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# Method Development And Validation For Simultaneous Estimation Of Amlodipine Besylate And Olmesartan In Bulk And Pharmaceutical Formulation By Rp-Hplc

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**ABSTRACT**

Objective: To develop and validate the RP-HPLC method for simultaneous estimation of amlodipine besylate and Olmesartan in bulk and pharmaceutical formulation. Materials and methods: Simultaneous estimation of amlodipine and Olmesartan were carried out by RP- HPLC using Acetonitrile: Phosphate buffer (pH 4.0) (58:42) and column Phenomenex Luna C-18 (150×4.6mm, 5µ) as a stationary phase and peak was observed at 235nm which was selected as a wavelength for quantitative estimation. Results: Method was developed and this method was validated as per ICH guidelines for specificity, linearity, precision, accuracy, robustness and ruggedness studies. **Conclusion:** All the validated parameters were within the limits. The method was found to be suitable for the estimation of amlodipine besylate and Olmesartan in bulk and pharmaceutical formulation.

**Keywords:** Amlodipine and Olmesartan Method development and validation;PDA Detection; RP- HPLC.ICH Guidelines.

**Abbreviations**

HPLC- High Performance Liquid Chromatography; $R^2$-Correlation coefficient; $R_t$-Retention time; LOD- Limit of detection; LOQ - Limit of quantification; RSD- Relative Standard deviation; AMLO-Amlodipine; OLMI – Olmesartan

## INTRODUCTION

Analytical chemistry is the science to analyze morphologies, compositions, and quantities of analytical targets. These analytical results have played critical roles from the understanding of basic science to a variety of practical applications, such as biomedical applications, environmental monitoring, quality control of industrial manufacturing, and forensic science.

Amlodipine Besylate[1] is a drug belongs to anti-hypertensive class used to treat hypertension by blocking the calcium channels. It has the structural formula and shown in Fig. 1. The chemical name of Amlodipine

Besylate is 2-[(2-aminoethoxy)-methyl]-4-(2- chlorophenyl)-1, 4-dihydro-6-methyl-3, pyridine dicarboxylic acid 3-ethyl 5-methyl ester benzene sulphonate. The molecular formula of Amlodipine Besylate is C20H25ClN2O5. C6H6O3S and it has the molecular weight of 567.1 g/mol. It is slightly soluble in water and in isopropyl alcohol, sparingly soluble in dehydrated alcohol, freely soluble in methyl alcohol.

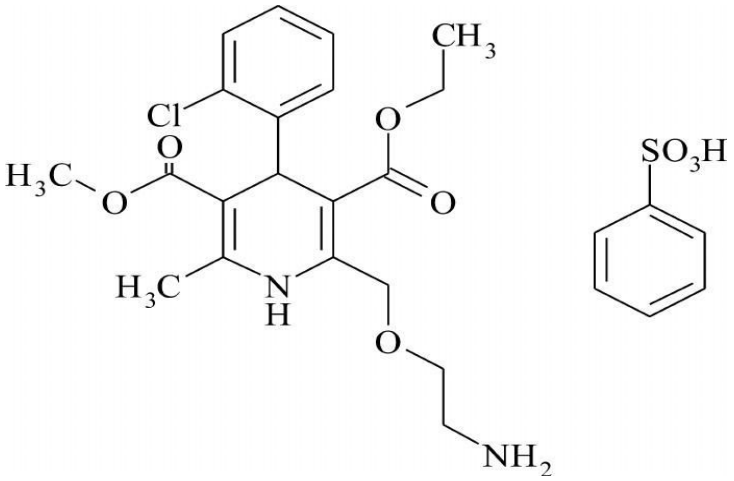


**Fig. 1: Chemical Structure of Amlodipine Besylate.**

Olmesartan is a drug belongs to anti-hypertensive class used to treat hypertension by blocking the angiotensin II receptors. It has the structural formula and shown in Fig. 2.

The chemical name Olmesartan 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]imidazole-4-carboxylic acid The molecular formula of Olmesartan is C33H30N4O2 and it has the molecular mass of 514.617 g/mol. It is soluble in methanol and in chloroform, insoluble in water.

