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# Development and Validation for Simultaneous Estimation of Drug in Combination from Pharmaceutical Formulation by RP-HPLC Method

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**Abstract :** In present work development and validation of new reverse phase high performance liquid chromatography method for estimation of Ivabradine (IVA) and Metoprolol Succinate (MET)from their combined tablet dosage form was carried out. The method was performed on Shimadzu SPD-10Avp, inbuilt with UV detector, UltraSil-MCX; 5µ, 100 X 2.1mm. ID Column and 15mM Ammonium Formate: MeOH (15:85 v/v) as mobile phase at ambient temperature. Detection was carried out at 223 nm and 230 nm. Concentration range 5-25 µg/ml for Ivabradine and 25-75 µg/ml for Metoprolol Succinate. The Percentage recovery of Ivabradine and Metoprolol succinate was found to be in the range of 98.06±1.70 % – 101.47±1.18 and 95.17±0.93 % - 101.2±1.00 % respectively. Correlation coefficient for Ivabradine and Metoprolol succinate was found 0.9995 and 0.9999 respectively. The Rt values for Ivabradine and Metoprolol succinate were found to be 1.78 min and 5.18 min respectively. The method was validated according to the guidelines of International Conference on Harmonization (ICH) and was successfully employed in the estimation of commercial formulations.

**Keywords :** Ivabradine, Metoprolol, Mobile Phase, Reverse-Phase High Performance Liquid Chromatography, Stability indicating method.



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## 1. Introduction

Reverse-Phase High Performance Liquid Chromatography is mostly recommended over other separation techniques for its versatile importance towards qualitative and quantitative analysis. In this technique most often, freshly prepared stock solution injected into a column packed with porous spherical silica phase (Normal phase HPLC) and when it is modified with non-polar bonded phases like C18, C8 then it termed as reverse phase HPLC. The mobile phase passed through the columns at high pressure, usually measured in bar or psi units. Thereafter, the retention parameters or separation behavior of sample (analyte/elute) is dependent on their diffusion through the column arising from different partition of the sample between the stationary and mobile phase. Thus, those compounds which have strongest retention, elute last and in contrast, those compounds have least retention will elute first.[1,2,3].

Importantly, so far, to our present knowledge, a literature survey revealed that, few publications reported the simultaneous analysis of both Ivabradineand Metoprolol on C18 column[3] and has mentioned the details of capacity factor and resolution which specifically have great importance in system suitability as per ICH guidelines. As reported in few articles the Metoprolol was eluted with void volume/solvent front ($t_0$) which is strictly not acceptable by ICH guidelines. In addition, the sensitivity of both Ivabradine and Metoprololwere found negligible in UV detection[4]. By considering such, in present investigation attempt has been made to develop new, accurate, precise and robust reverse phase high performance liquid chromatographic (RP-HPLC) method for successful development for the simultaneous estimation of both antihypertensive drugs Ivabradine[5,6]3-{3-[{[(7S)-3,4-Dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl]methyl}(methyl)amino]propyl}-7,8-dimethoxy-1,3,4,5- tetrahydro-2H-3-benzazepin-2-one (IVA, Fig. 1) and Metoprolol[1,7,,8,9], (±)-1-(Isopropylamino)-3-[p-(b-methoxyethyl) phenoxy]-2- propanol (MET, Fig. 2) in both standard and tablet formulation along with stability indicating studies or force degradation studies in 0.1 N HCl, 0.1N NaOH, 3% - 6% $H_2O_2$, and thermal degradation at $60^0$Ctemperature.

A stability indicating method[10-16] (SIM) is an analytical procedure used to quantitate the decrease in the amount of the active pharmaceutical ingredient (API) in drug product due to degradation. SIM measures the changes in active ingredients concentration without interference from other degradation products, impurities and excipients. Stress testing is carried out to demonstrate specificity of the developed method to measure the changes in concentration of drug substance when little information is available about potential degradation product. The addition of this analytical methods in the current practice would help the pharmaceutical industries in large to preserve the excellence of their products containing these active ingredients and also the enforcement agencies in general to monitor the quality of the marketed products.

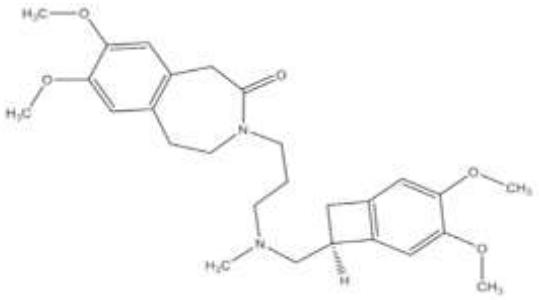

**Fig.1: Molecular structure of Ivabradine**

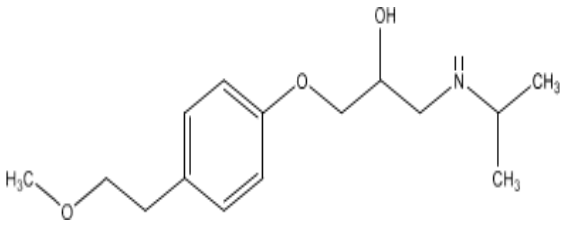

**Fig.2: Molecular structure of Metoprolol**

## 2. Materials and Methods

### Reagents and chemicals

Standard of Ivabradine and Metoprolol were obtained from Ajanta Pharma Ltd., Mumbai. IVAMET-XL®(Ajanta Pharma Ltd., Mumbai) tablets were purchased from medical store. IVA5mg and MET25mg were

used. All chemicals and reagents used were a HPLC grade and purchased from Merck specialties Pvt., Ltd., Mumbai.

**Ivabradine (IVA) standard stock solution (50 µg/ml)**

A sample of 50 mg of IVA was weighed and transferred to a 100 ml volumetric flask. Volume was made up to the mark with methanol-water (2:1 v/v). Take 10 ml from this solution, and transfer to 100 ml volumetric flask and volume was made up with methanol-water (2:1 v/v).

**Metoprolol (MET) standard stock solution (250µg/ml)**

A sample of 25 mg of MET was weighed and transferred to a 100 ml volumetric flask. Volume was made up to the mark with methanol-water (2:1 v/v).

