# RP-HPLC Method Development and Validation for the Simultaneous Estimation of Olmesartan and Clinidipine in Bulk and Pharmaceutical Dosage Form

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**ABSTRACT**

The main aim of the present work is to develop a rapid, simple, precise, speedy, accurate and validated chromatography method for the estimation of Olmesartan and Clinidipine quantitatively in fixed dosage form. Effective Chromatographic separation was achieved by using Hypersil $C_{18}$ Column (250 mm X 4.6 mm internal diameter, 5 µm particle size) using mobile phase composed of Methanol, Acetonitrile and Buffer (pH 4.5) in the proportion of 40: 40:20 (v/v) under controlled temperature. The Mobile phase was siphoned using a gradient HPLC system at a flow rate of 1.0 ml/min and quantification was based on peak area measurements at 274 nm. RT (Retention Time) for Olmesartan and Clinidipine was found to be 2.326 min and 4.344 min. The dimensionality of both the drugs found to be linear with a statistic value of 0.999. The acceptance criteria of precision was Relative variance should be less than 2.0% which shows that the method can be performed repeatedly. Reliability of the proposed method was assessed by evaluation of validation parameters like linearity, precision, specificity, accuracy, LOD, LOQ values as per ICH guidelines. The proposed chromatography method has been applied to formulation without additives interference and specific for the estimation of Olmesartan and Clinidipine.

**Key Words:** Olmesartan, Clinidipine, Validation, Accuracy, Precision

# INTRODUCTION

Hypertension or high blood pressure is a chronic condition in which the blood's force against the artery wall is high[1]. Olmesartan is in a class of medications called angiotensin II receptor antagonists. It works by blocking the action of certain natural substances that tighten the blood vessels, allowing the blood to flow more smoothly and the heart to pump more efficiently. High blood pressure is a common condition and when not treated, can cause damage to the brain, heart, blood vessels, kidneys and other parts of the body. Damage to these organs may cause heart disease, a heart attack, heart failure, stroke, kidney failure, loss of vision, and other problems. In addition to taking medication, making lifestyle changes will also help to control your blood pressure[2]. These changes include eating a diet that is low in fat and salt, maintaining a healthy weight, exercising at least 30 minutes most days, not smoking, and using alcohol in moderation. The chemical structure of Olmesartan is exhibited in figure 1.

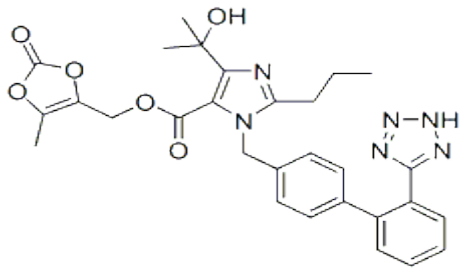


**Figure 1: Structure of Olmesartan**

Clinidipine is used in the treatment of Hypertension (high blood pressure), Angina (heart-related chest pain), Heart attack and Stroke. Clinidipine is a calcium channel blocker. It lowers blood pressure by relaxing blood vessels, which makes the heart more efficient at pumping blood throughout the body[3]. Clinidipine and Olmesartan is a combination of blood pressure-lowering medicine, and novel calcium channel blockers primarily used to treat hypertension[4] (high blood pressure).The chemical structure of Clinidipine is exhibited in figure 2.

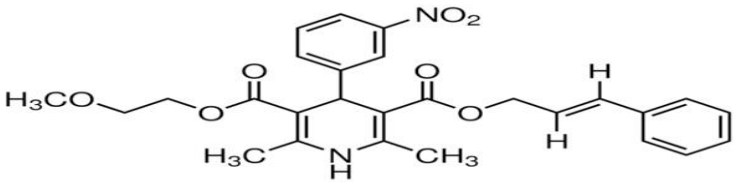


**Figure 2: Structure of Clinidipine**

The present strategy mainly focused on developing and validating a novel reversed-phase HPLC method for the estimation of Clinidipine and Olmesartan in bulk and pharmaceutical products. After performing extensive literature Survey[5-11] on chromatographic analysis of several dosage forms an attempt was made to develop a new novel, valid, speedy and accurate method for the estimation of Olmesartan and Clinidipine.

## MATERIALS AND METHODS

### Chemicals and Reagents

Olmesartan and Clinidipine were kindly gifted by Nutech Biosciences Pvt Ltd, Hyderabad having certified purity limits and were used without any chemical treatment. For separation of drugs solvents of HPLC Grade was used in analysis. Nexovas-O Tablets of Macleods Pharmaceuticals Pvt Ltd was utilized for analysis.

