VALIDATION OF DEVELOPED ANALYTICAL METHOD FOR METOPROLOL SUCCINATE FLOATING TABLETS BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ARTICLE INFO	ABSTRACT
Key Words Metoprolol Succinate, RP-HPLC, Validation, Method development	The present investigation was to validate a new analytical, simple, sensitive, selective and precise High Performance Layer Chromatograpic (HPLC) method for the estimation of Metoprolol Succinate in tablet dosage form. Metoprolol Succinate chemically (±) 1-(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1) (salt) used as an Anti-hypertensive agent. The mobile comprised of Acetonitrile: Phosphate buffer (0.05M phosphate buffer of pH 3.0) in the ratio of 350:650 and set at a flow rate of 1.2ml/minute. Detection was carried out at 222nm using pre-packed Symmetry C_{18}; 250x4.6mm, 5µm particle size column. The retention time of Metoprolol Succinate was found to be 1.825. The assay was linear over concentration range of 12.5µg/ml to 75µg/ml (R=0.99995). The limit of detection and the limit of quantification were found to be 2.68µg/ml and 4.46µg/ml respectively. The amount of Metoprolol Succinate was found to be 100.229±0.47 and the accuracy of Metoprolol Succinate was found to be 99.460% to 100.369%. The statistical analysis of the data showed that the method is reproducible and selective for the estimation of Metoprolol Succinate in tablet dosage form during routine analysis.

INTRODUCTION:

Metoprolol Succinate chemically (±) 1-(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1) (salt) used as an Anti-hypertensive agent. is a selective β1 receptor blocker used in treatment of several diseases of the cardiovascular system, especially hypertension. The active substance Metoprolol is employed either as *Metoprolol succinate* or *Metoprolol tartrate* (where 100 mg Metoprolol tartrate corresponds to 95 mg Metoprolol succinate), respectively as prolonged-release or conventional-release formulation. It has a relatively greater blocking effect on beta1-receptors (i.e. those mediating adrenergic stimulation of heart rate and contractility and release of free fatty acids from fat stores) than on beta2-receptors which are chiefly involved in broncho and vasodilatation. It has neither membrane-stabilising effect nor partial agonist (intrinsic sympathomimetic) activity. The stimulant effect of catecholamines on the heart is reduced or inhibited by Metoprolol. This leads to a decrease in heart rate, cardiac contractility and cardiac output. The literature survey [1-8] reveals that there is some Spectroscopic and HPLC methods have been reported. In this paper we describe a simple, inexpensive, sensitive and

validated HPLC method for the determination of Metoprolol Succinate in tablet dosage form.

EXPERIMENTAL WORK:

Working standard of Metoprolol Succinate, HPLC grade Acetonitrile, Sodium hydroxide, Potassium dihydrogen Phosphate, O-Phosphoric acid, 0.45µm PVDF membrane filter and Milli-Q water were procured from the market. The separation was carried out on isocratic HPLC system Shimadzu with UV detector with pre-packed Symmetry C18 250 x 4.6mm, 5.0µ m particle size using filtered and degassed Acetonitrile:Phosphate buffer (0.05M phosphate buffer of pH 3.0) in the ratio of 350:650 as mobile phase.

Mobile phase preparation

Diluent:. Mix Acetonitrile:Phosphate buffer (0.05M phosphate buffer of pH 3.0) in the ratio of 350:650

Mobile phase: Filtered and degassed mixture of Acetonitrile:Phosphate buffer (0.05M phosphate buffer of pH 3.0) in the ratio of 350:650

Standard preparation: Weighed accurately about 0.100g of Metoprolol Succinate working standard into a 100ml volumetric flask, added 70ml of diluent, shaked and sonicated to dissolve the content, made up the volume with diluent. Pipetted out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collected the filtrate after discarding the few ml of the filtrate.

Assay preparation: Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Metoprolol Succinate) in to a 100ml volumetric flask. Added 70ml of diluent sonicated for 30minutes, and made up the volume with diluent, pipetted out 5ml of filtrate to 100ml with diluent. Filtered the solution through 0.45micron membrane filter. Collected the filtrate after discarding the first few ml of the filtrate.

Chromatographic conditions: Flow rate 1.0ml/min; detection wavelength 222nm; injection volume 20µl; column used symmetry column (5µm, 250x4.6mm); column temperature: 25ºC; Acetonitrile:Phosphate buffer (350:650).

