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Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ASSAY AND DISSOLUTION OF METOPROLOL SUCCINATE EXTENDED RELEASE TABLETS

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ABSTRACT

Objective: In this study, a reverse phase high performance liquid chromatographic method have been developed and validated for dissolution profiling of Metoprolol Succinate in extended release tablet dosage forms.

Methods: As per official records, the dissolution media for ER Tablets is 6.8 PO_4 Buffer and for 20 hours time duration, on USP II (Paddle) Dissolution apparatus, at 50 RPM and at 37.0°C ± 0.5 °C. The chromatographic development for Dissolution samples was achieved in a SHISEIDO CAPCELLPAK, CAP C-18, 4.6mm X 250mm, 5µ, (Column No AKAB06451) as a stationary phase and ACN: Buffer in a ratio of 30: 70 (0.05M Phosphate Buffer of pH 3.0) as eluent, at a flow rate of 1.0 ml/min. UV detection was performed at 225 nm.

Results: The retention time of metoprolol was found to be 4.9 min. The results of analysis were validated statistically and by recovery studies. Linearity, accuracy and precision were acceptable in the ranges of 5-25µg/ml.

Conclusion: The method was found suitable for Dissolution profiling and the results of Percentage Drug Release were within the USP Dissolution Limits. The results of the studies showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which can be used for the routine Dissolution Profiling of Metoprolol Succinate in ER-Pharmaceutical dosage forms (Tablets).

Keywords: Dissolution, Liquid chromatography, RP-HPLC, Validation, Metoprolol Succinate, ER Tablets.

INTRODUCTION

Metoprolol Succinate is 1- isopropylamino-3-p-(2- methoxyethyl) phenoxypropane-2-ol Succinate, is a beta- adrenocepter antagonist. It is a official drug and also listed in the Merck Index with Merck no. 6151 [1]. Metoprolol (also named as metroprolol) works by reducing the amount of work the heart has to do and the amount of blood the heart pumps out, thereby reducing the demand for oxygen and lowering blood pressure. It is official in Indian Pharmacopoeia [2], British Pharmacopoeia [3] and United States Pharmacopoeia [4]. Literature survey reveals spectrophotometric method [5], [6] and RP-HPLC method [7], [8] for its assay but there is no RP-HPLC estimation for dissolution profiling of metoprolol in different dissolution media. This paper presents simple, accurate and reproducible an RP-HPLC method for determination of metoprolol in tablet dosage form in different dissolution media. The reported method is helpful in determination of metoprolol during dissolution study.

MATERIALS AND METHODS

Materials

Metoprolol was received as gift sample from Glenmark Pharmaceuticals Ltd., SEZ, Pitampura, Indore, Madhya Pradesh, India. All other chemicals used were of HPLC Grade or pharmaceutical or analytical grade purchased from local supplier (Merck). The marketed preparation of Metoprolol Succinate ER Tablets was purchased from local market.

Instrument

The Scan Graph for absorbance maxima measurements were made on double beam UV visible spectrophotometer (Shimadzu, Kyoto, Japan, model UV - 1800) with matched quartz cuvettes. Chromatographic data was acquired on Gradient controlled CYBER LAB, USA RP-HPLC, LC-P100 PLUS (Pump Serial No. 08090870) with UV Detector LC-UV100 PLUS (UV Detector Serial No.08090665). Stationary phase column used was SHISEIDO CAPCELLPAK, CAP C-18, 4.6mm X 250mm, 5µ, (Column No AKAB06451). Electrolab Tablet Dissolution Tester TDT-06P was used for dissolution studies.

Methods

Preparation of standard drug solution: The stock solution (100 µg/ml) of metoprolol was prepared by dissolving accurately about 10mg of pure drug in 25 ml of methanol and shake for 15 min and the volume was made up to 100 ml with phosphate buffer pH 6.8 and then further suitable aliquots were made in dissolution media, filtered through Millipore filter (0.45micron) and sonicated before use.

Mobile Phase

Finally optimized mobile phase was prepared by mixing ACN: Buffer in a ratio of 30:70. 0.05M Phosphate Buffer of pH 3.0, filtered through 0.45µ Millipore filter, sonicated and used.

Dissolution Media

Extended Release tablets are studied in dissolution media of 6.8 PO4 Buffer for 20 hours as per official records. So, the dissolution profile for Metoprolol Succinate ER Tablets was conducted in 6.8 PO4 Buffer for 20 hours. Dissolution parameters are given in Table 1.

Study of Absorbance Maxima

5ml of the stock solution was further diluted to 50ml with 6.8PO_4 buffer to obtain sample solutions of concentrations within Beer-Lambert's range and this solution was scanned in the wavelength range of 200-400nm. UV-spectra are presented in Figure 1.

