ISSN (O): 2349-5332
CODEN: IRJPHY



# DETERMINATION AND VALIDATION OF METOPROLOL AND ATORVASTATIN SIMULTANEOUSLY BY RP-HPLC METHOD IN TABLET DOSAGE FORM

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| Submitted on: 18.09.19; | Revised on: 22.09.19; | Accepted on: 24.09.19 |
|---|---|---|

**ABSTRACT:**

A new simple procedure was developed to simultaneously estimate metoprolol and atorvastatin and also validated by RP-HPLC method in tablet formulation. The separation of the peak was achieved on Inertsil ODS-3 (4.6Ã—150 mm, 5µm) column using the mixture of Phosphate buffer pH 3: Methanol 50:50v/v ratio as mobile phase. The injection flow rate was maintained at 1 ml/min, and run time was 10.0 mins. UV detection of both the drugs was achieved at 244nm at ambient temperature. The results obtained for this method were in the acceptance criteria and therefore can be employed to estimate metaprolol and Atorvastatin in other dosage forms as well.

**KEY WORDS:** Metoprolol, Atorvastatin, ICH guidelines, method development, validation

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Indian Research Journal of Pharmacy and Science; 22(2019)1952-1965;
Journal Home Page: https://www.irjps.in
DOI: 10.21276/irjps.2019.6.3.6

## INTRODUCTION

Metoprolol is chemically 1-[4-(2-methoxyethyl )phenoxy]-3-[(propan-2-yl)amino] propan-2-ol as shown in figure 1. It is selectively $\beta_1$ inhibitor, specifically to cardiac cells with minute effect on $\beta_2$ receptors. It causes -ve chronotropic and inotropic effects thereby decreasing the cardiac output and exhibits no activity towards membrane stabilization or intrinsic sympathomimetics.[1-3] and therefore produces reduction on heart-rate and cardiac output in dose dependent manner in normal subjects.

Atorvastatin is chemically (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid as shown in figure 2. It is an anti hyperlipedemic agent and is used in the treatment of individuals with high cholesterol levels. [4] Antihyperlipedemic activity of shown as it competitively inhibits the HMG-CoA reductase enzyme which is involved in cholesterol synthesis, thereby decreasing the hepatic cholesterol levels and also increase the HDL levels reducing the risk of cardiovascular mortality rate. [5,6] It is primarily used to prevent coronary heart disease (CHD), myocardial infarction and other cardio vascular disorder.

Literature survey reveals certain developed method to determine metoprolol as well as Atorvastatin which include LC-ESI-MS method [7,8], HPLC,[9-12] HPTLC method,[13-14] UPLC method,[15] and UV spectroscopic method. [16-18]. There was only one RP-HPLC method reported to estimate metoprolol.[19] However, there was no reported method to estimate metoprolol and Atorvastatin simultaneously, hence, a new simple, precise and accurate method was developed in the present study to determine the amount of metoprolol and Atorvastatin present in pharmaceutical dosage form.

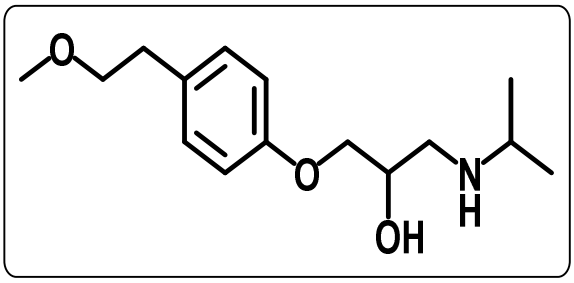


**Figure 1: Structure of Metaprolol**

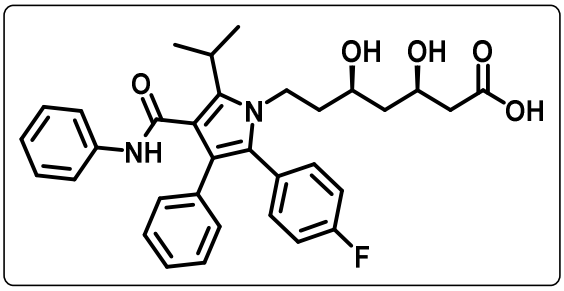


**Figure 2: Structure of Atrovastatin**

## MATERIALS AND METHODS

Metoprolol and Atorvastatin were obtained as gift sample from Pharmatrain lab, Hyderabad, India. Methanol as well as water of HPLC grade was purchased from MERCK. Potasium Dihydrogen phosphate and ortho phosphoric acid was obtained from Finar chemicals and Molychem respectively. MET XL ATF tablets were purchased from pharmacy.

### Instrumentation

HPLC system (WATERS) empowered with 2695 separation module was used for the separation of metoprolol and atorvastatin. Detection was achieved using UV/VIS spectrophotometer (LABINDIA UV

12.500$^{+}$) where instruments such as pH meter used was Adwa — AD 10100 and weighing machine belongs to Afcoset ER-1000A.

**Method development**

As there was no economical method observed for the determination of metoprolol as well as Atorvastatin simultaneously, a new method was felt to be developed using RP-HPLC method. Several trails were performed to optimize using various mixtures of solvents as mobile phase. Optimized trial was chosen considering the parameters such as theoretical plates, resolution and retention time.