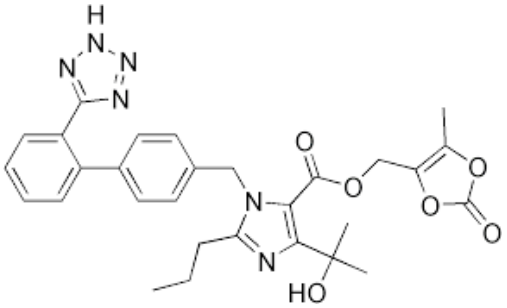


**Fig. 2: Chemical Structure of Olmesartan**

The two drugs which are mentioned above are official in Indian Pharmacopeia[3] and United States Pharmacopoeia.[4] From the literature survey, we found that Amlodipine Besylate and Olmesartan were estimated by different analytical methods like RP-HPLC[5-13], spectrophotometry[14-24] and HPTLC.[25] The availability of an HPLC method for simultaneous estimation of above mention cardiovascular drugs will be very much useful for the determination in bulk and pharmaceutical formulations. This study aimed to develop a simple, precise, accurate and validated Reversed-Phase HPLC method for the simultaneous estimation of Amlodipine Besylate and Olmesartan in bulk and pharmaceutical dosage form as per ICH guidelines.[26] The statistical analysis proved that method is reproducible and selective for the simultaneous analysis of Amlodipine Besylate and Olmesartan in bulk and formulations.

## MATERIALS AND METHODS

**Drug samples:** Amlodipine Besylate and Olmesartan were generously given by Lyka laboratories, Mumbai. Amlodipine (Assay: 99.80%) and Olmesartan (Assay: 99.65%) were used as standards.

**Tablets used:** Brand: AZOR; Amlodipine Besylate – 5mg and Olmesartan 40mg.

**Chemicals and solvents used:** All the solvents and reagents used were HPLC grade. Water, Acetonitrile, Disodium hydrogen phosphate, Methanol and Distilled water.

**Instruments used:** Shimadzu Isocratic HPLC system with following configurations, LC-10AT Vp series, Isocratic solvent delivery system (pump). Rheodyne 7725 injector with 20 µl loop. Spinchrome data station. Analytical column: Phenomenex – Luna, $C_{18}$ (150 x 4.6 mm i.d., 5µ). UV-Visible – SPD 10AVp series detector.

## PREPARATION OF MOBILE PHASE

**Preparation of Buffer:** 3.8954g of disodium hydrogen phosphate and 3.4023g of potassium dihydrogen phosphate in 1000 ml volume of distilled water and pH is adjusted to 4 with orthophosphoric acid.
**Mobile- phase:** 580 ml of acetonitrile and 420 ml of buffer were added in a beaker to give 1000ml. Finally the pH was adjusted to 4.0 by adding orthophosphoric acid.
**Mobile-Phase Ratio:** Buffer: Acetonitrile (42: 58% V/V).

### Preparation of Standard Stock Solution

An accurately weighed quantity of 5 mg of Amlodipine besylate and 40 mg of Olmesartan were transferred into a 100 ml volumetric flask. Dissolved with 25 ml of mobile phase and diluted to required volume with mobile phase, having the concentration of 0.4 mg/ml of Olmesartan and 0.05 mg/ml of Amlodipine besylate.

### Preparation of Standard Solution

From the standard stock solution 5 ml is pipetted out into 100 ml volumetric flask and made up the volume with mobile phase, having the concentration of 0.02 mg/ml of Olmesartan and 0.0025 mg/ml of Amlodipine besylate.

### Preparation of Sample Solution

Twenty tablets were weighed and ground to a fine powder. An amount of powder equivalent to 40 mg of Olmesartan and 5 mg of Amlodipine besylate were weighed accurately and transferred into a 100 ml volumetric flask containing 25 ml of mobile phase and sonicated for 30 min. and diluted to 100 ml with mobile phase, then the solution was filtered through 0.45 µm membrane filter and 5 ml of filtrate taken into 100 ml volumetric flask and made up to the volume with mobile phase.

The standard stock solution is diluted to the working concentration equivalent to that of sample. 20

µl of the standard and sample are injected separately and chromatograms are generated, with peak area obtained for standard and sample.

**Optimised Chromatographic Conditions**

After several trails with various solvents, Chromatographic separation was achieved by using Phenomenex Luna $C_{18}$ (150×4.6mm, 5µ) column as stationary phase and composition of Acetonitrile: pH 4.0 Phosphate buffer(58:42% v/v) as mobile phase. Flow rate was maintained at 1 ml/min at ambient temperature and the injection volume used was 20µl. The detection was carried out at 235 nm. Diluent was prepared by mixing 580 ml of Acetonitrile, 480 ml of Phosphate buffer filtered through Whattman filter paper (0.45µm) and degassed before use. Typical chromatogram of standard drug and sample is as shown in Fig. 3. and Fig.4.