### Instrument

Liquid Chromatography system used consists of Waters HPLC having Empower Software with 2695 separation module having PDA detector with universal loop injector of injection capacity 20 µL. The column used was Agilent $C_{18}$ Column, 5µ (250× 4.6 mm) at surrounding temperature. Several mobile phases were tested in order to search out the suitable conditions for separating the drugs.

### Optimized Chromatographic conditions

The mobile phase having Methanol, Acetonitrile and Phosphate buffer having pH 4.5 in proportion of 40:40:20 by volume was preferred because it ideally resolves the height with Retention Time (RT) of 2.326 min and 4.344 min for respectively. Standard drug were scanned over a large range of wavelength ranging from 200 nm to 390 nm and wavelength was selected at 274 nm because of showing reasonably good response with characteristic UV Spectrum exhibited in Figure 3

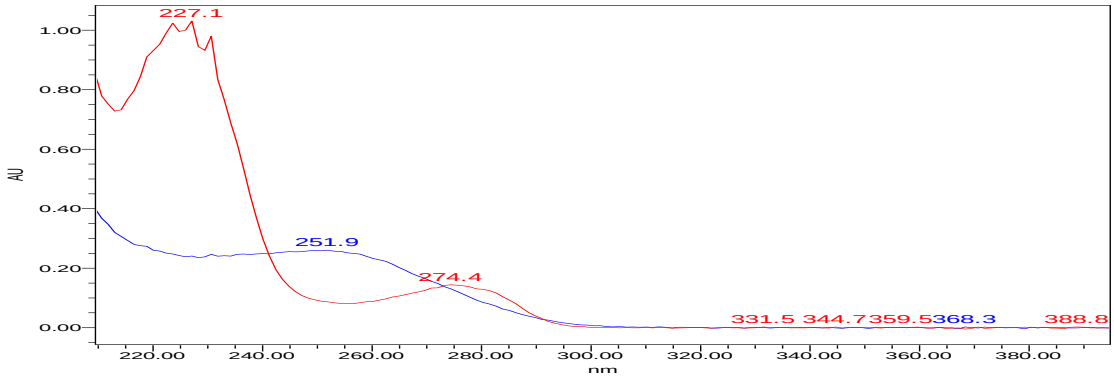


**Figure 3: Overlain UV spectrum of Olmesartan and Clinidipine**

### Preparation of Buffer

Accurately weighed and transferred 3.464 g of Di Potassium hydrogen Phosphate into 1000 ml clean dry volumetric flask. To this add 500 ml of HPLC water and sonicated for five minutes to dissolve it completely and make up the volume to mark with HPLC water and pH was adjusted to 4.5 by addition of few drops of Orthophosphoric acid.

### Preparation of Mobile Phase

Accurately measured 400 milliliter of Methanol (40%), 400 milliliter of Acetonitrile (40%) and 200 milliliter of phosphate buffer (20%) were mixed and kept for sonication in inaudible water tub for 10 minutes and after sonication filter the above solution using 0.45 µ membrane filter under vacuum prior its use and used as diluent

**Standard Stock Preparation**

Accurately weighed and transferred 10 mg of Clinidipine and 15 mg of Olmesartan working standard into 100 milliliter clean dry volumetric flask and add 70 ml of diluent and degassed for five min to dissolve Olmesartan and Clinidipine standards completely and make volume up to the mark with the identical solvent which gives 100 µg/ml of Clinidipine and 150 µg/ml of Olmesartan (Stock solution). From the above solutions 4.0 ml was pipette out into a ten milliliter volumetric flasks and then make up to the mark with diluent. The Chromatogram was exhibited in Figure 4.

