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Development and validation of a stability indicating RP-HPLC method for determination of metoprolol succinate in pharmaceutical dosage forms

[1]CH. Naveen Kumar*, [1]Saraswathi Mannuri, [1]Vijaya Kuchana and [2]N. Kannappan

[1]Teegala Krishna Reddy College of Pharmacy, Department of Pharmaceutical analysis & Quality Assurance, Medbowli, Meerpet, Saroornagar, Hyderabad, Telangana, India
[2]Department of Pharmacy, Faculty of Engineering and Technology, Annamalainagar, Annamalai University, Chidambaram, Tamilnadu, India

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ABSTRACT

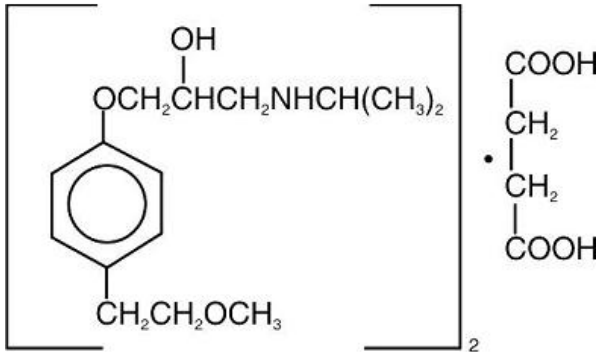
A simple, precise, rapid and accurate reverse phase HPLC method has been developed for the determination of Metoprolol succinate in pharmaceutical dosage form. An isocratic separation of metoprolol was achieved on Inertsil ODS-2, 150mm x 4.6 mm, 5.0 µm or equivalent column was used with Photo Diode Array detector. The mobile phase consisting of Buffer,Methanol, Acetonitrile (600:50:350) at a flow rate of 1.0ml/min at ambient temperature. The quantitation was achieved with UV detection at 223nm. The method was linear over the concentration range of 20-200µg/ml and the correlation coefficient was found to be (r^2 =0.999). The retention time of the drug was 11.37min.The method precision for the determination of assay was below 2% RSD, indicated a good precision of the analytical method. The percentage recovery of Metoprolol succinate was found to be 100.26%. Robustness of the method was performed by using different flow rates& temperatures and it was found as robust. The validation of method was done by using ICH Guidelines.

Keywords: Metoprolol succinate, Assay method, Stability indicating method, RP-HPLC

INTRODUCTION

Metoprolol block**s** the Beta [1] receptor, results in a decrease in heart rate, cardiac output, and also blood pressure. Metoprolol is the drug which competes with adrenergic neurotransmitters such as catecholamine for binding at beta-[1] adrenergic receptors in the heart. The blockage of this Beta receptor results in a decrease in heart rate, cardiac output, and blood pressure. This medication is a beta-blocker used to treat chest pain (angina), failure of heart, and high blood pressure. Lowering high blood pressure helps to prevent strokes, heart attacks, and kidney problems.

Figure. No.1: Metoprolol succinate



A detailed survey of analytical literature for metoprolol revealed some methods based on a variety of techniques such as UV-spectrophotometer[2] Liquid chromatographic –tandem mass spectrometric (LC-MS/MS) methods[3] have been developed for the estimation of metoprolol in biological fluids. HPLC is often the first choice for developing an analytical method as compared till date, none of the reported analytical procedure describes a simple, satisfactory and validated HPLC method for studying the effect of stress on pharmaceutical dosage forms as well as for assay of metoprolol in its pharmaceutical dosage forms.

According to an FDA guidance document, a stability-indicating method is "a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability-indicating method accurately measures the active ingredients, without interference from process impurities, degradation products, excipients or other potential impurities."[4]

Metoprolol succinate is white crystalline powder, designated chemically as (±)1(isopropyl amino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1) (salt) with an empirical formula of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$ and a molecular weight of 652.8 g/mol (Fig.1) It is freely soluble in water; soluble in methanol; sparingly soluble in ethanol; slightly soluble in dichloromethane and 2-propanol; insoluble in ethyl-acetate, acetone, diethyl ether and heptanes. It has a pKa of 6.8 [5-8]

The present work is aimed at development of new RP-HPLC method for the estimation of Metoprolol succinate in pharmaceutical dosage form and the results are validated according to ICH guidelines [9]. Only a very few HPLC estimations have been reported in the literature for the determination of purity to develop a method for Metoprolol succinate by RP-HPLC technique in-vitro [10]. Hence the present study has made an attempt to develop a new HPLC method for the determination of Metoprolol succinate in pharmaceutical solid dosage forms.