**Preparation of standard solution of binary mixtures of IVA (5µg/ml) and MET (25µg/ml)**

Take 1 ml from the IVA stock solution and 1ml from MET stock solution and transferred to 10 ml volumetric flask and volume made up to the mark by mobile phase which was used in trials.

**Preparation of Sample Stock Solution (IVA50 µg/mL, MET 250 µg/ml)**

Exactly 10 tablets of IVAMET-XL®, were separately weighed, powdered and mixed in a mortar. An accurately weighed amount of the finely powdered IVAMET-XL®5mg/25mg; Ajanta Pharma Ltd., Mumbai tablets; equivalent to 5 mg of IVA and 25 mg of MET were separately made up to 100 mL with methanol and sonicated until they dissolved and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

## Method Validation[17-26]

**Linearity/Calibrationstudies**

Accurately measured aliquots of stock solutions equivalent to 31.25-500 µg, of IVA and MET, respectively were transferred separately into a series of 10 mL volumetric flasks. The final volume was adjusted with same mobile phase, and then 20 µL were injected into HPLC. A calibration curve (linearity graph) was plotted by calculating peak area against concentration. The regression coefficient and regression coefficient ($r^2$) was determined.

**Precision of the proposedmethod**

Three similar concentrations of the mixture of IVA and MET (500, 250, 125 $\mu g.L^{-1}$) were analyzed three times for a single day (intraday precision) and three times for three subsequent days (inter-day/intermediate precision). The data evaluated for standard deviation and %RSD values.

**Accuracy/drug recovery**

IVA 5 µg/ml and MET 25 µg/ml drug solution were taken in three different flask label A, B, and C. Spiked 80%, 100%, and 120% of standard solution in it and diluted up to 10 ml in HPLC system. Two successive injections for each concentration were selected and data expressed in the form of % recovery ± % relative error were calculated.

**Limit of detection (LOD) and limit of quantitation (LOQ)**

The reproducibility profile of the analytical method was estimated by injecting five-times the standard solution and recording the parameters such as retention time, peak area, theoretical plates, and tailing factor. The limit of detection (LOD) may be defined as the lowest detectable concentration by any analytical method,

but not necessary to measure the exact amount. The limit of quantification (LOQ) may be defined as the lowest detectable concentration by any analytical method with a particular level of accuracy and precision.

The LOD was determined by the formula:

LOD = 3.3 (σ/S) Where σ = standard deviation of response; S = slope of the calibration curve. The slope S may be estimated from the calibration curve of theanalyte.

The LOQ is determined by the formula:

LOQ = 10 (σ/S) Where σ = standard deviation of response; S = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

**Robustness**

Robustness was attempted by deliberately changing the chromatographic conditions to evaluate the difference in resolution, capacity factor, peak height and peak width (tailing factor).The robustness of the method was judged by deliberately altering the mobile phase composition by ± 5% v/v (i.e., 65:35% v/v and 55.45% v/v), flow rate by ± 0.1 ml/minute (i.e., 0.9 and 1.1 ml/minute), and column temperature by ± 5°C (i.e., 25°C and 35°C), keeping the other chromatographic parameters constant. Finally, the effect of wavelength was monitored by making deliberate variation 223 to 230nm and the differences in system suitability parameters such as peak tailing, capacity factor, resolution and theoretical plates were evaluated.

## Forced degradation studies[25]

**Acid degradation**

Acid decomposition study was performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. A volume of 2 ml of 0.1 N HCl solutions was added, mixed well and then kept for 7 hours at 60˚C. Furthermore, the volume was adjusted with diluent to get the same concentration of drugs used for proposed method.After time period, the volume was adjusted with diluent to get 5µg/ml for IVA and 25µg/ml for MET.

**Base degradation**

Base decomposition study was performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. A volume of 2 ml of 0.1 N NaOH solutions was added, mixed well and then kept for 7 hours at 60˚C. After time period, the volume was adjusted with diluents to get 5 µg/ml for IVA and 25 µg/ml for MET.

**Thermal degradation**

Thermal degradation study was performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 80°C for 6 hours. Then, the volume was adjusted with diluents to get 5 µg/ml for IVA and 25 µg/ml for MET and the sample was analyzed using the same proposed method.

**Oxidative degradation**

Oxidation study was performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. A volume of 2 ml of 3-6% $H_2O_2$ solutions were added and mixed well and put for 12 hours at room temperature. After time period, the volume was adjusted with diluents to get 5 µg/ml for IVA and 25 µg/ml for MET for method development. The sample was then analyzed using the same proposed method.

## 3. Results and Discussion

### Selection of wavelength

Standard solution of IVA (5µg/ml) and standard solution of MET (25µg/ml) were scanned between 200nm and 400nm using UV-visible spectrophotometer Wavelength was selected from the overlay spectra of above solutions. UV detection was specifically carried out at 223nm and 230 nm at room temperature for selected IVA and MET as both compounds exhibit optimum absorption.The flow rate was adjusted to 1.2 $mL.min^{-1}$ to achieve better resolution, and peak symmetry.

### ChromatographicParameters[27]

Various chromatographic parameters are as follows,

1. Analytes: Ivabradine (250ppm) + Metoprolol(2500ppm)
2. Column: UltraSil-MCX; 5µ, 100 X 2.1mm.ID.
3. Mobile Phase: 15mM ammonium formate-MeOH(15:85v/v)
4. Flow rate:1.2$mL.min^{-1}$
5. Elution mode: Isocratic elutionmode
6. Wavelength selected:223nm
7. Temperature: Roomtemperature
8. Run time: 10minutes
9. Retention time: Ivabradine (1.78 min), Metoprolol (4.42min)

### System suitability tests for IVA andMET

System suitability test reveals the factors such as, theoretical plate (N), capacity factor (k'), resolution (R), separation factor (α), tailing factor (*T*), Mean±SD and RSD% and found to be in acceptable range for at least 6 successive injections of same analytes, as shown in Fig. 3. Table 1, represents the system suitability for IVA and MET.