Selection of Chromatographic Conditions

Various combinations of various solvents in different proportion were studied for best suitable chromatographic conditions. Finally optimized chromatographic conditions are given in Table 2.

Calibration Curve

Calibration curve was prepared in the concentration range of 5-25 ppm, for which the coefficient of regression was found to be near 1. The results are shown in Table 4 and Figure 3.

Table 1: Dissolution Parameters

Parameters	Description
Dissolution Apparatus Type	USP II (Paddle)
Dissolution Media	6.8 PO_4 Buffer, 500ml
RPM	50
Time Points	1,4,8, 20 Hours
Temperature	37.0°C ±0.5 °C
Sample Volume	10 ml
Filter	0.45µ Teflon Syringe filter

Table 2: Chromatographic Conditions

S. No.	Parameters	Optimized Conditions
1.	Column	ODS, 5 µ, 4.6X250 mm
2.	Mobile Phase	ACN: Buffer:: 30: 70
3.	Flow Rate	1.0 ml/min.
4.	Wavelength of Detection	225 nm
5.	Injection Volume	40 µl

System Suitability Parameter: System suitability parameters are shown in Table 3.

Table 3: System Suitability Parameters

Parameters	Observation	Limit
Retention Time	4.9	-
Theoretical Plates	3769.71	>2000
Asymmetry Factor	1.09	<1.5
Tailing Factor	1.48	<2.0

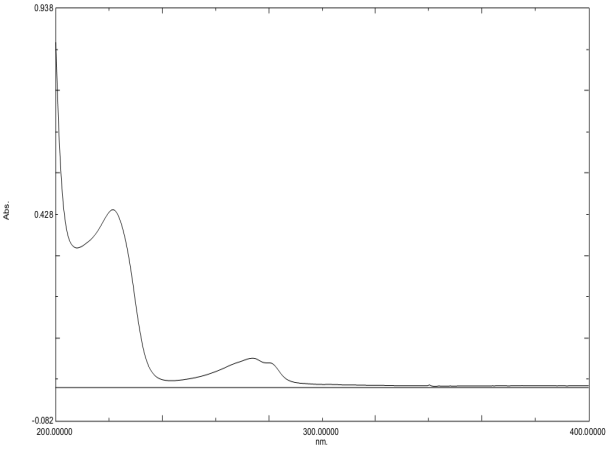

Fig. 1: UV-Spectra of Metoprolol Succinate in 6.8PO_4 buffer.

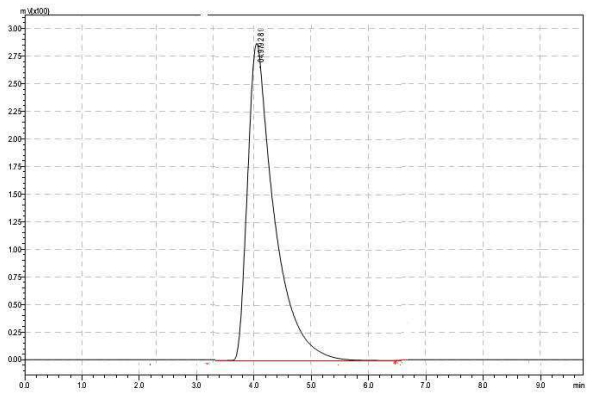

Fig. 2: A typical RP-HPLC Chromatogram for Metoprolol Succinate

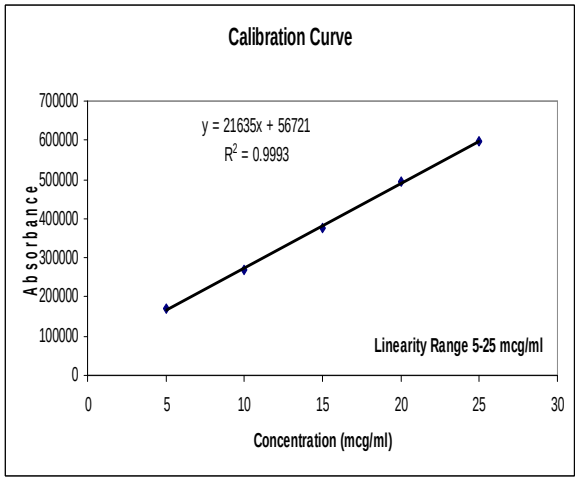

Fig. 3: Calibration curve of Metoprolol Succinate

Assay of Tablet Formulation

Twenty tablets were individually weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 10 mg Metoprolol was taken in 100 ml volumetric flask and dissolved in 25 ml of methanol and shake for 15 min; it was further diluted up to the mark with 6.8PO_4 buffer. The solution was mixed and filtered and 5ml of the filtrate was further diluted to 50ml with 6.8PO_4 buffer to obtain sample solutions of desired concentrations. The HPLC chromatogram of resulting solution was measured at 225nm wavelengths for the estimation of metoprolol. The results of the assay are shown in Table 5.

