Development of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Amlodipine and Olmesartan in Pure and Pharmaceutical Dosage Form

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Received: 20th Jun, 18; Revised: 4th Jan, 19, Accepted: 15th Feb, 19; Available Online: 25th Mar, 2019

ABSTRACT
A simple reverse phase HPLC method was developed for the simultaneous estimation of Amlodipine and Olmesartan in bulk and tablet form. Chromatography was performed by isocratic reverse phase separation on a stainless steel column 4.6 x 150mm, symmetry column packed with octa decyl silane bonded to porous silica (C18) with particle size 5 micron with mobile phase containing TEA Buffer of pH 3.0 and Acetonitrile in proportion of 25:75 respectively. The flow rate was 1.0 ml/min and effluent was monitored at 258 nm. The retention times were 2.39 min and 3.33 min respectively. The standard curve was linear over a working range of 0.5–35 µg/ml for both Amlodipine and Olmesartan and gave an average correlation coefficient of 0.999, and 0.999 for Amlodipine and Olmesartan respectively. The limit of quantitation (LOQ) of this method was 2µg/ml for Amlodipine and Olmesartan. The absolute recovery was 100% for Amlodipine and 100.3 for Olmesartan. Degradation products produced as a result of stress studies did not interfere with the detection of Amlodipine and Olmesartan and the assay can thus be considered stability-indicating.

Keywords: Amlodipine, Olmesartan, RP-HPLC, TEA Buffer: Acetonitrile, Validation.

INTRODUCTION
Amlodipine is a long-acting 1,4-dihydropyridine calcium channel blocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, amlodipine prevents calcium-dependent myocyte contraction and vasoconstriction. A second proposed mechanism for the drug’s vasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition of smooth muscle carbonic anhydrase. Some studies have shown that amlodipine also exerts inhibitory effects on voltage-gated N-type calcium channels. N-type calcium channels located in the central nervous system may be involved in nociceptive signaling and pain sensation. Amlodipine is used to treat hypertension and chronic stable angina¹. Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels. Calcium ions entering the cell through these channels bind to calmodulin. Calcium-bound calmodulin then binds to and activates myosin light chain kinase (MLCK). Activated MLCK catalyzes the phosphorylation of the regulatory light chain subunit of myosin, a key step in muscle contraction. Amlodipine is slowly and almost completely absorbed from the gastrointestinal tract. Peak plasma concentrations are reached 6-12 hour following oral administration. Its estimated bioavailability is 64-90%. Absorption is not affected by food. It is metabolized extensively (90%) to inactive metabolites via the cytochrome P450 3A4 isozyme². Olmesartan is an antihypertensive agent, which belongs to the class of medications called angiotensin II receptor blockers. It is indicated for the treatment of high blood pressure and is marketed under the name Olmetec®. The FDA label includes a black-box warning of injury and death to the fetus, so women of child-bearing age need to be warned and take the necessary precautions. Olmesartan is also contraindicated in diabetes mellitus patients taking aliskiren. Olmesartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Olmesartan is selective for AT1 and has a 12,500 times greater affinity for AT1 than the AT2 receptor. Also unlike the well-known ARB losartan, olmesartan does not have an active metabolite or possess uricosuric effects

MATERIALS AND METHODS:
Drugs
Pure pharmaceutical sample of AML and OLM was obtained from Yucca Pharma. Commercial tablet of amlodipine besilate (5mg), olmesartan

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Chemicals
Sodium dihydrogen phosphate (AR Grade), 85% Orthophosphoric acid (AR Grade), Acetonitrile (HPLC Grade), Hydrochloric Acid (AR Grade), Triethyl-Amine (AR Grade), Sodium Hydroxide (AR Grade) were purchased from Sd fine-Chem limited.

Instrument
Liquid chromatographic system from Waters alliance 2695 with Waters UV detector equipped with Empower software was used.

Preparation of mobile phase
Mobile phase was prepared by dissolving Buffer of pH 3 in Acetonitrile in the ratio of 25:75. The Mobile phase was filtered through 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

Preparation of TEA buffer (pH-3)
1.5 ml of Triethyl amine dissolved in 250 ml of HPLC Water. Adjusted pH 3.00 with ortho phosphoric acid.

