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Research Article

# HPLC Method Development and Validation for the Estimation of Amlodipine and Atorvastatin in Bulk and Formulation

Ranu Vishwakarma, Santosh Singh Bhadoriya*, Kavita R. Loksh

*Oriental College of Pharmacy, Raisen Rd, Patel Nagar, Bhopal, Madhya Pradesh 462022*

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*Address for Correspondence:

Prof. Santosh Singh Bhadoriya, Oriental College of Pharmacy, Raisen Rd, Patel Nagar, Bhopal, Madhya Pradesh 462022

## Abstract

In the present research work, a successful attempt was made for Validated UV and HPLC method development for the estimation of Amlodipine and Atorvastatin in marketed formulation which was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling. The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in marketed formulation. Liquid chromatographic system from waters comprising of manual injector, Waters 515 binary pump for constant flow and constant pressure delivery and U.V. detector connected to data ace software controlling the instrumentation as well as processing the data generated were used. The isocratic mobile phase consisted of 20 mM $KH_2PO_4$: Acetonitrile (pH 3 with OPA) in the ratio of 20:80 v/v at a flow rate of 1.0 ml $min^{-1}$. A thermo C-18 column (4.6 x 250mm, 5µ particle size) was used as the stationary phase, 237.0 nm was selected as the detection wavelength for UV-vis. detector. The proposed methods were found to be linear in the range of 1-5 µg/ml & 5-25 µg/ml with correlation coefficient close to one for amlodipine and atorvastatin respectively. Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing in the deliberate variation in solvent. The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.

**Keywords:** Amlodipine, Atorvastatin, Method development, HPLC, Validation

## INTRODUCTION

Atorvastatin calcium [1], chemically, [R-(R*, R*)]-2- (4-fluorophenyl) - β, δ-dihydroxy 5 (1- methylethyl)- 3- phenyl-4-[(phenyl amino) carbonyl]- 1H pyrrole- 1-heptanoic acid, calcium salt trihydrate (2:1) is an antihyperlipoproteinemic drug [2, 3], used for treatment of hypercholesterolemia (Figure 1). Amlodipine [4], chemically, 2- [(2-Aminomethoxy) methyl] - 4- (2- chlorophenyl) - 1, 4- dihydro-6-methyl-3, 5- pyridine dicarboxylic acid, 3- ethyl- 5-methyl ester, is a calcium channel antagonist, used as an anti-hypertensive drug [5] (Figure 2).

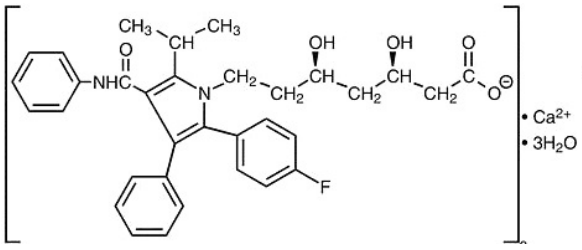


**Figure 1:** Structure of Atorvastatin calcium

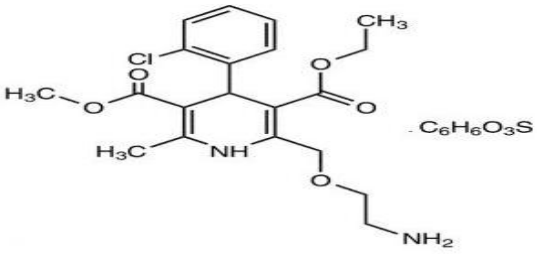


**Figure 2:** Structure of Amlodipine

Literature survey revealed that extractive spectrophotometery[6], GC-MS[7], LC-MS[8], LC- electrospray tendem mass spectrometry[9-11] and HPTLC[12] methods have been reported for the estimation of atorvastatin calcium. Amlodipine besylate is official in British Pharmacopoeia[13]. Different LC methods have been reported for the estimation of AML[14-17]. For the estimation of amlodipine and atorvastatin combination. Present study involves development of a stability indicating liquid chromatographic method for the estimation of ATV and AML in combination dosage form.

## MATERIALS AND METHODS

### Instrumentation

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data. Weighing was done on a Digital Micro Balance (CX-265) manufactured by Citizen Scale (I) Pvt. Ltd.

### Reagents and chemicals

Amlodipine besylate (AMD) and Atorvastatin (ATV) was obtained as a gift sample from Pharmaceutical Company. methanol, Acetonitrile, $KH_2PO_4$ purchased from CDH chemical Pvt. Ltd. New Delhi. Triple distilled water was used for whole experiment was generated in house. All other chemical were analytical grade.

### Selection of Mobile Phase

Initially to estimate Amlodipine besylate and Atorvastatin in fix dosage form number of mobile phase in different ratio were tried. A result was shown in Table 6.4.

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 20 MM $KH_2PO_4$: acetonitrile (pH-3 with Ortho phosphoric acid) in the ratio of 20:80v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

### Preparation of Stock Solution:

Accurately weighed 10 mg API of AMD and ATV was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A).

