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# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHODFOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND OLMESARTAN IN PHARMACEUTICAL DOSAGE FORM

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**ABSTRACT**

A simple, precise, rapid, specific and accurate stability indicating reverse phase high performance liquid chromatography method was developed for simultaneous estimation of Atorvastatin (ATV) and Olmesartan (OLM) in pharmaceutical dosage form. Chromatographic separation was performed onAgilent eclipse XDB C8 (150x4.6mm, 5µ)column, with mobile phase comprising of mixture of buffer (pH3.25, adjusted with ortho phosphoric acid), and methanol in the ratio of 60:40v/v, at the flow rate 0.8 ml/min. The detection was carried out at 287 nm. The retention times of OLM and ATV were found to be 2.47 and 3.77 mins respectively with a run time of 6 mins, theoretical levels for OLM and ATV were 6248 and 4867 respectively, with a resolution of 7.57. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness. Linearity of OLM was found in the range of 120-360 µg/mL and that for ATV was found to be 60-180 µg/mL. The correlation coefficient for OLM and ATV were 1.000 and 0.999 respectively. The LOD values for OLM and ATV were 2.845 and 2.927 µg/mL respectively. The LOQ values for OLM and ATV were and 9.486 and 9.756 µg/mL respectively. This demonstrates that the developed method is simple, precise, rapid, selective, accurate and reproducible for simultaneous estimation of OLM and ATV tablet dosage form.

**Keywords**: Atorvastatin (ATV), Olmesartan(OLM), RP-HPLC, Validation, Forced Degradation Studies.

## INTRODUCTION

Atorvastatin fig.(A1)is an antilipemic agent and chemical it is (3*R*,5*R*)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5- propan- 2-ylpyrrol-1-yl]- 3,5-dihydroxyheptanoic acid (MolWt- 558.64 g/mol), that competitively inhibits hydroxyl methyl glutaryl-coenzyme A (HMG-CoA) reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in cholesterol biosynthesis and it is used to reduce plasma cholesterol levels [1].

Olmesartan fig.(A2) is an antihypertensive agent and is chemically 5-methyl-2-oxo-2*H*-1,3- dioxol-4-yl)methyl4-(2-hydroxypropan-2-yl)- 2-propyl-1-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl) phenyl]phenyl}methyl)-1 *H*-imidazole-5- carboxylate (Mol Wt - 558.58g/mol). Olmesartanis a selective and competitive angiotensin II Type 1 (AT1) receptor antagonist and hence it blocks the vasocontrictor and aldosterone-secreting effects of angiotensin II. As a result of this blockage, olmesartan reduces vasoconstriction and the secretion of aldosterone. This lowers blood pressure by producing vasodilation, and decreasing peripheral resistance [2].

From the literature review it is found that there is no official stability indicating RP-HPLC method in Pharmacopoeia for the simultaneous estimation of Atorvastatin and olmesartan in pharmaceutical dosage form and commonly used methods for the estimation of these drugs include titrations, liquid chromatography, capillary electrophoresis and uv-spectrophotometer [3-11].

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Only very few HPLC estimations have been reported in the literature for the determination of Atorvastatin and olmesartan by RP-HPLC technique [5-12]. Hence here is an attempt to develop a precise and accurate stability indicating HPLC method for the simultaneous determination of Atorvastatin and olmesartan in pharmaceutical solid dosage forms. Various validation aspects of the analysis accuracy, precision, recovery, the limits of detection and quantification etc have been measured as per ICH guidelines [13].

## MATERIALSANDMETHODS

### Equipment

Chromatographic separation was performed on HPLC system-Water's 2690 series, PDA Detector 2695 series, equipped with a solvent delivery pump, sample injector and column thermostats. Empower software was applied for data collecting and processing.