Method development [09-21] : Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

Chromatogram
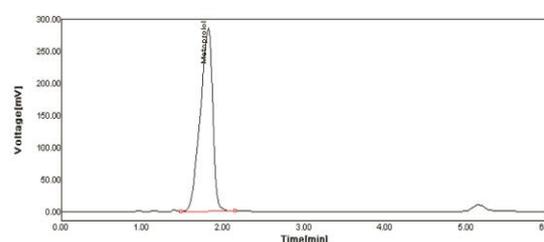
No.	Name	RT[min]	Area[mV*s]	Area%	TP	Height%	TF
1	Metoprolol	1.81	372414.96	100.00	4125	100.00	1.13
Sum			372414.96				

Chromatogram of Metoprolol Succinate

Validation of developed method [09-21]:

System Suitability Preparation: Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Metoprolol Succinate) in to a 100ml volumetric flask. Added 70ml of diluent sonicated for 30minutes, and made up the volume with diluent, pipetted out 5ml of filtrate to 100ml with diluent. Filtered the solution through 0.45micron membrane filter. Collected the filtrate after discarding the first few ml of the filtrate.

Procedure: Separately injected equal volumes (about 20 µl) of the standard preparation and the assay preparation into the chromatograph, recorded the chromatograms, and measured the responses for the Metoprolol Succinate peak.

Precision: To establish the precision of the analytical method by using the following two methods.

System Precision: Establish the repeatability of the analytical method by estimating the assay for six different sample preparations of the same batch. Calculate the assay for all six-sample preparations and report the %RSD for the same.

Preparation of Blank: Use diluent as blank.

Preparation of standard solution: Weighed accurately about 0.100g of Metoprolol Succinate working standard into a 100ml volumetric flask, added 70ml of diluent, shacked and sonicated to dissolve the content, made up the volume with diluent. Pipetted out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collected the filtrate after discarding the few ml of the filtrate.

Preparation of sample solutions: Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Metoprolol Succinate) in to a 100ml volumetric flask. Added 70ml of diluent and sonicated for 30minutes, and made up the volume with diluent, pipetted out 5ml of filtrate to 100ml with diluent. Filtered the solution through 0.45micron membrane filter. Collected the filtrate after discarding the first few ml of the filtrate.

Procedure: Injected separately 20µl of blank, standard and sample preparations into the chromatograph and measured the peak responses for the major peak. Calculated the content of Metoprolol Succinate in the individual solutions.

Intermediate precision (Ruggedness):

A different analyst using a different HPLC system with a different similar column on a different day should carry out this experiment. Estimating the assay for six different sample preparations of the same batch. Calculate the assay for all six-sample preparations and report the %RSD for the same.

Blank preparation: Use diluent as blank.

Preparation of standard solution: : Weighed accurately about 0.100g of Metoprolol Succinate working standard into a 100ml volumetric flask, added 70ml of diluent, shacked and sonicated to dissolve the content, made up the volume with diluent. Pipetted out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collected the filtrate after discarding the few ml of the filtrate.

Preparation of sample solutions: Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Metoprolol Succinate) in to a 100ml volumetric flask. Added 70ml of diluent and sonicated for 30minutes, and made up the volume with diluent, pipetted out 5ml of filtrate to 100ml with diluent. Filtered the solution through 0.45micron membrane filter. Collected the filtrate after discarding the first few ml of the filtrate.

Procedure: Separately injected 20µl of blank, standard and sample preparations into the chromatograph and measured the peak responses for the major peak. Calculated the content of Metoprolol Succinate in the individual solutions

Linearity & Range

Objective: To establish the linearity of the analytical method for assay using the following two methods.

Linearity & range for Metoprolol Succinate working standard: Demonstrate the linearity of the analytical method for assay by injecting the various concentrations of standard preparations prepared in the range of 25% to 150% into the chromatograph, covering 6 different concentrations. Draw a plot between the Concentrations vs. peak response of Metoprolol Succinate. Report the slope, intercept and regression coefficient from the plot obtained for concentration vs.

Peak response of Metoprolol Succinate in standard preparation.