### Preparation of Sub Stock Solution

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of AMD and ATV respectively (Stock-B).

### Preparation of Different Solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml, for AMD. In same manner 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml of ATV also prepared.

### Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 1-5 µg/ml for AMD and 5-25µg/ml for ATV were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 237 nm (Figure 3 & 4) and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived (Figure 5 & 6).

### Validation of developed Method

### Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 1 to 5 µg/ ml for AMD) and (5 to 25µg/ ml for (ATV) and areas for each concentration were recorded three times and mean area was calculated. The response ratio (response factor) was found by dividing the AUC with respective concentration.

### Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

### Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

### Precision

### Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5µg/ml for AMD and 5, 10, 15, 20 and 25µg/ml for ATV indicates the precision under the same operating condition over short interval time.

### Intermediate Precision

### Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations.

### Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 20mM $KH_2PO_4$: Acetonitrile (20:80% v/v) to (15:85% v/v). Results of robustness are reported in table 6.25-6.26.

### Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

### Analysis of both the drug in tablet sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 5mg of AMD and 10mg of ATV was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 5µg/mL AMD and 10µg/mL ATV respectively. The amounts of AMD and ATV in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with formulation.

## RESULTS AND DISCUSSION

The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drugs in marketed formulation.

### System Suitability

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of AMD 1µg/ml for AMD and 5µg/ml ATV was injected separately. The result of system suitability parameter is reported in table 1.

**Table 1: Results of system suitability parameters**

| Parameters | % Mean± SD* | |
|---|---|---|
| | AMD | ATV |
| No. of Theoretical Plates | 2371.667± 54.463 | 2118.442± 1025.756 |
| Tailing Factor | 1.120±0.035 | 1.243±0.025 |
| Retention time | 1.930±0.003 | 8.981±0.002 |

### Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. The results of linearity are reported in table 2.