**Preparation of buffer and mobile phase**

**Preparation of buffer**

Weigh accurately 3.5 gms of $KH_2PO_4$ mixed in 1L of HPLC water. pH was adjusted up to 3.0. Final solution was subjected to filtration through 0.44 µm membrane filter and was sonicated for 10 minutes.

**Preparation of the mobile phase**

0.5L (50%) of above buffer solution was mixed with 0.5L (50%) of Methanol HPLC. It was then degassed in a sonicator for around 10 minutes and subjected to filtration through 0.45µ filter under vacuum filtration. The same solution was used as diluents.

**Preparation of metoprolol and Atorvastatin solutions**

**Standard Solution Preparation:**

50 mg of Metoprolol and 10 mg of Atorvastatin working standard were weighed accurately and transferred into a 100 ml clean dry VF to which 7 mL of diluent was added and sonicated to dissolve completely. The volume was madeup to the mark with same solvent after which 1.5ml of this solution was pipetted into a 10ml VF and diluted up to the mark with diluent.

**Sample Solution Preparation:**

10 tablets of MET XL ATF, weighed accurately, crushed and powder containing equivalent amount of 50 mg of Metoprolol and 10 mg Atorvastatin sample were transfered into a 100 mL clean dry VF to which 7 mL of diluent was added and sonicated to dissolve completely and made the volume up to the mark with the mobile phase and filtered through 0.45 micron Injection filter after which 1.5ml of this solution was pipetted into a 10ml VF and diluted using diluent.

**Procedure**

Mixture of phosphate buffer pH3 and methanol in the ratio 50:50% v/v was used as mobile phase which was injected into the system for 30 minutes prior to injecting the prepared solutions of standard as well as sample. Detection of the drug was achieved at the wavelength of 230nm at 25°C. After several trials, method was optimized followed by validation of the method considering various validation parameters.

## RESULTS AND DISCUSSION

In the developed method, separation mode was isocratic and the column used to achieve the separation was Inertsil ODS (4.6 x 250mm) with particle size of 5.0µm. Binary solvent was used as mobile phase which is a mixture of Phosphate buffer pH3 and methanol in equal ratio. Flow rate was maintained at 1ml/min and runtime of 10 min with injection volume of 20µl at ambient temperature. The peaks obtained had good resolution and retention time of 2.478 mins for metoprolol and 4.169 for Atorvastatin respectively. The chromatogram of the optimized trial is shown in figure 3.

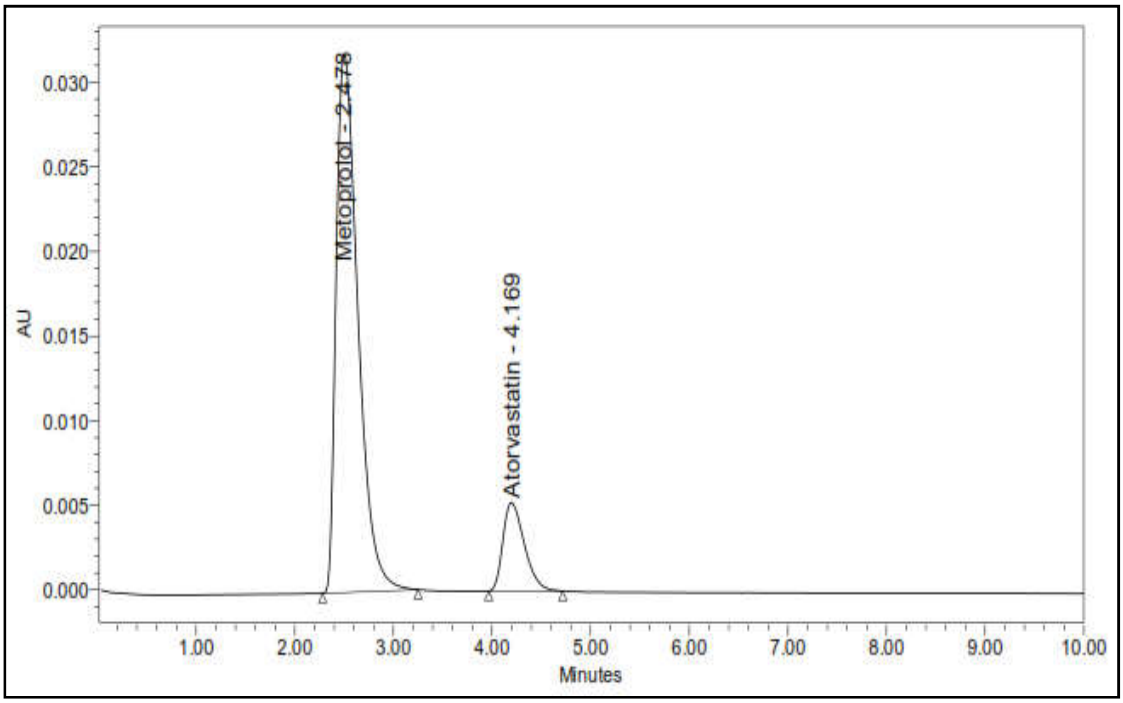


**Figure 3: Chromatogram of the optimized trial**

**System suitability**

Once the column was equilibrated, theoretical plates, asymmetric factor and resolution were evaluated by injecting the standard as well as sample solutions respectively into the chromatographic system and recorded the responses which determines the suitability of the chromatographic system for the analysis. The results are shown in the table 1.