MATERIALS AND METHODS

Reagents:
Water HPLC grade (Merck, pvt. Ltd. Mumbai), Methanol (Merck, pvt. Ltd. Mumbai) Acetonitrile (Merck,pvt.Ltd. mumbai), Phosphate buffer (Merck,pvt , Mumbai). Metoprolol pure drug substance was kindly supplied by Strides Arcolabs Limited,India.

Instrumentation:
HPLC (Schimazdu- SPD), UV- Spectrophotometer (Spectro 2060 plus), Analytical balance (Citizen), pH meter (Sisco), Sonicator (Sisco), Vaccum filter (Sisco)

Chromatographic conditions:
The chromatographic system was used to perform development and validation of this assay method. Chromatographic analysis was performed on Inertsil ODS-2, 150mmx4.6 mm, 5.0 µm or equivalent column which was used with Photo Diode Array, Separation was achieved by using a mobile phase which consist of Buffer, Methanol, Acetonitrile (600:50:350) at a flow rate of 1.0ml/min at ambient temperature. The eluent was monitored by using PDA detector at a wavelength 223 nm and injection volume of 10 µl was used. The mobile phase prepared was filtered through 0.45 µm filter prior to use.

PREPARATION OF MOBILE PHASE:
Preparation of Phosphate Buffer P^H (6.8):
Accurately weigh and transfer about 6.8g of potassium dihydrogen ortho phosphate of sodium hydroxide into a 1000ml of water and dissolve it. Adjust P^H of the solution to 6.8 with the sodium hydroxide solution.

Preparation of Standard Solution:
Transfer about 50mg of metoprolol succinate WS, accurately weighed, into a 50ml volumetric flask and add 20ml of methanol, sonicate to dissolve with methanol to volume and mix. Transfer 5.0ml of this solution to a 50ml volumetric flask, dilute with diluent up to the volume and mix.

Preparation of Sample Solution:
Transfer an accurately weighed quantity of the pellets equivalent to about 100.0mg of metoprolol succinate to a 100ml volumetric flask, and add 70 ml of methanol sonicate for 30minutes with intermittent shaking and dilute with methanol to volume, mix and filter. Transfer 5.0ml of this solution to a 50 ml volumetric flask, dilute with the diluents to volume and mix.

Procedure: Inject 20ul of diluent, five replicate injections of standard preparation and single injection of sample preparation into the chromatographic system and then measure the peak areas.

RESULTS AND DISCUSSION

System suitability Testing:

System suitability testing is used to verify that the reproducibility of the system is adequate for the analysis to be performed. System suitability is done by preparing and injecting the standard solution five times and calculating its RSD. Other parameters like theoretical plates and tailing should also be taken into consideration. Results are tabulated in Table No :1

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of some components which may be expected to be present. Typically these might include degradants, impurities, matrix, etc. Specificity was determined by injecting a blank, placebo and also standard solution. No interference was seen at the retention time of an analyte. The specificity was also demonstrated by induced degradation of Metoprolol formulation and placebo samples to acid degradation, alkali degradation, water degradation, peroxide degradation, thermal degradation, and U.V. degradation. The Purity angle is less than the purity threshold for all the stress conditions. The results are tabulated in Table No :2.Figures 6-11 represents different stress conditions.

Table No: 1

METOPROLOL SUCCINATE SYSTEM SUITABILITY

Injection No.	1	2	3	4	5	Mean	STDEV	RSD	Limits
Standard Area	3912794	3909278	3910385	3911485	3938798	3916548	10589	0.5	RSD NMT 2.0%
Theoretical Plates	2993	2982	2988	2985	2978	2989	5.71	0.1	NLT 2000
USP tailing	1.35	1.35	1.35	1.35	1.35	1.35	0	0.3	*NMT 2.0*
RT	11.31	11.31	11.3	11.27	11.31		0	0.3	

Table No: 2

STRESS CONDITION		DRUG PRODUCT	RESULTS
ACID /BASE	0.01 to 0.1N HCL	24 to 48h	No interference found
			Found
OXIDATIVE	0.3% H_2O_2	24 to 48 h	No interference
			Found
LIGHT	1200Lux h	>48h	No interference
			Found
TEMPERATURE	10^0C to 70^0C	Upto 3 weeks	No interference
			Found
TEMPERATURE / HUMIDITY	10^0C to 70^0C and 60 to 90 R.H	Upto 3 weeks	No interference
			Found