Preparation of analytical solutions for linearity & range for Metoprolol Succinate standard preparations:

a) **Blank preparation:** Use diluent as blank.

b) **Standard stock solution preparation:** Transfer an accurately weighed quantity of about 100 mg of Metoprolol Succinate working standard into 100 ml volumetric flask , add 20ml of diluent, sonicated for 10 minutes to dissolve and made to volume with mobile phase. From the stock solution 10 ml was pippeted out into the 100 ml volumetric flask and made to volume with mobile phase.

c) **25%Linearity Standard solution preparation (12.5 ppm):** Pipette out 6.25 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

d) **50%Linearity Standard solution preparation (25.0 ppm):** Pipette out 12.50 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

e) **75% Linearity Standard solution preparation (37.5 ppm):** Pipette out 18.75 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

f) **100% Linearity Standard solution preparation (50 ppm):** Pipette out 25 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

g) **125% Linearity Standard solution preparation (62.5 ppm):** Pipette out 31.25 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

h) **150% Linearity Standard solution preparation (75.0 ppm):** Pipette out 37.5 ml of Standard stock solution into 50 ml volumetric flaks and make up to volume with diluent.

Calculations: Draw a plot between the concentration vs. the average peak responses of Metoprolol Succinate peak for all the above studies. Calculate slope, intercept and regression coefficient from the plot obtained.

Accuracy / Recovery

Objective: To establish the accuracy of the analytical method is the closeness of sample results obtained by method to the true value by using recovery study.

Procedure: Perform the recovery studies by adding known quantity of Metoprolol Succinate working standard to known quantity of placebo (Metoprolol Succinate Tablet 100 mg excipient mixture) in the range of 50% to 150% of the sample concentration. Report the percentage recovery in relative standard deviation for all the values of % recovery.

a) **Blank preparation:** Use diluent as blank.

b) **Standard preparation:** Weighed accurately about 0.100g of Metoprolol Succinate working standard into a 100ml volumetric flask, added 70ml of diluent, shacked and sonicated to dissolve the content, made up the volume with diluent. Pipetted out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collected the filtrate after discarding the few ml of the filtrate.

c) **50% recovery solution preparation:** Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Metoprolol Succinate) in to a 100ml volumetric flask containing 50 mg of Metoprolol Succinate add 70ml of diluent. Sonicate for 30 minutes to dissolve and make up to the volume with diluent & mix. Filter through 0.45μ membrane filter. Diluted the above solution as 10ml to 50 ml with diluent. Repeat this

procedure for another two sample preparations.

d) 100% recovery solution preparation: Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Metoprolol Succinate) in to a 100ml volumetric flask containing 100 mg of Metoprolol Succinate add 70ml of diluent. Sonicate for 30 minutes to dissolve and make up to the volume with diluent & mix. Filter through 0.45μ membrane filter. Diluted the above solution as 10ml to 50 ml with diluent. Repeat this procedure for another two sample preparations.

e) 150% recovery solution preparation: Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Metoprolol Succinate) in to a 100ml volumetric flask containing 150 mg of Metoprolol Succinate add 70ml of diluent. Sonicate for 30 minutes to dissolve and make up to the volume with diluent & mix. Filter through 0.45μ membrane filter. Diluted the above solution as 10ml to 50 ml with diluent. Repeat this procedure for another two sample preparations.

Procedure: Separately inject 20μl of standard and sample preparations of recovery solutions into the chromatograph and measure the peak responses for the major peak. Calculate the % recovery in recovery solutions using the following expression.

Recovery Calculation:

% of recovery = mg of Metoprolol Succinate/ working standard addedX100

Stability of Analytical solutions

Objective: To establish the stability of analytical solutions by injecting the standard and sample solutions at periodic intervals up to 32hrs.

Preparation of analytical solutions

a) **Blank preparation:** Use diluent as blank.

b) Standard solution preparation: Weighed accurately about 0.100g of Metoprolol Succinate working standard into a 100ml volumetric flask, added 70ml of diluent, shacked and sonicated to dissolve the content, made up the volume with diluent. Pipetted out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collected the filtrate after discarding the few ml of the filtrate.

c) Sample solution preparation: Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 0.300g powdered tablets (equivalent to 0.100g of Metoprolol Succinate) in to a 100ml volumetric flask. Added 70ml of diluent and sonicated for 30minutes, and made up the volume with diluent, pipetted out 5ml of filtrate to 100ml with diluent. Filtered the solution through 0.45micron membrane filter. Collected the filtrate after discarding the first few ml of the filtrate.

