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# Stability Indicating RP-HPLC Method for Quantitative Estimation of S (-) Metoprolol Succinate in Tablet Dosage Form

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## ABSTRACT

A simple, precise, rapid and specific reversed phase high performance liquid chromatographic method for determination of S (-) Metoprolol Succinate was developed and validated. The chromatographic separation was achieved on Inertsil ODS column (125×4.6mm, 5µ), using a mixture of 20mM potassium dihydrogen phosphate (pH adjusted to 3.5 with ortho-phosphoric acid) and acetonitrile as mobile phase in the ratio of 80:20 at flow rate of 1.5 mL/min. The detection was performed at 220 nm. The calibration curve was linear in the range of 30 - 90 µg/mL ($r^2$ = 0.99999). Major impurities and degradation products were well separated from S(-) Metoprolol Succinate. Thus this assay method can be considered as stability-indicating.

**Keywords:** S (-) Metoprolol Succinate, Stability-indicating assay, RP-HPLC.

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## INTRODUCTION

S (-) Metoprolol Succinate chemically (2S)-1-[4-(2-Methoxyethyl) phenoxy]-3-[(1-methylethyl) amino]-2-propanol butanedioate (Figure-1) is a selective $\beta_1$receptor blocker used in treatment of several diseases of the cardiovascular system, especially hypertension [1-3]. The drug is available as IR Tablet dosage form and it is not official in any pharmacopoeia. From the Literature survey it was revealed that, several spectrophotometric and chromatographic methods are available for estimations of racemic drug in bulk, biological fluids and in their pharmaceutical formulations [4-7]. But no method was reported for S (-) Metoprolol Succinate immediate release (IR) tablet form; here the aim was to develop a specific, precise, rapid and economic assay method for estimation of S (-) Metoprolol Succinate by RP-HPLC.

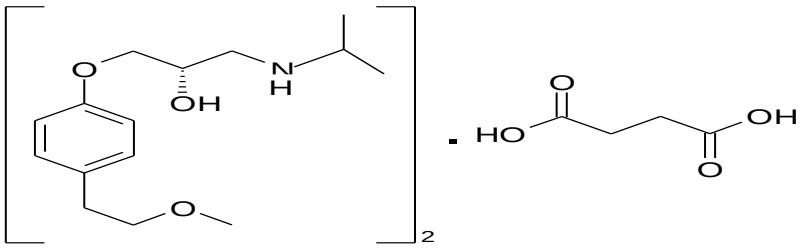


**Figure.1. Chemical structure of S (-) Metoprolol Succinate**

## MATERIALS AND METHODS

### Instrumentation

The separation of S (-) Metoprolol Succinate standard drug was carried out on a low pressure gradient reverse phase HPLC (Shimadzu Corporation, Tokyo, Japan) using Inertsil C18 column. UV and PDA detector were used in this RP-HPLC system. The column temperature was kept at 40° C. Data processing was carried out using LC Solution and Empower Pro software on LC-2010C HT system.

**Reagents and Chemicals:** S (-) Metoprolol Succinate Standard and S (-) Metoprolol Succinate IR tablets were procured from Emcure Pharmaceutical Limited, Bhosari, unit- II, Pune. Other chemicals like Acetonitrile (HPLC grade), Potassium di hydrogen phosphate ($KH_2PO_4$), Sodium Chloride (NaCl), Concentrated Hydrochloric Acid (HCl) were procured from Merck specialties Pvt. Ltd. Orthophosphoric acid (OPA) was purchased from RANKEM. Water used was of Milli Q grade.

### Chromatographic Condition

The analysis was carried out using 3.5 pH $KH_2PO_4$ buffer: Acetonitrile in the ratio of (80:20) as a mobile phase and Inertsil C18, 125 x 4.6 mm, 5 µm as a stationary phase at a flow rate 1.5 mL/min

in a low pressure gradient elution mode. Before delivering the mobile phase in to the system, it was degassed and filtered through 0.45 µm membrane Nylon filter and the detection was performed at 220nm.

**Procedure**

**Preparation of Mobile Phase**

5.44 gm of $KH_2PO_4$ was transferred into 2 liter beaker containing 2000 mL of water and sonicated for 5 minutes to dissolve $KH_2PO_4$ and pH was adjusted to 3.5 with O-phosphoric acid. Filter through 0.45µm Nylon filter and sonicated to degas for 20 minutes before use.