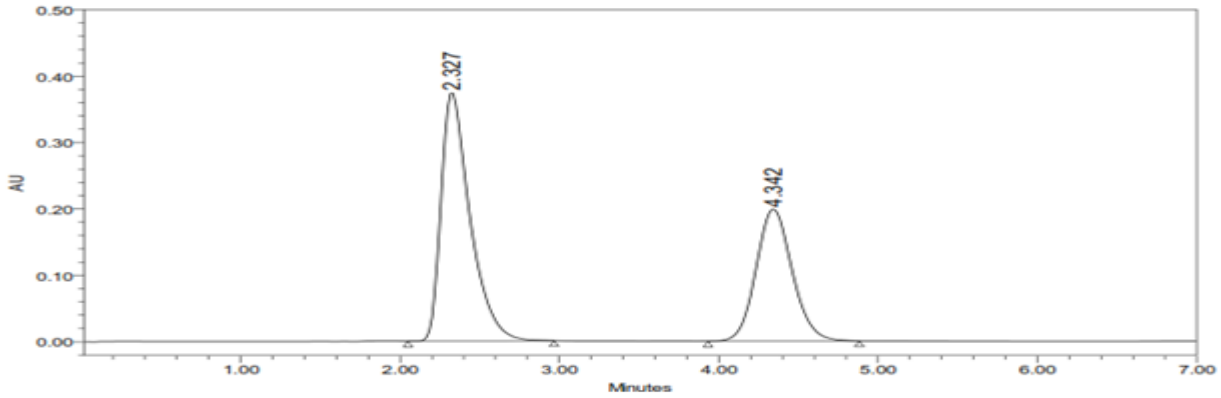


**Figure 4: Standard Solution Chromatogram of Olmesartan and Clinidipine**

**Sample Preparation**

A portion of powder equivalent to the weight of 10 mg of Clinidipine and 15 mg of Olmesartan was accurately weighed and transferred into 100 milliliter volumetric flask and diluent of 70 ml was added and degassed for five min to dissolve both the drugs completely and make up to the mark with identical solvent and then solution was filtered through a 0.45 µ membrane filter. From the above filtered solutions 4.0 ml was pipette out into a 10 milliliter volumetric flasks and then make up to the final volume with diluent. In similar manner sample was further diluted to get required concentration The Chromatogram was exhibited in Figure 5.

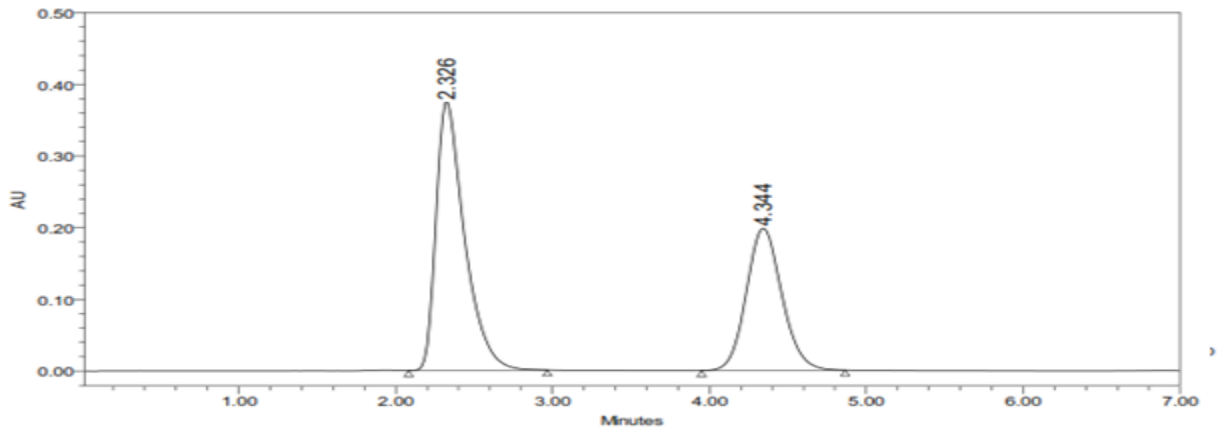


**Figure 5 : Sample solution Chromatogram of Olmesartan and Clinidipine**

## RESULTS AND DISCUSSION

### Preparation of Calibration Curves by HPLC

Serial dilutions of Clinidipine ranging from 18.75 to 112.5 µg/ml and Olmesartan ranging from 37.5 to 225 µg/ml were made and their chromatograms were recorded. Height space of drugs was calculated and also the individual activity curve was planned against quantitative of area underneath curve and their respective concentrations and results are reported in Table 1.

| Olmesartan | | Clinidipine | |
|---|---|---|---|
| **Conc (µg/mL)** | **Peak area** | **Conc (µg/mL)** | **Peak area** |
| 0 | 0 | 0 | 0 |
| 37.5 | 1097927 | 18.75 | 445769 |
| 75 | 2021264 | 37.5 | 966834 |
| 112.5 | 2970989 | 56.25 | 1414856 |
| 150 | 4008329 | 75 | 1913772 |
| 187.5 | 5135671 | 93.75 | 2348134 |
| 225 | 6087078 | 112.5 | 2776291 |

**Table 1: Linearity Data of Olmesartan and Clinidipine**

### HPLC Method Validation

The Developed method was validated for Linearity, Accuracy, Specificity, Precision, LOD, LOQ parameters as described in ICH Guidelines.