Diluent preparation
The Mobile phase was used as the diluent

Stock solutions and standards
A stock solution of drugs were prepared by transferring accurately weighed 25 mg of AML and OLM in two separate 25 ml volumetric flask and dissolved in 15 ml of mobile phase. The solutions were sonicated and the volumes were made up to mark with mobile phase to get concentration of 1000μg/ml of AML and OLM.

| S.No. | Peak Name | Rt | Area   | Height | USP Resolution | USP Tailing | USP plate count |
|-------|-----------|----|--------|--------|----------------|-------------|-----------------|
| 1     | Amlodipine| 2.316 | 1232142 | 194123 | 3.6            | 1.2         | 4651            |
|       | Olmesartan| 3.304 | 1491465 | 176582 | 5.6            | 1.5         | 3982            |

Figure 1: Amlodipine

Figure 2: Olmesartan

Figure 3: chromatogram of Amlodipine (Rt-2.395min).

Figure 4: chromatogram of Olmesartan (Rt-3.339min).
Preparation of Sub Stock Solution

1 ml was pipetted from Amlodipine stock solution and 4 ml from Olmesartan stock solution and transferred in 100 ml volumetric flask separately. The volume was made up to the mark with mobile phase it gives final concentration of 10 μg/ml and 40 μg/ml solution of AML and OLM respectively.

Preparation of Sample Solution

Accurately weighed ten tablets were taken and crushed in mortar and pestle. 100 mg equivalent weight of powdered

Table 1: System suitability parameters.

| Instrument used                           | Waters HPLC with auto sampler and UV detector |
|-------------------------------------------|-----------------------------------------------|
| Temperature                               | Ambient                                       |
| Column                                    | Symmetry C18 (4.6mm x 150mm, 5μm, Make: Waters) |
| Buffer                                    | 1.5ml of Triethyl amine dissolve in 250ml of HPLCwater. Adjust pH-3.00 with orthophosphoric acid. |
| pH                                        | 3                                             |
| Mobile phase                              | TEA Buffer (pH-3.00), Acetonitrile in proportion of 25:75 |
| Flow rate                                 | 1 ml per min                                   |
| Wavelength                                | 258 nm                                        |
| Injection volume                          | 20 μl                                         |
| Run time                                  | 6 min                                         |

Figure 5: chromatogram of Amlodipine (Rt-2.395min) and Olmesartan (Rt-3.339min).

| S.No. | Peak Name    | R<sub>t</sub> | Area   | Height  | USP Resolution | USP Tailing | USP plate count |
|-------|--------------|---------------|--------|---------|----------------|-------------|-----------------|
| 1     | Amlodipine   | 2.395         | 1242388| 197332  | 1.1            | 4741        |
| 2     | Olmesartan   | 3.339         | 1494848| 177825  | 5.2            | 1.2         | 3793            |

Figure 6: Chromatogram showing degradation for Amlodipine and Olmesartan in 0.1 N HCl

| S.No. | Peak Name    | R<sub>t</sub> | Area   | Height  | USP Tailing | USP plate count |
|-------|--------------|---------------|--------|---------|-------------|-----------------|
| 1     | Amlodipine   | 2.210         | 1113179| 198754  | 1.2         | 4854           |
| 2     | Olmesartan   | 3.138         | 1339383| 176582  | 1.3         | 3872           |
Table 3: Linearity results: (for Amlodipine).

| Concentration of AML in ppm | Peak area of Amlodipine |
|-----------------------------|-------------------------|
| 0                           | 0                       |
| 5                           | 224748                  |
| 10                          | 475848                  |
| 15                          | 692648                  |
| 20                          | 944621                  |
| 25                          | 1180741                 |
| 30                          | 1390935                 |
| 35                          | 1598929                 |

Table 2: Results of forced degradation studies of Amlodipine and Olmesartan API.