**Preparation of Diluent**

Accurately weigh and transfer 10 gm of NaCl to a 5 Liter beaker containing 5 Liter of Milli Q water, stir to dissolve and add 35 mL of HCl and mixed & used as diluent.

**Preparation of Standard**

30 mg of S (-) Metoprolol Succinate working standard was accurately weighed and transferred in 50 mL volumetric flask, dissolved and diluted up to the mark with diluents, to get 600 µg/mL of final concentration. 10 mL this solution was diluted to 100 mL with diluent and mixed well. (60 µg/ml)

**Preparation of Sample**

10 tablets or equivalent to 237.5 mg of S (-) Metoprolol Succinate were accurately weighed and transferred to 200 mL volumetric flask and 150 mL of diluent was added. The sample was sonicated for 20 minutes or until tablets disperse properly and made upto the mark, to get final concentration 1187.5 µg/mL and filtered through 0.45 µm Nylon disc filter. From this solution, 5 mL was transferred into 100 mL volumetric flask and volume was made upto the mark to get 59.4 µg/ml concentration.

**Selection of λ max by using UV Spectrophotometer**

**Table 1: Optimized chromatographic conditions**

| Parameter | Chromatographic Conditions |
|---|---|
| Column | Inertsil C18 (125 x 4.6 mm, 5 µm) |
| Mobile phase | Buffer : Acetonitrile (80: 20 %v/v) |
| Flow rate | 1.5 ml/min |
| Column Temperature | 40° C |
| Detection | 220 nm |
| Injection volume | 20 µl |
| Run time | 10 min |
| Retention time | 3.2 min |

Selection of λ max of S (-) Metoprolol Succinate drug was done by scanning standard solution in the range of 200 to 400 nm. The 220 nm being λ max was selected for further studies. Optimized chromatographic conditions are summarized in Table 1.

**Optimization Result**

The chromatographic conditions in Table 1 were set on RP-HPLC system and using 60 µg/mL concentration of standard solution. The retention time was achieved at 3.2 min and system suitability parameters were within the acceptable limit as per ICH guidelines. The HPLC procedure was optimized with a view to develop stability-indicating assay method. The method was optimized on Inertsil C18 ODS, 4.6 x 125 mm, 5 µm particle size. The column was saturated with the mobile phase (indicated by constant back pressure at the desired flow rate). During initial method development different combinations of mobile phases such as methanol: water: 50:50 V/V, Acetonitrile (ACN): water: 80: 20 V/V, 0.01M ammonium acetate buffer: Acetonitrile: 40:60 V/V were tried. The above mobile phase composition also tried on different columns like ACE3 C18,150X4.6mm.,Zorbax SB C8,150X4.6mm,5µ.Various flow rates like 0.8, 1, 1.2 ml/min were tried at low pressure gradient mode. The mobile phase and samples were filtered using 0.45 µm nylon membrane filters before injecting into the HPLC system. Out of the above trials it was found that 20 mM potassium dihydrogen phosphate buffer pH 3.5 (adjusted with dilute Ortho-phosphoric acid): ACN 80: 20% v/v at flow rate 1.5 ml/min with a runtime of 10 minutes provides acceptable retention time, tailing factor, theoretical plates and good peak shape of S (-) Metoprolol. Hence the method was validated as per ICH Guidelines.

## RESULTS AND DISCUSSION

**Validation of RP-HPLC Method**

The optimized RP-HPLC method was validated as per ICH guidelines [8-10]. From all the results obtained the method was found to be specific without any interference at retention time of Metoprolol, Linear in the range of 30 to 90ppm, %RSD of six assay determination in method precision was found to be 0.79, %Recovery was found in between 98-102% (98.17, 98.45 and 98.69 for 50%, 100% and 150% respectively) results of robustness were found well within limit (%RSD below 2.0%).

