Original Research Article

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF METOPROLOL TARTRATE

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ABSTRACT-

The objective of present work was to develop an UV spectrophotometric method for determination of Metoprolol Tartrate in bulk and Tablet dosage form. In this method Dist water, Phosphate Bufffer 6.8 and 0.1 N HCl used as a solvent. In all the solvent Metoprolol Tartrate shows absorption maxima at 222 nm. The Beer's law range for Distilled water, Phosphate buffer 6.8 and 0.1 N HCl was in 5-30 µg/ml. This method was validated as per International Conference on Harmonization (ICH) guidelines. Low values of %RSD for intra- and inter-day precision suggested reproducibility of the method. Satisfactory values of percent recovery indicated accuracy of the method. Sensitivity of the method was proved by low value of Limit of Detection and Limit of Quantitation. Regression analysis of the calibration data showed a good correlation coefficient. The method was found to be simple, accurate, precise and economical.

Keyword: Metoprolol Tartrate, UV, Phosphate Buffer 6.8.

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INTRODUCTION-

Chemically Metoprolol Tartrate (MT) is 1-[4-(2-Methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-2-propanol hemitartrate with Molecular formula $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ & molecular weight 684.81 [1]

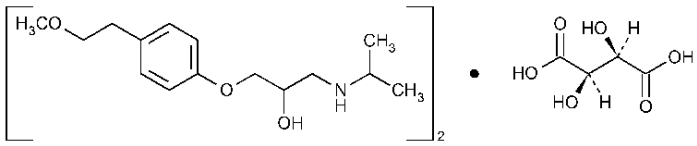

FIG 1- Structure of Metoprolol Tartrate

Metoprolol Tartrate is a cardioselective β1-adrenergic blocking agent used for acute myocardial infarction (MI), heart failure, angina pectoris and mild to moderate hypertension. It may also be used for supraventricular and tachyarrhythmias and prophylaxis for migraine headaches. At low doses, metoprolol selectively blocks cardiac β1-adrenergic receptors with little activity against β2-adrenergic receptors of the lungs and vascular smooth muscle.Membrane-stabilizing effects are only observed at doses much higher than those needed for β-adrenergic blocking activity. The drug is quite sensitive, even a small dose of the drug giving a sufficient blockade of the beta-adrenergic receptors..[2].
It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP). USP [3] , BP [4] and IP [5]

From Literature survey several methods have been reported for determination of Metoprolol succinate by using spectrophotometry [8], Capillary Electrophoresis [10], HPLC [11] and Bioanalytical methods[13].Very

few methods reported for estimation of MT using HPLC [12]. No single method was reported for Metoprolol tartrate using UV Detection.

Analysis is an important component in the formulation and development of any drug molecule. A suitable and validated method has to be available for the analysis of drugs in bulk, in drug delivery systems, release dissolution studies and in biological samples.
Thus the present study involved development of spectrophotometric method for determination of Metoprolol Tartrate in Bulk sample. The report describes simple, sensitive, accurate, precise, rapid and economic spectrophotometric method. For Metoprolol tartrate the quantitative determination of the drug is important and a simple validated method such as the UV-VIS absorption for the assay would be of a great interest.

MATERIALS AND METHODS

Equipment-

UV-Vis spectrophotometer - Shimadzu Corporation with model no 1800,

Software- UV-probe 3.43 and was employed with Wavelength Range: 190 to 1100nm.

All weighing were done on single pan balance (Shimadzu).

The gift sample of Metoprolol Tartrate was produced from Ipca Laboratories Limited Kandivli (West) Mumbai. The commercial Tablet dosage form was available from Metolar (25 Mg metoprolol Tartrate) of Cipla limited

Preparation of Standard Solutions-

Aqueous solutions of phosphate buffer of pH 6.8, 0.1N hydrochloric acid were prepared as per Indian Pharmacopoeia (edtn) .

