ejpmr, 2018,5(9), 333-342

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

A RAPID AND SENSITIVE VALIDATED HIGH PERFORMANCE LIQUIDCHROMATOGRAPHY METHOD FOR DETERMINATION OF RELATED SUBSTANCES IN METOPROLOL SUCCINATE (API)

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Article Received on 09/07/2018 **Article Revised on 30/07/2018** **Article Accepted on 20/08/2018**

ABSTRACT

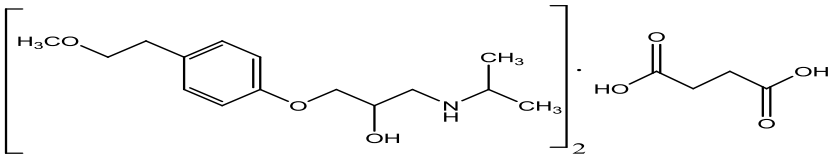
The purpose of this research study is to develop simple, precise, accurate and economical method for determination of related substances in Metoprolol Succinate API. The chromatographic method was developed on Durashell C18, (250 mm x 4.6 mm, 5 µm) column with binary gradient system and the mobiles phase used for speperation was (a) Dipotassium hydrogen phosphate buffer with pH 3.0 ± 0.05 adjusted with ortho phosphoric acid and mobile phase (b) was mixture of 5 volumes of Acetonitrile, 4 volumes of Methanol and 1 volume of Water. The detection of all related substances observed at lower walength 223 nm and The peak response of all related substances are very good, all impurities were detected at about 0.112ppm and quitified at 0.340ppm.The relative standard deviation of all impurities was below five percentage. Developed method was validated as per ICH guideline and found to be linear, accurate, specific, precise, and robust. The correlation coefficient of all the impurities was below 0.999 and impurites responces where liner from 0.340 ppm to 3.500 ppm. The obtained recovery of all the impurities between 90 to 110 percentage and solution stability of the sample solution was found to be stable up to 24 hrs, hence this method can be successfully applied for the determination of related substances in Metoprolol Succinate API.

KEYWORDS: Metoprolol Succinate API, Binary Gradient, HPLC, method development, validation.

1. BACKGROUND OF THE INVENTION

Metoprolol was first made in 1969 and most important medications needed in a basic health system.World Health Organization's it was listed as Essential Medicines, It is available as a generic drug. In 2013, Metoprolol was the 19th-most prescribed medication in the United States. Metoprolol Succinate is in form of succinate salt of metoprolol, a cardio selective competitive beta-1 adrenergic receptor antagonist with antihypertensive properties and devoid of intrinsic sympathomimetic activity. Metoprolol, marketed under the trade name Lopressor among others, is a medication of the selective β1 receptor blocker type. It is used to treat high blood pressure, chest pain due to poor blood flow to the heart, and a number of conditions involving an abnormally fast heart rate. It is also used to prevent further heart problems after myocardial infarction and to prevent headaches in those with migraines.

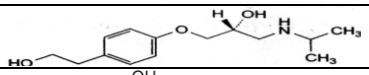
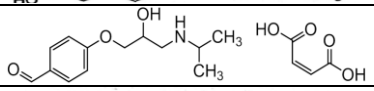
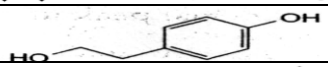
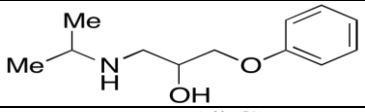
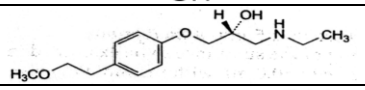
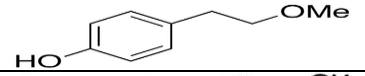
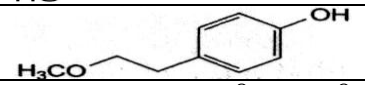
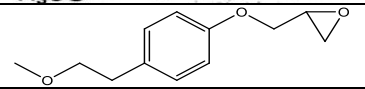
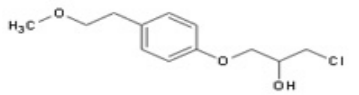
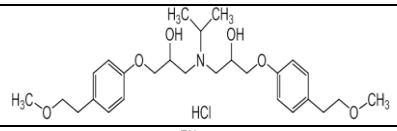
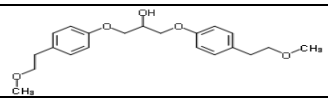
Metoprolol is used for a number of conditions, including hypertension, angina, acute myocardial infarction, supraventricular tachycardia, ventricular tachycardia, congestive heart failure, and prevention of migraine headaches. Metoprolol is sold in formulations that can be taken by mouth or given intravenously. The medication is often taken twice a day. The extended-release formulation is taken once per day. Metoprolol may be combined with hydrochlorothiazide in a single tablet.The active substance metoprolol is employed either as Metoprolol Succinate or as Metoprolol Tartrate.The tartrate is an immediate release formulation and the succinate is an extended-release formulation. Metoprolol is a beta blocker and is banned by the world anti-doping agency in some sports. Beta blockers can be used to reduce heart rate and minimize tremors, which can enhance performance in sports such as archery. All beta blockers are banned during and out of competition for archery and shooting. In United state and European pharmacopoeia Metoprolol Succinate API release analysis for related substance two different method was given, but those methods was not suitable for process impurities and listed impurities in pharmacoipa.To harmonized these pharmacopial method and possible process impurities we had developed a single method of analysis and then validate as per ICH guidline. The structure of Metoprolol Succinate is as below.