Procedure: Inject 20μl of blank, resolution solution, standard preparation and sample preparations into the chromatograph and record the chromatograms. Measure the peak responses for major peak for all solutions. Continue the chromatography with periodic injections in duplicate for standard and sample preparations in the interval of 4hrs or suitable interval depending on the instrument utilization and sequence of injections.

Calculation: Calculate the average peak response and %RSD for initial 5 replicate injections of standard preparations. Calculate the %RSD for average peak responses of standard and sample preparations for periodical intervals.

RESULTS AND DISCUSSION:

The assay values for Metoprolol Succinate tablets 100mg obtained from six samples were found to be within the acceptance criteria. The RSD of assay values from 6 samples is not more than 2.0%. Therefore, the method is considered precise.

Table no 1: Data for system suitability

Injections	RT	Peak area	USP Plate	USP Tailing
1	1.81	375841.38	4005	1.12
2	1.83	365756.27	4025	1.14
3	1.81	366748.63	4054	1.15
4	1.83	364785.35	4056	1.16
5	1.85	372726.96	4012	1.13
6	1.82	369765.32	4089	1.12
Mean	**1.825**	**369270.652**	**4040.167**	**1.137**
Std deviation	**0.015**	**4340.383**	**31.896**	**0.016**
% RSD	**0.831**	**1.175**	**0.789**	**1.437**

The % RSD of all the parameters like retention time, area, theoretical plates and tailing factor was within the limit. So the method passes these system suitability parameters.

Table no 2: Data for System Precision

Injections	Retention Time	Peak Area
1	1.79	365725.36
2	1.82	368558.69
3	1.83	375485.65
4	1.81	372754.21
5	1.82	375675.24
Mean	**1.814**	**371639.830**
Std deviation	**0.015**	**4381.526**
% RSD	**0.836**	**1.179**

The % RSD of five replicate injections of standard solution is within the specified acceptance criteria.

Table no 3: Data for Method Precision

Samples	Peak Area	Weight of sample	Assay in mg	in %
1	375656.25	300.25	99.99	99.99
2	382745.68	300.25	101.88	101.88
3	372414.96	300.56	99.13	99.13
4	382985.69	300.48	101.94	101.94
5	372774.46	300.95	99.22	99.22
6	372762.24	300.56	99.22	99.22
Mean			**100.229**	**100.229**
Std deviation			**1.338**	**1.338**
% RSD			**1.335**	**1.335**

Table no 4: Data for Intermediate Precision

Day	Sample Inj	Peak Area	Weight Of Sample	Assay in mg	in %
1	1	375754.26	300.25	100.02	100.02
	2	372785.65	300.65	99.23	99.23
2	3	382749.38	300.48	101.88	101.88
	4	376715.34	300.95	100.27	100.27
3	5	373784.59	300.48	99.49	99.49
	6	372765.18	300.48	99.22	99.22
Mean				100.017	100.017
Std deviation				1.006	1.006
% RSD				1.006	1.006

Table no 5: Linearity study for Metoprolol Succinate

Sample no	%level	concentration (µg/ml)	area
1	25	12.5	94182.59
2	50	25.0	188105.18
3	75	37.5	283874.76
4	100	50.0	375694.35
5	125	62.5	471130.94
6	150	75.0	570373.53

Linearity curve:
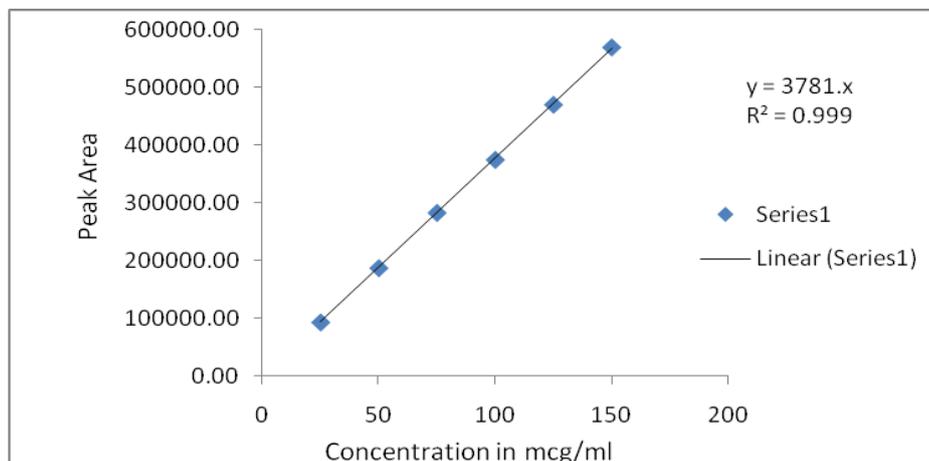
Linearity Curve of Metoprolol Succinate