**TABLE 1: RESULTS OF SYSTEM SUITABILITY PARAMETERS**

| S.No | Name | RT (min) | Area (µV sec) | Height (µV) | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | Metoprolol | 2.478 | 536575 | 32710 | | 1.43 | 5584.11 |
| 2 | Atorvastatin | 4.169 | 85809 | 5298 | 4.07 | 1.38 | 6560.51 |

**Validation of the method**

The proposed method was validated by evaluating various validation parameters such as specificity, linearity, accuracy and precision, detection and quantitation limit, robustness as well as stability of the method. Evaluation was done following the ICH guidelines. The method was specific as there were no interferences found due to the excipients. Linearity results obtained had good correlation and the assay result obtained was good and is shown in table 2.

**TABLE 2: ASSAY RESULTS**

| | Label Claim (mg) | % Assay |
|---|---|---|
| **Metoprolol** | 50 | 100.03 |
| **Atorvastatin** | 10 | 99.82 |

**Linearity**

The linearity range was found to lie from 25µg/ml to 125µg/ml of Metoprolol, 5µg/ml to 25µg/ml of Atorvastatin. Correlation coefficient was observed to be greater than 0.999 for both the drugs. The absorbance values for both the drugs and their calibration curve data are shown in table 3 and the linearity graphs are shown in figure 4 and 5 respectively.

**TABLE 3: RESULTS OF LINEARITY**

| S. No. | Metoprolol | | Atorvastatin | |
|---|---|---|---|---|
| | Concentration (µg/ml) | Area | Concentration (µg/ml) | Area |
| 1 | 25 | 177826 | 5 | 28052 |
| 2 | 50 | 357746 | 10 | 56700 |
| 3 | 75 | 539448 | 15 | 85185 |
| 4 | 100 | 717271 | 20 | 113978 |
| 5 | 125 | 890665 | 25 | 143962 |
| Slope (m) | 7140.8 | | 5782 | |
| Intercept | 1030.3 | | 1154 | |
| $R^2$ | 0.999 | | 0.999 | |

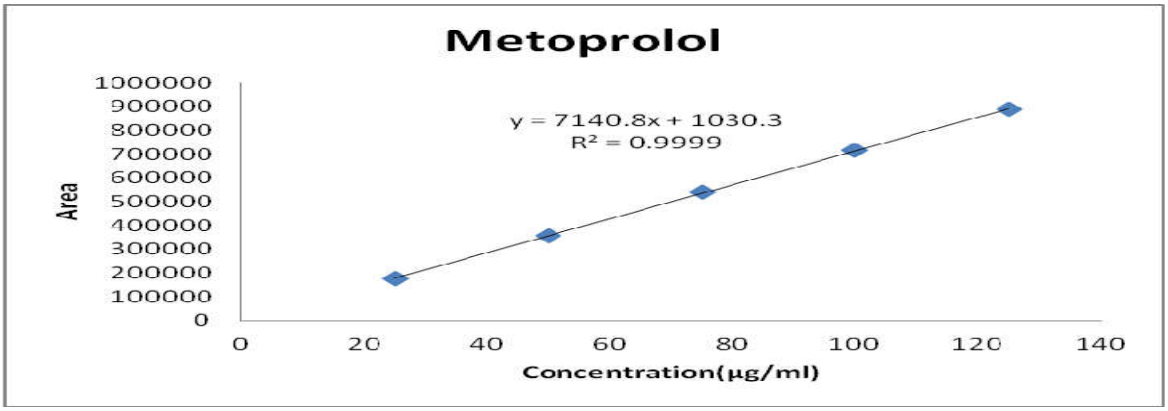


**Figure 4: Calibration graph for Metoprolol**

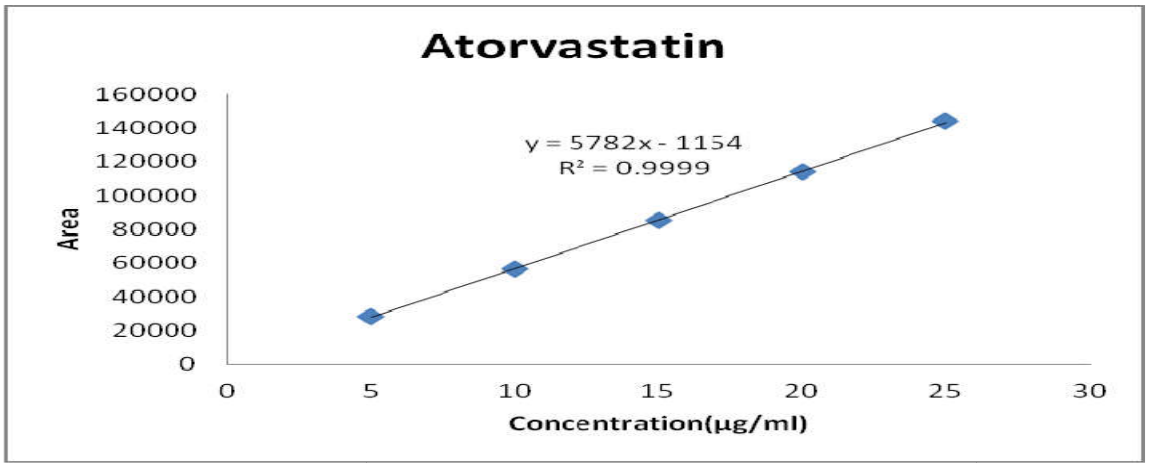


**Figure 5: Calibration graph for Atorvastatin**

**Accuracy**

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and these concentration samples were spiked into the chromatographic system in triplicates and response were recorded. The % recovery was calculated and the method was found to be accurate as the results were within the limits. Accuracy results are shown in table 4.