### Linearity and Range

One-dimensionality in a strategy is its ability to induce to take a glance at results and was constructed using the mean areas at their respective concentrations over a given range. Linearity for Clinidipine was within the range of 18.75 to 112.5µg/ml and Olmesartan was in the range of 37.5 to 225 µg/ml respectively. The coefficient of correlation value for calibration plot of Olmesartan and Clinidipine was 0.999 which shows good linearity for the drugs. The Linearity curves were exhibited in figure 6 and 7. Linearity results are tabulated in Table 1

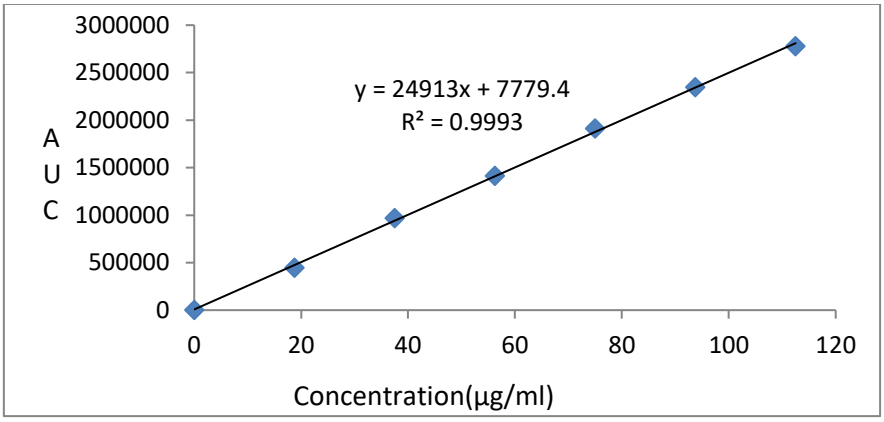


**Figure 6: Linearity Curve of Clinidipine**

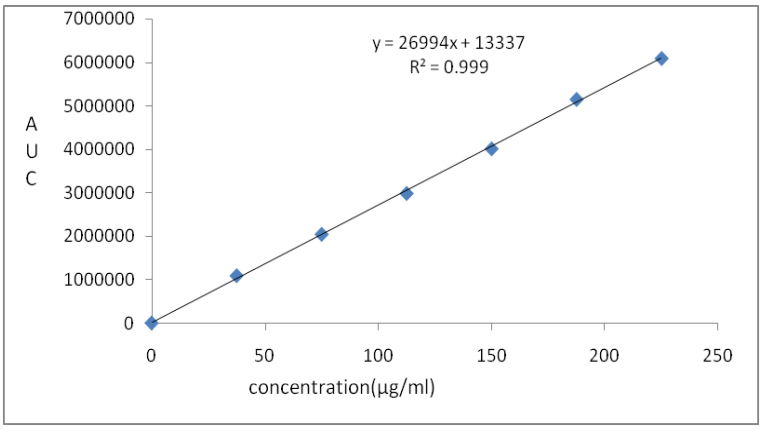


**Figure 7: Linearity Curve of Olmesartan**

**Accuracy**

The Certainty of an approach is that the intimacy of the measured worth to actuality worth for the sample. The mean recovery was found to be 99.85% for Olmesartan and 99.42% for Clinidipine respectively. The %RSD of the sample was found to be below 2 and results were tabulated in Table 2 and Table 3

**Table 2: Accuracy Report of Olmesartan**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | **Mean %Recovery** |
|---|---|---|---|---|
| 50% | 75 | 75.09 | 100.12 | 99.85% |
| | 75 | 74.03 | 98.70 | |
| | 75 | 75.46 | 100.62 | |
| 100% | 150 | 150.30 | 100.20 | |
| | 150 | 150.35 | 100.23 | |
| | 150 | 150.52 | 100.35 | |
| 150% | 225 | 224.68 | 99.86 | |
| | 225 | 223.21 | 99.21 | |
| | 225 | 223.59 | 99.38 | |

