EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 7
363-367
Year: 2019

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF METOPROLOL SUCCINATE IN FORMULATED PRODUCT USING RP-HPLC

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Article Received on 20/05/2019 **Article Revised on 11/06/2019** **Article Accepted on 01/07/2019**

ABSTRACT
The present work is concern with the utilization of simple, precise, accurate, reproducible and specific RP-HPLC method for estimation of metoprolol succinate in tablet formulation. Separation was achieved on X Bridge shield RP 18(4.5× 250mm, 5µm) column in an isocratic mode. Using trifluroacetic acid (0.1%):acetonitrile (70:30v/v) at a flow rate of 1ml/min and peak was observed at 200nm, with retention time of 3.9minutes. the method was validated and found to be linear within the concentration range of 100µg/ml-500µg/ml. the value of correlation coefficient and LOD and LOQ was found to be 0.999, 2.29 µg/ml and 6.96 µg/ml. the method was validated under ICH guidelines for various parameters like precision, accuracy, linearity and robustness.

KEYWORDS: MTS, RSD, HPLC, STD.

INTRODUCTION
Metoprolol succinate(MTS) is a beta-selective adrenoceptor blocking agent available as extended release tablets for oral administration.[1] Use for the treatment of broad spectrum cardiovascular disorders.[2] Metoprolol succinate is official in united state(USP) and british pharmacopoeia.[3,4] Chemically MTS is a 1-(isopropylamino)-3-[4-(2-methoxyethyl)phenoxyl]-2-propanol succinate.[5] It is white crystalline powder with a molecular weight 652. It is freely soluble in water soluble in methanol and sparingly soluble in 2-propanol.[6] Metoprolol is a beta-1-adrenergic receptor inhibitor unequivocal to cardiovascular cells with insignificant effect on beta-2 receptors. This restriction lessens cardiovascular yield by conveying negative chronotropic and inotropic impacts without presenting activity towards layer change or natural sympathomimetics.[7] Literature survey reveals that a few HPLC methods, UV spectroscopy, and LCMS method has been used.[8,9] The objective of the present work was to develop simple, precise and economical RP-HPLC method. The aim of the present work was to develop and validate a simple and reliable method for estimation of metoprolol succinate in tablet formulation. However, there was some report aviable method.[10] The important feature of this method include simple small amount of powder sample was treated with sonication at ambient temperature. Short retention time (less than 5 min.) good precision value (RSD less than 2%) and good recovery (greater than 98%). The purposed method was validated under ICH guidelines.[11]

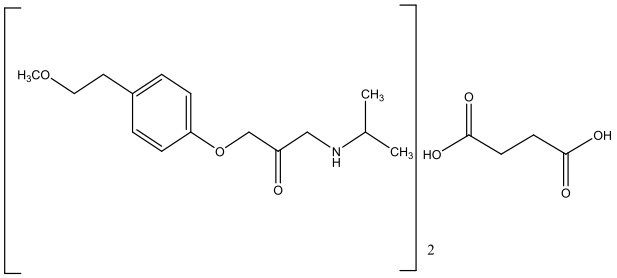

Fig. 1. Chemical Structure of Metoprol Succinate.

MATERIAL AND METHOD
Chemicals: The metoprolol succinate was obtained from jubilant generic (Noida) as a gift and used as such without further purification. The commercial formulation available as prolomet-xl (50mg) was purchased from the local pharmacy (Delhi, India) having batch number BST2027 manufactured by sun pharmaceuticals Ltd., India.

Reagents: Reagents used in the study includes gradient grade Acetonitrile (Merck Ltd., Mumbai, India) and HPLC grade trifluro acetic acid(TFA) (Merck Ltd., Mumbai, India) water used for HPLC analysis was purified using Millipore Milli-Q plus water purified system (Merck Ltd., Mumbai, India).

Methods

Solubility: From the literature review as well as self performed Metoprolol succinate is soluble in Acetonitrile and Water.

Table. 1. Mobile phase ratio with flow rates

Time (min)	0	2	3.5	4	5	6	6.10	8
Flow (ml/min)	1	1	1	1	1	1	1	1
A%	70	70	50	20	2	2	70	70
B%	30	30	50	80	98	98	30	30

A: Trifluoro acetic acid B: Acetonitrile.

Preparation of buffer

Pipette out 1ml measured amount of Trifluro Acetic Acid in 1000ml Milli-Q water was sonicated for 15 minute and filter through 0.2µ 6, 6 nylon membrane filter.