Table No 6: Method accuracy study of Metoprolol Succinate

Sample No	Theoretical (%)	Mean Peak area	Recovery In (mg)	Recovery In (%)	Mean (%) Recovery	%RSD
1	50	188638.175	50.21	100.42		
2	50	191452.675	50.96	101.92	100.369	1.571
3	50	185530.62	49.38	98.77		
1	100	377164.35	100.39	100.39		
2	100	372769.35	99.22	99.22	99.460	0.843
3	100	371061.24	98.77	98.77		
1	150	566461.525	150.78	100.52		
2	150	568809.025	151.40	100.93	99.856	1.525
3	150	552911.86	147.17	98.11		

Table No 7: Data for LOD and LOQ

Sample no	%Level	Concentration (µg/ml)	Area
1	25	12.5	94182.59
2	50	25.0	188105.18
3	75	37.5	283874.76
4	100	50.0	375694.35
5	125	62.5	471130.94
6	150	75.0	570373.53
Slope			3796.40
Standard Deviation			46.77
Correlation co-efficient			0.99995
LOD			2.68 µg/ml
LOQ			4.46 µg/ml

Table No 8: Data for standard solution stability

S. No.	Time In Hours	RT	Peak Area
1	0	1.83	383364.25
2	4	1.85	374568.36
3	8	1.81	372715.56
4	12	1.81	366256.35
5	16	1.83	375568.25
Mean		**2.94**	**2.398**
Std deviation		**0.05**	**0.034**
% RSD		**1.76**	**1.426**

The RSD of obtained standard area is not more than 2.0%. Therefore, the solution is considered stable.

Table no 9: Data for sample solution stability

S. no.	Time In Hours	RT	Peak Area
1	0	1.83	362769.26
2	4	1.83	374154.35
3	8	1.82	371745.25
4	12	1.83	377755.65
5	16	1.85	372749.78
Mean		2.84	2.256
Std deviation		0.27	0.032
% RSD		9.66	1.423

The RSD of obtained sample area is not more than 2.0%. Therefore, the solution is considered stable.

Table no 10: Data of validation parameters for Metoprolol Succinate

PARAMETERS	METOPROLOL SUCCINATE
Specificity	No interference between blank, standard and sample peak
System Suitability Retention time Peak Area Theoretical Plates Tailing Factor	 1.825 369270.652 4040.167 1.137
Precision System precision (% RSD) Method precision (% RSD)	 1.179% 1.335%
Intermediate Precision (% RSD)	1.006%
Linearity and Range Slope Standard deviation Correlation co-efficient	12.5 µg/ml to 75.0 µg/ml 3796.40 46.7707 0.99995
% Recovery 50% 100% 150%	 100.369% 99.460% 99.856%
LOD	2.68 µg/ml
LOQ	4.46 µg/ml
Solution Stability Standard (% RSD) Sample (% RSD)	 0.916% 0.598%

The assay values for Metoprolol Succinate tablets 100mg obtained from six samples were found to be within the acceptance criteria. The RSD of assay values from 6 samples is not more than 2.0%. Therefore, the method is considered precise. The assay values for the range of recovery levels from 50%-150% of the Metoprolol Succinate working concentration (25micron/ml) conform to the acceptance criteria. The percentage Metoprolol Succinate recovered at each of the levels falls between 99.460%-100.369% and the %RSD of all determinations at each level was not more than 2.0% .therefore the method is considered accurate.

The LOD and LOQ of the Metoprolol Succinate was calculated from the following formula,

$$LOD = 3.3\sigma / S$$

Where the σ = the standard deviation of the response,

S = the slope of the calibration curve

$$LOQ = 10\,\sigma / S$$

Where the σ = the standard deviation of the response,

S = the slope of the calibration curve

SUMMARY AND CONCLUSION:

