A NOVEL STABILITY INDICATING VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF AZILSARTAN AND AMLODIPINE BESYLATE HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT
A simple, precise, accurate and economical stability-indicating RP-HPLC assay method was developed and validated for simultaneous estimation of Azilsartan (AZN) and Amlodipine Besylate (AMD) in bulk drugs and their combined commercial tablets. The method has shown adequate separation of AZN and AMD from their degradation products. Separation was achieved on a Symmetry C18 (4.6 x 250mm, 5µm, Make: Waters) or equivalent) column using a mobile phase consisting of a mixture of Acetonitrile: Potassium Dihydrogen Phosphate buffer Adjust pH 3.0 with ortho phosphoric acid (70:30, v/v).a isocratic elution mode at a flow rate of 1 ml/min. The retention times for AZN and AMD were found to be 3.5 and 2.3 min respectively. Both drugs and their combination drug product were subjected to acid, base, hydrolysis, thermal and photolytic stress conditions. Thus stressed samples were analyzed by the proposed analytical method. Validation of the proposed analytical method was carried out as per ICH guidelines Q2R1. Quantitation was achieved with PDA detection at 254 nm based on peak area with linear calibration curves at concentration ranges 2.5-20µg/ml for AZN and 10-80µg/ml for AMD (R² > 0.9999 for both drugs). The LOD and
LOQ were 3.09μg/ml, 2.90μg/ml and 10.1μg/ml for Azilsartan and Amlodipine Besylate respectively. The method was found to be specific and stability indicating as no interfering peaks of degradents and excipients were observed. The proposed method is suitable for application in quality-control laboratories for quantitative analysis of both the drugs individually and in combination dosage forms.

KEY WORDS: RP-HPLC Method Azilsartan and Amlodipine Besylate and PDA Detector; Forced degradation studies; Tablet dosage forms

INTRODUCTION

1. Azilsartan

This fixed-dose combination of Azilsartan Medoxomil\(^1\) is found to show superior antihypertensive efficacy in blood pressure reduction in patients with stage 2 hypertension when compared with the maximum approved dose of olmesartan/hydrochlorothiazide.\(^2\)

Azilsartan Medoxomil is an Angiotensin II receptor antagonist which has the chemical name (5 – Methyl – 2 – o xo -1,3 – dioxol -4 – yl) methyl 2 – ethoxy -1 – {[ 2’- ( 5 - o xo -4,5 – dihydro - 1, 2, 4 – oxadia zol -3 - yl) biph enyl – 4 – yl ] methyl } - 1H – benzimidazole -7 - carboxylate monopotassium salt. It is a white crystalline powder which is practically insoluble in water, freely soluble in methanol, dimethyl sulfoxide and dimethyl formamide, soluble in acetic acid, slightly soluble in acetone and Acetonitrile and very slightly soluble in Tetra Hydro furan and 1- octanol. Azilsartan is a potent and lasting angiotensin II receptor blocker ("ARB") that lowers blood pressure by inhibiting the action of angiotensin II, a vasopressor hormone. Amlodipine is a calcium channel blocker ("CCB") having a hypotensive action by blocking inward calcium ion channels mainly in vascular smooth-muscle cells, resulting in peripheral arteriolar vasodilation. In a phase 3 clinical trial, the anti-hypertensive effect in diastolic blood pressure in the sitting position as the primary endpoint, of “Zacras” was statistically significant compared to monotherapy with either azilsartan or amlodipine.

![Chemical structure of Azilsartan medoxomil.](image)

Fig.No.1.Chemical structure of Azilsartan medoxomil.
Amlodipine: 3-ethyl 5-methyl-2-[(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate\(^{[1-4]}\) (Fig.2), is a potent dihydropyridine calcium channel blocker used in the treatment of hypertension and angina pectoris\(^{1,2}\) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. It is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure.

![Fig. No. 2. Chemical structure of Amlodipine.](image)

The literature survey shows that spectroscopic and chromatographic methods\(^{[3, 4, 5, 6]}\) for individual drugs but there is no methods were reported only a single method available for quantitation of Azilsartan Medoxomil and Amlodipine in solid dosage forms simultaneously. Thus it is inevitable to develop such a sensitive, accurate, precise, rapid and economical method for routine analysis of this combination in pharmaceutical dosage form successfully.

**MATERIALS AND METHOD**

**Instrumentation**

RP-HPLC waters 2695 separation module equipped with 2996 Photodiode Array Detector was employed in this method. The Empower 2 software was used for LC peak integration along with data acquisition and data processing. High performance liquid chromatograph waters 2695 equipped with Quaternary constant flow pump, Auto injector with injection volume of 10 μl. 2696 Photo diode Array detector and Empower-2 software, Phenomenex ODS C18 column (150 mm × 4.6 mm I.d., 5 μ size particle) forms the stationary phase.

**Reagents and Chemicals**

The Azilsartan and amlodipine standard drugs provide as gift samples by KP Labs Pvt. Ltd. Dilsukhnagar, Hyderabad. The “Zacras®” (The Combination Tablets LD and Zacras® Combination Tablets HD. 20mg azilsartan / 2.5mg amlodipine, or 20mg azilsartan / 5mg
Amlodipine, is orally administered once a day). formulation procured from Takeda Pharmaceuticals India Pvt. Ltd. at Mumbai. And all the HPLC grade Solvents Methanol, Water, Potassium dihyrogen ortho phosphate and orthophosphoric acid were purchased from Merck Specialities Private Limited, Mumbai, India

METHOD VALIDATION
The proposed method was developed and validated as per ICH Guidelines.[18] The parameters assessed were specificity, linearity, precision, accuracy, stability, LOD and LOQ, Ruggedness and Robustness.