**Table 1: System suitability of IVA and MET**

| System suitability parameters | Ivabradine (IVA) | Metoprolol (MET) | Acceptable Values |
|---|---|---|---|
| Theoretical plates (N)* | 421 | 570 | > 2000 |
| Capacity Factor (K') | 3.312 | 10.472 | 1-10 |
| Resolution (R) | ---- | 4.929 | ≥ 2 |
| Selectivity/Separation factor (α) | 6.613 | 3.495 | > k' |
| Asymmetry/Tailing factor (T) | 1.58 | 1.43 | ≤ 2 |
| Retention time (tR) | 1.78 min. | 5.18 min. | > k' |
| Wavelength of Detection (nm) | 223 nm | 223 nm | > 200 nm |
| Repeatability (% RSD) | 0.43 | 0.69 | < 2 |
| Intra-Day Precision (%RSD) | 0.31-0.90 | 0.20 -1.10 | < 2 |
| Inter-Day Precision (%RSD) | 0.30 - 0.35 | 0.20 - 0.75 | < 2 |
| Linearity range | 32.5 – 500 µg.ml-1 | 32.5 – 500 µg.ml-1 | NA |
| Regression equation | Y=26007x | Y= 24771x – 1840.3 | NA |
| SE of intercept (Se) | 74893.49814 | 35707.37211 | NA |
| SD of intercept (Sa) | 167466.3555 | 79843.82648 | NA |
| Correlation Coefficient (r2) | 0.9995 | 0.9999 | NA |
| LOQa (µg.mL−1) | 21.25 µg.ml-1 | 10.63 µg.ml-1 | NA |
| LODa (µg.mL−1) | 64.34 µg.ml-1 | 32.23 µg.ml-1 | NA |

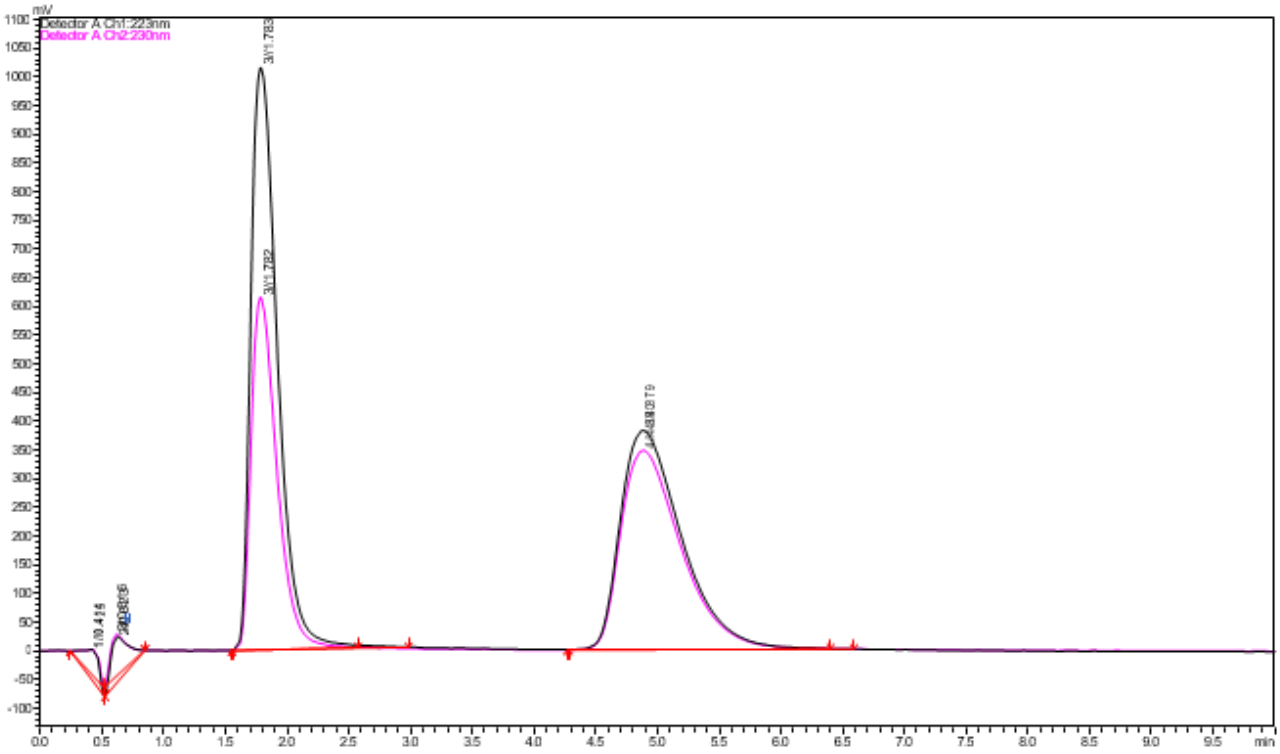


**Fig. 3:Simultaneous chromatographic analysis of Ivabradine and Metaprolol by RP-HPLC**

**Repeatability**

The data for repeatability of peak area measurement for Ivabradine and Metaprolol was based on six replicates of same concentration of selected drugs which is summarized in Table 2. The percentage RSD (% RSD) for Ivabradine and Metaprolol was found to be 0.43 and 0.69, respectively.

**Table 2: Repeatability data of IVA and MET**

| Sr. No. | Drug Name; Ivabradine<br>Peak Area; Conc. 250 ppm | Drug Name; Metaprolol<br>Peak Area; Conc. 250 ppm |
|---|---|---|
| 1 | 8624251 | 6244281 |
| 2 | 8661926 | 6215073 |
| 3 | 8682663 | 6276217 |
| 4 | 8705093 | 6287503 |
| 5 | 8721047 | 6269341 |
| 6 | 8641373 | 6343276 |
| **Mean** | **8672725.5** | **6272615.167** |
| **STD. DEV.** | **37231.06** | **43248.63065** |
| **RSD (%)** | **0.43** | **0.69** |

**Intraday Precision:**

The data for intraday precision by implementing the procedure mentioned, the homologous mixture of both IVA and MET of 250 ppm concentration was tested and evaluated within the same day (intra-day precision) for Ivabradine and Metaprolol(shown in Table 3 and Table 4). The percentage RSD (% RSD) for intraday precision was found to be in the range 0.31–0.90 for Ivabradine and 0.20 - 1.10 for Metaprolol.