2-propanol,1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, (±)-, butanedioate (2:1)

Figure 1: Metoprolol Succinate.

Table 1: Chemical name of Metoprolol Succinate and its related substances.

Sr. No.	Component	Chemical Name	Structure
1	Impurity H	*{2RS)-I-* [4-(2-hydroxyethyl)phenoxy)-3- [(l-rnethylethyl)-amlnolpropen-z-ol,	
2	Impurity C	(±)4-[2-Hydroxy-3-(1-methylethyl) aminopropoxy] benzaldehyde hydrochloride.	
3	Impurity G	2-(4-hydroxyphenyl)ethanol	
4	Impurity F	(2RS)-1- [(1-methylethyl)amino)- 3-phenoxypropan- 2-ol	
5	Impurity A	(±)1-(ethylamino)-3-[4-(2methoxyethyl) -phenoxy]-propan-2-ol.	
6	Impurity D-EP	3-[4-(2-methoxyethyl)phenoxy]-1,2-propanediol	
7	4-MEP	4-(2-methoxyethyl)phenol	
8	MTS1	2-((4-(2-methoxyethyl)phenoxy)methyl) oxirane	
9	Impurity B	(±)1-Chloro-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]-propane	
10	Impurity D-USP	(±)N,N-bis-[2-hydroxy-3-[4-(2-methoxyethyl)phenoxy] propyl](1-methylethyl)amine hydrochloride	
11	Bisether	1,3-bis(4-(2-methoxyethyl) phenoxy) propan-2-ol.	

MATERIALS

2.1 Reagent and Chemicals

Metoprolol succinate working standard, Test sample and its impurites working standards received from Analytical research and development department of indoco research centre (Navi Mumbai). HPLC ultra gradient solvents like Acetonitrile, Methanol are purchased from J.T. Baker (India) and Dipotassium hydrogen phosphate AR grade buffer was purchase from Merck (India)

2.2 Instrumentation

Waters, Alliance 2695 series HPLC system comparising a quaternary pump, an autosampler, a thermostatted column compartment, a solvent cabinet with degasser along with photodiode array (PDA) 2998 detector and ultraviolet (UV) 2487 detectors were used for separation and detection. Data acquisition and calculations were carried out using Waters Empower3 software (Milford). Sartorius (Germany) analytical balance was used for weighing material.

3. METHODOLOGY

3.1 Method optimization

Metoprolol was most important medications needed in a basic health system, It was listed in World Health Organization's as Essential Medicines.The Metoprolol succinate API was avaiable in pharmacopeia Indian, United State and Europe, pharmacopeia has given method of analysis for impurites testing, in pharmacopeia Indian and United State has given same method of analysis where as pharmacopeia Europe has given differnent method of analysis.We run Metoprolol succinate API for both method of analysis and observed the raw data. The Indian and United State pharmacopeial has reserve phase chromatography.In this method analysis pharmacopial listed impurities are well