Table No: 3

METOPROLOL SUCCINATE-LINEARITY

Run	% Conc.	Conc. Of Metoprolol succinate (µg/mL)	Area of Metoprolol succinate	Slope	Y-intercept	R^2
1	20%	0.041	8220	24100	1151.51	0.999
	50%	0.1024	18825			
	80%	0.1638	29308			
	100%	0.2048	37494			
	200%	0.4096	71750			
2	20%	0.041	8239	23126	12535.2	0.999
	50%	0.1024	18826			
	80%	0.1638	29358			
	100%	0.2048	37384			
	200%	0.4096	71744			
3	20%	0.041	8245	24564	11258.2	0.999
	50%	0.1024	18825			
	80%	0.1638	29310			
	100%	0.2048	37487			
	200%	0.4096	71752			
Average				23930	28028.8	0.999
Standard Deviation				55.66	2931.59	0
Acceptance criteria: Coefficient of correlation shall be NLT 0.999						

Table No: 4				
METOPROLOL SUCCINATE- LIMIT OF DETECTION (LOD) & LIMIT OF QUANTIFICATION (LOQ)				
S.No.	Injection No.	Slope	Standard deviation	R^2
1	Inj-1	23930	16053.32	0.999
σ = Standard deviation of y-intercepts of regression line				
S= slope of the linearity curve				
LOD	2.21378	µg/ml.		
LOQ	6.70844	µg/ml.		
Acceptance Criteria:LOD & LOQ values shall be less than the minimum linearity concentration				

Table No: 5						
METOPROLOL SUCCINATE BENCH TOP STABILITY OF STANDARD SOLUTION						
Time(Hrs)	Day	Std. Wt.	Response	Fresh Std Wt.	Response of fresh std.	Similarity Factor
Initial	Initial	50.28	2300776			
24 Hrs	Day-1	50.28	2311087	50.16	2316978	1
48 Hrs	Day-2	50.28	229268	50.43	2268919	0.99
METOPROLOL SUCCINATE BENCH TOP STABILITY OF TEST SOLUTION-1						
Time(Hrs)	Day	Weight(mg)	Response of sample	% Assay	Difference from Initial	Difference in Assay results of Initial,24 & 48 Hrs shall be NMT 2.0
Initial	Initial	1252.55	2337253	101.29	NA	
24 Hrs	Day-1	1252.55	2331891	100.6	0.7	
48 Hrs	Day-2	1252.55	2305665	101.01	0.3	
METOPROLOL SUCCINATE BENCH TOP STABILITY OF TEST SOLUTION-2						
Time(Hrs)	Day	Weight(mg)	Response of sample	% Assay	Difference from Initial	Difference in Assay results of Initial,24 & 48 Hrs shall be NMT 2.0
Initial	Initial	1246.46	2321437	100.6	NA	
24 Hrs	Day-1	1246.46	2320785	100.12	0.5	
48 Hrs	Day-2	1246.46	2327739	101.99	1.4	

Table No: 6					
Standard	50.43	mg	5	Potency	99
Preparation	50		50		
Sample	Wt. of sample taken in mg		5	Label Claim	100
Preparation	100		50		
Standard Area		2316978	Average Wt. in mg		250

Table No:7								
Conc.		Inj-1	Inj-2	Inj-3	Mean	**%** recovery	STD	%RSD
80	80%	261673	262254	263628	262518	98.75626	1003.95	0.38243
100	100%	532547	531537	531754	531946	100.0559	531.67	0.09995
120	120%	813627	813517	812677	813274	101.9813	519.647	0.0639
Acceptance criteria: % Average recovery shall be between 95.0% -105.0%								

Table No: 8								
METOPROLOL SUCCINATE ANALYTICAL MEHTOD VALIDATION-ASSAY								
Method Parameter			METHOD PRECISION					
Std. wt. & Dilution	50.33	5	Capsule Wt.	Spl. wt. & Dilution	Wt.of sample taken	5	Label claim (mg)	100
	50	50	250		100	50	Potency (%)	99
Std. No.	Standards	USP Tailing	Weight of sample taken	Area of sample	Assay %	Average (%)	STDEV	% RSD
1	2310915	1.35	1250	2337254	101.23	100.71	0.51552	0.51
2	2290693	1.35	1250	2321427	100.55			
3	2300684	1.35	1250	2317128	100.36			
4	2300777	1.35	1250	2341249	101.41			
5	2300755	1.35	1250	2324067	100.66			
			1250	2310208	100.06			
Average	2300765	1.54	1250	2325222	100.71			
STDEV	7149.73	0	% RSD of 6 replicate injections is not more than 2					
%RSD	0.31	0						