**Linearity**

Linearity of the method was performed by preparing and injecting in triplicate a series of Standard preparations over a range starting from 50% to 150% of the Standard preparations of S (-) Metoprolol Succinate. Stock solution of 600 µg/mL of S (-) Metoprolol was prepared and this

stock solution was diluted with simulated gastric fluid to obtain final concentration of 30-90 µg/mL (50%-150% of test concentration). 20 µl volume of each concentration was injected into HPLC in triplicate. Average peak areas of each level were plotted against drug concentration in ppm and linear regression analysis was performed on the resulting plot. The results are depicted in Table 2.and Figure 2.

**Table 2: Data of Linearity**

| Concentration in µg/mL (ppm) | Area |
|---|---|
| 30 | 689049 |
| 42 | 964651 |
| 48 | 1106988 |
| 60 | 1379131 |
| 72 | 16502993 |
| 90 | 2060117 |

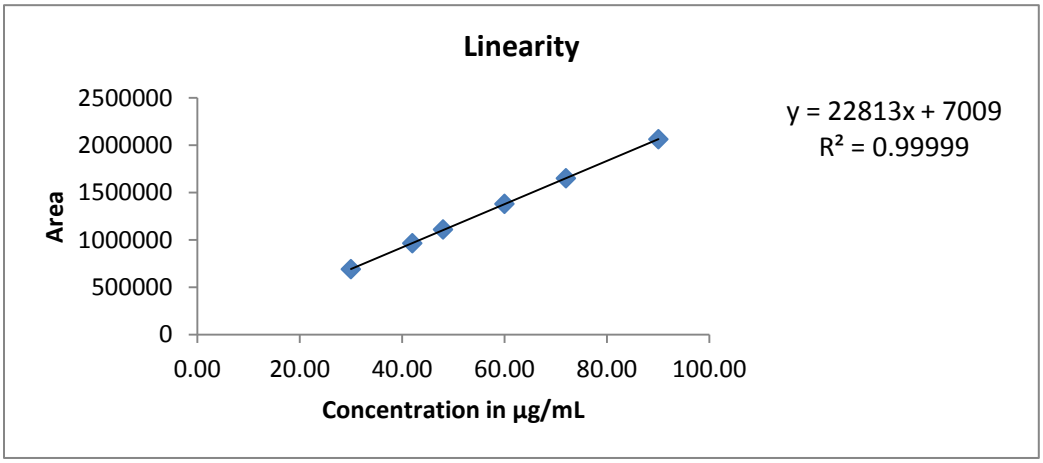


**Figure 2: Linearity graph of S (-) Metoprolol**

**LOD and LOQ**

Limit of detection and Limit of quantification were calculated from linearity data by using the following formula LOD=3.3*σ/S and LOQ=10*σ/S Where σ=the standard deviation of the response and S=Slope of the calibration curve. The LOD and LOQ Values for S(-)Metoprolol were found to be 0.419µg/mL and 1.271µg/mL respectively.

**Precision**

For determining method precision, six samples were prepared as per sample preparation in analytical method and injected for determination of assay value. The %RSD of Assay results were calculated. %RSD of six results should not be more than 2. The result obtained was 0.79.For determination of intermediate precision same experiment was repeated as per method precision on different day using different instrument, different column and different analysist. The assay values

of six preparations were calculated. %RSD of six results was found to be 1.25 which is well within the limit of 2.0%.

**Accuracy (%Recovery)**

The accuracy of the method may often expressed by % recovery.% Recovery was performed by adding known amount of API(Active pharmaceutical ingredient) to Placebo at three different concentrations labels of 50%, 100%, 150% of test concentration in triplicate preparation. 60µg/mL is considered as 100%. The samples were prepared as per the preparation method for sample and analyzed by optimized method. The percentage recovery data are summarized in Table 3.