**Table 3: Intraday Precision data of IVA**

| Drug Name: Ivabradine (IVA) | | | | |
|---|---|---|---|---|
| **Sr. No.** | **Conc. (µg.mL$^{-1}$)** | **Area** | **Mean ± SD** | **%RSD** |
| 1 | 250 PPM | 8703984 | 31199.82 | 0.35 |
| | 250 PPM | 8762006 | | |
| | 250 PPM | 8713111 | | |
| 2 | 250 PPM | 8760236 | 27657.52 | 0.31 |
| | 250 PPM | 8755758 | | |
| | 250 PPM | 8805744 | | |
| 3 | 250 PPM | 8565025 | 78482.72 | 0.90 |
| | 250 PPM | 8662103 | | |
| | 250 PPM | 8720384 | | |
| Range of %RSD | | | | 0.31 – 0.90 |

**Table 4: Intraday Precision data of MET**

| Drug Name: Metoprolol (MET) | | | | |
|---|---|---|---|---|
| **Sr. No.** | **Conc. (µg.mL-1)** | **Area** | **Mean ± SD** | **%RSD** |
| 1 | 250 ppm | 6348110 | 48383.93 | 0.75 |
| | 250 ppm | 6403713 | | |
| | 250 ppm | 6444499 | | |
| 2 | 250 ppm | 6433125 | 13252.68 | 0.20 |
| | 250 ppm | 6455762 | | |
| | 250 ppm | 6456384 | | |
| 3 | 250 ppm | 6228002 | 69910.26 | 1.10 |
| | 250 ppm | 6335132 | | |
| | 250 ppm | 6359379 | | |
| Mean %RSD | | | | 0.20 – 1.10 |

**Interday (intermediate)precision:**

The data for interday precision by implementing the procedure mentioned, the homologous mixture of both IVA and MET of 250 ppm concentration was tested and evaluated within the same day (inter-day precision) for Ivabradine and Metaprolol (shown in Table 5 and Table 6). The percentage RSD (% RSD) for intraday precision was found to be in the range 0.30 –0.35 for Ivabradine and 0.20 – 0.75 for Metaprolol.

**Table 5:Interday (intermediate) Precision data ofIVA**

| Drug Name: Ivabradine (IVA) | | | | |
|---|---|---|---|---|
| **Sr. No.** | **Conc. (µg.mL$^{-1}$)** | **Area** | **Mean ± SD** | **%RSD** |
| DAY 1 | 250 ppm | 8720384 | 26970.8859 | 0.30 |
| | 250 ppm | 8774001 | | |
| | 250 ppm | 8742074 | | |
| DAY 2 | 250 ppm | 8703984 | 31199.82809 | 0.35 |
| | 250 ppm | 8762006 | | |

| | | | | |
|---|---|---|---|---|
| | 250 ppm | 8713111 | | |
| DAY 3 | 250 ppm | 8760236 | 27657.52081 | 0.31 |
| | 250 ppm | 8755758 | | |
| | 250 ppm | 8805744 | | |
| Range of %RSD | | | | 0.30 – 0.35 |

**Table 6:Interday (intermediate) Precision data ofMET**

| **Drug Name: Metoprolol (MET)** | | | | |
|---|---|---|---|---|
| **Sr. No.** | **Conc. (µg.mL-1)** | **Area** | **Mean ± SD** | **%RSD** |
| DAY 1 | 250 ppm | 6359379 | 39320.73 | 0.61 |
| | 250 ppm | 6434446 | | |
| | 250 ppm | 6417212 | | |
| DAY 2 | 250 ppm | 6348110 | 48383.93 | 0.75 |
| | 250 ppm | 6403713 | | |
| | 250 ppm | 6444499 | | |
| DAY 3 | 250 ppm | 6433125 | 13252.68 | 0.20 |
| | 250 ppm | 6455762 | | |
| | 250 ppm | 6456384 | | |
| Range of % RSD | | | | 0.20 – 0.75 |

**Linearity and range**

Under linearity or calibration studies, a linear relationship between area under peak values and selected drug concentration (µg.mL.min$^{-1}$) was plotted for five-six chosen concentrations of Ivabradine (shown in Fig.4) and Metaprolol(shown in Fig.5). The regression equations, correlation coefficient values ($r^2$), standard error of intercept (Se), standard deviation of intercept (Sa), limit of detection (LOD) and limit of quantification (LOQ) have been calculated. The linearity of the calibration curves was validated by the high value of correlation coefficient, acceptable values of regression coefficient, standard deviation of the slope and standard deviation of the intercept; shown in (Table 7 and Table 8).

