase. Further transfer 2ml of this solution into a 100ml of volumetric flask, dissolve and dilute to a volume with mobile phase. 5, 10, 25, 50, 75, 100 ng/ml of solution were prepared for linearity from this solution.

Results And Discussion

The resultant UV spectrum of metoprolol succinate solution (100 µg/ml) exhibited maximum wavelength of absorbance at 222nm, this complies with the reported one for the drug.

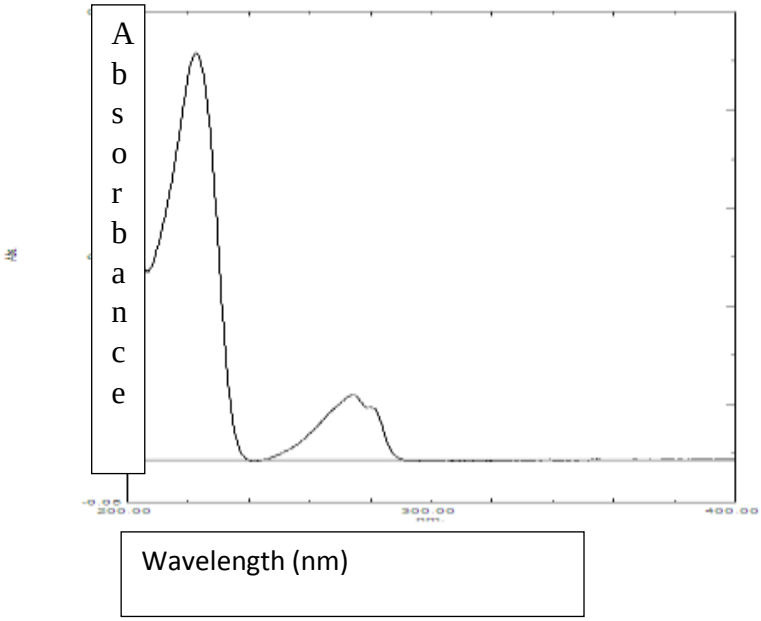

Fig.1: UV spectrum of MS in 0.1 N HCL

The high value of correlation coefficient ($R^2 = 0.9997$) indicates linearity and obeys Beer's law

Linearity study:

The linearity for the drug was determined between the range of 2-10(µg/ml).This study was done by preparing various dilutions of the drug solution in 0.1N HCL.[7]

Table. 1: Absorbance of metoprolol succinate in 0.1N HCL

Sr.No.	Concentration(µg/ml)	Absorbance
1	2	0.088
2	4	0.142
3	6	0.196
4	8	0.251
5	10	0.31

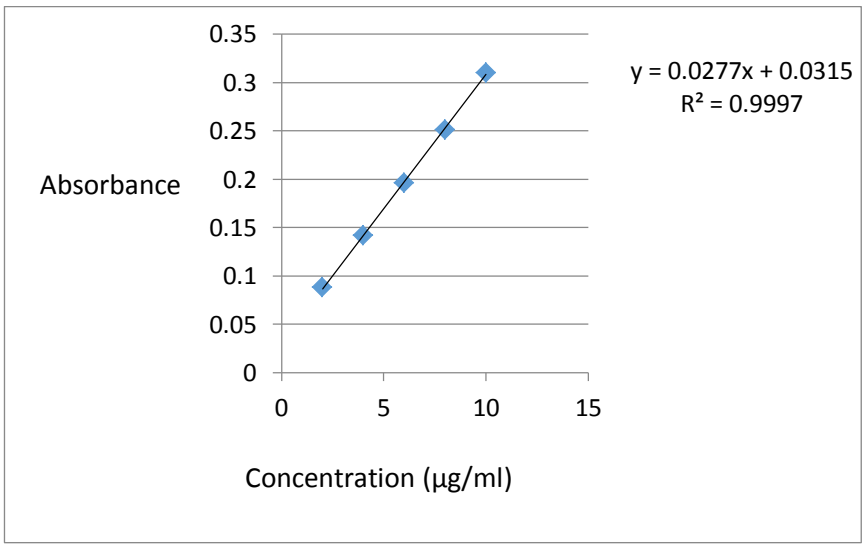

Fig.2: Calibration curve of Metoprolol succinate

Table. 2: Standard calibration curve statistics for metoprolol succinate

Sr.No.	Parameters	Observations
1	Maximum absorbance	222 nm
2	Slope	0.0315
3	Intercept	0.0277
4	Correlation coefficient (r^2)	0.9997
5	Equation	y = 0.0277x + 0.0315

Precision

Absorbance of the prepared dilutions for calibration curve were determined for three times in a day (morning, afternoon, evening) and the percent relative standard deviation was calculated for intraday study (which should be less than 2%).