**Table 3: Accuracy Report of Clinidipine**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 37.5 | 36.94 | 98.51 | 99.42% |
| | 37.5 | 36.98 | 98.61 | |
| | 37.5 | 36.90 | 98.40 | |
| 100% | 75 | 74.16 | 98.87 | |
| | 75 | 73.67 | 98.22 | |

| | | | |
|---|---|---|---|
| | 75 | 74.69 | 99.58 |
| 150% | 112.5 | 113.47 | 100.86 |
| | 112.5 | 113.39 | 100.79 |
| | 112.5 | 113.53 | 100.91 |

**Precision**

Precision of the strategy was evaluated by performing repeatability in the same day and inter-day studies. The Percentage Relative Standard Deviation of each study was calculated and was found to be less than 2 showing the strategy was precise and the results were shown in Table 4.

| S. No | Area of Olmesartan | Area of Clinidipine |
|---|---|---|
| 1. | 4030687 | 1933598 |
| 2. | 4013852 | 1929068 |
| 3. | 4021562 | 1934776 |
| 4. | 4033893 | 1931245 |
| 5. | 4034091 | 1924732 |
| 6. | 4015898 | 1925861 |
| Mean | 4024982 | 1929898 |
| S.D | 9060.2 | 4065.6 |
| %RSD | 0.2 | 0.2 |

**Table 4: Precision Report of Olmesartan and Clinidipine**

**LOD & LOQ**

Limit of Detection, Limit of Quantitation values of the method were 0.03 and 0.08 for Clinidipine, 0.22 and 0.71 for Olmesartan respectively. The results obtained are within the limits.

**Robustness and Ruggedness**

Robustness and Ruggedness studies were carried out by injecting five replicate injections of Olmesartan and Clinidipine on different days and variations was calculated in terms of percentage relative variance which was found to be less than 2% and results were reported in Table 5 and 6.

| S.No | Flow Rate (ml/min) | System Suitability Parameters | |
|---|---|---|---|
| | | Plate Count | Tailing Factor |
| I | 0.8 | 4512 | 1.7 |
| II | 1.0 | 4339 | 1.6 |
| III | 1.2 | 4212 | 1.6 |

**Table 5: Results of Robustness**

| S. No | Mobile phase composition change | Results | |
|---|---|---|---|
| | | Plate Count | Tailing factor |
| I | <5% | 4628 | 1.6 |
| II | *Actual | 4546 | 1.6 |
| III | >5% | 4878 | 1.6 |

**Table 6: Effect of change in mobile phase Composition**

**Recovery studies**

Standard addition method is performed at 50,100,150 % levels and interference of formulation additives was tested. The Recovery was calculated based on amount of Drug found and results are reported in Table 7 and Table 8.

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 4030625 | 4025748 | 99.82 |
| 2 | 4013874 | 4027767 | 99.87 |
| 3 | 4021592 | 4018426 | 99.64 |
| 4 | 4033815 | 4029788 | 99.92 |
| 5 | 4034095 | 4025517 | 99.81 |
| 6 | 4015891 | 4022579 | 99.74 |
| Avg | 4024982 | 4024971 | 99.80 |
| Stdev | 9060.2 | 4012.0 | 0.099 |
| %RSD | 0.3 | 0.2 | 0.2 |

**Table 7: Recovery Data of Olmesartan**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 1933592 | 1918227 | 99.20 |
| 2 | 1929075 | 1915135 | 99.04 |
| 3 | 1934777 | 1910180 | 98.78 |
| 4 | 1931264 | 1929603 | 99.79 |
| 5 | 1924751 | 1919061 | 99.24 |
| 6 | 1925878 | 1921673 | 99.38 |
| Avg | 1929890 | 1918980 | 99.24 |
| Stdev | 4065.6 | 6525.3 | 0.3374 |
| %RSD | 0.2 | 0.3 | 0.3 |

**Table 8: Recovery Data of Clinidipine**

## CONCLUSION

A systematic and practical approach was utilized to develop an efficient and robust RP-HPLC method for the separation of drugs. Different trials were carried out to determine the optimized chromatographic conditions and initial attempt was performed by utilizing low proportion of organic solvents for the elution of compounds by reducing retention time of the

compounds. The acceptable results are achieved by the proposed chromatographic conditions. The proposed method is easy, speedy and measurably substantial. During the analysis of drug no interfering peak was found within the chromatogram indicating that there is no excipient interference.

**Conflict of Interest**

There is no Conflict of Interest among the authors regarding the publication of this paper

**ACKNOWLEDGEMENT**

The authors are thankful to Seven Hills College of Pharmacy (Autonomous) and Nutech Biosciences, Hyderabad for providing financial assistance under R&D Funds and providing necessary Infrastructure to carry out the research work.

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