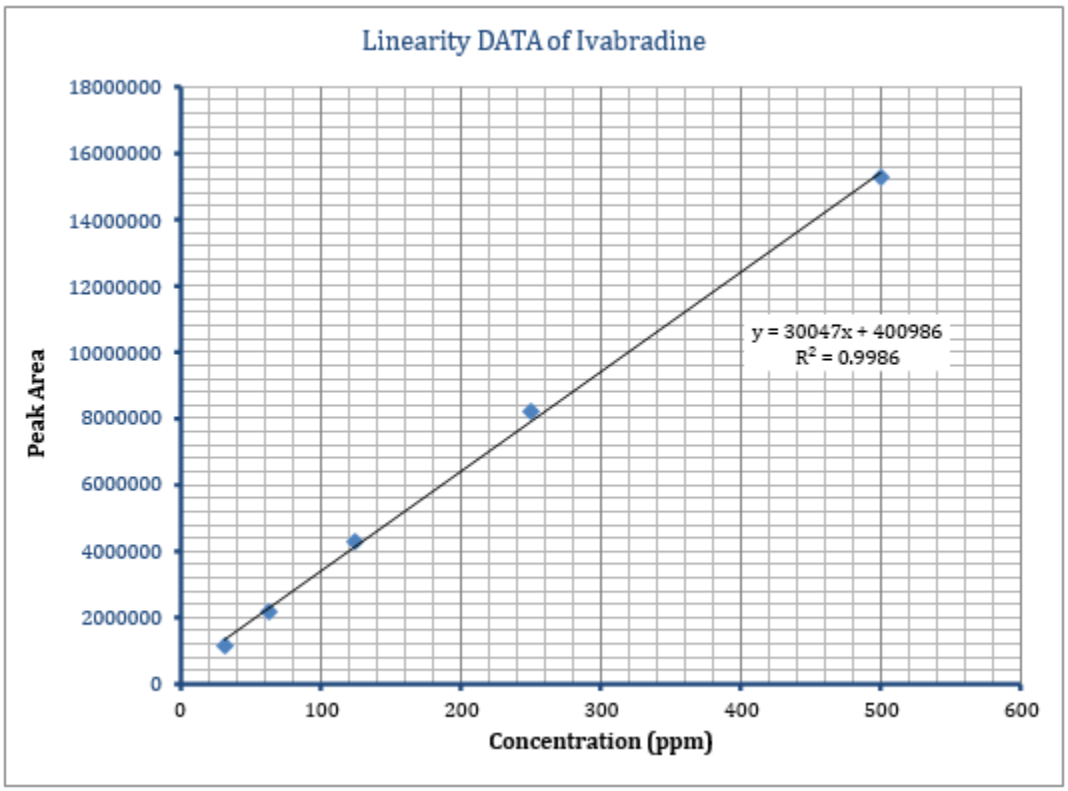


**Fig. 4: Calibration data ofIvabradine (IVA)**

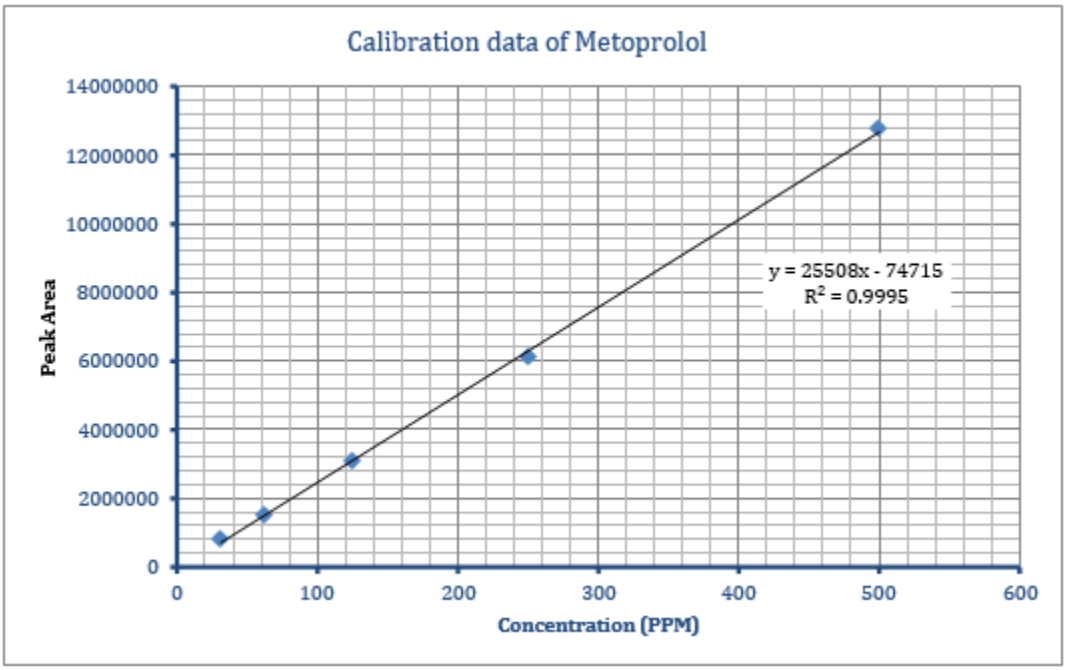


**Fig. 5: Calibration curve ofMetoprolol (MET)**

**Table 7: Linearity data for Ivabradine**

| **Name of Drug:Ivabradine** | | | |
|---|---|---|---|
| **Sr. No.** | **Concentration (µg.mL$^{-1}$)** | **Area** | **Average (Mean)** |
| 1 | 500 PPM | 15259138 | 15259138 |
| | 500 PPM | 14536854 | |
| 2 | 250 PPM | 8232682 | 8232682 |
| | 250 PPM | 8375791 | |
| 3 | 125 PPM | 4402525 | 4267689 |
| | 125 PPM | 4267682 | |
| 4 | 62.5 PPM | 2192869 | 2186284 |
| | 62.5 PPM | 2186284 | |
| 5 | 31.25 PPM | 1179230 | 1167092 |
| | 31.25 PPM | 1167092 | |
| 6 | Regression Equation | | Y=26007x |
| 7 | Correlation coefficient ($R^2$) | | 0.9995 |
| 8 | Std. Error of intercept | | 74893.49814 |
| 9 | Std. Dev. of intercept | | 167466.3555 |
| 10 | LOQ | | 21.25 µg.ml$^{-1}$ |
| 11 | LOD | | 64.34 µg.ml$^{-1}$ |

**Table 8: Linearity data of Metoprolol**

| Name of Drug**:Metoprolol** | | | |
|---|---|---|---|
| Sr. No. | Concentration (µg.mL$^{-1}$) | Area | Average (Mean) |
| 1 | 500 PPM | 13115556 | 12765757 |
| | 500 PPM | 12415958 | |
| 2 | 250 PPM | 6133352 | 6133352 |
| | 250 PPM | 6330190 | |
| 3 | 125 PPM | 3177086 | 3069754 |
| | 125 PPM | 3069754 | |
| 4 | 62.5 PPM | 1552484 | 1538751 |
| | 62.5 PPM | 1538751 | |
| 5 | 31.25 PPM | 838102 | 829853 |
| | 31.25 PPM | 829853 | |
| 6 | Regression Equation | | Y= 24771x – 1840.3 |
| 7 | Correlation coefficient ($R^2$) | | 0.9999 |
| 8 | Std. error of intercept | | 35707.37211 |
| 9 | Std. Dev. Of intercept | | 79843.82648 |
| 10 | LOQ | | 10.63 µg.ml$^{-1}$ |
| 11 | LOD | | 32.23 µg.ml$^{-1}$ |

**Limit of detection (LOD/LOQ)**

Limit of detection represents the concentration of analyte at S/N ratio of 3.3 and limit of quantification (LOQ) at which S/N is 10 were determined and results are given in Table 7 and Table 8. Low values of LOD and LOQ indicate sensitivity of the applied method for determination of mentioned drugs intablets.