The *in vitro* drug release rate method of tablet is official in USP. It was carried out using USP dissolution testing apparatus II (paddle type) at 50 rpm. The dissolution test was performed using 500 ml of phosphate buffer (6.8) as described in the USP monograph. [4] Dissolution test was carried out 6.8PO_4 buffer

for a period of 20 Hrs. The temperature of the dissolution medium is maintained at 37 ± 0.5°C. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at regular intervals and replaced with the same volume of pre-warmed fresh dissolution medium. The samples were filtered through a 0.45 µm membrane filter and diluted to 10ml to get a suitable concentration with respective media. The amount of drug release was determined from the comparison with standard response of pure drug. The results of *in vitro* dissolution are shown in Table 6 and Figure 3.

Precision

Precision of UV and RP-HPLC method were checked by analyzing the samples at three different time intervals of the same day (intraday precision) as well as on different days (interday precision).

Accuracy

To check the degree of accuracy of UV and RP-HPLC method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%.

Robustness

Robustness for RP-HPLC method was determined by analysis of samples under deliberately changed chromatographic conditions. The flow rate of the mobile phase was changed from 0.9 mL/min to 1.0 mL/min and 1.1 mL/min. The ratio of the mobile phase was changed by ± 2%. The effect on retention time and peak parameter were studied.

Limit of Detection and Limit of Quantitation

LOD, LOQ given RP-HPLC method was calculated by using the values of slopes and intercepts of the calibration curves for the given drug.

Table 4: Calibration curve

Conc.	AUC						Average	Slope	Intercept	(r^2)
µg/ml	1	2	3	4	5	6	AUC			
5	169006	169023	169117	169059	168973	169080	169043	21635	56721	0.9993
10	269042	269173	268975	269126	269007	269151	269079			
15	376444	376573	376411	376387	376540	376597	376492			
20	493882	493618	494637	494832	494896	493977	494307			
25	596724	597549	597880	597055	597679	596925	597302			

r^2 - Regression coefficient

Table 5: Results for assay of Metoprolol Succinate in pharmaceutical preparation

Labeled amount (mg)	Observed amount ± SD	%RSD
50	99.78±0.55	0.5471
25	100.09±0.31	0.3143

In vitro dissolution studies

Table 6: In-Vitro Dissolution Data for Metoprolol Succinate

		HPLC Response Data For Dissolution				
Std. Area	**1**	**2**	**3**	**4**	**5**	**6**
	427373	**427502**	**427596**	**427556**	**427626**	**427573**
	Average	**427531**	**SD**	**99.6**	**%RSD**	**0.02**
Tab. No	**Tab. Weight (mg)**	**0 min**	**1Hr**	**4Hr**	**8hr**	**20Hr**
1.	157.30	0	61101	131178	201827	405249
2.	158.90	0	63637	138564	210219	372700
3.	158.60	0	62445	138660	207677	370533
4.	157.40	0	63296	141320	208544	401574
5.	157.80	0	63537	139743	207656	392395
6.	158.00	0	66379	147360	217743	398392
Percentage Drug Release						
Tab. No	**Tab. Weight (mg)**	**0 min**	**1Hr**	**4Hr**	**8hr**	**20Hr**
1.	157.30	0	14.2	30.6	47.0	94.4
2.	158.90	0	14.8	32.3	49.0	86.8
3.	158.60	0	14.5	32.3	48.4	86.3
4.	157.40	0	14.7	32.9	48.6	93.5
5.	157.80	0	14.8	32.6	48.4	91.4
6.	158.00	0	15.5	34.3	50.7	92.8
Percentage Cumulative Drug Release						
Tab. No	**Tab. Weight (mg)**	**0 min**	**1Hr**	**4Hr**	**8hr**	**20Hr**
1.	157.30	0	14.2	30.8	47.9	96.2
2.	158.90	0	14.8	32.6	49.9	88.7
3.	158.60	0	14.5	32.6	49.3	88.2
4.	157.40	0	14.7	\33.2	49.5	95.5
5.	157.80	0	14.8	32.8	49.3	93.3
6.	158.00	0	15.5	34.6	51.7	94.8
Average		**0.0**	**14.8**	**32.8**	**49.6**	**92.8**
Minimum		0.0	14.2	30.8	47.9	88.2
Maximum		0.0	15.5	34.6	51.7	96.2
SD		0.0	0.4	1.2	1.2	3.5
%RSD		**-**	**2.7**	**3.7**	**2.5**	**3.8**
USP Limits		**-**	**NMT 25%**	**20-40%**	**40-60%**	**NLT 80%**