Preparation of diluents

Mixed well Milli-Q water: acetonitrile in a ratio (80:20) sonicated degassed and filter through 0.2µ 6, 6 nylon membrane filter.

Preparation of standard

Weighed accurately and transferred about 50mg of metoprolol succinate standard in 100ml volumetric flask. Added a little quantity of diluents to dissolve sonicated and degassed, make the volume upto 100ml and sonicate.

Selection of chromatographic method

On the basis of the sample nature (ionic/ionisable/neutral molecules), its molecular weight and solubility the proper selection of the method development depends. The drug selected i.e. Metoprolol succinate for the study is polar in nature and hence reverse phase chromatography or ion exchanged chromatography method may be used. The reversed phase HPLC was selected because of its simplicity and suitability.

Selection of wavelength

The sensitivity of any LC method which uses UV detection depends upon the proper selection of wavelength. The maximum absorbance of Metoprolol succinate at 200nm was determined in the mobile phase by utilizing the Photodiode Array Detector. During condition optimization we found that 200nm is appropriate wavelength for this analysis.

Chromatographic conditions

WATERS HPLC, model Aliance 2695 with photodiode array detector was used to perform the chromatographic separation. The output signals was monitored and integrated by using Empower3 software. The chromatographic column is X Bridge shield RP 18(4.6×250,5µ). The mobile phase of 0.1% TFA and Acetonitrile in the ratio 70:30v/v at a flow rate of 1ml/min. the injection volume was 5µL and the chromatographic run time of 8.0 min was used. A linear gradient elution method was applied as follows.

Preparation of sample

10 tablets of Metoprolol Succinate (ProlometXL) each containing of 50mg metoprolol succinate, were weighed and powdered. To prepare 200µg/ml concentration of sample solution quantity of powder equivalent to 229.6mg(50mg) was weighed approximately and transfer to 100ml volumetric flask. The sample was dissolved in diluents and sonicated for 15 min. volume was make upto 100ml and than 5ml of this solution was further diluted to 25ml with diluents to get the final concentration of 200µg/ml.

RESULTS AND DISCUSSION

Validation of developed HPLC method

Different chromatographic conditions such as mobile phase, wavelength, column and column temperature were experimented to achieve efficiency of chromatographic system. Different gradients of buffer and solvents were checked in order to attain optimum retention of the API. Minimization of run time and cost were the major tasks while developing the method.

Table. 3: Linearity data.

Concentration (%)	Peak Area		Average
	Injection-1	Injection-2	
100	1473911	1469721	1471816
200	3141644	3098954	3120299
300	4612656	4621564	4617110
400	6241535	6214652	6228093
500	7832634	7799897	7816265

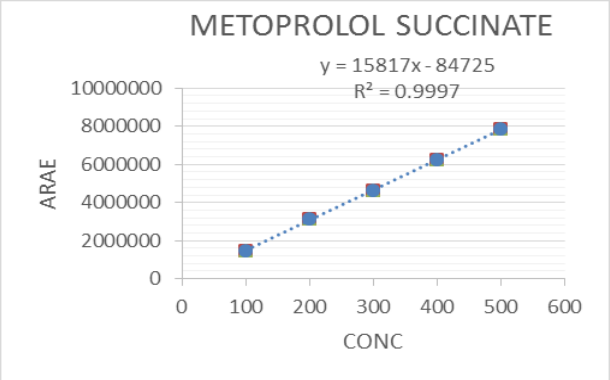

Fig. 2: Calibration curve of Metoprolol succinate.

Accuracy

Recovery of assay method for Metoprolol succinate was established by three determinations of test sample using tablets at 50%, 100% and 150% concentration. Each solution was injected thrice (n=3) into HPLC system and the average peak area was calculated to obtain % recoveries. All the individual recoveries were found to be between 100.96 -102.7% all individual recovery level were found to be within 0.16 to 0.37(%RSD). The results are summarized in table 3.

Table. 3: Recovery studies of Metoprolol succinate.

Conc.	Sample area	Average area	Sample Wt. (mg)	Amount added (µg)	Amount recovered(µg)	% Recovery	Average % Recovery	SD	% RSD
50%	1435491	3054718	11.4	114.80	114.80	100.50	100.96	0.16	0.16
	1429093				114.80	100.60			
	1531428				114.80	100.78			
100%	304550/2	3094512	22.9	229.60	229.60	104.30	104.45	0.40	0.38
	3058437				229.60	104.90			
	3179597				229.60	104.15			
150%	4597422	4590743	34.4	344.40	344.40	102.34	102.77	0.38	0.37
	4584064				344.40	102.34			
	4631428				344.40	102.34			

Precision (system and method): The precision of the system was evaluated by carrying out six independent injection of standard. The % RSD of peak area of the standard was found to be **0.23.** The results are summarized in table 4.