Standard drug solution was prepared (1mg/ml) in Distilled water, 0.1NHcl,

Phosphate Buffer6.8.

Standard solutions were scanned in the UV-VIS spectrophotometer in the wavelength range

200-400 nm. The λmax was determined in respective solvent and found to be 222 nm.

Stock Solutions were prepared by 10 mg of Metoprolol tartrate was dissolved in 100 ml of

Distill water,0.1 N HCl , Phosphate Buffer 6.8.

Stock solutions were further diluted in Dist. water, 0.1 N HCl, Phosphate buffer 6.8. to obtain

Desired concentration 5, 10, 15, 20, 25, 30 µg/ml respectively.

The absorbance was measured in respective solvents against 222 nm & calibration curve was plotted as concentration versus absorbance over the range of 5-30 µg/mL with correlation

Coefficient 0.999, 0.999, and 0.997 respectively.

Method Validation Validation of an analytical procedure is the process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirements for the intended analytical application. The proposed method was validated for various parameters such as linearity, precision, accuracy, Limit of detection (LOD), Limit of Quantitation (LOQ) according to ICH Q2 (R1) guidelines [16].

Linearity and range Linearity was studied by diluting stock standard solutions of MT with respective solvents to give a concentration range of 5 to 30 µg/ml. Calibration curve of Absorbance vs. Concentration was plotted using standard solutions of 5µg/ml to 30µg/ml and regression line equation and correlation coefficient was determined. The range of solution has been decided according to statistical analysis of regression equation. Calibration curve for drugs are shown in Table No (1, 2), Figure no (1, 2, 3)

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [16]. Precision of the method was studied by intra- and inter-day variations method . Intraday precision was evaluated by assaying six different sample

preparations on the same day. Interday precision was performed by assaying six different sample preparations on different days at different time intervals. The percentage relative standard deviation (%RSD) was calculated (table no 3, 4).

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness [16]. The method was applied to drug sample and accuracy of the method was determined by calculating recovery of Metoprolol at 80%, 100% and 120% level of label claim. Percentage recovery was calculated using equation for the method and the results are presented in (Table no 5).

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Six sets of known concentrations (5-30 µg/ml) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the formulae as

***LOD*=**

LOQ=

Where, S is value of slopes of calibration plot and SD is calculated using values of y intercepts of regression equations. The results of LOD and LOQ are given in (table no 2)

RESULTS AND DISCUSSION

The maximum absorption for Metoprolol Tartrate in distilled water, Phosphate buffer 6.8, and in 0.1N HCL were observed at 222nm .Hence λmax for Metoprolol Tartrate is 222nm.

The high values of correlation coefficient in distilled water, Phosphate buffer and in 0.1N HCL indicates linearity for Metoprolol Tartrate in all three solvents. Beer's law was obeyed for distilled water, Phosphate buffer 6.8 and 0.1N HCl in the range of 5-30 µg/ml.

The accuracy of method was determined by calculating mean percentage recovery and

(% relative error). It was determined at 80,100 and 120 % level of standard dilution 10 µg/ml.

Precision was calculated as repeatability , inter and intraday variations for Metoprolol Tartrate, %RSD was found to be less than 1.

The repeatability data are presented in [Table no 3 and 4]. LOD was found to be 0.1619 µg/ml for detection of Metoprolol Tartrate in water, 0.1112 µg/ml in phosphate buffer 6.8 and 0.4278 µg/ml in 0.1 N

HCl. LOQ was found to be 0.4908 µg/ml in water 0.3372 in phosphate buffer 6.8 and 1.2964 µg/ml in 0.1 N HCl.