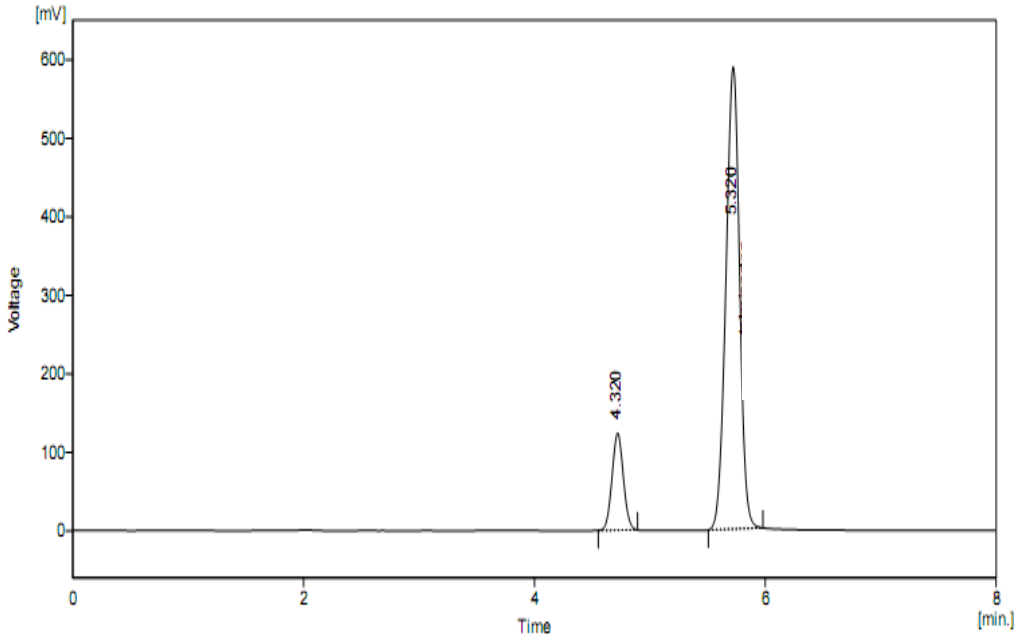


**Fig. 3: Typical Chromatogram of Standard drug.**

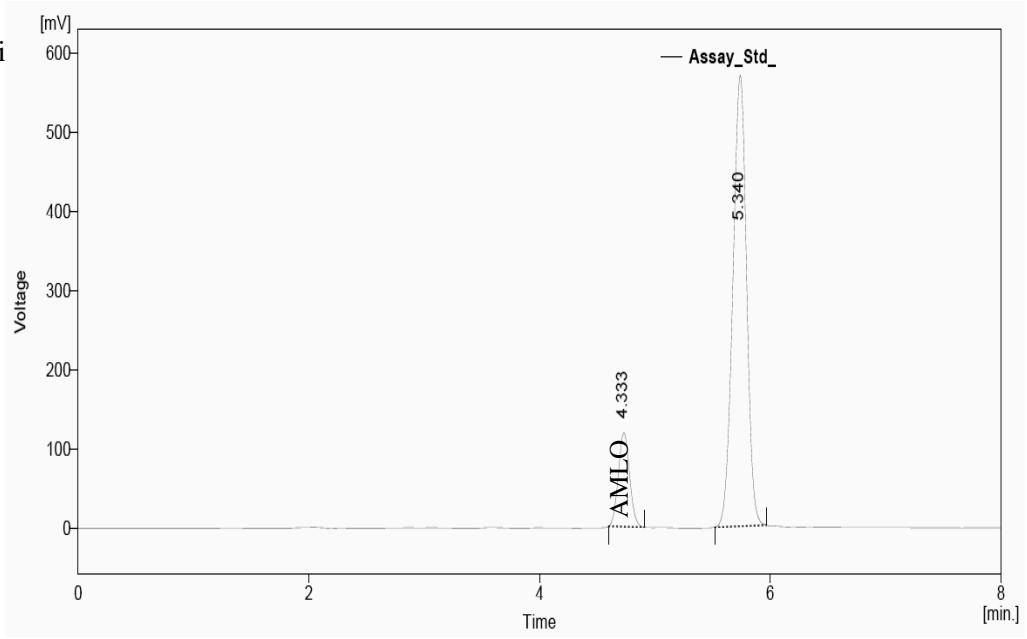


**Fig. 4: Typical Chromatogram of Sample.**

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**RESULTS AND DISCUSSION**

**Assay**

Estimation of Amlodipine Besylate and Olmesartan in tablet dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared. The chromatograms were recorded. The peak area ratio of standard and sample solutions was calculated. The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation. The tablet shows percentage purity values ranging from 99.71% for Amlodipine Besylate and 99.02% for Olmesartan respectively. Assay results were shown in table: 1.