separated where as process and unspecificed pharmacopial impurites are not well separated with each other. The coloum used for pharmacopeial method of analysis was very short column, i.e 15cm to 12.5cm lenghth and partical size was 5µ to 3µ. Due to short column length impurities may not be well separated therfore we take a trial with longer column and observe the analysis data, but no impurities separation are observed.The Metoprolol Succicinate molecule and its impurities are pH sensetive therefore we mentained the pH of mobile phase through out the analysis but no effect was observed in pharmacopial method of analysis. Hence we take further trial with change in mobile phase pH and observed raw data. The change in pH of mobile phase was working for impurities seperation. Hence further trial was taken with mobile phase in composition rato, but still seperation of impurities are not getting properly.Finally we change the system from isocratic to binary gradient. The gradient method was working properly,then we tried with differernt column stationary phases like C8, C18, cyno and phenyl for impurities separation,base on raw data outcome and our valuable observation we decide that Durashell C18, (250 mm x 4.6 mm, 5 µm) column was working for impurities separation. Then we optimized buffer concentration and ratio of buffer concentration.The used buffer was di-potassium hydrogen phosphate buffer and it show a very good separation and responces of impurites.The solvent like methanol, acetonitrile and water as secondary mobile phase, Futher flow was optimizing to1.0ml/min. The optimized parameters in chromatographic condition details are given in Table 2. For system suitability we injected mixture of Metoprolol Related compound A ,B, C and D and Metoprolol succinate standard and observed the resolution, then inject low load of Metoprolol succinate working standard and observed the Therotical plate and relative standard deviation.The relative standard deviation for replicate injections should not be more than 5 percentage, whereas retention time conformation was done by injecting working standard and test sample.

Preparation of Mobile Phase

Mobile Phase-A

Transfer about 0.5 g of Dipotassium hydrogen phosphate into 1L bottle, dissolve in 1000 mL of water, mix well then adjust to pH 3.0 ± 0.05 with ortho phosphoric acid. Filter this solution through a 0.45µm membrane filters, and degas by sonication for 2 minutes.

Mobile Phase-B

Mixture of 5 volumes of Acetonitrile, 4 volumes of methanol and 1 volume of water and degas by sonication for 2 mins.

Diluent

Mixture of 8 volumes of mobile phase-A and 2 volumes of acetonitrile and degas by sonication for 2 minutes.

Preparation of Blank

Use diluent as a blank.

Preparation of solutions

Reference solution (a)

Transfer about 20 mg of Metoprolol Succinate working standard into 10 mL volumetric flask, dissolve in 5 mL of diluent and make upto the mark with diluent. Transfer 1.0 mL of this solution in to 100 mL volumetric flask and make upto mark with diluent. Further transfer 1.0 mL of this solution to 10 mL volumetric flask and make upto mark with diluent.

System suitability solution

Transfer about 5 mg each of Metoprolol related compound A, B, C, D and Metoprolol Succinate working standard into 100 mL volumetric flask, dissolve in 50 mL of diluent and make upto the mark with diluent.

Test solution

Transfer about 20 mg of Metoprolol Succinate sample into 10 mL volumetric flask, dissolve in 5 mL of diluent and make upto the mark with diluent.

Chromatographic Conditions

Column	Durashell C18, (250 mm x 4.6 mm, 5 µm) or equivalent		
Column Temperature	30°C ± 2°C		
Flow Rate	1.0 mL/min		
Gradient Programme	Time (min)	Mobile phase-A (%)	Mobile phase-B (%)
	0	80	20
	35	15	85
	40	80	20
	45	80	20
Injection Volume	10 µL		
Detector Wavelength	223 nm		
Run Time	45 minutes		
Retention Time	MTS about 10.5 minutes, RRT 1.00.		
Needle wash	Water : Acetonitrile (80:20)		

Note: Disregard the peaks due to isopropyl amine at RRT about 0.2 and Succinic acid at RRT about 0.3.

Injection sequence

Sr No.	Description	No. of Injections
1	Blank	1
2	System suitability solution	1
3	Blank	1
4	Reference solution (a)	5
5	Test solution	2

Procedure

Equilibrate the HPLC system with the initial composition until a steady baseline is obtained. Inject Blank and System suitability solution and ensure that all the system suitability parameters are meet the requirements. Then inject blank, reference solution (a) and Test solution as per injection sequence and record the chromatograms. Make blank correction if necessary.

Sr.No.	Components	Relative Retention Time	Relative Response factor
1	Metoprolol	1.00	-
2	Impurity C	0.56	1.12
3	Impurity G	0.64	1.39
4	Impurity F	0.86	0.52
5	Impurity A	0.89	0.89
6	Impurity H	0.52	0.78
7	Impurity D-EP	1.23	1.33
8	4-MEP	1.39	1.37
9	MTS1	2.10	1.12
10	Impurity B	2.20	1.10
11	Impurity D-USP	2.50	1.04
12	Bisether	2.90	1.58

System suitability

Acceptance criteria

Resolution: The resolution between the peaks due to Metoprolol Succinate and Metoprolol related compound-A in the chromatogram obtained with system suitability solution should not be less than 4.0.

Tailing factor: The tailing factor for peak due to Metoprolol succinate in the chromatogram obtained with system suitability solution should not be more than 2.0.

%RSD: The percent relative standard deviation for five replicate injection due to Metoprolol Succinate peak in the chromatogram obtained with reference solution (a) should not be more than 5.0.