Table 1-Absorbance of MT at 222 nm for Dist water, phosp. buffer 6.8 and 0.1 N HCl-

Concentration (µg/ml)	**Absorbance at 222 nm**		
	Dist water	**Phosphate Buffer 6.8**	**0.1 N HCl**
5	0.134	0.178	0.16
10	0.262	0.325	0.273
15	0.405	0.469	0.401
20	0.516	0.648	0.522
25	0.646	0.803	0.66
30	0.802	0.955	0.819

Table 2- Linearity Parameters-

Parameters	**Result**		
	Dist water	**Phosphate Buffer 6.8**	**0.1 N HCl**
λ max	222 nm	222 nm	222 nm
Slope	0.0260	0.0317	0.0262
Intercept	0.0045	0.0071	0.0148
Correlation Coefficient	0.999	0.999	0.997
LOD	0.161991	0.111289	0.427827
LOQ	0.490881	0.337239	1.296446

Precision-

Table -3 Intraday variability-

Concentration (µg/ml)	**Absorbance**			**Mean**	**Standard deviation**	**%RSD**
	Trial I	**Trial II**	**Trail III**			
10 (DW)	0.262	0.261	0.261	0.2616	0.0027	0.2206
10 (Phos. Buff 6.8)	0.325	0.323	0.325	0.3243	0.00115	0.356
10 (0.1 N HCl)	0.273	0.272	0.273	0.2726	0.00057	0.2117
20 (DW)	0.515	0.516	0.517	0.516	0.001	0.1938
20 (Phos. Buff 6.8)	0.648	0.65	0.648	0.6486	0.0011	0.178
20 (0.1 N HCl)	0.521	0.523	0.522	0.516	0.001	0.1916
30 (DW)	0.802	0.800	0.802	0.8013	0.0011	0.1441
30 (Phos. Buff 6.8)	0.953	0.955	0.954	0.954	0.001	0.1048
30 (0.1 N HCl)	0.818	0.820	0.819	0.819	0.001	0.1221

Table 4- Interday variability -

Concentration (µg/ml)	Absorbance			Mean	Standard deviation	%RSD
	Trial I	Trial II	Trail III			
10 (DW)	0.262	0.264	0.266	0.264	0.002	0.7576
10 (Phos. Buff 6.8)	0.324	0.327	0.328	0.3263	0.00208	0.6308
10 (0.1 N HCl)	0.273	0.275	0.278	0.2753	0.002517	0.9142
20 (DW)	0.515	0.522	0.517	0.518	0.003606	0.6961
20 (Phos. Buff 6.8)	0.648	0.651	0.653	0.6506	0.002517	0.00416
20 (0.1 N HCl)	0.522	0.525	0.527	0.5246	0.01257	0.2396
30 (DW)	0.802	0.809	0.806	0.8056	0.003512	0.4359
30 (Phos. Buff 6.8)	0.953	0.957	0.959	0.957	0.002	0.2089
30 (0.1 N HCl)	0.818	0.822	0.821	0.8206	0.00152	0.1852

Accuracy- Table 5-

- **Distill Water**
- **Phosphate Buffer 6.8**
- **0.1 N HCl**

Parameters	Leval Of Recovery		
Conc. used µg/ml	08	10	12
Conc. found	7.96	9.92	12.08
%Recovery coefficient $_x$	99.5	99.2	100.6
%Relative error $_y$	0.5	0.8	0.666

(a)

Parameters	Leval Of Recovery		
Conc. used µg/ml	08	10	12
Conc. found	7.99	9.94	11.99
%Recovery coefficient $_x$	99.87	99.4	99.91
%Relative error $_y$	0.125	0.6	0.0866

(b)

Parameters	Leval Of Recovery		
Conc. used µg/ml	08	10	12
Conc. found	7.95	9.98	11.99
%Recovery coefficient $_x$	99.37	99.8	99.87
%Relative error $_y$	0.625	0.2	0.0833

(c)

x recovery coefficient =100 x (amount found)/(amount known) [r]

y % relative error [=100 × (predicted concentration – nominal concentration)/ nominal concentration]. [r]

Fig 1- Calibration curve of MT In Dist water

Fig 2- Calibration curve of MT In Phosphate Buffer 6.8

Fig 3- Calibration curve of MT In 0.1 N HCl

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