Table. 4: Result of system precision.

S. No	Replicate	RT	Standard Area
1	Replicate-1	3.9	3108573
2	Replicate-2	3.9	3092371
3	Replicate-3	3.9	3098001
4	Replicate-4	3.9	3095481
5	Replicate-5	3.9	3082397
6	Replicate-6	3.9	3075699
	Average	**3.9**	**3092087.0**
	SD	**0.0**	**7032.02**
	%RSD	**0.0**	**0.23**

The precision of the method was evaluated by carrying out six independent injections of test samples against a qualified reference standard. The % RSD of peak area of the standard was found to be **0.25**. The results are summarized in table 5.

Table. 5: Results of method precision.

Sample	Injection	Area	Avg. Area	Retention Time	% Assay
1	INJ-01	3088744	3065403	3.9	98.2
	INJ-02	3042061		3.9	
2	INJ-01	3116539	3163690	3.9	101.3
	INJ-02	3210841		3.9	
3	INJ-01	3156022	3160587	3.9	101.2
	INJ-02	3165152		3.9	
4	INJ-01	3056255	3090950	3.9	99.0
	INJ-02	3125645		3.9	
5	INJ-01	3139433	3126183	3.9	100
	INJ-02	3112933		3.9	
6	INJ-01	3019472	3202844	3.9	102.6
	INJ-02	3102844		3.9	
Mean				**3.9**	**100.2**
SD				**0.0**	**7751.46**
%RSD					**0.25**

Reproducibility (Intermediate precision): An assay was performed by analyzing six samples of Metoprolol succinate against a qualified reference standard. The % RSD obtained from these samples was observed as **0.61** and % RSD of peak area of reference standard was observed as **0.43**. The results are summarized in table 6.

Table. 6: Result of intermediate precision.

S No.	Injection	Std. Area	Test Area		Avg. Test Area
			Inj.1	Inj.2	
1	INJ-01	3105661	3074488	3024016	3049252
2	INJ-02	3142726	3115639	3218014	3166827
3	INJ-03	3117496	3150622	3151626	3151124
4	INJ-04	3106522	3065552	3126554	3096053
5	INJ-05	3126354	3134933	3125651	3130292
6	INJ-06	3151639	31203254	3102844	3202844
	MEAN	3125066.3			3132732
	SD	**18959.95**			**15345.59**
	%RSD	0.61			**0.43**

Specificity: The specificity of the method was determined by comparing the chromatograms obtained from the sample containing Metoprolol succinate standard stock with those of the test samples. The specificity test reveals the interference of impurity with the drug, since no extra peak appeared at the same time. The RSD for six replicates measurements of peak area of standard preparation was found to be **0.59**. The results are summarized in table 7.

Table. 7: Results of specificity.

S. No.	RT	Standard Area	Test Area
1	3.9	3131918	3112242
2	3.9	3095445	3078956
3	3.9	3101717	
4	3.9	3092359	
5	3.9	3098252	
6		3052229	
Mean		**3095320.0**	**3095599**
SD		**18128.54**	**23536.76**
RSD		**0.59**	**0.76**

Robustness

Robustness is the capacity of the method to remain unaffected by small deliberate variations in method parameters. Such as change in flow rate (±0.10ml/min), buffer concentration (±10%), column temperature (±5◦c). in all the above varied conditions, the component of mobile phase was held constant, but no marked changes were observed in the chromatograms, which confirmed that the developed HPLC method is robust.

Limit of detection (LOD)

Limit of detection is the lowest amount of an analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The minimum concentration at which the analyte can be detected was determined by visual examination of signals to noise ratio which should be 3:1 with respect to height.

CONCLUSION

A new method has been developed to determined Metoprolol succinate efficiently and accurately within a relatively short period by using reverse phase HPLC method. It shown a good precision (RSD<2%) and recovery (100.96-102.77) and proved to be simple linear price accurate robust and rapid. It gives faster elution, maintaining good separation. Short retention time allows the analysis of a large no of samples in short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. This method can be directly used for HPLC analysis n need basis.

ACKNOWLEDGEMENT

Authors greatfully acknowledge the Director Dr. Avjit Mazumdar and H.O.D. Dr. salahuddin of Noida Institute of Engineering and Technology (Pharmacy College), Greater Noida for their kind help and providing all necessary facilities and also thank jubilant chemsys Noida, for providing the gift sample of Metoprolol Succinate.

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