Calculation

Calculate impurities content by formula given below:

$$\%\ \text{Known impurities} = \frac{AI \times WS \times 1 \times 1 \times 1}{AR \times WT \times 100 \times 10 \times RRF} \times P$$

$$\%\text{Single impurity} = \frac{AU \times WS \times 1 \times 1}{AR \times WT \times 100 \times 10} \times P$$

$$\%\text{Total unknown impurity} = \frac{AS \times WS \times 1 \times 1}{AR \times WT \times 100 \times 10} \times P$$

% Total impurities = % known impurities + %Total unknown impurities

Where,

AI = Average peak area for respective known impurities in test solution.

AR = Average peak area of Metoprolol Succinate in reference solution (a).

AU = Average peak area of single unknown largest impurity in test solution.

AS= Sum of peak area of all unknown impurities in test sample preparation.

WS = Weight of Metoprolol Succinate working standard (mg) taken for reference solution (a)

WT = Weight of Metoprolol Succinate sample taken (mg) for test solution preparation.

P = Potency of Metoprolol Succinate working standard.

RRF= Relative response factor of respective impurities.

4.0 ANALYTICAL METHOD VALIDATION

The developed method is subjected to analytical method validation, which is conducted according to the International Council for Harmonisation (ICH) guidelines [5-10].The parameter which was taken for analytical method validation as specificity, limit of detection, limit of quantitation, linearity, accuracy, precision, robustness and sample solution stability.

5.0 RESULTS AND DISCUSSION

5.1 System suitability

The System suitability test represents as an integral part of the method and used to ensure adequate performance of the chromatographic system.To check the system suitability, system suitability solution was injected and observed the resolution between Metoprolol Succinate peak and Metoprolol related compound A peak, then injected five replicate injections of reference solution(a) and calculate percentage relative standard deviation for the Metoprolol succinate peak.The area details of reference solution, relative standard deveation and

resolution were recored in Table 2. The percentage relative standard deviation should be less than 5.0 and resolution should not be less than 4.0.The system suitability was checked before each validation parameter.

Table 2: System suitability data.

Name	No of injection	Area
Reference Solution (a)	Injection-1	35825
	Injection-2	34992
	Injection-3	35788
	Injection-4	34799
	Injection-5	35045
	Avg. Area	35290
	Std. Deviation	480.34
	% RSD	1.36
Theoretical plate	49747	
Resolution between Metoprolol succinate and Metoprolol related compound-A	6.20	

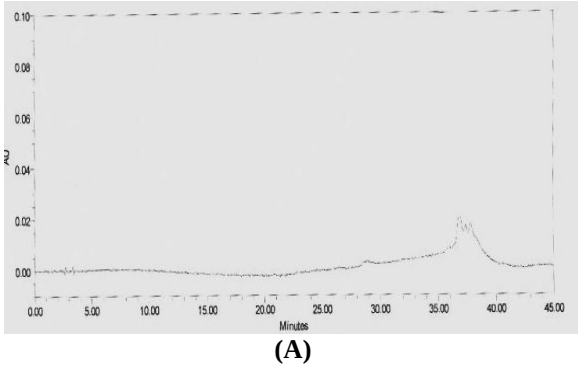
5.2 Specificity

Specificity is the capability of the method to measure the analyte response in the presence of impurities. Figure 1 shows the typical chromatograms of the blank solution, system suitability solution, reference solution (a),test solution and impurites spiked test sample.The results indicated that all impurities are well separated under the optimized chromatographic conditions. Also,there was no interference of peaks due to blank solution and the samples solution within the retention time of impurities obtained and Metoprolol succinate peak. The peak purity for all impurities and Metoprolol succinate were passing as acceptance critria. The retention times for each impurity and peak purity refer Table No.03.

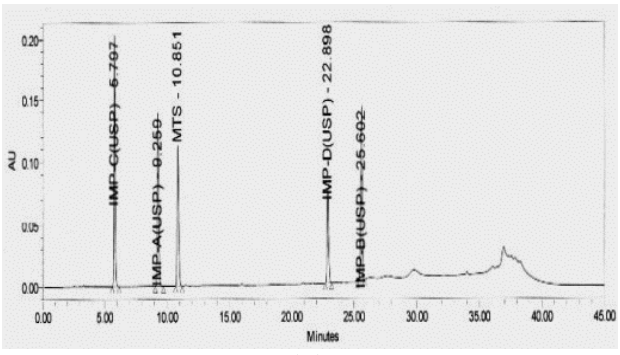
Table 03: Peak purity for spiked test solution.