| Stress condition          | Time   | Assay of degraded products | Assay of active substance | Mass Balance (%) |
|---------------------------|--------|----------------------------|----------------------------|------------------|
| Acid Hydrolysis (0.1 M HCl)| 24Hrs. | 10.4                       | 89.6                       | 100              |
| Basic Hydrolysis (0.1 M NaOH)| 24Hrs. | 7.18                      | 92.82                     | 100              |
| Thermal Degradation (50 °C)| 24Hrs. | 4.92                      | 95.08                     | 100              |
| UV (254nm)                | 24Hrs. | 2.44                      | 97.56                     | 100              |
| 3 % Hydrogen peroxide     | 24Hrs. | 9.78                      | 90.22                     | 100              |

Table 3: Linearity results: (for Olmesartan).

| Concentration of OLM in ppm | Peak area of Olmesartan |
|-----------------------------|-------------------------|
| 0                           | 0                       |
| 5                           | 1234613                 |
| 10                          | 2472924                 |
| 15                          | 3570426                 |
| 20                          | 4853049                 |
| 25                          | 6053925                 |
| 30                          | 6990601                 |
| 35                          | 7817235                 |

Table 4: Linearity results: (for Olmesartan).

| Concentration of OLM in ppm | Peak area of Olmesartan |
|-----------------------------|-------------------------|
| 0                           | 0                       |
| 5                           | 1234613                 |
| 10                          | 2472924                 |
| 15                          | 3570426                 |
| 20                          | 4853049                 |
| 25                          | 6053925                 |
| 30                          | 6990601                 |
| 35                          | 7817235                 |

Stability Study

Drug containing OLM and AML (marketed formulation-dose of OLM is 20 mg, dose of AML is 5 mg in combination tablet) were transferred to 250 mL round bottomed flask and treated under acidic, alkaline, oxidizing, thermal and photolytic stress conditions. When degradation was complete, the solution were left to equilibrate to room temperature and diluted with diluents to furnish solutions of concentration equivalent to 40 μg/ml OLM and 10 μg/ml AML. The specific conditions are described below. In acidic degradation drug was heated under reflux with 1M hydrochloric acid for 30 min at 80 °C and the drug was treated with 0.1N NaOH at room temperature for 2 h in alkaline degradation. Then resulting solution was neutralized. The drug was treated with 2% (v/v) H2O2 at room temperature for 2 h in oxidative degradation. Thermal degradation was performed by exposing the solid drug to dry heat in a convection oven at 70 °C for 72 h and photolytic degradation was performed by exposing the drug to sunlight for 72 h.

Apparatus and Chromatographic conditions

Quantitative HPLC was performed on Waters HPLC system with UV detector. Empower software is used along with a stainless steel column 4.6 x 150mm, packed with Octa decyl silane bonded to porous silica (C18) with particle size 5 micron. To develop a suitable and robust HPLC method for the determination of OLM and AML, different mobile phases containing TEA buffer and Acetonitrile were used in different compositions like (30:70, 40:60, 50:50, 70:30, 80:20) at different flow rates (0.5, 0.75, 1.0, 1.2, 1.5 ml/min). The mobile phase TEA buffer and Acetonitrile with a flow rate of 1.0 ml/min gave peaks of good resolution and were eluted at retention times...
around 2.39 min, 3.33 min with symmetric peak shape. The detection is performed at the wavelength 258 nm.  

Running the standard solution of Amlodipine 1 ml of stock solution (1000ppm) was pipetted out into a 100 ml volumetric flask. The volume was made up to the mark with mobile phase. The solution was filtered through the 0.45 μm membrane filter and degassed under.
ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure 3.

**Running the standard solution of Olmesartan**

4 ml of stock solution was pipetted into a 100 ml volumetric flask. The volume was made up to the mark with mobile phase. The solution was filtered through the 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure 4.

**Running the standard solution of Amlodipine and Olmesartan**

1 ml of AML stock solution and 4 ml OLM stock solution was pipetted into a 100 ml volumetric flask. The volume was made up to the mark with mobile phase. The solution was filtered through the 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure 5.

**RESULTS AND DISCUSSION**

**Method development and optimization**

The main target of the chromatographic method is to get the separation of closely eluting drugs Amlodipine and Olmesartan. The drugs were co-eluted by using different stationary phases like C18, C8 with varying lengths and different mobile phases containing buffers like phosphate, sulphate and acetate with different pH (2-7) and using organic modifiers like acetonitrile, methanol and ethanol in the mobile phase. pH of the buffer has played a significant role in achieving the separation between drugs.

