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Validated RP-HPLC Method for the Estimation of Metoprolol Succinate in Dosage Formulations

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ABSTRACT

A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the determination of Metoprolol Succinate in dosage formulation. The analyte was resolved by using a mobile phase (Acetonitrile, water and 1 % ortho phosphoric acid in the ratio 70:27:3 v/v/v) at a flow rate 2.0 ml/min on an isocratic HPLC system (Agilent 1100 series with Chemstation software) consisting UV lamp detector, Aligent C-8, RP column (4.6 mm i.d x250 mm) at a wavelength of 280 nm. The linear dynamic range for Metoprolol Succinate was 10 g/mL–200µg/mL. The limit of detection [LOD]and Limit of quantification[LOQ] for Metoprolol Succinate was 0.0284µg/mL and 0.094µg/mL respectively.

Keywords: Metoprolol Succinate , HPLC, linearity, validation.

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INTRODUCTION

Metoprolol Succinate[1-4], (Rs)-1-(Isopropylamino)-3-[4-(2-methoxyethyl) phenoxy] propan-2-ol is a cardio selective drug used in the treatment of hypertension and various cardio vascular disorders. Literature survey revealed that chromatographic[5] and spectrophotometric methods [6-10] were reported for estimation of metoprolol succinate individually or in combination with other drugs. The present work describes a simple RP-HPLC method using Aligent C_8 column for determination of Metoprolol Succinate in tablet dosage form. The method was validated as per ICH guidelines (2006).

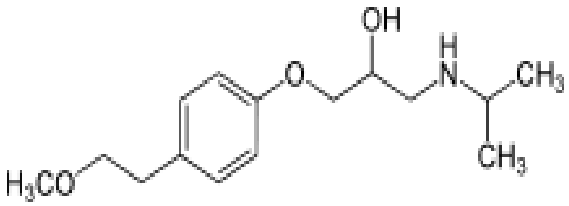

Figure.1: Structure of Metoprolol Succinate

MATERIALS AND METHODS

Chemicals and materials:

The pharmaceutical grade pure sample of Metoprolol Succinate (99.28%) was procured from CELON Laboratories limited, Andra Pradesh. Acetonitrile solvent of analytical grade was obtained from E Merck Ltd, Mumbai, India. Ortho phosphoric acid (AR grade) was procured from Qualigens Fine Chemicals, Mumbai, India. The HPLC grade water was obtained from a Milli-QRO water purification system, sonicated and used.

Instrumentation:

The development and validation of the method was performed on a isocratic HPLC system Agilent 1100 series) consisting of Isocratic liquid pump, LC 8200 variable wavelength UV detector with Chemstation software on a Dell computer. The analytical column used to achieve chromatographic separation was a stainless steel Aligent C_8 RP column (4.6mmx250mm)) purchased from Waters Corporation (Bedford, MA, USA) protected by a guard column of the same material. The work was carried out in an air-conditioned room maintained at temperature 25±2 °C. The flow rate was 2.0 mL min-1.The analytes were monitored at 280 nm and run time was 3.688min.

Standard stock solution:

Prepared by dissolving 50.0mg of Metoprolol Succinate to 100ml with mobile phase. A series of working standard solutions (10µg/mL -200µg/mL were obtained by diluting the stock

solutions with mobile phase (acetonitrile, water and 1% ortho phosphoric acid in the ratio of 70:27:3 v/v/v). All the volumetric flasks containing Metoprolol Succinate were wrapped with aluminium foil and stored in the dark.

Preparation of solution containing the drug in tablets:

50 mg of Metoprolol Succinate tablet powder was transferred into 100 mL volumetric flask.To this 5.0 mL of water was added to dissolve the tablet powder. 20 ml of 0.1N Hydrochloric acid was added and sonicated for 30 Minutes and then made upto the mark with 0.1N Hydrochloric acid. 10ml of above solution was diluted to 100ml with mobile phase. A sample of 40µL of this solution was directly injected. The average content of the tablets was determined either from the calibration graph or using the corresponding regression equation.

Chromatographic conditions:

The mobile phase was filtered by passing through a 0.45µm membrane filter (Millipore, Bedford, MA, USA).Chromatographic analysis was carried out at ambient temperature. The compounds were separated isocratically with a mobile phase consisting of acetonitrile, water and 1% ortho phosphoric acid in the ratio of 70:27:3 v/v/v). The flow rate was 2.0 mL/min. The effluent was monitored spectrophotometrically at a wavelength of 280nm. The optimized chromatographic conditions for the determination of Metoprolol Succinate are represented in Table.1.

Table 1: Optimized chromatographic conditions

Parameters	PEAK HPLC
Elution	Isocratic
Mobile phase	Acetonitrile: water : 1 % ortho phosphoric acid (70:27:3 v/v/v)
Column	Agilent C_8 (250 x 4.6 mm) column
Flow rate	2.0 ml/ min
Detection	UV at 280 nm
Injection volume	40 micro liters
Temperature	Ambient
Retention time	3.94 minutes
Run time	10 minutes
Area	199617.27 mAU
pH	3.0

RESULTS AND DISCUSSION

METHOD DEVELOPMENT:

Several tests were performed in order to get satisfactory separation-resolution of Metoprolol Succinate in different mobile phases with various ratios by using C_8 column. The ideal mobile phase used is acetonitrile, water and 1% ortho phosphoric acid in the ratio of 70:27:3 v/v/v to obtain satisfactory and good resolution. The retention of Metoprolol Succinate on analytical

column was evaluated at a flow rate of 2.0 mL.min^{-1}. The injection volume was 40µL. 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.