Sr.No	Peak Name	RT	Area	RT Ratio	Purity Angle	Purity Threshold
1	MTS	9.96	2077315	1.00	1.44	2.85
2	Impurity C	5.75	2878149	0.58	0.35	2.52
3	Impurity G	7.30	3026532	0.73	2.05	2.64
4	Impurity F	8.76	1544468	0.88	1.41	2.85
5	Impurity A	9.17	2257806	0.92	1.46	2.80
6	Impurity H	5.36	1844079	0.54	2.05	2.77
7	Impurity D-EP	14.07	2858847	1.41	1.66	2.72
8	4-MEP	15.85	3168695	1.59	1.52	2.67
9	Impurity D-USP	22.66	1539467	2.28	1.42	2.96
10	MTS1	24.06	2543125	2.42	1.45	2.77
11	Impurity B	25.35	2341309	2.55	1.45	2.79
12	Bisether	33.65	3360076	3.38	1.47	2.69

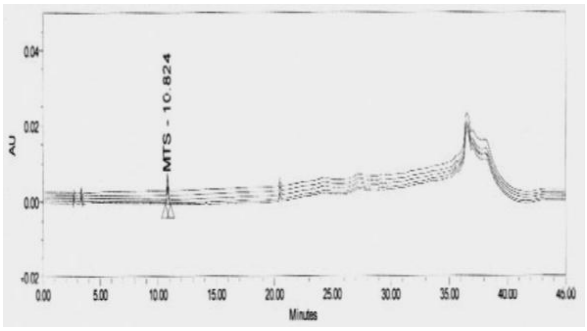
Typical chromatrogram



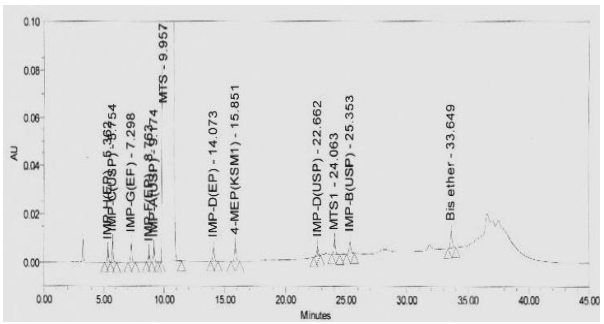
(A)



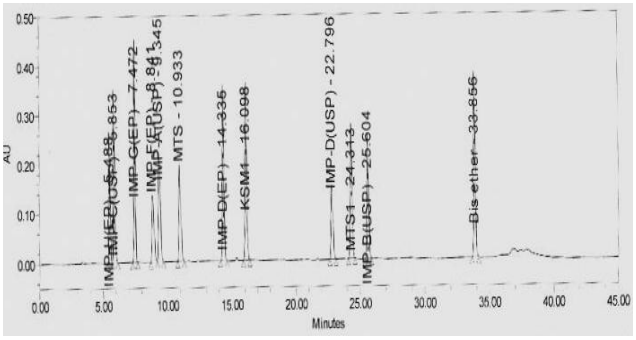
(B)



(C)



(D)



(E)

Figure 2: Specificity.

A) Blank, B) System suitability solution, C) Reference solution, D) Sample spiked with impurities, E) Mixture of impurities solution.

5.3 Limit of detection and limit of quantitation

A series of standard solutions of Metoprolol succinate and its impurities were prepared and injected in concentration ranging from 25% to 150% of target concentration and calculate the Limit Of Detection (LOD) and Limit Of Quantitation (LOQ) based on regression line i.e residual standard deviation (STE_{YX}) and slope.The calculated LOD and LOQ was well within limit as per ICH guidline and it show lowest 0.112ppm LOD and 0.340 ppm LOQ for all the impurities and Metoprolol succinate API (Table 04).

Table 04: Limit of detection and quantitation.

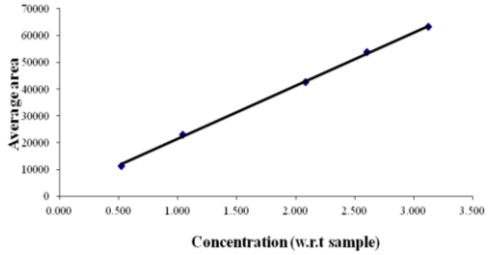
Sr. No	Name of impurities	Parameter	
		LOD (PPM)	LOQ (PPM)
1	Metoprolol Succinate	0.112	0.340
2	Impurity C	0.041	0.125
3	Impurity G	0.034	0.102
4	Impurity F	0.031	0.093
5	Impurity A	0.031	0.094
6	Impurity H	0.038	0.116
7	Impurity D-EP	0.022	0.067
8	4-MEP	0.048	0.146
9	MTS1	0.039	0.118
10	Impurity B	0.070	0.212
11	Impurity D -USP	0.072	0.218
12	Bisether	0.083	0.252

5.4 Linearity

A series of linearity solution of Metoprolol Succinate and its impurities solution were prepared from 25% to 150% of target concentration.The linearity curves were drawn by plotting the peak responce of Metoprolol succinate and impurities against its corresponding concentration. The regression coefficient, slope and % y intercept are calculate and reported in Table05.Observed regression coefficient was greater than 0.998 and % y intercept was less than 5.0%.