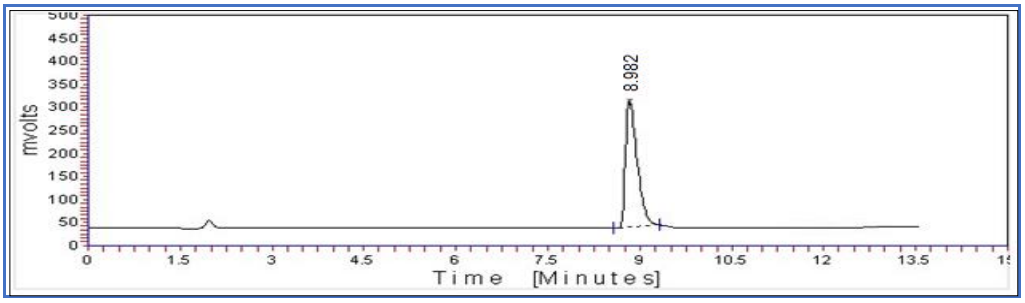


**Figure 3: Chromatogram of ATV**

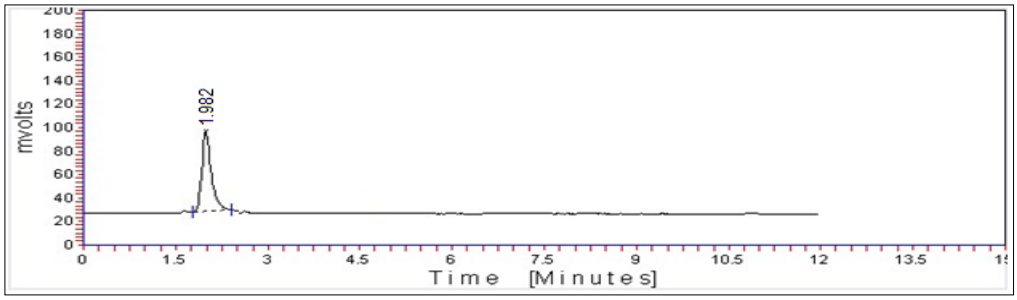


**Figure 4: Chromatogram of AMD**

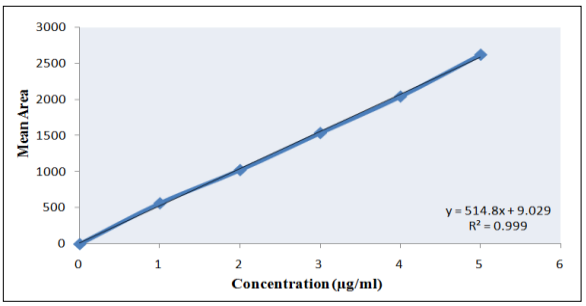


**Figure 5: Calibration Curve of ATV**

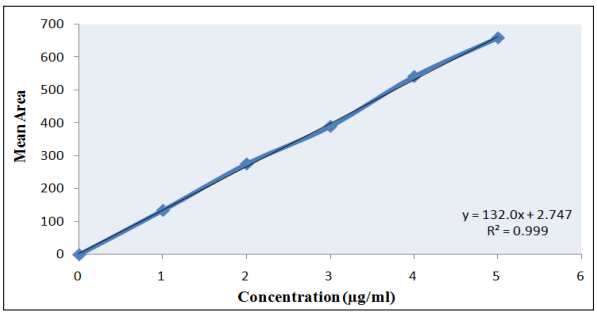


**Figure 6: Calibration Curve of AMD**

**Table 2: Results of linearity of AMD and ATV**

| Parameter | AMD | ATV |
|---|---|---|
| Concentration (µg/ml) | 1.5 | 5-25 |
| Correlation Coefficient ($r^2$)* | 0.999 | 0.999 |
| Slope (m)* | 132.0 | 514.8 |
| Intercept (c)* | 2.747 | 9.029 |

*value of six replicate

**Specificity**

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components Figure 7 & 8.

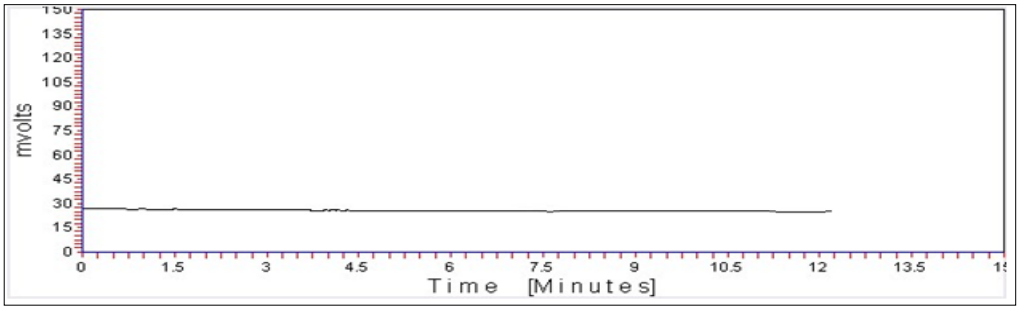


**Figure 7: Chromatogram of Blank**

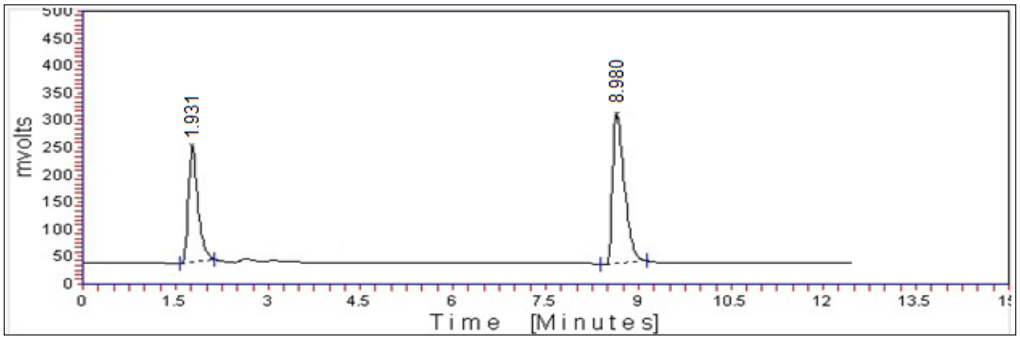


**Figure 8: Chromatogram of Both the drug**

**Accuracy**

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table 3.

**Table 3: Results of recovery study**

| % Level | % MEAN±SD* | |
|---|---|---|
| | AMD | ATV |
| 80% | 99.12±0.350 | 99.03±1.117 |
| 100% | 98.52±0.257 | 98.85±0.243 |
| 120% | 99.04±0.527 | 98.62±0.495 |

* Value of three replicate and three concentrations.

**Precision**

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less then 2 indicate the precision of method. Result of precision shown in table 4.

**Table 4: Results of precision**

| Parameter | % MEAN±SD* | |
|---|---|---|
| | AMD | ATV |
| Repeatability | 98.252±0.040 | 99.365±0.079 |
| Day to day precision | 97.688±0.061 | 99.708±0.046 |
| Analyst to Analyst | 98.497±0.024 | 99.474±0.075 |
| Robustness | 97.940±0.042 | 99.281±0.082 |

* Value of five replicate and five concentrations

**LOD and LOQ**

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

**Table 5: Results of LOD and LOQ**

| Name | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|
| AMD | 0.10 | 0.30 |
| ATV | 0.15 | 0.45 |

**Assay of Tablets formulation**

The results of the analysis of Tablets formulation were reported. The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipient in the estimation of drugs table 6.

**Table 6: Assay of capsule formulation**

| | AMD* | ATV* |
|---|---|---|
| Label Claim (mg) | **5mg** | **10mg** |
| % Found (mg) | 4.98 | 9.95 |
| % Assay | 99.6 | 99.50 |
| % RSD | 0.145 | 0.214 |

## CONCLUSION

The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and %RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.

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