The chromatographic separation was achieved on a stainless steel column (4.6 x 250mm) column packed with Octa decyl silane bonded to porous silica (C18) with particle size 5 micron, by using solutions TEA Buffer and Acetonitrile in the ratio of (25:75), pH adjusted to 3 using ortho phosphoric acid. The flow rate of the mobile phase was maintained at 1.0 ml/min. At 25°C of column temperature, the peak shape of AML AND OLM was found symmetrical with mobile phase 60:40 ratio. In the optimized conditions AML AND OLM were well separated with a good resolution and the typical retention times of AML AND OLM were about 2.3 min and 3.3 min, respectively. The system suitability results are given in the table no.1 and the developed LC method was validated.

### Table 5: Results of method precession for Amlodipine.

| S. No. | Peak name  | Rt    | Area (µV*sec) | USP Plate Count | USP Tailing |
|-------|------------|-------|---------------|-----------------|-------------|
| 1     | Amlodipine | 2.234 | 1010585       | 1.0             | 3802        |
| 2     | Amlodipine | 2.261 | 1011075       | 1.1             | 3546        |
| 3     | Amlodipine | 2.183 | 1011924       | 1.4             | 4633        |
| 4     | Amlodipine | 2.244 | 1014299       | 1.1             | 4812        |
| 5     | Amlodipine | 2.458 | 1022159       | 1.0             | 3802        |

### Table 6: Results of method precession for Olmesartan.

| S.No. | Peak Name  | Rt    | Area (µV) | USP Tailing | USP Plate Count |
|-------|------------|-------|-----------|-------------|-----------------|
| 1     | Olmesartan | 3.294 | 1513391   | 1.2         | 4759            |
| 2     | Olmesartan | 3.191 | 1513391   | 1.1         | 3695            |
| 3     | Olmesartan | 3.076 | 1526673   | 1.1         | 4741            |
| 4     | Olmesartan | 3.166 | 1560819   | 1.2         | 3793            |
| 5     | Olmesartan | 3.319 | 1560819   | 1.1         | 4741            |

### Table 7: Accuracy studies for Amlodipine.

| % Concentration (at specification Level) | Area  | Amount Added (mg) | Amount Found (mg) | % recovery | Mean Recovery |
|-----------------------------------------|-------|-------------------|-------------------|------------|---------------|
| 80%                                     | 605652.5 | 4                  | 4.0               | 100.0%    | 99.9%         |
| 100%                                    | 1246314  | 5                  | 4.94              | 98.0%     |               |
| 120%                                    | 1869868  | 6                  | 6.1               | 101.6%    |               |

### Table 8: Accuracy results for Olmesartan.

| % Concentration (at specification Level) | Area  | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|-----------------------------------------|-------|-------------------|-------------------|------------|---------------|
| 80%                                     | 774787.7 | 16                 | 15.9              | 99.37%    | 99.8%         |
| 100%                                    | 1537580  | 20                 | 19.9              | 99.5%     |               |
| 120%                                    | 2285575  | 24                 | 24.1              | 100.4%    |               |
Table 9: Results of specificity studies for Amlodipine and Olmesartan.

| % Concentration (at specification Level) | Drug Added (mg) | Excipient Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|-----------------------------------------|----------------|---------------------|------------------|------------|--------------|
| 50%                                     | 444310         | 2.5                 | 4.98             | 99.6%      |              |
| 100%                                    | 885413         | 5                   | 4.97             | 99.7%      |              |
| 150%                                    | 131923         | 7.5                 | 4.96             | 99.7%      |              |

Table 10: Results of robustness for Amlodipine.

| Change in parameter | % RSD |
|---------------------|-------|
| Flow (1.1 ml/min)   | 1.03  |
| Flow (0.9 ml/min)   | 0.68  |
| Temperature (27°C)  | 0.42  |
| Temperature (23°C)  | 0.57  |
| Wavelength of detection (250 nm) | 0.23 |
| Wavelength of detection (266 nm) | 0.12 |

Stability Studies

Acid Hydrolysis

An accurately weighed 25 mg of pure drugs AML and 100 mg of OLM were transferred to a clean & dry 25 ml volumetric flask separately. To which 0.1 N Hydrochloric acid was added & made up to the mark & kept for 24 hrs. From both drug solutions 0.5 ml was taken and transferred in to a 50 ml volumetric flask & made up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl (after all optimized conditions).