**TABLE 4: ACCURACY RESULTS**

| %Concentration | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| **Results for metoprolol** | | | | | |
| 50% | 267451.7 | 25 | 24.96 | 99.82 | 100.03 |
| 100% | 532332.3 | 50 | 49.67 | 99.34 | |
| 150% | 811114.3 | 75 | 75.68 | 100.91 | |
| **Results for Atorvastatin** | | | | | |
| 50% | 42711.7 | 5 | 4.99 | 99.83 | 99.82 |
| 100% | 85512 | 10 | 9.99 | 99.93 | |
| 150% | 127978.3 | 15 | 14.96 | 99.71 | |

**Precision**

Precision of the method was determined by evaluating the repeatability and intermediate precision/ruggedness. The was done by preparing 75ppm of Metoprolol and 15ppm Atorvastatin respectively and injecting into the chromatographic system six times. Their responses were recorded and the %RSD was calculated for metoprolol and Atorvastatin respectively and the results were found to be within the limits. The results are presented in table 5 and 6.

**TABLE 5: REPEATABILITY RESULTS**

| Injection | Area | |
|---|---|---|
| | **Metoprolol** | **Atorvastatin** |
| Injection-1 | 536587 | 85514 |
| Injection-2 | 536645 | 85722 |
| Injection-3 | 534973 | 85615 |
| Injection-4 | 539939 | 85728 |
| Injection-5 | 538130 | 85268 |
| Injection-6 | 539250 | 85258 |
| **Average** | 537587.3 | 85517.5 |
| **Standard Deviation** | 1860.8 | 212.2 |
| **%RSD** | 0.3 | 0.2 |

**TABLE 6: INTERMEDIATE PRECISSION RESULTS**

| Injection | Area | |
|---|---|---|
| | **Metoprolol** | **Atorvastatin** |
| Injection-1 | 530543 | 85499 |
| Injection-2 | 539435 | 85366 |
| Injection-3 | 530808 | 85790 |
| Injection-4 | 534588 | 85997 |
| Injection-5 | 531979 | 85525 |
| Injection-6 | 532150 | 85121 |
| **Average** | 533250.5 | 85549.7 |
| **Standard Deviation** | 3351.5 | 309.4 |
| **%RSD** | 0.6 | 0.4 |

**Detection and quantitiation limit**

For limit of detection and quantitation**,** the lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. The chromatograms of LOD and LOQ are shown in figure 6 and 7 where as the results are shown in table 7.