The validated method was to quantitatively estimate the amount of Metoprolol Succinate in Pharmaceutical tablet dosage form using HPLC method. The calibration curve for Metoprolol Succinate was found to be linear in the range of 12.5μg/ml to 75μg/ml (r2=0.99995) indicating a good linearity. The percentage recovery of sample was found to 99.460 to 100.369 %w/w for Metoprolol Succinate indicating the good accuracy of the method. To evaluate the validity and reproducibility of the method, known amount of pure drug was added to previously analysed samples and these samples were reanalysed by proposed method, the percentage recovery was found to be close to 100% for all the methods. The limit of detection and limit of quantification was done by using linearity data, slope and standard deviation of the linearity samples were found to 2.68μg/ml and 4.46μg/ml respectively. The % relative standard deviation (%RSD) values for system precision was 1.179%, method precision was 1.335% and Intermediate precision was 1.006. The system precision and method precision were found to be less than 2% and so the method is said to be precise. The developed RP-HPLC method is simple and selective for estimation of Metoprolol Succinate in tablet dosage form was found to be accurate, rapid and sensitive. The values of coefficient of variance were satisfactory low and recovery was close to 100% indicating reproducibility of the method. The linearity was observed within limit hence method is linear.

REFERENCES:

1. Budavari S, editor. The Merck Index. 14th ed. Whitehouse Station, NJ: Merck and co., Inc; 2006. p. 1060.
2. Indian Pharmacopoeia; The Indian Pharmacopoeia Commission, Vol. II, Govt. of India Ministry of Health and Family Welfare: Ghaziabad, 2010. p.1681.
3. British Pharmacopoeia, 5th ed.; The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA), The Department of Health: Great Britain, 2005. p.1327-28.
4. United States Pharmacopoeia; United States Pharmacopoeial Convention, Inc.: Rockville, MD, 2009.p. 377-379, 2963-2965.
5. Kulkarni MN, Kshirsagar RV, Sakarkar DM.: Development and validation of spectrophotometric method for determination of metoprolol succinate. Int J ChemTech Research 2009;1(4):1273-7.
6. Jadhav AS, Tarkase KN, Deshpande AP.: Quantitative determination of Metoprolol Succinate in bulck and tablet dosage form through comparative study of UV and Derivative Spectroscopy. Der Pharmacia letter 2012;4(3):763-7.
7. Gosai , Tanna, Thumar K, Chikhalia: RP-HPLC method for simultaneous estimation of Simultaneous estimation of Metoprolol succinate and clopidogrel bisulphate in tablet dosage. Inventi Rapid 2012;3:1-6.
8. Kalisetty S, Reddy TS, Reddy AM, Palnati JB, Rao DV, Manikandan R.: Stability Indicating Reverse phase liquid chromatographic method for determination of metoprolol succinate in pharmaceutical dosage

forms. J Chem Pharm Res 2012; 4(9):4420-5.

9. A.H. Beckett, S. K. Jain , and J.B. Stenlake, Practical pharmaceutical chemistry, 4th edn, part-II, CBS publisher and distributors, New Delhi, 275-300.

10. Sethi PD, High performance liquid chromatography, New Delhi: CBS publisher: 2001; 101.

11. Willard, Merrit , Dean JJ, Frank AS. Instrumental method of analysis, 7th edn, New Delhi: CBS Publishier and Distributors: 1986; 2-5.

12. International Conference on Harmonization (ICH), Validation of Analytical procedures: Methodology Q2B, 1996.

13. Mendum J. Denny, R.C., and Thomas, M.N., Vogel's Text book of Quantitative Analysis, 6[th]Edn., Pearson education ltd., 2004, 268.

14. Sethi P.D. Quantitative Analysis of Drugs in Pharmaceutical Formulations, 3[rd]Edn. CBS Publishers and Distributors, New Delhi, 1997, 51.

15. Schirmer R. E. Modern Methods of Pharmaceutical Analysis, 2[nd]Edn. Vol. I, CRC Press, 1991, 31.

16. Scott. Liquid Chromatography for the Analyst, Vol. 67, Marcel Decker Inc., 1994, 4.

17. Meyer, Practical High Performance Liquid Chromatography, 4[th]Edn, John Wiley and Sons, 2004, 87.

18. Loyd, R. Snyder, Joseph. J. Kirkland, Joseph. L. Glajch, Practical HPLC Method Development, 2[nd]Edn, 2004, 345-54.

19. United States Pharmacopoeia, NF 21, Asian Edn., United States Pharmacopoeial Convention, Inc. Webcom Ltd., 2003, 2278.

20. ICH, Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization, Geneva, October, 1994, 1-7.

21. ICH, Q2B, Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Geneva, November, 1996, 1-12.

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