**Table 1: Assay Results.**

| Inj. No | Area of Amlodipine Besylate | Area of Olmesartan | % of AMLO Recovered | % of OLMI Recovered |
|---|---|---|---|---|
| **1** | 808.439 | 4715.886 | 98.55 | 98.11 |
| **2** | 820.048 | 4793.930 | 99.96 | 99.73 |
| **3** | 825.462 | 4771.852 | 100.62 | 99.22 |
| **Mean** | 817.983 | 4760.556 | 99.71 | 99.02 |
| **S.D** | 8.697344 | 40.22954 | 1.05740 | 0.82831 |
| **%R.S.D** | 1.06326 | 0.84506 | 1.06048 | 0.83651 |

**System suitability**

System suitability studies were carried out in which the resolution between the peaks, tailing factor and number of theoretical plates was found and are presented in Table: 2.

**Table 2: System suitability Parameters.**

| System Suitability Parameters | Amlodipine Besylate | Olmesartan |
|---|---|---|
| Resolution | 3.746 | |
| Tailing Factor | 1.0402 | 0.918 |
| Number of theoretical Plates | 3594 | 3334 |
| Retention time | 4.348 | 5.342 |

**Linearity**

The Linearity for the both drugs, From the calibration curve constructed by plotting concentration vs. peak area, it was found that there exists a linear relationship in the concentration range 2.5 to 15 µg/ml and 20 to 120 µg/ml for Amlodipine Besylate
and Olmesartan with 0.999 and 0.999 as the value of correlation coefficient for the both drugs respectively. Linearity plots of Amlodipine and Olmesartan are shown in the Fig. 5. and Fig. 6.

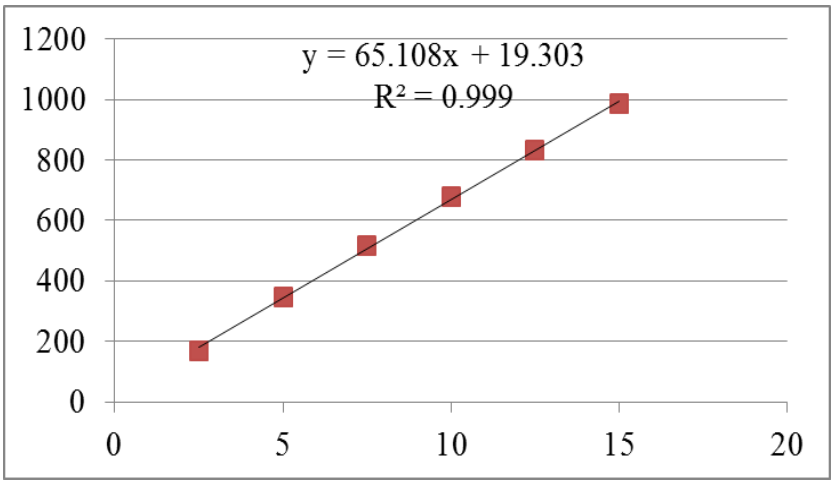


**Fig. 5: Linearity plot for Amlodipine Besylate.**

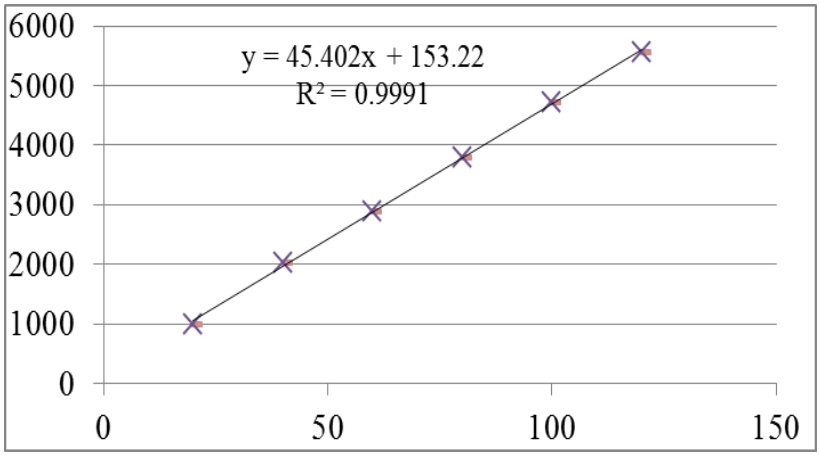


**Fig. 6: Linearity plot for Olmesartan**

**Precision**

For System Precision studies, the standard solution was prepared at working concentration and analysis was carried for five replicated injections. The percentage relative