Basic Hydrolysis

An accurately weighed 25 mg of pure drugs AML and 100 mg of OLM were transferred to a clean & dry 25 ml volumetric flask separately. To which 0.5N Sodium hydroxide was added & make up to the mark & kept for 24 hrs. From both drug solution 0.5 ml was taken in to a 50 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of NaOH (after all optimized conditions).

Dry Heat Degradation

An accurately weighed 25 mg of pure drugs AML and 100 mg of OLM were transferred in to a 25 ml volumetric flask, volume was made up to the mark with mobile phase & maintained at 50°C for 24 hrs. From both drug solutions 0.5 ml was taken in to a 50 ml volumetric flask & make up to the mark with mobile phase. Further it is injected into the HPLC system against a blank of mobile phase.

Photolytic Degradation

Approximately 25 mg of pure drugs AML and 100 mg of OLM were taken in a clean & dry Petri dish. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg of AML and 4 mg of OLM the UV exposed drug was transferred to a clean & dry 100 ml volumetric flask. First the UV exposed drug was dissolved in methanol & make up to the mark. Then injected into the HPLC system against a blank of mobile phase (after all optimized conditions).

Oxidation With (3%) H₂O₂

Accurately weighed 1 mg of AML and 4 mg of OLM of pure drugs were taken in a clean & dry 100 ml volumetric flask. 30 ml of 3% H₂O₂ and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to prepare 10 ppm and 40 ppm of AML and OLM solution respectively. The above sample was injected into the HPLC system.

Results of forced degradation studies

The results of the stress studies indicated the specificity of the method that has been developed. Amlodipine and Olmesartan were stable in photolytic, thermal and basic stress conditions. The result of forced degradation studies are given in the following table 2.

Results of method validation

Linearity

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 1- 3 µg/ml for Amlodipine and 2µg/ml to 30µg/ml for Olmesartan and the correlation coefficient obtained was greater than 0.999. The results show that an excellent correlation existed between the peak area and concentration of the analyte which is given in table 3 and 4.

Intermediate precision/ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Recovery and accuracy

The percentage recovery of AML and OLM in bulk drugs samples was ranged from 99.4 - 99.6% which indicates that the method was accurate which is given in table no.7.

Accuracy results

The accuracy of the method was determined by preparing solutions of different concentrations of AML and OLM that is 80%, 100% and 120% in which the amount of marketed formulation (AML and OLM 5 mg and 20 mg respectively) was kept constant and the amount of pure drug was varied that is 4 mg, 5mg and 6mg for AML and
Development of Stability...  

| Change in parameter | % RSD |
|---------------------|-------|
| Flow (1.1 ml/min)   | 0.03  |
| Flow (0.9 ml/min)   | 0.08  |
| Temperature (27°C)  | 0.19  |
| Temperature (23°C)  | 0.73  |
| Wavelength of Detection (250 nm) | 0.82 |
| Wavelength of detection (266 nm) | 0.46 |

16mg, 20mg and 24 mg for OLM i.e. 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy. Similarly was indicated by % recovery in table 7 and 8.

Specificity
5mg/ml of AML was spiked with 50% (2.5mg), 100% (5mg), and 150% (7.5mg) of excipient mix (Magnesium Stearate). Further 01 ml is pipetted out from the all three samples and diluted to 100 ml in three separate volumetric flask, and analysed for % recovery of AML. Similarly 20 mg/ml OLM sample were prepared and analysed.

LOD and LOQ
Detection limit and Quantitation limit of described method were observed as 0.653 mg/ml and 1.959 mg/ml for AML, 0.646 mg/ml and 1.638 mg/ml for OLM.

Robustness
Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of wavelength (235 and 239 nm) and mobile phase flow rate by 0.1 ml/min (0.9 and 1.1ml/min) had no significant effect on the retention time and chromatographic response of the 50 μg/ml solution, indicating that the method was robust.