**Table 3: Recovery study by RP-HPLC**

| Recovery Levels | Amount of API Added (mg) | Actual Amount Added (mg) | Amount of Placebo Added (mg) | Area of sample | Amount Recovered (mg) | Recovery (%) | Average Recovery (%) | SD | %RSD |
|---|---|---|---|---|---|---|---|---|---|
| **50%** | 119.10 | 118.04 | 813.2 | 653136 | 115.00 | 97.42 | **98.17** | 0.66 | **0.67** |
| | 119.04 | 117.98 | 813.2 | 659812 | 116.18 | 98.47 | | | |
| | 119.33 | 118.27 | 813.0 | 662511 | 116.65 | 98.63 | | | |
| **100%** | 238.26 | 236.14 | 813.3 | 1319322 | 232.30 | 98.37 | **98.45** | 0.40 | **0.41** |
| | 237.93 | 235.81 | 813.5 | 1324176 | 233.16 | 98.88 | | | |
| | 238.36 | 236.24 | 813.4 | 1316147 | 231.74 | 98.10 | | | |
| **150%** | 357.18 | 354.00 | 813.4 | 1995962 | 351.44 | 99.28 | **98.69** | 0.61 | **0.62** |
| | 357.68 | 354.50 | 813.1 | 1987773 | 350.00 | 98.73 | | | |
| | 356.90 | 353.72 | 813.4 | 1969992 | 346.87 | 98.06 | | | |

**Specificity and Forced degradation**

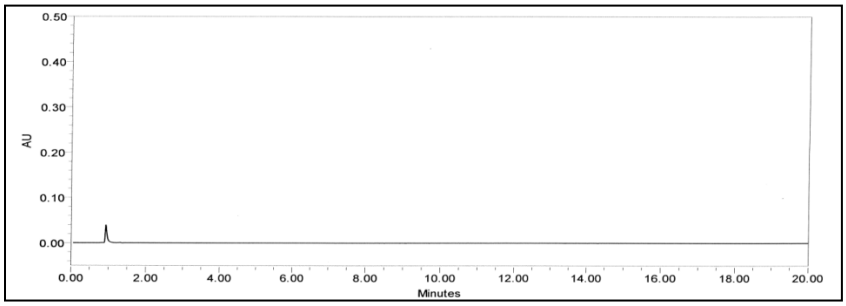


**Figure 3: A Typical chromatogram of Blank**

Specificity study is the ability of method to measure the analyte response in the presence of impurities. Forced degradation studies were performed to demonstrate selectivity and stability indicating capability of the proposed RP-HPLC method. Figure shows that there is no interference at the RT (retention time) of S (-) Metoprolol Succinate standard drug and IR tablet sample due to blank and placebo and degradation products. Diluent blank, Placebo, standard, Unspiked sample,

and Peroxide treated sample chromatograms are presented in Figures 3, 4, 5, 6, 7, and 8 respectively. System suitability data are summarized in Table 4.

**Table 4: System Suitability**

| Peak Name | Retention time | Plate count | Tailing factor | Purity angle | Purity Threshold |
|---|---|---|---|---|---|
| Sample | 2.750 | 3838 | 1.32 | 0.20 | 1.07 |
| Impurity | 5.260 | 4530 | 1.09 | 11.43 | 14.18 |