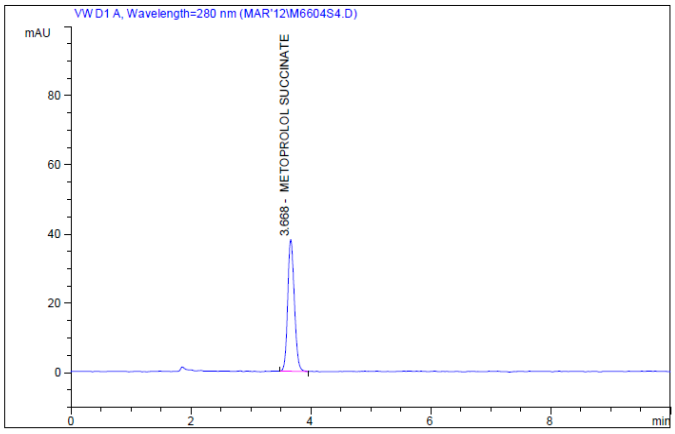

Figure 2: HPLC Chromtogram of Metoprolol Succinate

METHOD VALIDATION:

Linearity:

The linearity for HPLC method was determined at eight concentration levels ranging from 10 - 200.0µg.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 an correation coefficient (R^2 = 0.9996) for Metoprolol Succinate, where Y represents the ratio of peak area ratio of analyte and X represents analyte concentration. The results were satisfactory shown that significant correlation exists between response factor and concentration of drug within the concentration range indicated on Y-axis(Table.2).

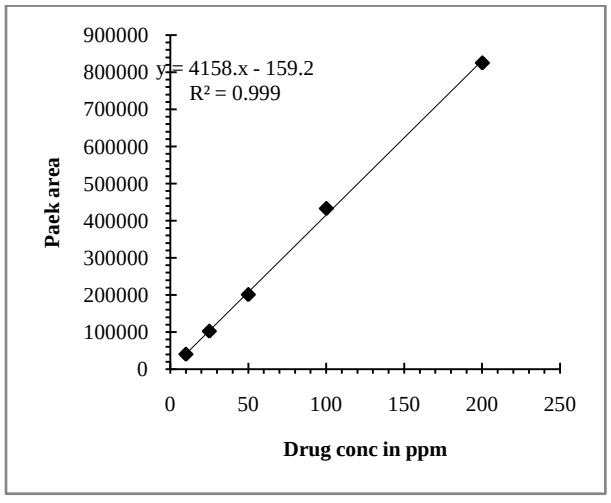

Figure 3:Calibartion Curve of Metoprolol Succinate

Table 2:Calibration of the RP HPLC for the estimation of Metoprolol Succinate

Concentration in µg.mL^{-1}	**Area (mAU)**
10	39844.400
25	101946.700
50	200561.450
100	432657.540
200	825065.140
Regression equation	Y = a X + b
Slope (a)	159.2
Intercept (b)	4158.2
Correlation coefficient	0.9996

Sensitivity:

The Limit of Detection (LOD) was determined as lowest concentration giving response and Limit of Quantification (LOQ) was determined as the lowest concentration analyzed with accuracy method were 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 0.028µg.mL^{-1} and 0.094µg.mL^{-1} respectively.

Precision:

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies, three repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the interday 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.3. From the data obtained, the developed RP-HPLC method was found to be precise.

Table 3: Precision data

Injection No.	**Area response**
1	191879.280
2	191512.220
3	192002.890
Mean	191798.130
%RSD	0.133
STDEV	255.202

All the values are the averages of three determinations

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 found in formulation, mean, standard deviation in formulation were calculated. The results of the recovery analysis were found to be 99.83 ± 1.120 to 100.07 ± 0.134 reported in Table.4. The results of analysis (Table 5) shows that the amounts of drug were in good agreement with the labeled claim of the formulation.

Table 4:Recovery studies of the proposed HPLC method

Labeled amount µg/ml	Amount added µg/ml	Total amount µg/ml	Amount found µg/ml	% of Recovery	Mean
10	2	12	11.98	99.83%	
10	4	14	14.01	100.07%	99.88%
10	6	16	15.96	99.75%	

All the values are the averages of three determinations

Table 5:Results of analysis of tablet containing Metoprolol Succinate

Pharmaceutical formulation	Amount of Metoprolol Succinate		% of recovery
	Labeled	Found*	
(TOPROL-XL)	10.0 mg	9.97	99.70 %

*Values are the averages of three determinations

Ruggedness and Robustness

Ruggedness test was carried out between two analysts, instruments and columns. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation (Table 6) indicating that the method was rugged and robust.

Table 6: Ruggedness of the proposed HPLC method

S.No	Parameter	Analyst-1	Analyst-2
1	Linearity	0.999	1.000
2	Repeatability(STD)	0.475	0.531
3	Repeatability(TEST)	0.487	0.230

CONCLUSION

A new, reversed-phase HPLC method has been developed for the determination of Metoprolol Succinate in dosage formulations. It was shown above that, the proposed method was linear, accurate, reproducible, repeatable, precise, selective and specific proving the reliability of the method. The run time is relatively short, i.e. 3.688 min, which enable rapid determination of many samples in routine and quality control analysis of tablet formulations. More over same solvent was used throughout the experimental work and no interference from any excipient was observed. Hence, the proposed method was successfully applied to analyze pharmaceutical preparation containing Metoprolol Succinate.

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