### Chemicals andreagents

Methanol, Water of HPLC grade was purchased from Merck Chemicals (Mumbai, India). Buffer used was pH-3.25 (pH adjusted with analytical reagent (AR) grade o-phosphoric acid from LOBA Chemie Pvt). Reference standards Atorvastatin and Olmesartan were obtained from Rainbow labs. Olmesar Av, Tablets with Atorvastatin (10 mg) and Olmesartan (20 mg) manufactured by Macleods pharmaceuticals Pvt Ltd, were procured from local market.

### Preparation ofstandardsolutions

Accurately weighed and transferred about 10 mg of Atorvastatin and 20 mg of Olmesartan working standard into a 50 ml clean dry volumetric flask and added about 30mL of diluent.. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution) (200, 800 µg/mL). From this, 5ml of the solution was pipetted into another 25ml volumetric flask and diluted up to the mark with diluent (20, 80 µg/mL).

### Preparation of samplesolution

Accurately weighed and transferred tablet powder equivalent to10 mg of ATV and 20 mg of OLM into a 50mL clean dry volumetric flask and added about 30mL of diluent. It was sonicated to dissolve completely and made volume up to themark with the same diluent. (Stock solution)(200, 1500µg/mL)From this, 5 mL of the solution was pipette into another 25ml volumetric flask and diluted up to the mark with diluent.

### Preparationofbuffer

Take 1000mL of HPLC grade water. The pH was adjusted to 3.0 with orthophosphoric acid.

### Optimizedchromatographicconditions

Diluent:Methanol

Mobile phase: Buffer: O-$H_3PO_4$ (PH- 3.25)(60): Methanol (40).
Flow rate: 0.8 mL/min
Column: Agilent eclipse XDB C8 (150x4.6mm, 5µ)
Detector wavelength: 287 nm
Injection volume: 10µL
Run time: 6 min
Mode of Pump: Isocratic

## METHODVALIDATION

### Linearity

Solutions were prepared containing 60µg/ml, 90µg/ml, 120µg/ml, 1 50µg/ml, 180µg/ml, concentrations of Atorvastatin and 120µg/ml, 180µg/ml, 240 µg/ml, 300µg/ml, 360 µg/ml, concentrations of olmesartan, which corresponding to 50, 75, 100, 125 and 150% respectively of the test solution concentration. Each solution was injected, linearity was evaluated by linear-regression analysis.

### Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected.

### Precision

Intraday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

### Robustness

The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. Thefactors chosen for thisstudy were theflow rate (±0.1ml/min) and temperature (±5 $^0$C).

### Limit of detection (LOD)and Limit of quantification (LOQ)

LOD and LOQ was calculated from linear curve using formulae
LOD=3.3*σ /slope, LOQ=10*σ /slope
(Where σ = the standard deviation of the response
And, S = Slope of calibration curve).
The LOD was 2.845 and 2.927 µg mL-1 for OLM and ATV respectively and, the respective LOQ were 9.486 and 9.756 µg mL-1

### Specificity

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting

sample solution under optimized chromatographic conditions to demonstrate separation of both Atorvastatin and Olmesartan from impurities.

**Forced Degradation of ATV and ATV**

To determine whether the analytical method and assay were stability-indicating OLM and ATV bulk powder were stressed under different forced degradation conditions in forced degradation studies. OLM (20 mg) and ATV (10 mg) were weighed and dissolved with 50 mL of mobile phase. These stock solutions were used for forced degradation studies.

**Acidic and Alkaline Degradation**

HCl (1N, 10 mL) and NaOH (1N, 10 mL) were separately added to 10 mL stock solutions of OLM and ATV. These mixtures were separately heated under reflux for 4 h at 70°C in the dark (to exclude the possible degradative effect of light). The solutions (10 mL) were transferred separately to volumetric flasks, neutralized by addition of 10 mL 1N NaOH and 1N HCl, and suitable volume was diluted with mobile phase.

**Oxidative Degradation**

Hydrogen peroxide ($H_2O_2$; 3% v/v, 10 mL) was added to 10 mL stock solutions of OLM and ATV. These solutions were separately heated under reflux for 3 h at 70°C in dark. The solutions were then transferred separately to volumetric flasks, neutralized by addition of 1N NaOH and diluted to suitable volume with mobile phase.