Acid and alkali hydrolysis:

The hydrolytic degradation of a new drug in acidic and alkaline condition can be studied by refluxing the drug in 0.1 N HCl / 0.1 N NaOH. If reasonable degradation is seen, testing can be stopped at this point. However in case no degradation is seen under these conditions the drug should be refluxed in acid/alkali of higher strength & for longer duration of time. Alternatively if total degradation is seen after subjecting the drugs to initial condition, acid/alkali strength can be decreased with decrease in reaction temperature.

Oxidation:

To test for oxidation, it is suggested to use hydrogen peroxide in the concentration range of 3 to 30 %. In some drugs extensive degradation is seen when exposed to 3% of hydrogen peroxide for very shorter time period at room temperature. In other cases exposure to high concentration of hydrogen peroxide, even under extreme condition does not cause any significant degradation. The behavior is on expected lines, as some drugs are in fact oxidisable, while there are others that are not. The latter are not expected to show any change even in the presence of high dose of oxidizing agent.

Table No: 9

METOPROLOL SUCCINATE ANALYTICAL MEHTOD VALIDATION-ASSAY

Method Parameter			INTERMEDIATE PRECISION					
Std. wt. & Dilution	50.25	5	Capsule Wt.	Spl. wt. & Dilution	Wt.of sample taken	5	Label claim (mg)	100
	100	20	250		100	200	Potency (%)	99
Std. No.	Standards	USP Tailing	Weight of sample taken	Area of sample	Assay %	Average(%)	STDEV	% RSD
1	2315498	1.52	1250	2303175	98.9	99.29	0.373	0.38
2	2302693	1.52	1250	2318575	99.56			
3	2314434	1.52	1250	2314650	99.4			
4	2321577	1.52	1250	2305262	98.99			
5	2330688	1.52	1250	2325271	99.85			
			1250	2306776	99.06			
Average	2316978	2	1250	2312285	99.29			
STDEV	10269.35	0	Limits	% RSD of 6 replicate injections is not more than 2				
%RSD	0.4	0						

Table No: 10

METHOD & INTERMEDIATE PRECISION COMBINEDLY

Method Precision		Intermediate Precision					
S.No.	% Drug content	S.No.	% Drug content	Difference	Average of both Method & Intermediate precision	STDEV of both Method & Intermediate precision	%RSD of both Method & Intermediate precision
1	101.23	1	98.9	2.3	100	0.856	0.86
2	100.55	2	99.6	1			
3	100.36	3	99.4	1			
4	101.41	4	99	2.4			
5	100.66	5	99.9	0.8			
6	100.06	6	99.1	1			

Table No: 11

METOPROLOL SUCCINATE ANALYTICAL MEHTOD VALIDATION-ASSAY

Method Parameter			ROBUSTNESS		
Change in Flow Rate(0.9mL/min)			Change in Flow Rate(1.1mL/min)		
Std. No.	Standards	USP Tailing	Std. No.	Standards	USP Tailing
1	2743768	1.4	1	1973844	1.3
2	2774675	1.4	2	1943356	1.3
3	2740839	1.4	3	1960267	1.3
4	2732443	1.4	4	1952045	1.3
5	2734278	1.4	5	1958534	1.3
Average	2745155	1.4	Average	1957656	1.3
STDEV	17118.59	0	STDEV	11255.34	0
%RSD	0.62	0	%RSD	0.57	0
Change in temperature (20ºC)			Change in temperature (30ºC)		
Std. No.	Standards	USP Tailing	Std. No.	Standards	USP Tailing
1	2271434	1.3	1	2263432	1.4
2	2252256	1.3	2	2258745	1.4
3	2249455	1.3	3	2276007	1.4
4	2244184	1.3	4	2272585	1.4
5	2241567	1.3	5	2276135	1.4

Photolytic degradation:

Sunlight: The photolytic studies should cover the exposure of drug solution to sunlight. The drug solution should be exposed to sunlight for 4 days.