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2°C), Wavelength of detection (±2nm) & Acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-19, % RSD < 2%) the developed RP-HPLC method for the analysis of Amlodipine.

CONCLUSION
High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Amlodipine and Olmesartan was done by RP-HPLC. The proposed method was found to be simple, precise, accurate and rapid for determination of AML and OLM in pure and dosage form. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement within the limit. Hence, this method can be easily and conveniently adopted for routine analysis of AML and OLM in pure form and dosage form.

ACKNOWLEDGEMENTS
I, Dhiraj Kumar, thankful to Dr. P. Suresh, Associate Director, School of Pharmacy, GNITC Campus, Ibrahimpattnam, Hyderabad, for providing necessary facilities to carry out the research work.

REFERENCE
1. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf
2. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, (1995), PP 1126.
3. Aniruddha R. Chabukswar*a, Bhanudas S. Kuchekara, Swati C. Jagdale, Dipali M. Mehetre, Archana S Morea and Pradeep D. Lokhande, Development and validation of a RP-HPLC method for simultaneous estimation of Olmesartan Medoxomil and Amlodipine Besylate in tablet dosage form, Scholars Research Library Archives of Applied Science Research, 2010, 2 (4): 307-312.
4. Janhavi R Rao*, Milindkumar P Rajput, Savita S Yadav, Toufik S. Mulla, Vishal V.Bharekar, Simultaneous Quantitation of Olmesartan medoxomil, Amlodipine besylate and Hydrochlorothiazide in Pharmaceutical dosage form by using HPLC, International Journal of PharmTech Research 2011; 3(1): 1435-1440.
5. K. Krishna Chaitanya, D. Gowri Sankar, D. Samson Israel, RP-HPLC Method development and validation of Amlodipine and Losartan in binary mixture, Journal of Global Trends in Pharmaceutical Sciences 2013; 4(3): 1144-1152.
6. Pourmina S.Patil, Harinath N. More, Sachin A. Pishvikar, RP-HPLC Method for simultaneous estimation of Amlodipine besylate and Olmesartan medoxomil from tablet, International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(3): 146-149.
7. Srinivas and Y. Sneha, stability indicating forced degradation RP-HPLC method development and validation of olmesartan medoxomil, international journal of pharmaceutical sciences and research 2014; 5(7): 2841-2847.
8. Nirmal M. Thakker, Haresh B. Panchal,1 Dinesh R. Rakhkholiya, R. Murugar,1 Vishnu P. Choudhari, and Bhanudas S. Kuchekar, Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of Olmesartan Medoxomil and Metoprolol Succinate in pharmaceutical dosage form. Pharm Methods. 2012 Jul-Dec; 3(2): 84–89.
9. Deepak Kumar Jain, development and validation of RP-HPLC method for estimation of amlodipine besylate, olmesartan medoxomil and hydrochlorothiazide in tablet dosage form, International
10. B. Jyothirmai, Satyadev Tnvss, T. Santosh and B. Syama Sundar, Development and validation of an RP-HPLC method for the determination of Olmesartan in human plasma, International Journal of Research in Pharmacy and Chemistry 2014; 4(2): 457-466.

11. Buchi N. Nalluri, D. Venkateswara Naik, B. Sunandana and K. Sushmitha, Development and validation of RP-HPLC-PDA method for the simultaneous estimation of Hydrochlorothiazide, Amlodipine besylate and Olmesartan medoxomil in bulk and pharmaceutical dosage forms, Journal of Chemical and Pharmaceutical Research 2013; 5(1): 329-335.

12. Nirmal M. Thakker, Vishnu P. Choudhari, Bhanudas S. Kuchekar, Haresh B. Panchal, Dinesh R. Rakholiya, R. Murugan, Pharmaceutical Methods 2012; 3(2): 84-89.

13. Jain PS, Patel MK, Gorle AP, Chaudhari AJ, Surana SJ, Journal of Chromatographic Science 2012; 50(8): 680-7.

14. Napa raj, Sockalingam ambazhagan, Kunapareddy anudeep babu, Sunkara nareendra babu, Chusena narasimharaju bhimanadhuni, International Current Pharmaceutical Journal 2012; 1(11): 336-341.