**Accuracy**

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in Tables 9 and 10. Percentage recovery for IVA was 98.06±1.70 %–101.47±1.18 %, while for MET, it was found to be in range of 95.17±0.93 %- 101.2±1.00 %

**Table 9: Accuracy data of Ivabradine (IVA)**

| Conc. (%) | S. No. | S. amt. (µg/mL) | D. added (µg/mL) | Amt. rec. (µg/mL) | % recovery | Mean±SD | % RSD |
|---|---|---|---|---|---|---|---|
| 80% | 1 | 5 | 4 | 9.11 | 101.22 | 99.77±1.26 | 1.26 |
| | 2 | 5 | 4 | 8.90 | 98.88 | | |
| | 3 | 5 | 4 | 8.93 | 99.22 | | |
| 100% | 1 | 5 | 5 | 9.90 | 99.00 | 98.06±1.70 | 1.73 |
| | 2 | 5 | 5 | 9.20 | 96.10 | | |
| | 3 | 5 | 5 | 9.91 | 99.10 | | |
| 120% | 1 | 5 | 6 | 10.23 | 102.30 | 101.47±1.18 | 1.16 |
| | 2 | 5 | 6 | 10.91 | 109.10 | | |
| | 3 | 5 | 6 | 10.88 | 108.80 | | |

**Table 10: Accuracy data of Metoprolol (MET)**

| Conc. (%) | S. No. | S. amt. (µg/mL) | D. added (µg/mL) | Amt. rec. (µg/mL) | % recovery | Mean±SD | % RSD |
|---|---|---|---|---|---|---|---|
| 80% | 1 | 25 | 20 | 43.23 | 96.06 | 96.72±1.13 | 1.17 |
| | 2 | 25 | 20 | 44.12 | 98.04 | | |
| | 3 | 25 | 20 | 43.24 | 96.08 | | |
| 100% | 1 | 25 | 25 | 50.87 | 101.74 | 101.2±1.00 | 0.99 |
| | 2 | 25 | 25 | 50.91 | 101.82 | | |
| | 3 | 25 | 25 | 50.02 | 100.04 | | |
| 120% | 1 | 25 | 30 | 52.23 | 94.96 | 95.17±0.93 | 0.98 |
| | 2 | 25 | 30 | 52.91 | 96.20 | | |
| | 3 | 25 | 30 | 51.90 | 94.36 | | |

**Robustness for the chromatographic method**

The changes incurred by varying the flow rate, eluent composition and wavelength was found within the acceptance criteria and have not made any large variations,results wereshown in Table 11 and Table 12.

**Table 11: Robustness data of IVA: Calculated for resolution, tailing and capacity factor**

| Sr. No. | F.(-0.2ml.mL$^{-1}$) | F.(+0.2ml.mL$^{-1}$) | A (-2ml) | A (+2ml) | WL (-2nm) | WL (+2 nm) |
|---|---|---|---|---|---|---|
| Resolution | ---- | ----- | ---- | ---- | ---- | ---- |
| Tailing factor | 1.41 | 1.82 | 1.43 | 1.47 | 1.28 | 1.31 |
| Capacity factor | 3.775 | 3.32 | 2.76 | 4.56 | 3.83 | 2.78 |

**Table 12: Robustness data of MET; calculated for resolution and tailing factor**

| Sr. No. | F.(-0.2ml.mL$^{-1}$) | F(+0.2ml.mL$^{-1}$) | A (-2ml) | A (+2ml) | WL (-2nm) | WL (+2nm) |
|---|---|---|---|---|---|---|
| Resolution | 2.38 | 3.006 | 3.86 | 2.24 | 3.005 | 2.73 |
| Tailing factor | 1.58 | 1.46 | 1.08 | 1.51 | 1.24 | 1.53 |
| Capacity factor | 4.38 | 4.64 | 4.33 | 5.037 | 3.005 | 3.44 |

**Force degradation/Stability indicating method**[27,28]

Stability of both drugs are studied utilizing different parameter. In this study, the area of standard for stability and degradation of sample and standard were compared. The standard area of MET and IVA is 649.883 and 2649.948 respectively. Result showedMET has highest degradation in oxidation and thermal as compare to others. IVA shows highest degradation in oxidation and basic environment.The standard area of IVA and MET as well as peaks of all parameters were given in Fig 6-9. The percent degradation of all parameters is given below in Tables 13 and 14.