Figure. No.2: Blank-Diluent

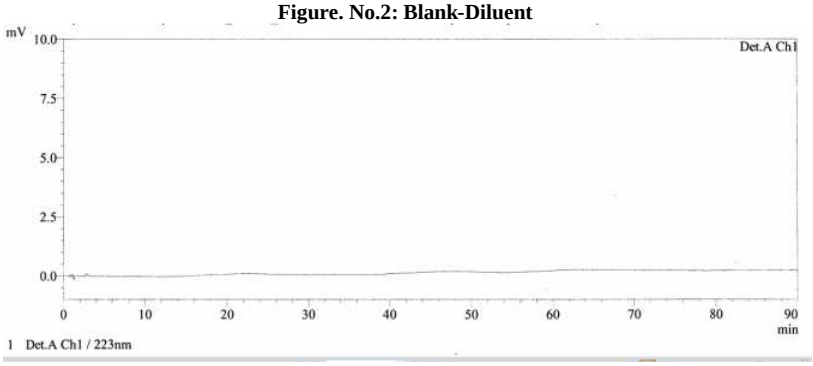


Figure.No.3: Standard

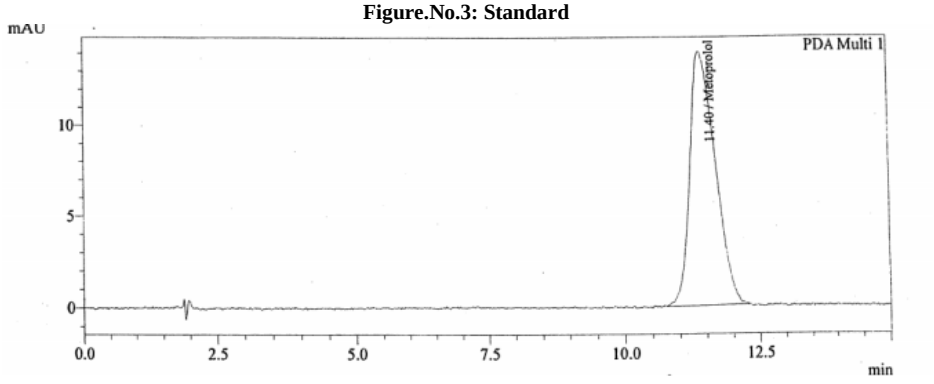


Figure No.4: Sample

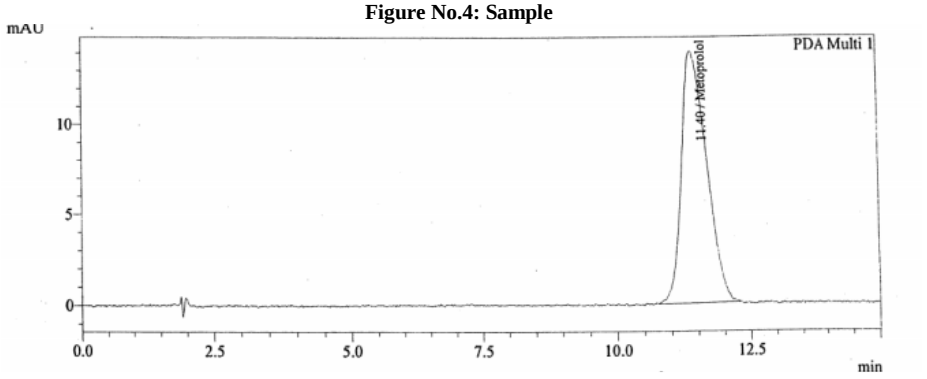
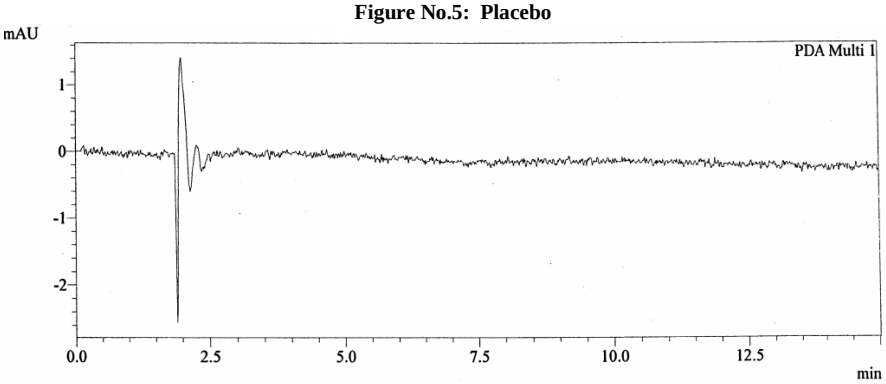


Figure No.5: Placebo



Specificity

UV light: The drug solution should be exposed to UV radiation, in UV chamber for 4 days to study the photolytic stability off drug.