The same procedure was repeated for three consecutive days for the interday study and the percent relative standard deviation should be less than 4% for interday study.[8]

Interday precision (n=3)

Table. 3: Interday precision

Concentration (µg/ml)	±Standard deviation n=3	%Relative Standard deviation
2	±0.00152	1.732
6	±0.0020	1.030
10	±0.0015	0.487

Intraday Precision (n=3)

Table.4: Intraday precision

Concentration (µg/ml)	±Standard deviation n=3	%Relative Standard deviation
2	±0.00057	0.655
6	±0.00057	0.292
10	±0.001	0.323

Accuracy

Accuracy study was done by spiking the drug (80%, 100%, 120% of the dose) into the placebo solution containing all other excipients of the formulation. The average % recovery was found to be 99.61%.

Accuracy (n=3)

Table. 5: Accuracy

Tablet Amount (µg/ml)	Level of Addition (%)	Amount Added (µg)	Drug found (µg/ml)	%recovery	Mean % recovery
10	80	8	7.93	99.21	99.61
10	100	10	9.93	99.37	
10	120	12	12.03	100.26	

Limit of detection (LOD) and Limit of quantitation (LOQ)

It was calculated from standard deviation and slope value using a formulae:

LOD=3.3*σ/slope

LOQ=10* σ/slope

Where σ is standard deviation.

LOD & LOQ (n=3)

Table. 6: LOD & LOQ

LOD(µg/ml)	LOQ(µg/ml)
0.261	**0.793**

Linearity study and calibration curve by HPLC method:

The calibration curve was plotted as response factor against concentration of MS. The equation for calibration curve is Y= 231.51X- 142.64 with a correlation coefficient of R^2 = 0.9998 for MS, where Y represents peak area and X represents analyte concentration.

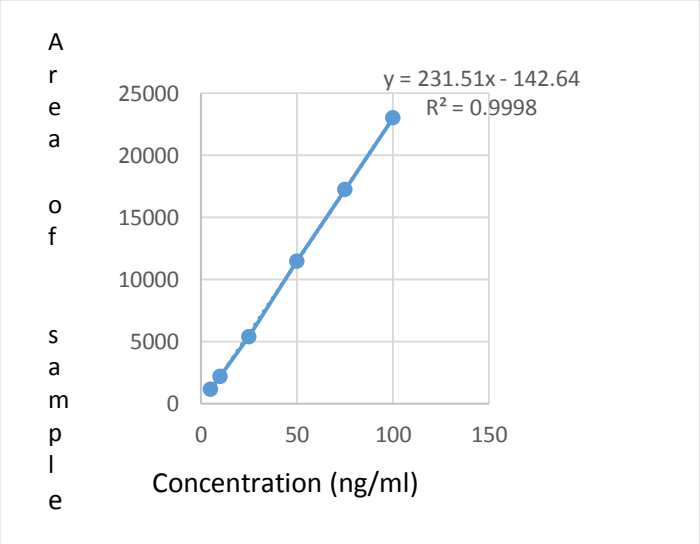

Fig. 3: Calibartion Curve of Metoprolol Succinate

Table. 7: Calibration curve data of the RP HPLC

Concentration ng/ml	Area mAU
5	1150
10	2200
25	5400
50	11485
100	17240
200	23020

Precision

Precision was determined by interday and intraday studies. Intraday studies were performed in the same day, whereas interday studies were done for three consecutive days. Response factor i.e area of the peak was determined in each study and % RSD of them were determined, for doing so the dilutions were prepared of

particular concentration and injected to the column of HPLC. The data reveals that the RP-HPLC method for MS was precise.

Table. 8: Intraday and interday % RSD

Sr.No.	%RSD	Observed value
1	intraday	0.654
2	interday	0.907

Accuracy:

The accuracy of an analytical method is the closeness of the test results obtained by the method to the true value.

The accuracy of the method can be determined by adding known amount of analyte to cover both above and below (80,100, 120%) the normal levels expected in the sample.

Accuracy should be assayed using a minimum of nine determinations over a minimum of 3 concentration levels. It should be reported as %recovery by assay of known added amount of analyte in the sample or as the difference between the mean and accepted true value together with the confidence intervals. RSD of each level must not be more than 2-3%.[9]

Table. 9: Accuracy study

Tablet Amount (µg/ml)	Level of Addition (%)	Amount Added (µg)	Drug found (µg/ml)	%recovery	Mean % recovery
10	80	8	7.85	98.12	99.56
10	100	10	9.99	99.90	
10	120	12	12.08	100.66	

Sensitivity

The Limit of Detection (LOD) is the lowest concentration giving response and Limit of Quantification (LOQ) is the lowest concentration analyzed with accuracy method and is determined by injecting progressively lower concentrations of the standard solution using the developed RP-HPLC method. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) for Metoprolol Succinate was found to be 25.5 ng.mL-1 and 0.096.22 ng.mL-1 respectively.

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