Table 7: Linearity

Linearity range	5-25 µg/mL
R2	0.9993

Table 8: Intra-day and Inter-day Precision

Precision*	Metoprolol Succinate
Interday (% RSD)	0.2709
Intraday (% RSD)	0.2860

Table 9: Data for Recovery Studies

Level of % Recovery	Method	*% Recovery	% RSD	SE
80	RP-HPLC	100.19	0.6581	0.3807
100	RP-HPLC	100.03	0.5352	0.3091
120	RP-HPLC	100.39	0.7036	0.4034

Table 10: Data for Robustness Studies

Parameter	Level	Retention time*	Tailing factor*
Flow rate (±0.1 mL/min)			
0.9	-0.1	4.58	1.05
1.0	0	4.55	1.06
1.1	+0.1	4.49	1.06
	(±) SD	0.0458	0.0057
	% RSD	0.4345	0.5428
Mobile phase Change			
73	-2	4.61	1.05
75	0	4.55	1.06
77	+2	4.51	1.07
	(±)SD	0.0503	0.0100
	% RSD	0.4767	0.9433

Table 11: LOD and LOQ

LOD (µg/mL)*	0.2441
LOQ (µg/mL)*	0.7399

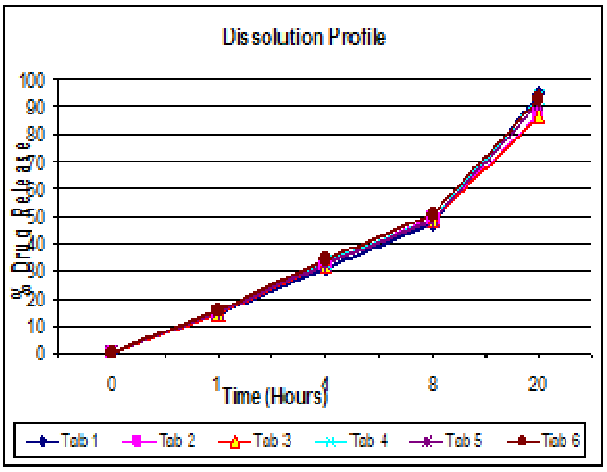

Fig. 4: Dissolution Profile

Validation of analytical method

The methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures.

Linearity

The calibration curve for RP-HPLC method was obtained with concentrations of the standard solutions 5-25 µg/mL of Metoprolol Succinate. The solutions were prepared in triplicate. Linearity was evaluated by regression analysis, which was calculated by the least square regression method.

RESULT AND DISCUSSION

Different proportions of acetonitrile and 0.05M phosphate buffer was tried for selection of mobile phase. Ultimately, 0.05M phosphate buffer (pH was adjusted to 3.0 using orthophosphoric acid) and acetonitrile in a proportion of 70:30 v/v respectively was finalized as the mobile phase. Figure 2 shows typical chromatogram obtained from the analysis of standard solution of Metoprolol Succinate using the proposed method. The Rt for Metoprolol Succinate was found to be 4.9, at a flow rate of 1.0 mL/min. The chromatogram was recorded at 225 nm.

The proposed RP-HPLC method, allows a rapid and accurate quantitation of Metoprolol Succinate in tablet preparation. The absorption spectrum of Metoprolol Succinate in 6.8PO4 buffer is shown in Figure 1. Wavelengths selected for analysis are 225 nm (λmax of Metoprolol Succinate). Calibration curves were constructed in the concentration range of 5-25 µg/mL. Beer's law was obeyed over this concentration range, and the coefficient of regression of the drug was found to be nearer to 1 (Table 4 and Figure 3). Precision was calculated as interday and intraday variations given drug. Percent relative standard deviations for estimation of Metoprolol Succinate under intraday and interday variations were found to be less than 2 (Table 8). The accuracy of proposed method was determined by recovery studies (Table 9), indicating an agreement between the true value and found value. For robustness studies in all deliberately varied conditions percent relative standard deviations were found to be less than 2 % (Table 10).

CONCLUSION

The proposed RP-HPLC method were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy for the proposed methods. The proposed method was found to be simple, accurate and reproducible for routine estimation of metoprolol in different dissolution media. The standard deviation, percentage recovery indicates precision and accuracy of the method.

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