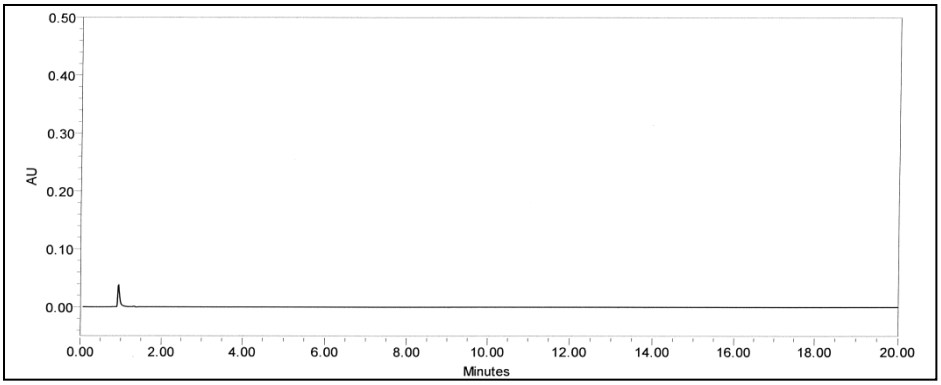


**Figure 4: A Typical chromatogram of Placebo**

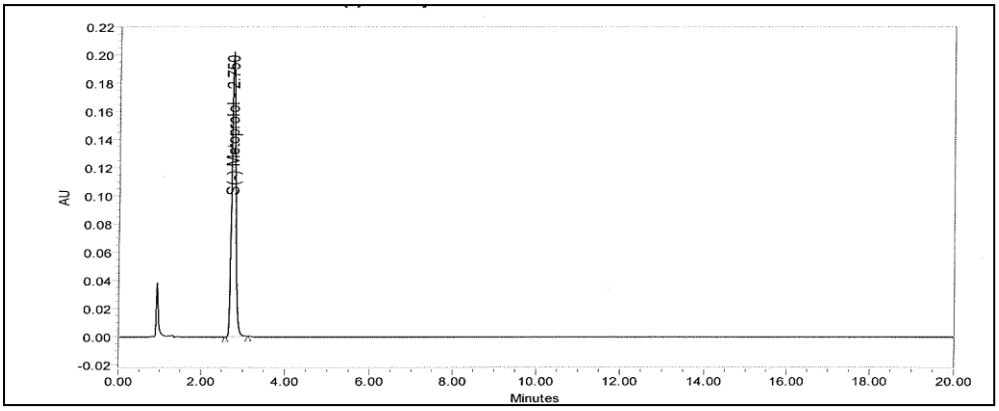


**Figure 5: A Typical chromatogram of Standard**

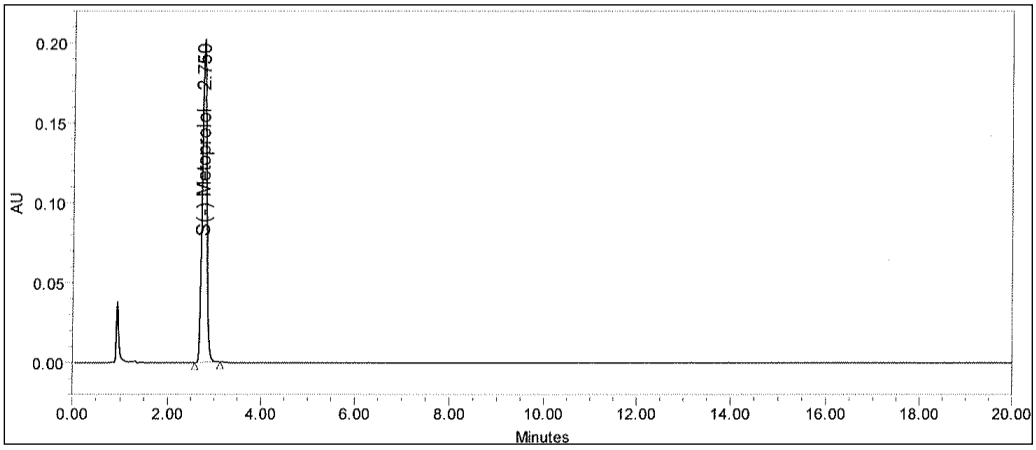


**Figure 6: A Typical chromatogram of Unspiked Sample**

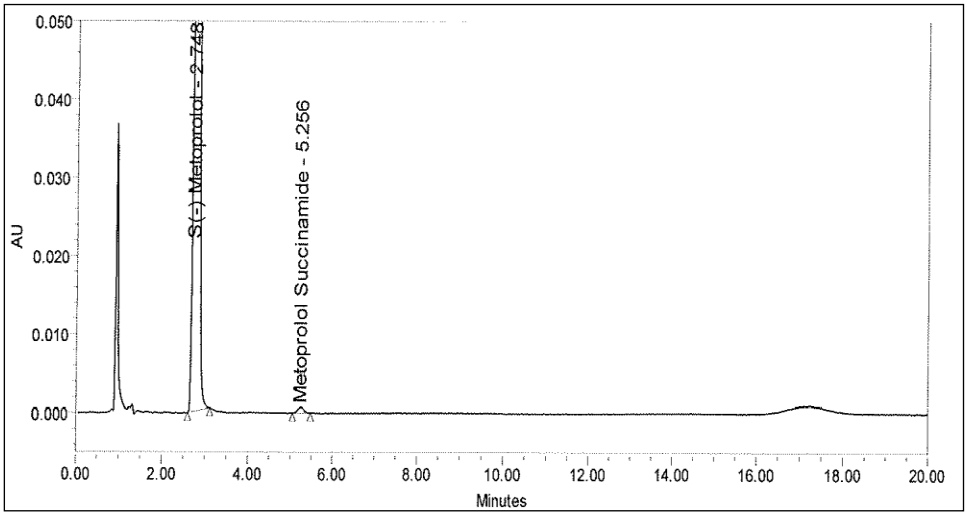


**Figure 7: A Typical chromatogram of Spiked Sample**

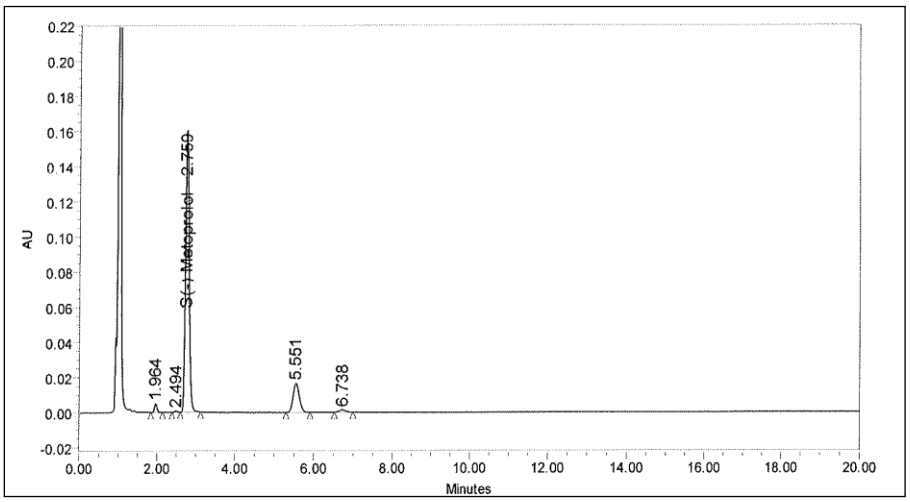


**Figure 8: A Typical Chromatogram of $H_2O_2$ treated Sample**

**Forced degradation**

Significant degradation was observed when the drug product and drug substance were subjected to oxidation (30% v/v $H_2O_2$, 80°C, 2 hrs). Where as no or negligible degradation was observed when Placebo, drug product and drug substance were subjected to acid hydrolysis (1N HCl, at 80°C/2Hrs), base hydrolysis (1N NaOH, at 80°C/2hrs), photolytic degradation at 1.2 million lux hours and 200 watt hours/square meter UV light as per ICH and thermally exposed to 80° C for 5 hrs. Peaks due to Samples were investigated for spectral purity in the chromatogram of all exposed samples and found spectrally pure. Peak purity angles were less than peak purity thresholds for sample. The purity and assay of S (-) Metoprolol Succinate drug and IR tablets were unaffected by the presence of impurities and degradation products, thus confirming the stability-indicating power of the developed method. Results from forced degradation study are given in Table 5.