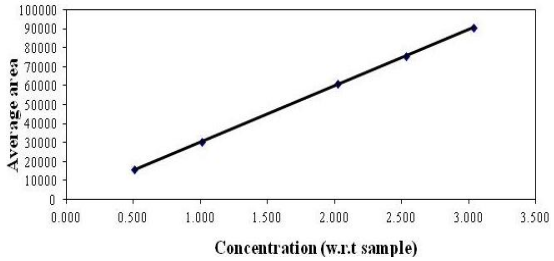
Table 05. Linearity table and figures.

Metoprolol Succinate



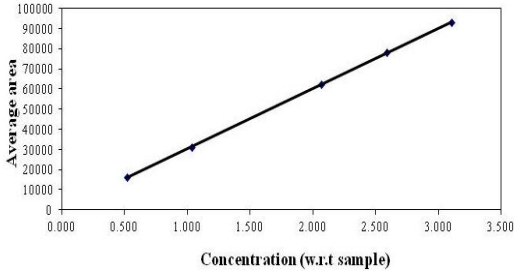
Slope	19806.72
Intercept	1598.51
%y-Intercept	3.76
Regression coefficient (R^2)	0.9993

Impurity C



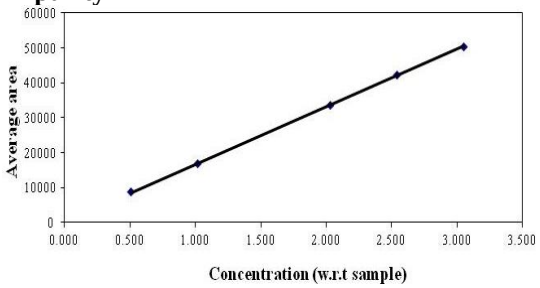
Slope	29553.13
Intercept	802.81
%y-Intercept	1.32
Regression coefficient (R^2)	0.9999

Impurity G



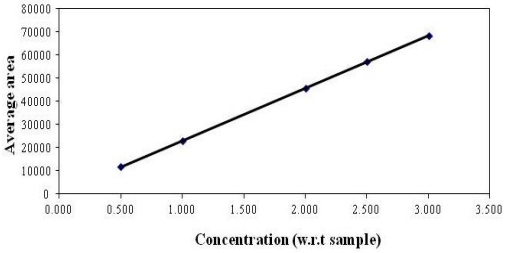
Slope	29869.88
Intercept	505.81
%y-Intercept	0.81
Regression coefficient (R^2)	0.9999

Impurity F



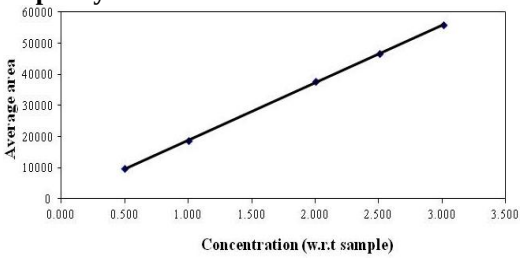
Slope	16495.47
Intercept	190.49
%y-Intercept	0.57
Regression coefficient (R^2)	0.9999

Impurity A



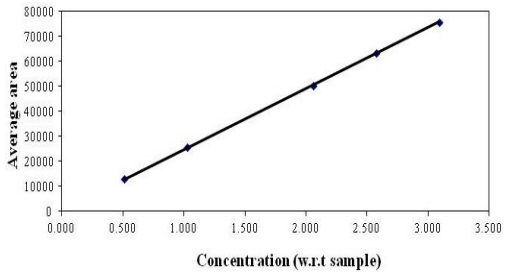
Slope	22607.95
Intercept	427.91
%y-Intercept	0.94
Regression coefficient (R^2)	0.9999

Impurity H

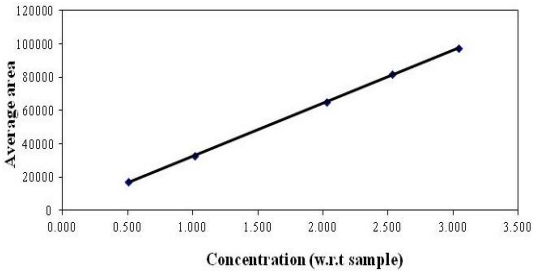


Slope	18473.92
Intercept	289.53
%y-Intercept	0.77
Regression coefficient (R^2)	0.9999

