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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC AND RP-HPLC METHOD FOR METOPROLOL SUCCINATE

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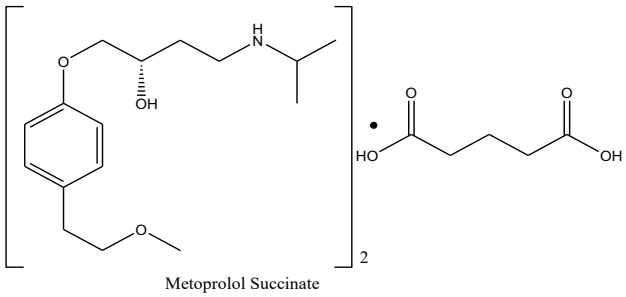
ABSTRACT

The aim of recent study was to develop a combined UV Spectrophotometric method and RP-HPLC method for metoprolol succinate, UV Spectrophotometric method was performed at 270 nm and samples were prepared with phosphate buffer solution pH 6.8, RP-HPLC method was performed using Alligent-1100 C18 column (Chemstation-32 software) at 30^0c with flow rate of 1.0 ml/min. at detection wavelength of 215 nm. Both the methods were validated as per ICH guidelines and various validation parameters like accuracy, precision, LOD, LOQ, recovery study and range were determined. The proposed methods were simple, rapid, precise, and accurate, and can be used for routine analysis of metoprolol succinate in bulk and combinations.

Keywords: Metoprolol succinate, UV Method, RP-HPLC Method

INTRODUCTION

Metoprolol Succinate is chemically (+) 1- (isopropyl amino)-3-[p-(2-methoxyethyl) phenoxy]-2- propanol succinate. It is white crystalline powder with formula C H NO and molecular weight is 652.8. It is a cardio selective drug used in the treatment of hypertension and various cardiovascular disorders. In the past various chromatographic and Spectrophotometric methods were reported for estimation of metoprololsuccinate individually or in combination with other drugs. In the present study UV spectroscopic and RP-HPLC methods were developed.[1-3]



Metoprolol Succinate

Figure 1

MATERIALS AND METHODS

UV Spectroscopic Method
Instrumentation

A UV-Visible Spectrophotometer (UV-1700 SHIMADZU) with 10 mm matched quartz cells was used for Spectrophotometric method. All weighing was done on electronic balance (Model Shimadzu AUW-220D).

Reagents and chemicals

Metoprolol succinate was received as gift sample from WOKHARDT Research Centre, Aurangabad. Tablet formulation manufactured by Biocon limited was purchased from local market Actiblock-IPR containing metoprolol succinate 50 mg per tablet.

Preparation of standard stock solution:

Standard drug solution of metoprolol succinate was prepared by accurately weighing 10 mg of the drug, and dissolved in phosphate buffer pH 6.8 and the volume was made up to 100 ml to obtain stock solution (100 µg/ml).[4]

Determination of Analytical Wavelength

From the standard stock solution 0.1 ml was pipette out into 10 ml volumetric flask. The volume was made up to 10 ml with phosphate buffer pH 6.8. The resulting solution containing 10 µg/ml was scanned between 200-400 nm.[4]

Preparation of Calibration Curve

Aliquots of 0.05 to 0.3 ml portions of stock solutions were transferred to a series of 10 ml volumetric flasks and volume made up to the mark with phosphate buffer pH 6.8. The serial dilutions in the range of 5, 10, 15, 20, 25 and 30 µg/ml were prepared. The absorbance was measured at λ_{max} 270 nm.[4]

UV Method Validation

Linearity and Range

The linearity of the response of the drug was verified at 5 to 30 µg/ml concentrations. The calibration curve was obtained by plotting the absorbance versus the concentration data and was treated by linear regression analysis. The equation of the calibration curve for metoprolol was obtained. [4-6,8-10]

Precision

The accuracy of the method was determined by recovery experiments. Each solution was repeated in triplicate and the percentage recovery was calculated. The precision of the method was demonstrated by intra-day and inter-day variation studies.[4]

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

LOD and LOQ were calculated by the equations;

$LOD = 3.3\sigma / S$ and $LOQ = 10\sigma/S$

Where S is the slope of the calibration curve and σ is the residual standard deviation.[4]

Recovery Study

Accuracy of the method was studied by recovery experiments. The recovery was performed at three levels, 80, 100 and 120% of metoprolol standard concentration. The recovery samples were prepared in a before mentioned procedure for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve.[4]

RP-HPLC Method

Instrumentation

Agilent 1100 Series HPLC Value System by Agilent Technologies Hewlett-Packard-Strasse 8 76337 Waldbronn Germany with UV detector was used for metoprolol with chemostation-32 software; the analytical column used to achieve chromatographic separation was a stainless-steel Agilent C18 RP column at 30^0c with flow rate of 1.0 ml/min.[2,7,11,12]

Preparation of buffer solution (0.05 M ammonium acetate)

Ammonium acetate about 3.85 gram was accurately weighed and dissolved in water and the volume was made up to 1000 ml. The pH was adjusted to 4.4 with H_3PO_4 (Orthophosphoric acid).