Neutral hydrolysis:
Stress testing under neutral condition can be started by refluxing the drug in water for 12 hours. Refluxing time should be increased or decreased as per the degradation obtained in 12 hours.

Figure.No.6: Acid Stressed Sample

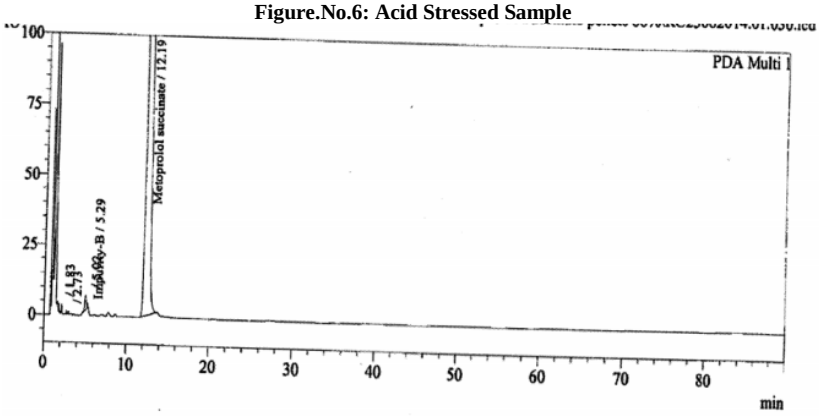


Figure.No.7: Alkali Stressed Sample

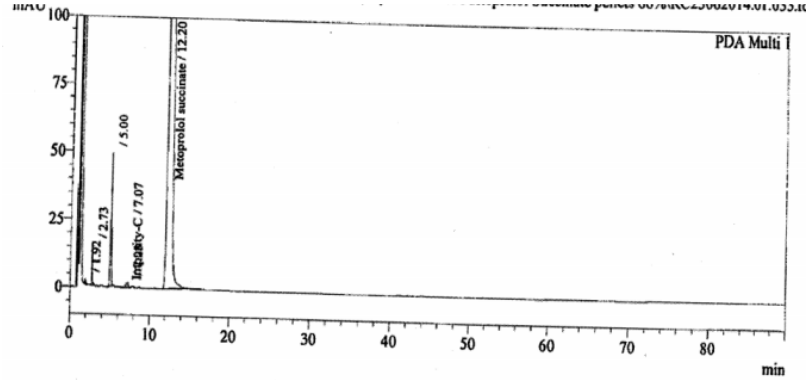


Figure.No.8: Peroxide Stressed Sample

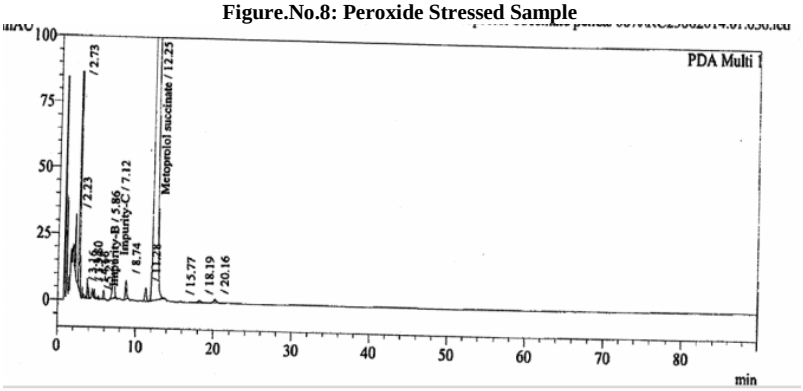
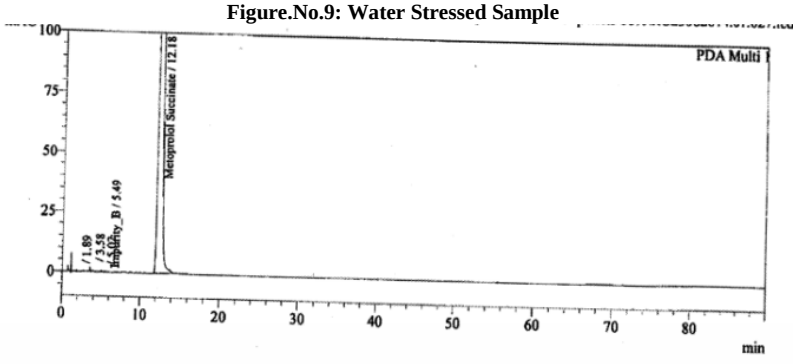


Figure.No.9: Water Stressed Sample



Dry heat:

Heating the drug powder at high temperature in oven can carry out stress testing for dry heat degradation. The heating time can be increased up to 12 hrs and above if there is no sufficient degradation seen in initial studies.