Impurity D-EP



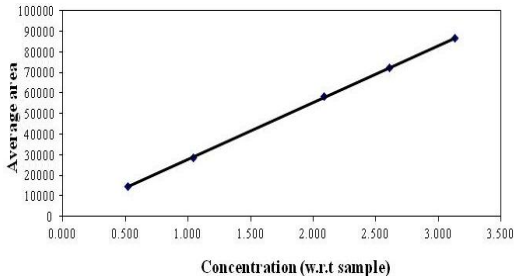
4-MEP



Slope	31833.91
Intercept	612.95
%y-Intercept	0.94
Regression coefficient (R^2)	0.9999

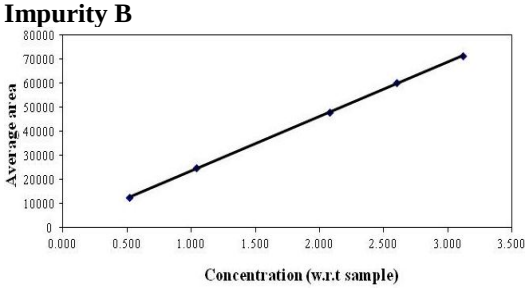
Slope	24344.68
Intercept	446.56
%y-Intercept	0.89
Regression coefficient (R^2)	1.0000

MTS1



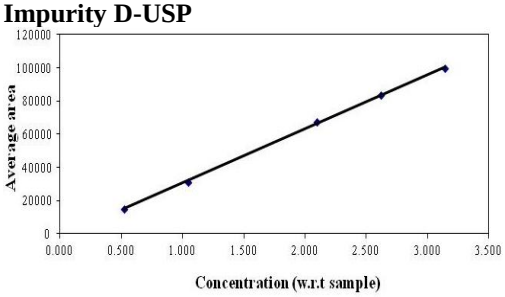
Slope	27630.34
Intercept	180.67
%y-Intercept	0.31
Regression coefficient (R^2)	0.9999

Impurity B



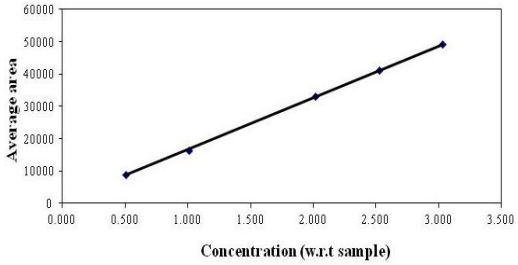
Slope	22667.55
Intercept	961.14
%y-Intercept	2.00
Regression coefficient (R^2)	0.9997

Impurity D-USP



Slope	16088.00
Intercept	463.21
%y-Intercept	1.39
Regression coefficient (R^2)	0.9997

Bisether



Slope	32630.28
Intercept	-2229.16
%y-Intercept	-3.32
Regression coefficient (R^2)	0.9996

5.5 Precision

System precision was carried out by injecting six reference solutions (a) of Metoprolol Succinate sample at limit level concentration. The relative standard deviation for the peak area of Metoprolol Succinate was calculated and found to be 1.36%. Precision at LOQ was analysed by preparing a mixture of impurities at concentration of LOQ level and injecting six times and observed the relative standard deviation. Relative standard deviation for impurities peaks where below 2.34 % (Table 06).

Table 06: System precision and precision at LOQ.

Parameter	Peak name	% RSD for peak area
System precision	Metoprolol Succinate	1.36%
Precision at LOQ	All the impurites	Below 2.34%

5.6 Accuracy

Accuracy of the method was established by carrying out the recovery or doping studies of impurities. The test solution was spiked with impurities solution at specific limit level concentrations 50%, 100% and 150%. Each spiked test solution was analysed for recovery study and observed the percentage recovery. Recovery obtained for impurities should be between 90% to 110% (Table-07,08 and 09).

Table 07: Recovery of Impurities-50%.