Mobile Phase

600 ml of buffer solution and 400 ml of methanol was measured separately and transfer into 1000 ml volumetric flask. Mixed and sonicated for 5 minutes. Degas and filtered through 0.45 um membrane filter.

Diluents

Mobile phase

Preparation of standard solution

Metoprolol succinate 10 mg was weighed accurately and transfer to 100 ml volumetric flask and volume was made with mobile phase

Sample Preparation

Accurately weighed 10 mg of sample metoprolol succinate was transferred in 100 ml volumetric flask. Mixed, sonicated and diluted to volume with mobile phase.

Chromatographic System

Table 1

Column	C18
Column temperature	30^0 C
Flow rate	1 mL per minute
Wavelength	215 nm
Injection volume	20 uL

RESULTS AND DISCUSSION

U V Spectroscopic Method

Precision

Precision of the method was evaluated for metoprolol. The reproducibility (inter-day precision) of the method and repeatability (intra-day precision) was evaluated in the same laboratory. The values obtained were 0.1935 and 0.2539 respectively (Table 2).

Table 2: Precision determinations

Precision	Intra-Day Precision*	Inter-Day Precision*
Result	0.2539	0.1935

Accuracy (Recovery Study)

Accuracy of the method was studied by recovery experiments. The recovery was performed at three levels 80, 100 and 120% of metoprolol standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery value for metoprolol was 99.71 ± 0.493 and RSD was 0.495 which is less than 2, which shows that the method has good reproducibility.

Table 3: Recovery Study (UV)

Formulation stock	Total conc	Drug Recovered	% Recovery	Mean % Recovery	SD	%RSD
8	18	18	100	99.62962963	0.641500299	0.6438851
8	18	17.8	98.88888889			
8	18	18	100			
10	20	19.8	99	99.66666667	0.577350269	0.5792812
10	20	20	100			
10	20	20	100			
12	22	22	100	99.84848485	0.262431941	0.2628302
12	22	22	100			
12	22	21.9	99.54545455			
			Mean	99.7149270	0.4937	0.4953

Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection and limit of quantification was found to be 0.104900 and 0.317880 respectively

Specificity

Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components (excipients). The results were compared with the analysis of a standard metoprolol and tablet formulations. Excipients of the solid dosage form did not interfere with the analyte.

Table 4: UV Method Validation Parameters

Parameter	Result
Linearity range	5-30 mcg/ml
Regression eq.	y = 0.0082 x -0.0037
Correlation coefficient	0.999961399
Slope (m)	0.02906
Avg % RSD	0.37461569
SD Average	0.00092376
λ_{max}	270 nm
LOD	0.104900531
LOQ	0.317880396
Molar absorptive	7792.273
Sandal's sensitivity	0.038167939
Intraday precision	0.253932495
Inter day precision	0.193590897

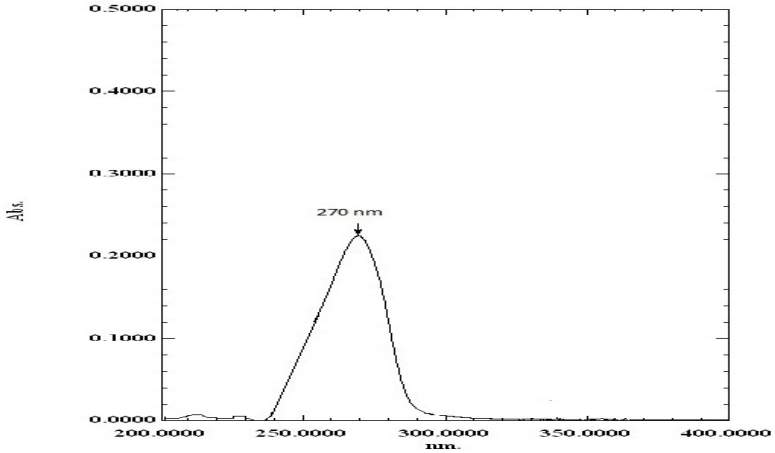


Figure 2: UV Spectrum of Metoprolol

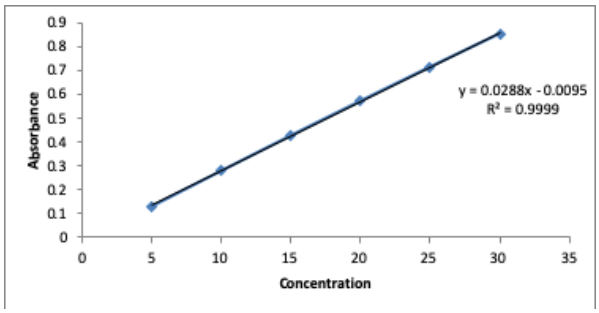


Figure 3: Calibration Curve (UV)

RP-HPLC Method

For getting the best resolution different mobile phase with different concentrations were tested on C18 column. From this the mobile phase selected was ammonium acetate buffer and methanol in the ratio of 60:40 v/v to obtain satisfactory and good resolution. The retention of Metoprolol Succinate on analytical column was evaluated at a flow rate of 1.0 mL.min^{-1}. The injection volume was 20 µL. The typical chromatogram of Metoprolol Succinate is shown in Figure 2. The retention time of standard and sample for Metoprolol Succinate was satisfactory with good resolution.