standard deviation (% RSD) was calculated for the peak areas for Amlodipine and Olmesartan and it was found to be not more than 2.0%. The acceptance criterion of method precision was found to be RSD NMT 2.0% and the Method Precision for Amlodipine and Olmesartan shows 0.9949 and 0.9619.

**Accuracy**

For the Accuracy of the method was determined by performing recovery studies at 80%, 100%, 120%. The recovery study was carried out and results were expressed in terms of the percentage recovery range found to be within the limit. The mean average recovery of amlodipine besylate and Olmesartan for accuracy was shown in table: 3.

**Table: 3 Mean Average Recovery of Amlodipine Besylate and Olmesartan for Accuracy.**
**of**

| Accuracy level | Mean Recovery of Amlodipine Besylate | Mean Recovery Olmesartan |
|---|---|---|
| Accuracy 80% | 99.76 | 99.6 |
| Accuracy 100% | 99.77 | 99.99 |
| Accuracy 120% | 99.70 | 99.89 |

**LOD and LOQ**

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) Of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The detection limit (LOD)
was found to be 0.04791 µg/ml for Amlodipine Besylate and 0.4496 µg/ml for

Olmesartan respectively.

The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The quantitation limit (LOQ) was found to be 0.1452 µg/ml for Amlodipine Besylate and 1.3625 µg/ml for Olmesartan respectively.

**Robustness**

For demonstrating the robustness of the developed method experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes in chromatographic conditions and allow routine analysis of the sample. Effect of column temperature, and Effect of buffer pH were carried out and standard was injected. There was no change in system suitability parameters.

**Ruggedness**

Defined by USP, The Ruggedness is the degree of reproducibility of test results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory and from analyst to analyst.

**Stability Studies**

Stability of the sample and standard used in HPLC method is required for a reasonable time to generate reproducible and reliable results. The stability of the sample spiked with drug was subjected to short term stability at room temperature (Initial & after 8 hours). Solution stability after 8 hours was presented in table: 4.

**Table. 4: Solution Stability after 8 hours.**

| Time | Area of Amlodipine Besylate | Area of Olmesartan |
|---|---|---|
| Initial | 845.034 | 4826.725 |
| After 8 hours | 846.390 | 4829.735 |
| Deviation | 1.356 | 3.010 |

The values obtained were satisfactory and in accordance with guideline limits. The values were show in table 5**.**

**Table: 5: Results From Evaluation of The Suitability of The Method.**

| Parameter | Amlodipine | Olmesartan | Limits |
|---|---|---|---|
| Accuracy | 99.70-99.77 | 99.60-99.99 | % Recovery range 98 –102 % |
| System precision | 0.7703 | 0.9619 | RSD NMT 2.0% |
| Method precision | 0.9949 | 0 .9619 | RSD NMT 2.0% |
| Linearity range | 2.5-15µg/ml | 20-120µg/ml | R<1 |
| Correlation Coefficient | 0.999 | 0.999 | |
| Slope (m) | 65.10 | 45.402 | |
| Intercept | 10.34 | 82.08 | |
| Regression equation (Y = mx +c) | y =65.108x+19.30 | y=45.402x+153.22 | |
| Specificity | Specific | Specific | No interference |
| Limit of Detection | 0.04791 | 0.4496 | NMT 3 |
| Asymmetry factor | 1.85 | 1.06 | NMT 2% |
| Number of Theoretical Plates | 3594 | 3334 | NLT 2000 |
| Assay | 99.71 | 99.02 | 90-110% |

**CONCLUSION**

A simple, accurate, precise, selective and sensitive RP- HPLC assay method with DAD detection for simultaneous estimation of Amlodipine Besylate and Olmesartan in pharmaceutical dosage form has been developed and validated. The method will be extensively used for the simultaneous estimation of Amlodipine Besylate and Olmesartan in bulk and pharmaceutical formulation.

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