Sr. No	Imp Name	Wt Of Imp.	Test Area	Observed Area	Std Area (0.10%)	Theoretical added imp (%)	Observed imp (%)	% Recovery
1	Impurity C	10.13	0	30513	30258	0.506	0.051	100.7
2	Impurity G	10.35	0	31160	31074	0.500	0.050	100.2
3	Impurity F	10.16	244	17039	16794	0.493	0.049	99.9
4	Impurity A	10.02	1015	24022	22801	0.487	0.049	100.6
5	Impurity H	10.04	0	18872	18626	0.497	0.050	101.2
6	Impurity D-EP	10.30	334	25912	25550	0.200	0.050	100.0
7	4-MEP	10.14	0	33080	32458	0.050	0.051	101.8
8	MTS1	10.44	0	27196	28674	0.522	0.049	94.8
9	Impurity B	10.40	0	27476	24829	0.518	0.057	110.6
10	Impurity D-USP	10.10	0	18521	16236	0.488	0.056	114.0
11	Bisether	10.48	0	35618	31232	0.518	0.059	113.9

Table 08: Recovery of Impurities-100%.

Sr. No	Imp Name	Wt Of Imp.	Test Area	Observed Area	Std Area (0.10%)	Theoretical added imp (%)	Observed imp (%)	% Recovery
1	Impurity C	10.13	0	61282	60994	0.506	0.101	100.0
2	Impurity G	10.35	0	62893	62168	0.500	0.101	100.7
3	Impurity F	10.16	244	34224	33597	0.493	0.099	100.6
4	Impurity A	10.02	1015	47237	45702	0.487	0.098	100.5
5	Impurity H	10.04	0	37896	37667	0.497	0.100	100.1
6	Impurity D-EP	10.30	334	51372	50363	0.200	0.101	100.8
7	4-MEP	10.14	0	65960	65000	0.100	0.102	101.0
8	MTS1	10.44	0	56879	58268	0.522	0.101	97.1
9	Impurity B	10.40	0	49749	48124	0.518	0.106	102.9
10	Impurity D-USP	10.10	0	34411	33209	0.488	0.101	103.1
11	Bisether	10.48	0	67043	67064	0.518	0.103	99.5

Table 09: Recovery of Impurities-150%.

Sr.No	Imp Name	Wt Of Imp.	Test Area	Observed Area	Std Area (0.10%)	Theoretical added imp (%)	Observed imp (%)	% Recovery
1	Impurity C	10.13	0	91339	90507	0.506	0.153	100.9
2	Impurity G	10.35	0	94129	93284	0.500	0.151	100.9
3	Impurity F	10.16	244	51195	50484	0.493	0.149	100.9
4	Impurity A	10.02	1015	70144	68381	0.487	0.147	101.0
5	Impurity H	10.04	0	56341	55843	0.497	0.150	100.9
6	Impurity D-EP	10.30	334	71135	75683	0.200	0.140	93.5
7	4-MEP	10.14	0	98099	97217	0.150	0.153	100.9
8	MTS1	10.44	0	85837	86641	0.522	0.155	99.1
9	Impurity B	10.40	0	71586	71206	0.518	0.156	100.5
10	Impurity D-USP	10.10	0	50562	49151	0.488	0.151	102.9
11	Bisether	10.48	0	99660	99650	0.518	0.155	100.0

5.7 Solution stability

Test solution stability was established by injecting the test solution after every six hours time interval up to 24 hours. The result obstained was will within specified limit with and the relative standard deviation should be less than 5.0 %, thus solution stability was established up to 24 hours at 25 °C (Table 10).

Table 10: Solution stability of Metopropal Succinate.

Sr. No.	Imp Name	Impurities content in %			
		6 Hrs	12Hrs	18 Hrs	24 Hrs
1	Impurity C	0.00	0.00	0.00	0.00
2	Impurity G	0.00	0.00	0.00	0.00
3	Impurity F	0.00	0.00	0.00	0.00
4	Impurity A	0.00	0.00	0.00	0.00

5	Impurity H	0.00	0.00	0.00	0.00
6	Impurity D-EP	0.00	0.00	0.00	0.00
7	4-MEP	0.00	0.00	0.00	0.00
8	MTS1	0.23	0.27	0.17	0.22
9	Impurity B	0.00	0.01	0.01	0.00
10	Impurity D-USP	0.00	0.00	0.00	0.00
11	Bisether	0.10	0.14	0.12	0.12
12	Single max	0.02	0.05	0.02	0.02
13	Total Impurities	0.35	0.42	0.40	0.36

6.0 CONCLUSION

The reverse phase HPLC method was developed for quantitative determination of related substances of Metoprolol succinate API. This method was validated and found out to be linear, accurate, precise, robust and specific. The test solution was stable up to 24 hrs at room temperature.The data was acceptable for all method validation parameters tested and found out to be satisfactory. The developed method can be use for quality control department for determine the related substances in commercial sample analysis and stability samples analysis of Metoprolol succinate API.

7.0 ACKNOWLEDGMENT

The author wishes to thank to the management of Indoco Remedies Limited for supporting this work by providing the samples of Metoprolol Succinate API and impurites working standard required for this research.

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