Method validation

Linearity

The linearity for HPLC method was determined at eight concentration levels ranging from 5-30 µg.mL^{-1} for Metoprolol Succinate. The calibration curve was constructed by plotting response factor against concentration of Metoprolol Succinate (Figure 3). The slope and intercept for calibration curve were Y = 4158.x-159.2 with a correlation coefficient (R^2 = 0.9996) for

Metoprolol Succinate, where Y represents the ratio of peak area ratio of analyte and X is the analyte concentration. From the results it was found that shown that significant correlation exists between response factor and concentration of drug in the range shown on Y-axis.

Sensitivity

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined at least concentration with accuracy method was determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) for Metoprolol Succinate was found to be 0.109 µg.mL^{-1} and 0.364 µg.mL^{-1} respectively.

Precision

The precision of the method was obtained by inter day and intraday variation studies. In the intraday studies, samples were injected same day from which the response factor of drug peaks and percentage RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated and presented in Table 5 from the data obtained, the developed RP-HPLC method was found to be precise.

Table 5: Precision

Precision	Intra-Day Precision*	Inter-Day Precision*
Result	0.3802	0.3619

Accuracy [Recovery studies]

Recovery study carried out for the drug was performed by spiking the known standard drug in powdered formulations. The assay procedure was repeated for standard and sample six times and mean peak area ratio and concentration of drug was calculated. The percentage of individual drug is found in formulation, mean, standard deviation in formulation were calculated. The results of the recovery analysis were found to be 99.7795 ± 0.169179 with %RSD of 0.205833 reported in Table 4. The results of analysis (Table 6) show that the amounts of drug were in good agreement with the labeled claim of the formulation.

Table 6: Recovery Study (HPLC)

Formulation stock	Total conc	Drug Recovered	% Recovery	Mean % Recovery	SD	%RSD
8	18	17.99	99.9657	99.7656	0.176177	0.2142
8	18	17.91	99.537			
8	18	17.96	99.7942			
10	20	20	100	99.8199	0.158549	0.1928
10	20	19.92	99.6142			
10	20	19.96	99.8457			
12	22	21.99	99.9647	99.7530	0.172811	0.2105
12	22	21.94	99.7531			
12	22	21.89	99.5414			
			Mean	99.7795	0.169179	0.205833

Specificity

Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components (excipients). The results were compared with the analysis of a standard metoprolol and tablet formulations. Excipients of the solid dosage form did not interfere with the analyte.

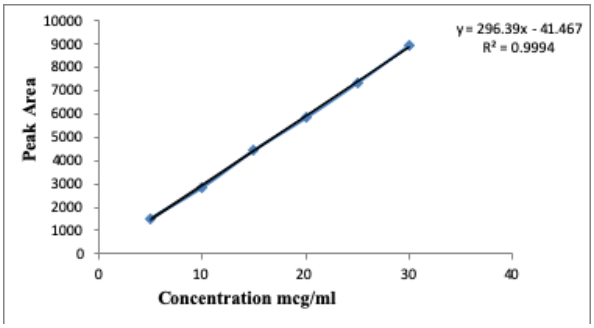


Figure 4: Calibration Curve HPLC

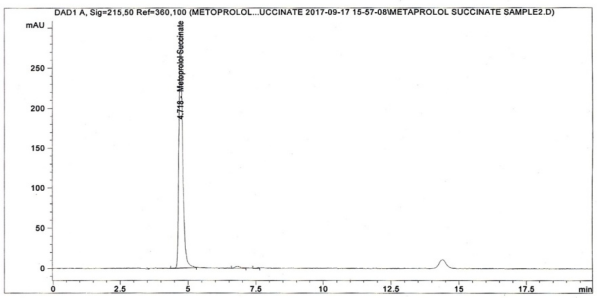


Figure 6: HPLC Chromatogram

CONCLUSION

A combined UV Spectrophotometric method and RP-HPLC method for metoprolol succinate, was developed and validated as per ICH guidelines for the determination of Metoprolol Succinate in dosage formulations. It was shown above that, the proposed method was linear, accurate, reproducible, repeatable, precise, selective, specific and cost effective proving the reliability of the method. Moreover, same solvent was used throughout the experimental work and it was found that no interference from any excipients was observed in both the methods. Hence, the proposed method was successfully applied to routine analysis of Metoprolol Succinate in bulk and combined formulations.

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