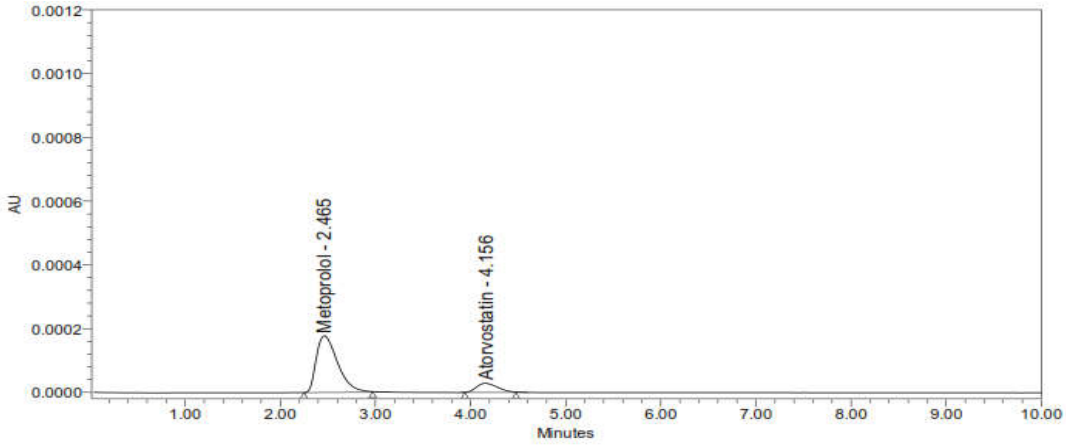


**Figure 6: Chromatogram of Metoprolol, Atorvastatin showing LOD**

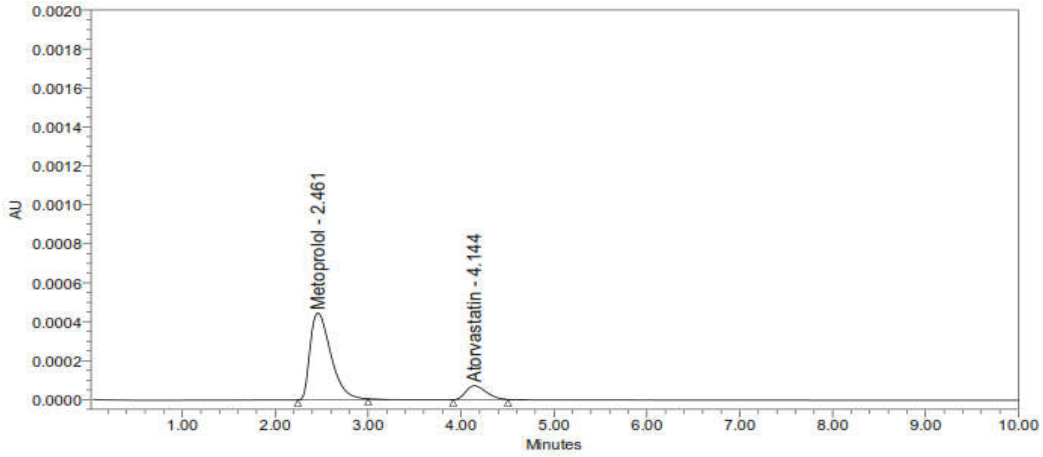


**Figure 7: Chromatogram of Metoprolol, Atorvastatin showing LOQ**

**TABLE 7: DETECTION AND QUANTITATION LIMIT RESULTS**

| Drug name | Baseline noise (µV) | Signal obtained (µV) | S/N ratio |
|---|---|---|---|
| **Results of LOD** | | | |
| Metoprolol | 58 | 174 | 3.00 |
| Atorvastatin | 58 | 173 | 2.98 |
| **Results of LOQ** | | | |
| Metoprolol | 58 | 579 | 9.98 |
| Atorvastatin | 58 | 580 | 10.00 |

**ROBUSTNESS**

The standard and sample solution of Metoprolol and Atorvastatin were injected by changing the conditions of chromatography such as flow rate and mobile phase composition. No significant variation was observed in the parameters such as resolution, tailing factor, asymmetric factor, and plate count. Results are shown in table 8 and 9 and their respective chromatograms are depicted in figure 8-11.

**Variation in flow rate**

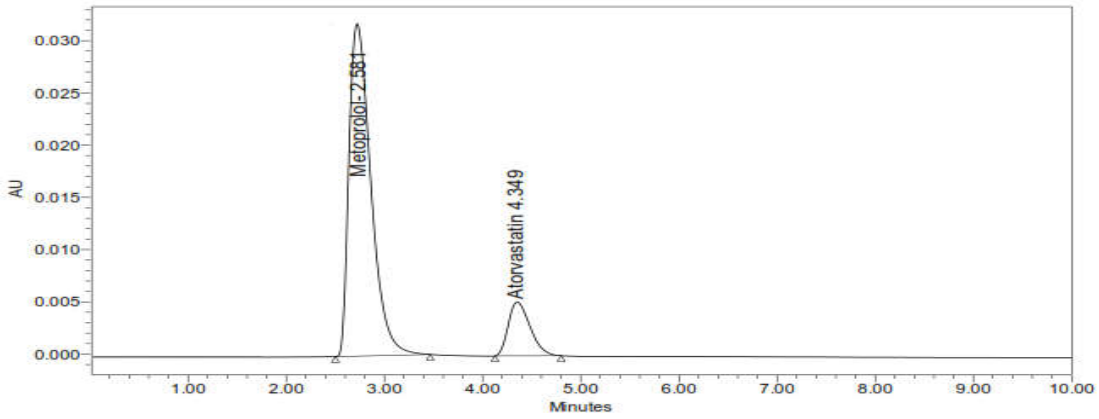


**Figure 8: Chromatogram (less flow)**

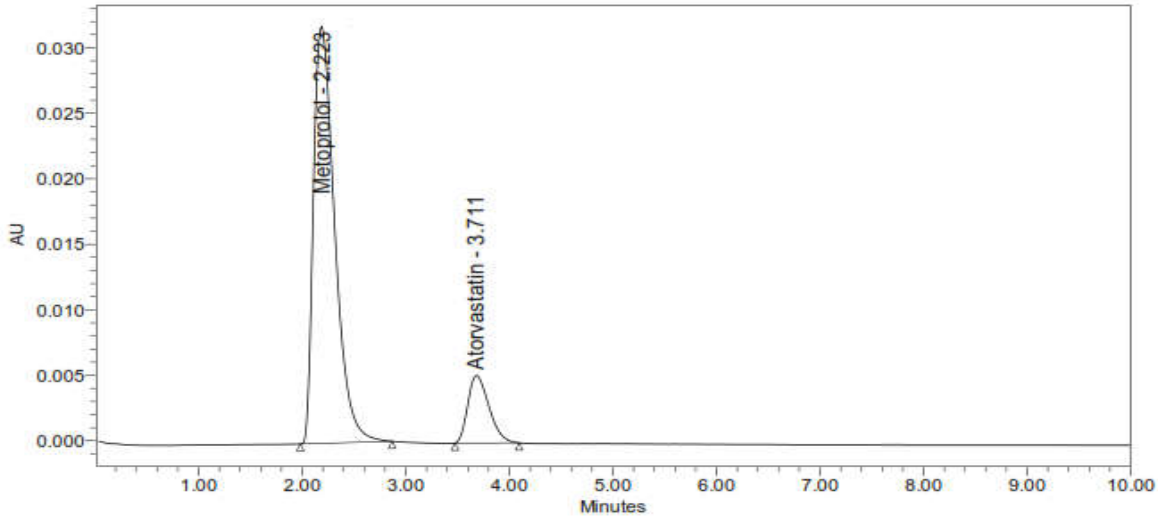


**Figure 9: Chromatogram (more flow)**

**TABLE 8: RESULTS FOR VARIATION IN FLOW RATE**

| S. No | Flow Rate (ml/minutes) | System Suitability Results | | | |
|---|---|---|---|---|---|
| | | Metoprolol | | Atorvastatin | |
| | | USP Tailing | USP Plate Count | USP Tailing | USP Plate Count |
| 1 | 0.9 | 1.42 | 5615.00 | 1.28 | 6652.00 |
| 2 | 1 | 1.43 | 5584.11 | 1.38 | 6560.51 |
| 3 | 1.1 | 1.42 | 5611.61 | 1.24 | 6503.15 |