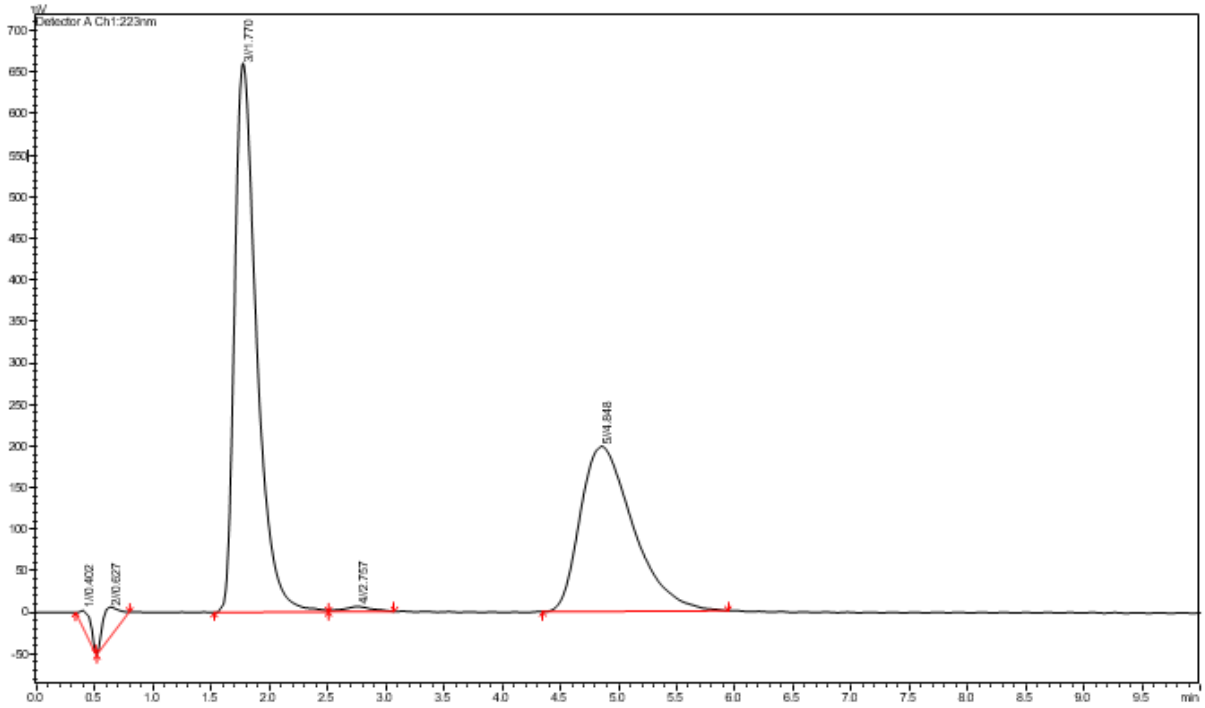


**Fig. 6: Neutral hydrolysis of IVA and MET at 60˚C carried out for 12 Hours**

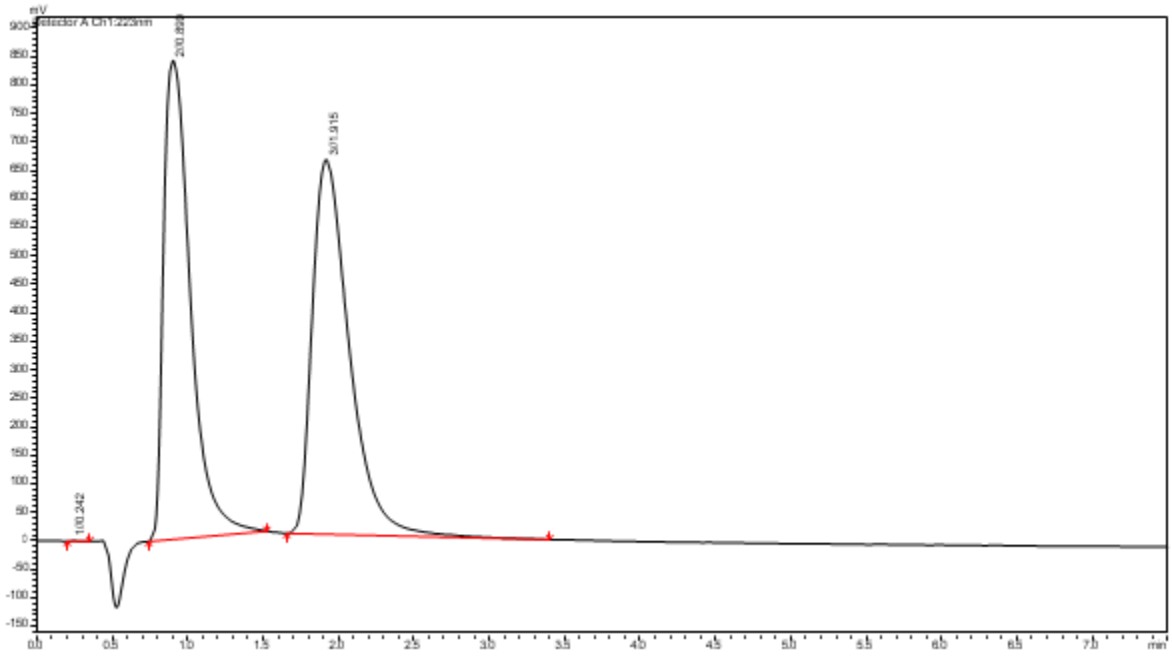


**Fig. 7: Acid induced degradation studies using 0.1N HCl for 12 Hours for IVA and MET at 60˚C**

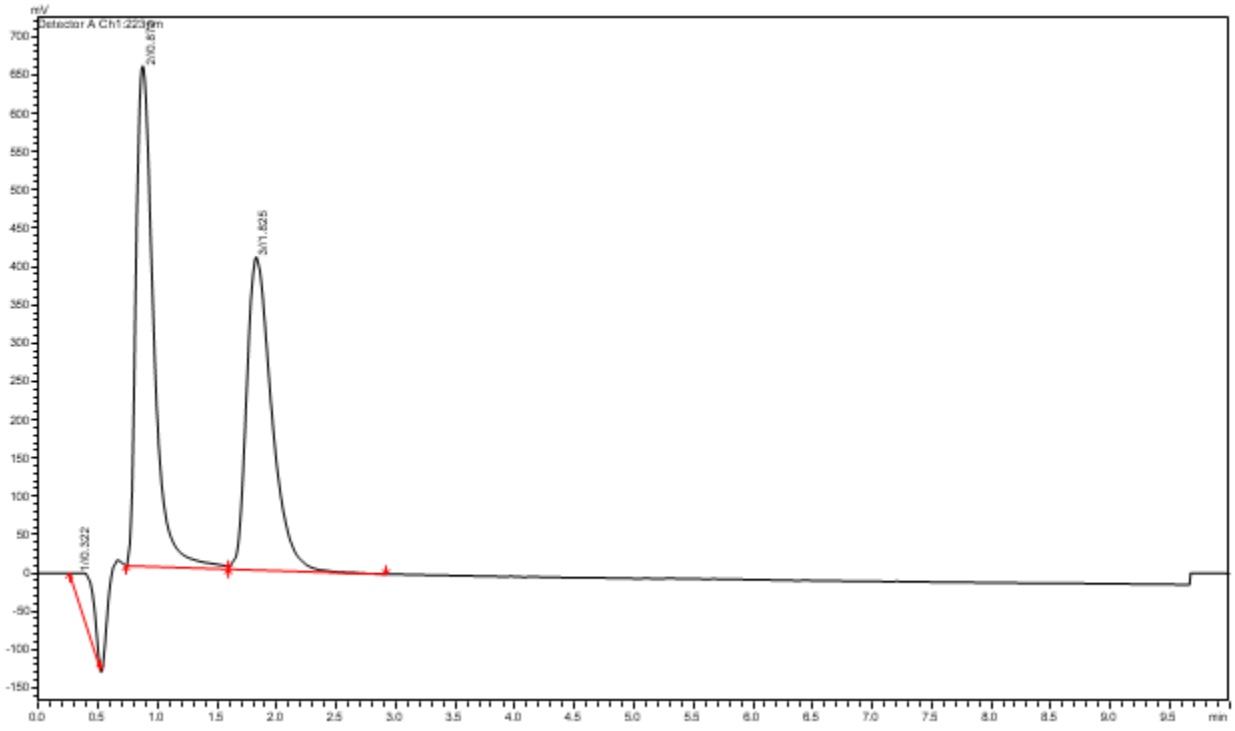


**Fig. 8: Base induced degradation studies using 0.1N NaOH for 12 Hours for IVA and MET at 60˚C.**

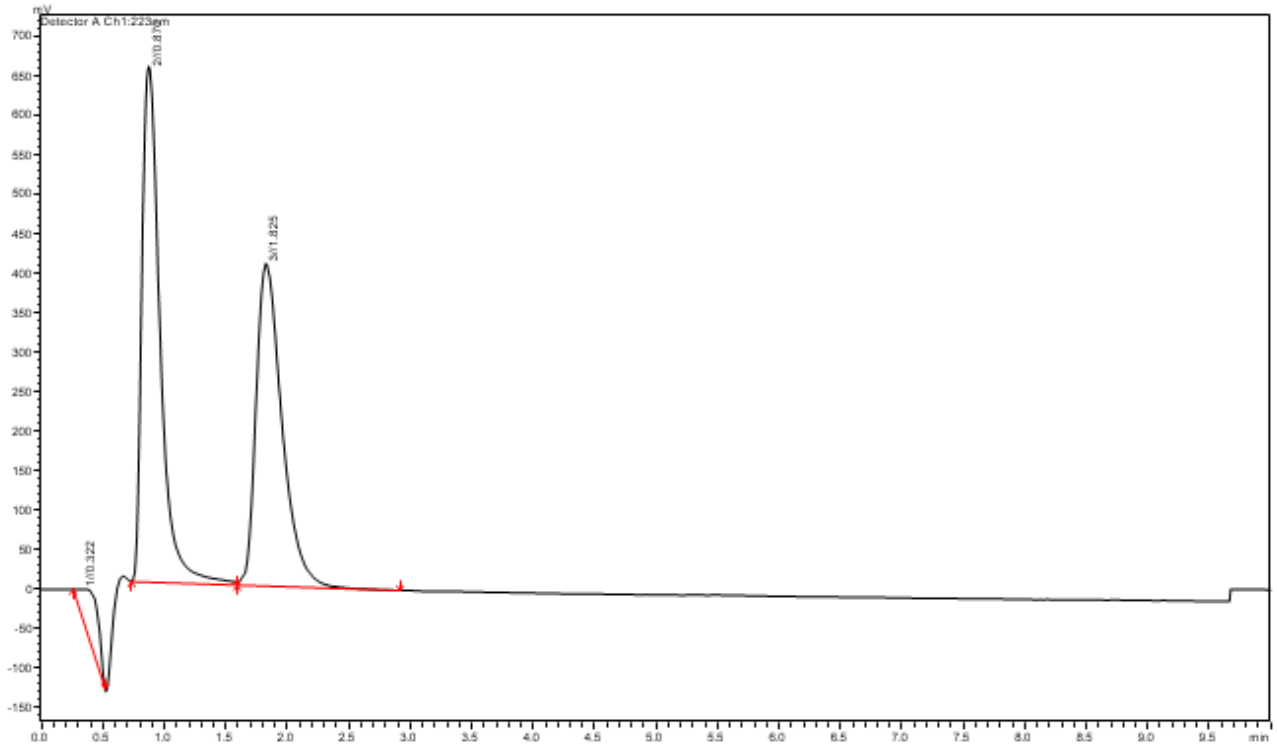


**Fig. 9: 6% H2O2 induced degradation studies for 12 Hours for IVA and MET at room temperature**

**Table 13:Force Degradation/Stability indicating studies of IVA**

| | Ivabradine | | Degradants of IVA |
|---|---|---|---|
| **Conditions** | **% Area Std.** | **% degradation** | **No. of degradants** |
| Acid (0.1N/M HCl) + 60˚C + 12 Hrs. | 88% | 12% | 1 |
| Base (0.1N/M NaOH) + 60˚C + 12 Hrs. | 100% | 0% | 0 |
| Thermal (60˚C) + 12 Hrs. | 100% | 0% | 0 |
| Oxidation (3-6% H2O2) + Room Temp. | 47.44% | 52.56 | Not distinguished |

**Table 14:Force Degradation /Stability indicating studies of MET**

| | Metoprolol | | Degradants of MET |
|---|---|---|---|
| **Conditions** | **% Area Std.** | **% degradation** | **No. of degradants** |
| Acid (0.1N/M HCl) + 60˚C + 12 Hrs. | 100% | 0% | 0 |
| Base (0.1N/M NaOH) + 60˚C + 12 Hrs. | 100% | 0% | 0 |
| Thermal (60˚C) + 12 Hrs. | 100% | 0% | 0 |
| Oxidation (3-6% $H_2O_2$) + Room Temp. | 100% | 0% | 0 |

## 4. Conclusion

From all above results and discussion, it has been concluded that the developed analytical method for the simultaneous estimation of Ivabradine (IVA) and Metoprolol (MET) in both bulk and tablet formulation has comply all relevant ICH guidelines. As per the ICH guidelines, the developed method has complied the

linearity range, accuracy (%), repeatability, precision (intraday and interday/intermediate), and robustness. As per the ICH guidelines, the system suitability test carried out for Ivabradine and Metaprolol has followed all given criteria; for instance, tailing factor, separation factors, theoretical plates, capacity factor, resolution and RSD (%) values with optimum requirements of US-FDA monograph. The validated stress degradation studies under thermal, oxidative, alkali and acid ascertained no possible degradation products developed for both Ivabradine and Metaprolol. Hence, this developed method by ion-exchange chromatography can be used for routine analysis of simultaneous of Ivabradine and Metaprolol for its high precision, reproducibility, and accuracy for any marketed formulation containing either or both of Ivabradine and Metaprolol.

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