Wet heat:
Wet heat degradation can be studied by refluxing the drug solution for several hours.

Linearity:
The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of an analyte in the sample. The linearity of the test method was performed by plotting a graph between concentration of the test solution on X-axis and the response of the corresponding solutions on Y-axis from 20% to 200% of test concentration and calculated the correlation coefficient, it was found to be 0.999.The results are tabulated in Table No: 3 and the graphs are represented as Fig No: 12.

Limit of detection (LOD) and limit of quantification (LOQ):
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Calculated the LOD & LOQ, with the calculations obtained from evaluation of the calibration curve of the linearity.LOD and LOQ values are less than the minimum linearity concentration. The calculations and results are tabulated in Table. No: 4, Fig No: 13, 14.

Bench top stability of standard & test preparation:
Performed the assay of Metoprolol succinate as per the test method in duplicate and kept the test and standard solutions on the bench top for 48 Hrs. Injected at initial, 24 Hrs and 48 Hrs. Calculated the difference between initial and bench top stability samples for % assay of Metoprolol succinate for the test solutions and similarity factor for standard solutions were found to be within limits. The results are tabulated in Table No: 5

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 which was found. Performed the accuracy of test method using metoprolol placebo at 80%, 100%, 120%, spike levels. The % assay at each spike level was found to be between 95.0-105.0% of the labeled amount. The results are tabulated in Table No: 7

Figure.No.10: Heat Stressed Sample

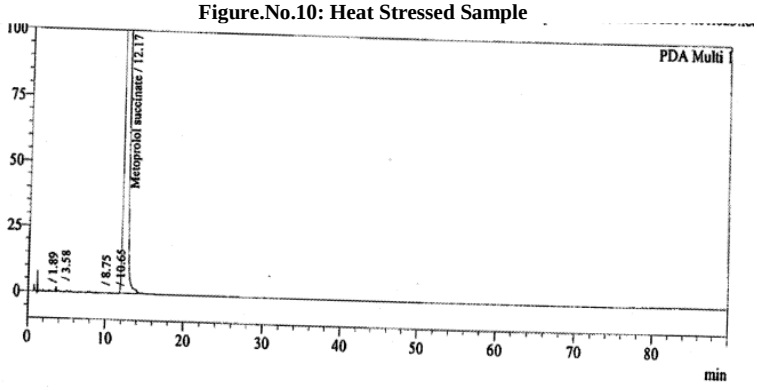
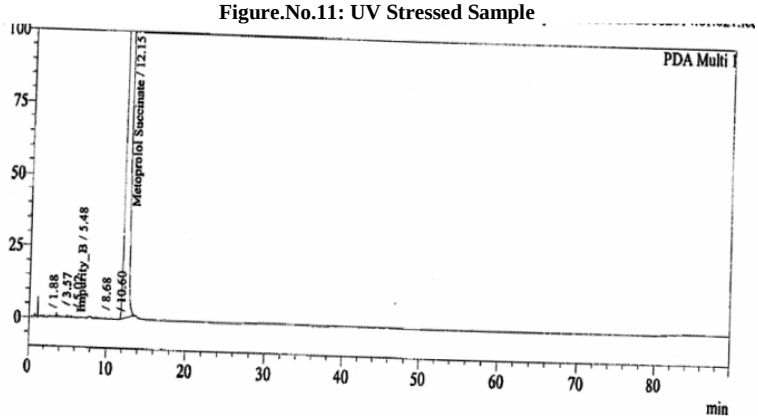


Figure.No.11: UV Stressed Sample



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. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Figure.No.12: Linearity