**Dry heat Degradation**

For dry heat degradation, solid drugs were kept in petriplates in an oven at 100°C for 5h. Thereafter, 10 mg and 75 mg of each OLM and ATV were weighed and transferred into 10 mL volumetric flask and suitably diluted with mobile phase.

**Photochemical Degradation**

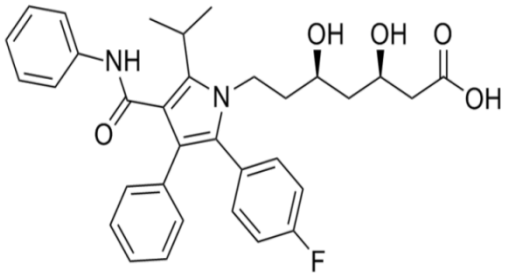
10 mL stock solutions of OLM and ATV were separately subjected to natural sunlight for 5 h to study the effect of photo degradation. Suitable volumes from above stock solutions were diluted with mobile phase.

**RESULTANDDISCUSSION**

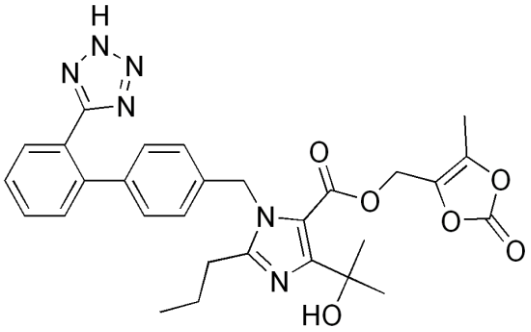
Several mobile phase compositions were tried to resolve the peak of OLM and ATV. The mobile phase containing buffer: Water: Methanol in proportion of 60:40 v/v was found ideal to resolve the peak of OLM and ATV. Retention time of OLM and ATV were 2.47 and 3.77 min respectively (Figure 1&2). Result of assay is shown in Table-3. The proposed method was found to be linear in concentration range 60-180µg/ml for ATV and 120 -360 µg/ml for OLM. The data was shown inTable-1and Figure-3 & 4. System suitability parameters were evaluated and results shown in (Table-2), which were with in acceptance criteria. The mean percentage recovery for ATV and OLM was found to be 100.01% and 99.94% respectively, which are well within the limit and hence the method was found to be accurate (Table-3). LOD and LOQ values were 2.927µg/mL and 9.756 µg/mL for ATV and 2.845µg/mL and 9.486 µg/mL for OLM (Table-1). Results of intraday precision were shownin (Table-4). The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and column temperature. The result obtained implies method is robust for routine qualitative analysis (Table-5).

**Degradation Studies**

The degradation studies indicated that both ATV and OLM degraded in acidic and basic hydrolysis, oxidation, photolysis and dry heat degradation under experimental conditions with maximum degradation under acidic hydrolytic condition. The percent degradation of ATV and OLM, at photolytic, dry heat, oxidative, basic and acid stress conditions, was found to be 25, 24, 25, 31, 34% and 26, 27, 26, 32, 38 % degradation respectively. Both ATV and OLM were highly susceptible towards acidic hydrolysis, resulting in more than 30% degradation, however no additional degradation peak was observed. Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of the drugs under non degradation condition. Summary of degradation studies of the drugs were shown in Table 6.




Atorvastatin (A1)




Olmesartan (A2)