**Variation of mobile phase organic composition**

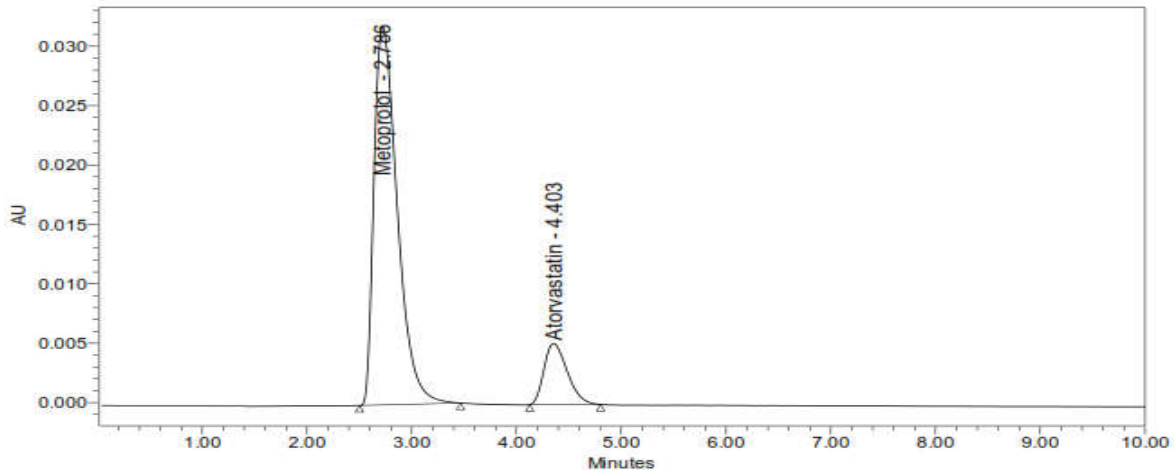


**Figure 10: Chromatogram (less organic composition)**

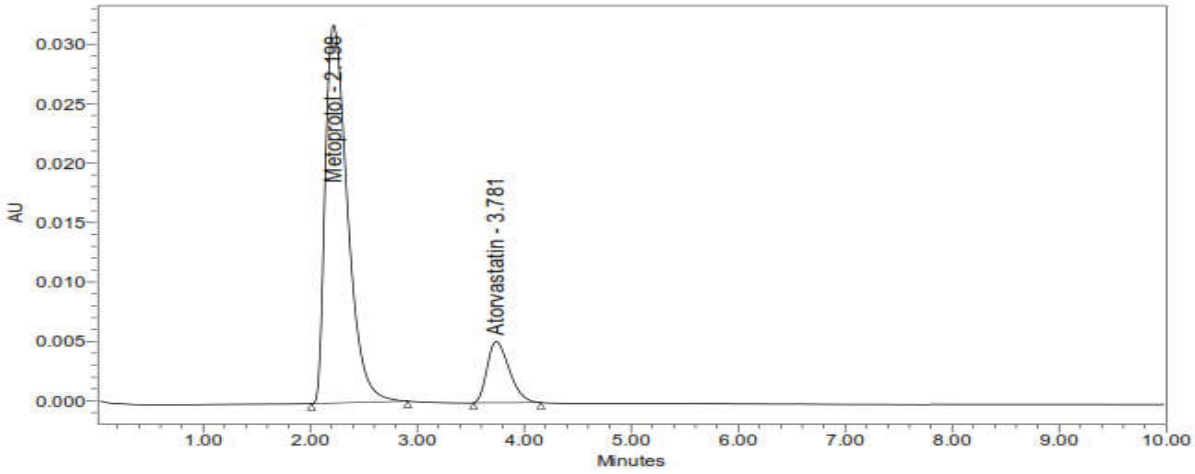


**Figure 11: Chromatogram (more organic composition)**

**TABLE 9: RESULTS FOR VARIATION OF MOBILE PHASE ORGANIC COMPOSITION**

| S. No | Change in Organic Composition in the Mobile Phase | System Suitability Results | | | |
|---|---|---|---|---|---|
| | | Metoprolol | | Atorvastatin | |
| | | USP Tailing | USP Plate Count | USP Tailing | USP Plate Count |
| 1 | 10% less | 1.37 | 5577.00 | 1.15 | 6337.00 |
| 2 | *Actual | 1.43 | 5584.11 | 1.38 | 6560.51 |
| 3 | 10% more | 1.46 | 5625.00 | 1.28 | 6720.00 |

**STABILITY OF THE METHOD**

The developed method to be stable was evaluated by adopting degradation studies of the metoprolol as well as Atorvastatin in presence of various stress conditions such as acid, base, peroxide, thermal degradation and photo degradation. The chromatograms of various degradation studies are shown in figure 12-16 and the results are depicted in table 10.