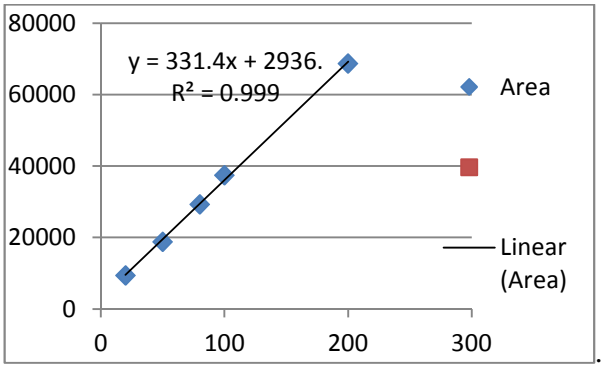


Figure No.13*:* Limit of Detection

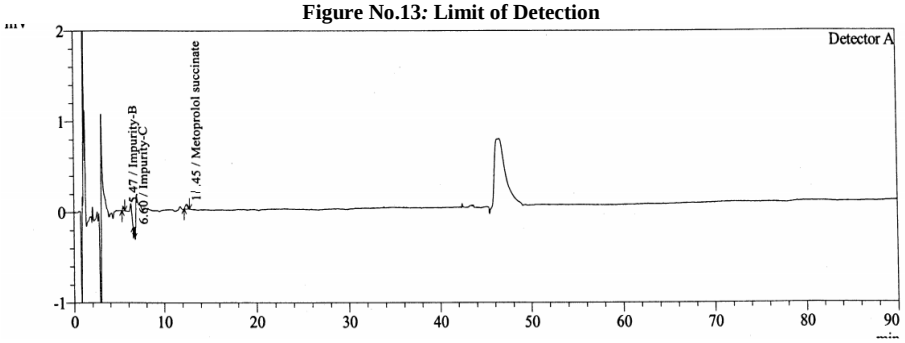
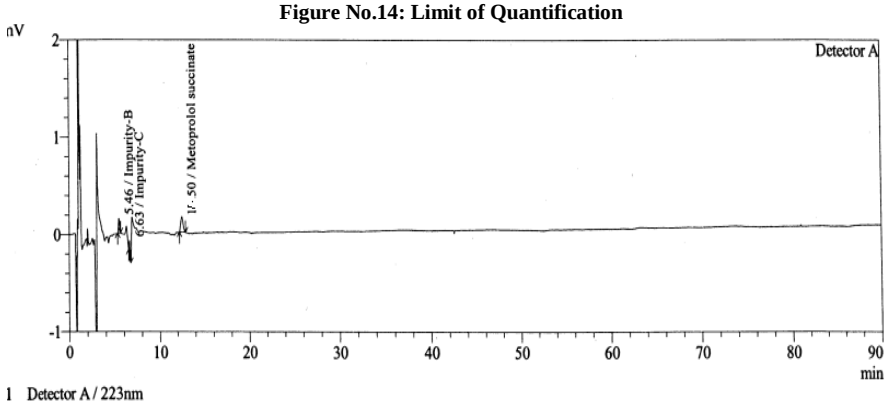


Figure No.14: Limit of Quantification



Method precision:
Determined the precision of the test method by preparing & injecting 6 test solutions of Metoprolol succinate formulations in to the chromatograph and recorded the results. The average % assay was found to be 100.4with % RSD of 0.62. The results are tabulated in Table No: 8

Intermediate precision:
Performed the assay of Metoprolol succinate by following the same procedure as that of Method precision but on a different day and by a different analyst. The average % assay was found to be 99.4%with % RSD of 0.39.Overall RSD when compared with Method precision is 0.73. The results are tabulated in Table No: 9&10

Robustness:
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [10]. Robustness was performed by injecting the Metoprolol succinate standard solution in to the RP-HPLC by altering the Flow rate,

Column oven temperature and also by changing the pH of the buffer & composition of the organic solvent from the normal chromatographic conditions. The results are tabulated in Table No: 11

Calculation:

$$\% \text{ Assay: } \frac{AT}{AS} \times \frac{WS}{50} \times \frac{5}{50} \times \frac{100}{WT} \times \frac{50}{5} \times \frac{P}{100} \times 100$$

Where,
AT = Peak Area of metoprolol succinate in sample solution.
AS = Average Peak Area of metoprolol succinate in standard Preparation.
WS = Weight metoprolol succinate of working standard taken in mg.
WT = Weight of sample taken in mg.
P = Percentage purity of working standard.

CONCLUSION

The reported RP-HPLC method was proved to be simple, rapid with a runtime of 11.37 min & reproducible. The validation data indicates good specificity, accuracy, precision & reliability of the method. The developed method has many advantages like isocratic mode of elution, easy sample preparation, and can be used for routine quality control analysis of Metoprolol succinate formulations.

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