**Fig 1. Chromatogram of OLM (20µg/mL) and ATV (10µg/mL) standard**

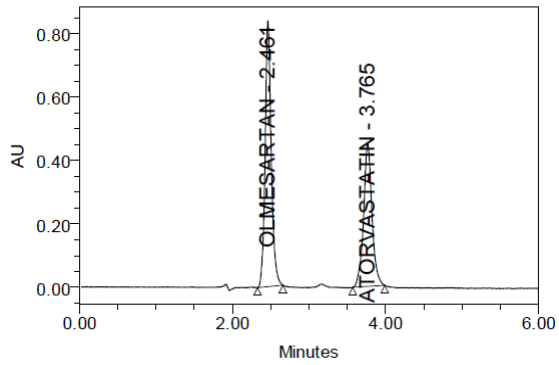



**Fig 2. Chromatogram of OLM (20µg/mL) and ATV (10µg/mL) sample**

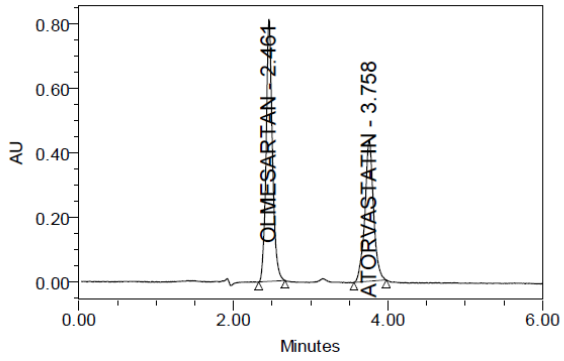



**Fig 3.Calibration curve for Atorvastatin**

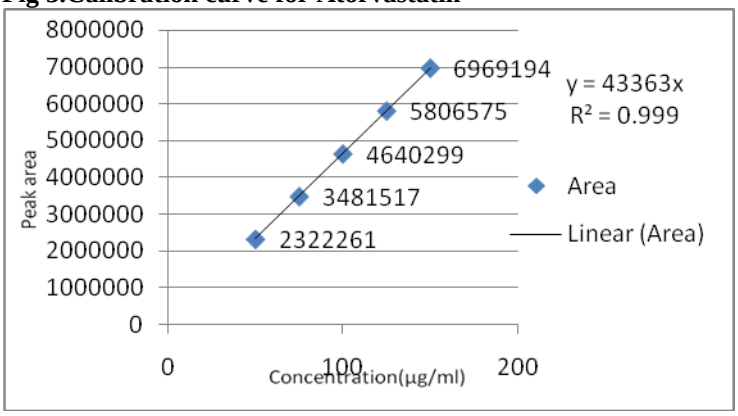



**Fig 4. Calibration curve for Olmesartan**

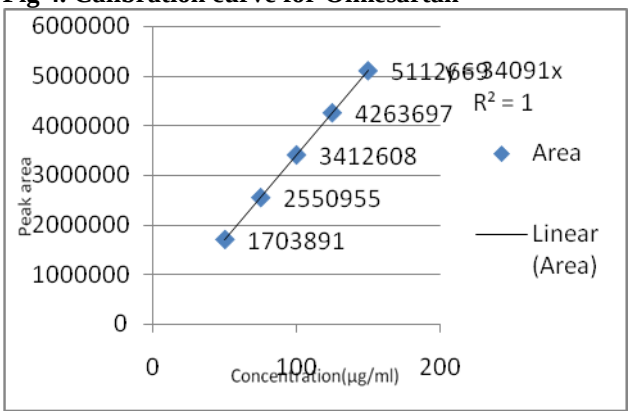



**Table 1.Result of Linearity**

| Linearity Conc(%) | Volume of stock taken(mL) | Diluted to (mL) | Final Conc (µg/mL) | | Peak Area | | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|---|---|---|---|---|---|
| | | | OLM | ATV | OLM | ATV | OLM | |
| 50 | 0.5 | 10 | 120 | 60 | 1703891 | 2322261 | | |
| 75 | 0.75 | 10 | 180 | 90 | 2550955 | 3481517 | 2.845 | 9.486 |
| 100 | 1 | 10 | 240 | 120 | 3412608 | 4640299 | ATV | |
| 125 | 1.25 | 10 | 300 | 150 | 4263697 | 5806575 | | |
| 150 | 1.5 | 10 | 360 | 180 | 5112669 | 6969194 | 2.927 | 9.756 |

**Table 2. System suitability studies**

| Parameters | Olmesartan | Atorvastatin | Acceptance criteria |
|---|---|---|---|
| Theoretical plates | 5480 | 6774 | Not less than 2000 |
| Tailing factor | 1.30 | 1.20 | Not more than 2 |
| Resolution | - | 7.91 | Not less than 2 |