**Filter Selection**

To confirm the filter compatibility in proposed method, filtration recovery experiments were carried out by filtration techniques. The 5mL of sample stock solution was filtered by using 0.45 Nylon Disc filter and 0.45 PVDF Disc filter by discarding 1 mL, 2 mL, 3 mL and 4 mL by both disc filters and % assay was calculated against centrifuged sample. The results shows that % amount of sample at centrifuged, 0.45 Nylon disc filter, 0.45 PVDF disc filter were 102.39, 100.92, 99.86, respectively. In the result obtained 0.45 Nylon disc filter had better compatibility. So on the basis of data generated the final Disc filter was selected.

**Table 5: Force degradation study by RP-HPLC**

| Stress condition | % Degradation | Purity angle | Purity Threshold |
|---|---|---|---|
| Acid hydrolysis | 0.0 | 0.15 | 1.10 |
| Base hydrolysis | 0.0 | 0.23 | 1.09 |
| Oxidative degradation | Sample-19.97 API-19.53 | Sample-0.40API-0.21 | Sample-1.19API-1.09 |
| Photolytic degradation | 0.0 | 0.19 | 0.29 |
| Thermal degradation | 0.0 | 0.18 | 0.29 |

**Robustness**

The robustness is ability to remain unaffected by small deliberate changes in parameters. Robustness of the method was determined by preparing standard and sample solution as per method of analysis and carrying out the analysis under different conditions in which Flow rate was changed by ± 0.2 mL/min, wavelength was changed by ± 2 nm, and column temperature was changed by ± 5°C. Based on the results obtained it was found that the proposed method had no significant change in the system suitability. Hence the method was found robust at small but deliberate changes .The results of robustness are summarized in Table-6.