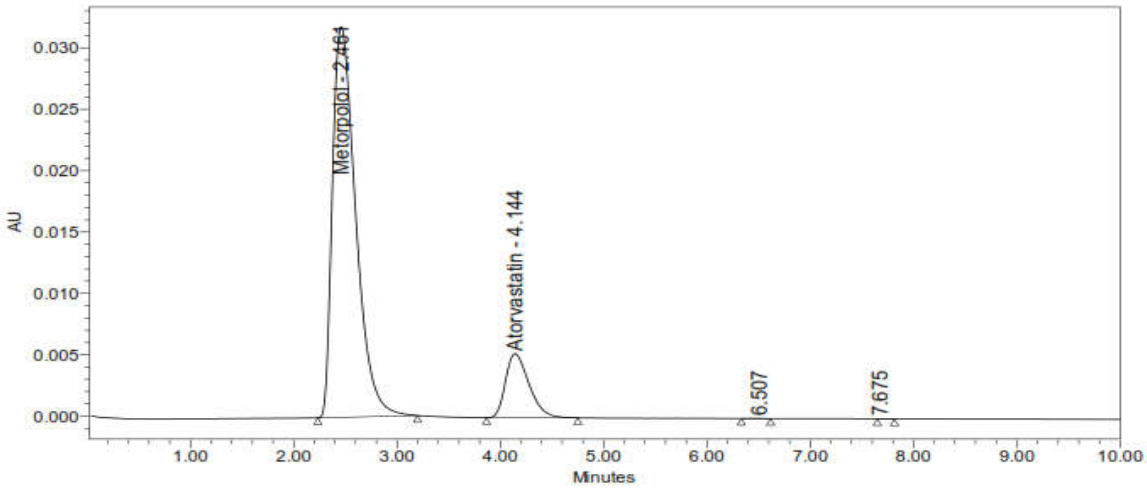


**Figure 12: Acid degradation**

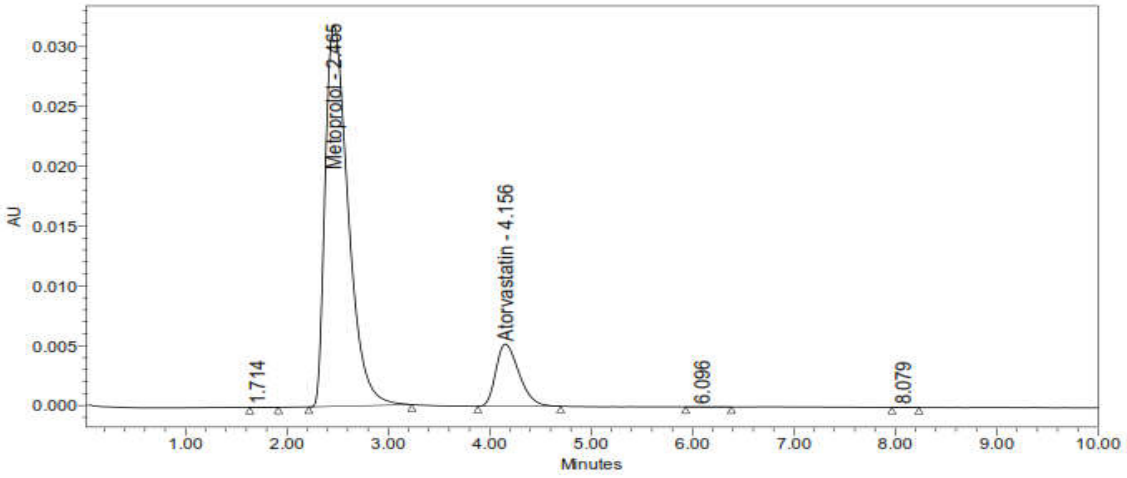


**Figure 13: Base degradation**

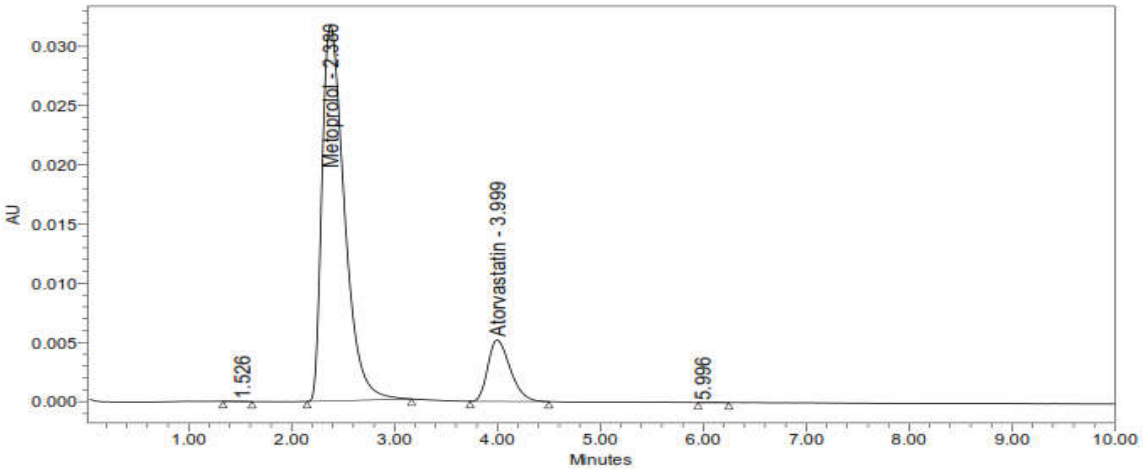


**Figure 14: Peroxide degradation**

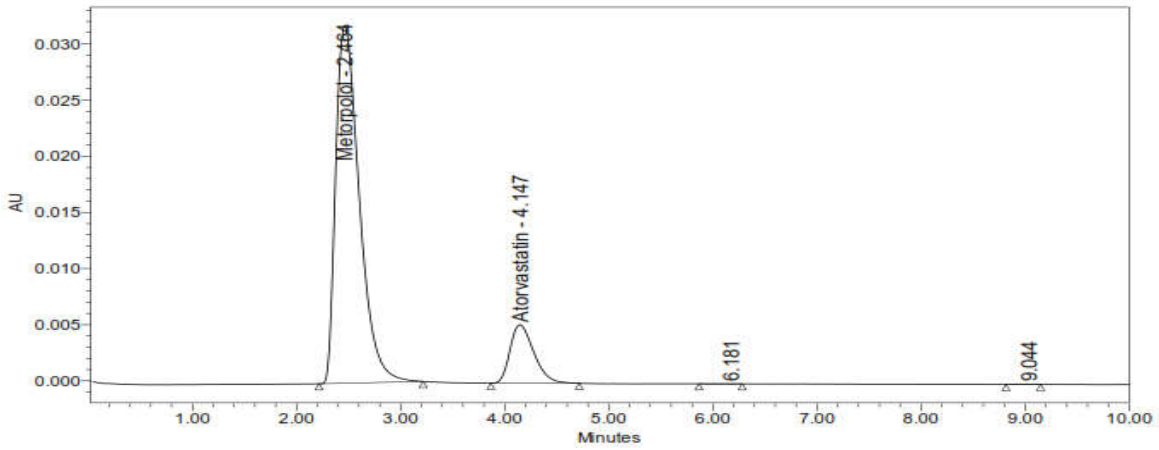


**Figure 15: Thermal degradation**

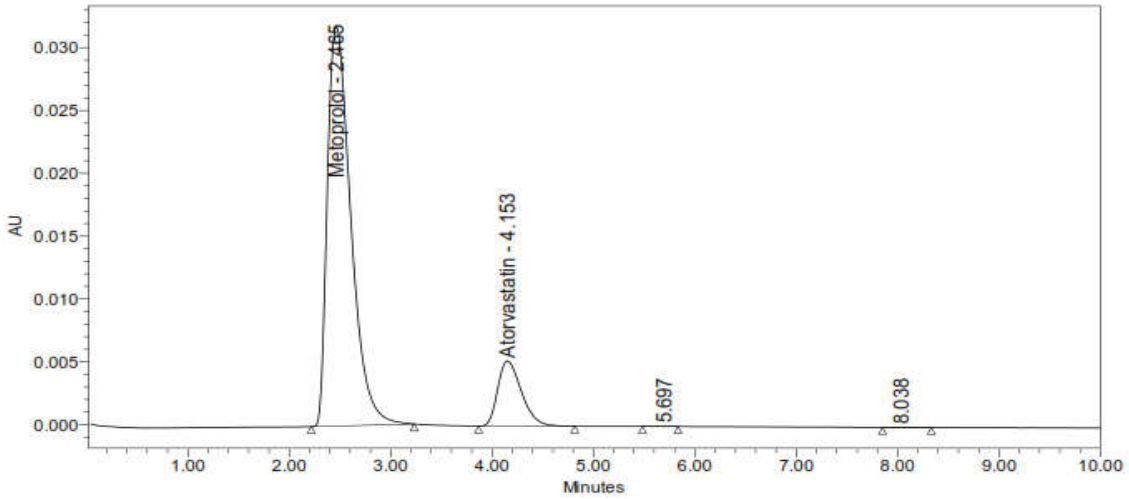


**Figure 16: Photo degradation**

**TABLE 10: RESULTS FOR STABILITY OF METOPROLOL AND ATORVASTATIN**

| Sample Name | Metoprolol | | Atorvastatin | |
|---|---|---|---|---|
| | Area | % Degraded | Area | % Degraded |
| **Standard** | 534777.3 | | 85398.3 | |
| **Acid** | 496035 | 7.24 | 81506 | 4.56 |
| **Base** | 499639 | 6.57 | 80134 | 6.16 |
| **Peroxide** | 479014 | 10.43 | 78314 | 8.30 |
| **Thermal** | 504407 | 5.68 | 80534 | 5.70 |
| **Photo** | 467350 | 12.61 | 77687 | 9.03 |

## CONCLUSION

The present research analytical study was aimed to develop a simple, precise, accurate, robust and stable analytical method for the estimation of metoprolol and atorvastatin simultaneously and validate it by RP-HPLC method in tablet dosage

form. The chromatographic separation was clear at the flow rate of 1 ml/min, and analysis time of 10.0 min, UV detection was at 244 nm. The assay for metoprolol and Atorvastatin were found to be 100.03 and 99.82 respectively. The linearity results of the developed method was greater than 0.999. From the recovery studies, the method was found to be accurate as the % recovery of the drugs were within the acceptable range. From the forced degradation studies, the method was found to be stable as the results were NMT 15%. Therefore, from the results obtained, it can be concluded that this can be employed for estimation of Atorvastatin and metoprolol in other dosage forms.

**ACKNOWLEDGEMENT**

The authors are thankful to Mr. MD Muzaffar ur Rehman for helping in the preparation of manuscript.

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CONFLICT OF INTEREST REPORTED: NIL ; SOURCE OF FUNDING: NONE REPORTED