**Table 3. Recovery studies for Atorvastatin and Olmesartan**

| Drug | Spiked level% | Amount taken(µg/ml) | Amount found(µg/ml) | Percent recoveryn=3 | Mean recovery |
|---|---|---|---|---|---|
| ATV | 50 | 59.486 | 59.583 | 100.13 | |
| | 100 | 118.972 | 119.012 | 99.98 | 100.01 |
| | 150 | 178.467 | 178.42 | 99.94 | |
| OLM | 50 | 119.692 | 119.37 | 99.73 | |
| | 100 | 239.384 | 239.615 | 100.09 | 99.94 |
| | 150 | 359.094 | 359.059 | 99.99 | |

*n- Number of replicate injections*

**Table 4. Results of Intraday Precision**

| S.No | Sample Weight | Atorvastatin sample area | Olmesartan Sample area | Atorvastatin % Assay | Olmesartan % Assay |
|---|---|---|---|---|---|
| 1 | 687.30 | 4641536 | 3414412 | 98.993 | 99.735 |
| 2 | 687.30 | 4649770 | 3417410 | 99.169 | 99.823 |
| 3 | 687.30 | 4643971 | 3418496 | 99.045 | 99.855 |
| 4 | 687.30 | 4645505 | 3411716 | 99.078 | 99.657 |
| 5 | 687.30 | 4644610 | 3414828 | 99.059 | 99.747 |
| 6 | 687.30 | 4649524 | 3411134 | 99.163 | 99.640 |
| Avg Assay | - | 4645819.33 | 3414666 | 99.084 | 99.743 |
| STD | - | 3245.209 | 2948.448 | 0.069 | 0.086 |
| %RSD | - | 0.070 | 0.086 | 0.070 | 0.086 |

**Table 5. Results of Robustness study**

| S.No | Parameter changing | Area | USP Tailing | USP Plate count | Rt | |
|---|---|---|---|---|---|---|
| | | | | | ATV | OLM |
| 1 | Temp1 | 4630692 | 1.01 | 1722 | 2.48 | --- |
| 2 | Temp2 | 4631543 | 1.00 | 1514 | 2.46 | --- |
| 3 | Flow1 | 6259844 | 1.07 | 3152 | 3.23 | --- |
| 4 | Flow2 | 6259844 | 1.07 | 3152 | 3.23 | --- |
| 5 | Temp1 | 3421511 | 0.90 | 1613 | --- | 3.79 |
| 6 | Temp2 | 3426563 | 0.90 | 1645 | --- | 3.75 |
| 7 | Flow1 | 4598991 | 0.95 | 4375 | --- | 4.92 |
| 8 | Flow2 | 4598991 | 0.95 | 4375 | --- | 4.92 |

**Table 6. Forced Degradation Studies**

| Parameters | Sample Area | | % Assay | | %Deg | |
|---|---|---|---|---|---|---|
| | ATV | OLM | ATV | OLM | ATV | OLM |
| ACID | 3053797 | 2124335 | 65 | 62 | -34 | -38 |
| BASE | 3207744 | 2305421 | 68 | 67 | -31 | -32 |
| PEROXIDE | 3472850 | 2536116 | 74 | 74 | -25 | -26 |
| LIGHT | 3451072 | 2531756 | 74 | 74 | -25 | -26 |
| HEAT | 3530252 | 2490905 | 75 | 73 | -24 | -27 |
| Avg Assay**:** | 2785952.6 | 1998089 | - | - | - | - |
| STD | 1376907.6 | 991957 | - | - | - | - |

## CONCLUSION

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of ATV and OLM using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific. The results of the degradation studies indicated the suitability of the method to study stability of ATV and OLM under various forced degradation conditions viz. acid, base, dry heat, photolytic degradation. The developed method can be applied to the analysis of stability samples of combination dosage form of ATV and OLM.

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