**Table 6: Robustness study by RP-HPLC**

| Sr. No. | Parameter | Theoretical plate | Tailing factor | % RSD |
|---|---|---|---|---|
| 1 | At Flow rate 1.3 mL/min | 3565 | 1.42 | 0.06 |
| 2 | At Flow rate 1.7 mL/min | 3159 | 1.37 | 0.29 |
| 3 | At wavelength 218 nm | 3332 | 1.41 | 0.04 |
| 4 | At wavelength 222 nm | 3319 | 1.41 | 0.09 |
| 5 | At Column temperature 35° C | 3312 | 1.39 | 0.04 |
| 6 | At Column temperature 45° C | 3451 | 1.42 | 0.16 |

**Solution Stability**

The stability of sample solution was determined by its storage at ambient temperature for 24 hrs. The assay were determined and compared against Initial sample and sample kept at ambient temperature for 24 hrs. The difference obtained in % assay of initial and 24 hrs sample is 1.9 %, hence it can be concluded that the sample is stable at ambient temperature for 24 hrs.

## CONCLUSION

A low pressure gradient RP-HPLC method was successfully developed for the estimation of S (-) Metoprolol Succinate in IR tablet dosage form. The experimental part of our analysis describes the important RP-HPLC components including mobile phase, column, and flow rate and column temperature. This approach ensures better design of product. The method validation part has proved that the method is precise, linear, selective, accurate, robust, filter selective and stability indicating. The method was robust described by using design of experiments, taking consideration into the selectivity of the RP-HPLC and from the stress study drug is stable in acidic, basic, thermal and photolytic conditions and degrades in oxidative conditions. The short run time (10.0 min) enables rapid determination of drug. Moreover, it may be applied for characterization of degradants and determination of content uniformity and in vitro test profiling of IR dosage forms, where sample load is higher and high throughput is required for faster delivery of results.

## REFERENCE

1. European Pharmacopoeia 5.0 (01/2005) 2032-2034
2. USP 36 NF 31, Volume 3, The Us Pharmacopeia Convention, Twin brook Parkway, Rockville, MD, 20852
3. British Pharmacopeia Congress, Volume II , MHRA, Buckingham Palace Road, London
4. Jadhav AS, Tarkase KN, Deshpande AP. Quantitative Determination of Metoprolol succinate in bulk and tablet Dosage form through comparative study of UV and derivative Spectroscopy, Der Pharmacia Lettre 2012; 4 (3): 763-767
5. Hapse SA, Bhagat B.V, Mogal SA, Kamod AC. Spectrophotometric Estimation and Validation of Metoprolol Succinate and Amlodipine Besylate By Different Method From Pure And Tablet Dosage Form. Int J PharmTech Res 2013; 5(1): 126-131.
6. Satyanarayana Rath, Susanta Kumar Panda, Rashmi Ranjan Sarangi, Arun Kumar dash, Sangram Kumar Rath, Srikant Nayak, UV - Spectrophotometric method for simultaneous estimation of Metoprolol and Amlodipine in bulk and their formulation., International J biological & Pharmaceutical Research., 2(2), 2011; 50-54.
7. V.Rajamanickam1, B. Stephen Rathinaraj, N.Thangavelpandian, Ananda Rajagopal Pandian, A validated RP-HPLC method of Metoprolol Succinate and Amlodipine Succinate from bulk drugs. Scholars Research Library, Der Pharmacia Lettre, 2(4), 2010; 40-46

8. ICH Harmonized Tripartite Guideline, Validation Of Analytical Procedures: Text And Methodology, Q2 (R1) Current Step 4 version November 2005
9. ICH Harmonized Tripartite Guideline, Stability testing of new drug substances and Products, Q1A(R2),Current Step 4 version February 2003
10. ICH Harmonized Tripartite Guideline, Pharmaceutical Development, Q8(R2),Current Step 4 version August 2009