Chromatographic condition:
Equipment : High performance liquid chromatography equipped with Auto Sampler and PDA detector
Column : Symmetry C18 (4.6 x 250mm, 5μm, Make: Waters) or equivalent
Flow rate : 1.0 ml per min
Wavelength : 254 nm
Injection volume : 20 μl
Column oven : Ambient Temp.
Run time : 6min.

Preparation of Phosphate buffer :(pH:3.0)
Weighed 7.0 grams of KH₂PO₄ into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjust Ph3.0 with ortho phosphoric Acid.

Preparation of mobile phase
Mix a mixture of above Buffer 300 mL (30%),700 mL of Acetonitrile HPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 μ filter under vacuum filtration.

Diluents Preparation
The Mobile phase used as diluents.

Preparation of Standard Solution
Accurately weigh and transfer 10 mg of Amlodipine and 20mg of Azilsartan working standard into a 10&25mL clean dry volumetric flask add about 7&20mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
Further pipette 1.0ml of the above Amlodipine stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 0.8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Sample Solution Preparation**
Accurately weigh and transfer to 231.6mg of Amlodipine and Azilsartan Tablet powder into a 100mL clean dry volumetric flask add about 70mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 1.6ml of Amlodipine and Azilsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Selection of ($\lambda_{max}$) Maximum wavelength.**
Azilsartan Medoxomil showed absorption maxima at 241.6 nm and Amlodipine showed at 234 nm. For simultaneous estimation of both the drugs Azilsartan Medoxomil and Amlodipine a common wavelength was selected as absorption maxima at 254nm. Fig3.

**Procedure**
Inject 20 μL of the standard, sample into the chromatographic system and measure the areas for Amlodipine and Azilsartan peaks and calculate the %Assay by using the formulae.

**System Suitability**
Tailing factor for the peaks due to Amlodipine and Azilsartan in Standard solution
Should not be more than 2.0
Theoretical plates for the Amlodipine and Azilsartan peaks in Standard solution
Should not be less than 2000

**Calculation: (For Amlodipine)**
Assay % =

\[
\frac{AT}{WS} \times \frac{DT}{P} \times \frac{Avg. \ Wt}{Avg. \ Wt} \times \frac{AS}{DS} \times \frac{WT}{100} \times \frac{Label \ Claim}{Label \ Claim} \times 100
\]

Where:
AT = average area counts of sample preparation.
As= average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P     = Percentage purity of working standard
LC = LABEL CLAIM OF Amlodipine in mg/ml.

RESULTS
System Suitability Results:
1). Tailing factor Obtained from the standard injection is 1.2
2). Theoretical Plates Obtained from the standard injection is 4488.5

Assay Results

\[
\begin{array}{cccccccc}
180433.3 & 10 & 1 & 0.8 & 100 & 10 & 99.8 & 231.6 \\
\hline
\end{array}
\]

\[
\begin{array}{cccccccc}
179945.2 & 10 & 10 & 10 & 231.6 & 1.6 & 100 & 5 \\
\hline
\end{array}
\]

Calculation: (For Azilsartan)
Assay % =

\[
\frac{AT \times WS \times DT \times P \times Avg. Wt}{AS \times DS \times WT \times 100 \times Label \ Claim} \times 100
\]

Where:
AT = average area counts of sample preparation.
As= average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P     = Percentage purity of working standard
LC = LABEL CLAIM OF Azilsartan mg/ml.

RESULTS
System Suitability Results:
1). Tailing factor Obtained from the standard injection is 1.1
2). Theoretical Plates Obtained from the standard injection is 7617.1

Assay Results

\[
\begin{array}{cccccccc}
2493958 & 20 & 0.8 & 100 & 10 & 99.8 & 231.6 \\
\hline
\end{array}
\]

\[
\begin{array}{cccccccc}
2493642 & 25 & 10 & 231.6 & 1.6 & 100 & 40 \\
\hline
\end{array}
\]
**Accuracy:** The accuracy was determined by calculating % recoveries of Alogliptine and pioglitazone. It was carried out by adding known amounts of each analyte corresponding to three concentration levels (50, 100, and 150%) of the labelled claim to the excipients. At each level, six determinations were performed and the accuracy results were expressed as percent analyte recovered by the proposed method.

**Precision:** Precision of an analytical method is usually expressed as the standard deviation. The repeatability studies were carried out by estimating response of alogliptine and pioglitazone six times. The intra-day and inter-day precision studies (intermediate precision) were carried out by estimating the corresponding responses three times on the same day and on three different days for three different concentrations and the results are reported in terms of relative standard deviation.

**Linearity:** The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits a linear response and is directly proportional over the relevant concentration range for the target concentration of the analyte. The linear regression

![Calibration Plot](image-url)

**Fig.No.3 Calibration curve of Amlodipine.**
data for the calibration plot is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance.

**Robustness:** Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase, flow rate and temperature. This deliberate change in the method has no affect on the peak tailing, peak area and theoretical plates and finally the method was found to be robust.

**Limit of Detection & Limit of Quantitation:** The LOD can be defined as the smallest level of analyte that gives a measurable response and LOQ was determined as the lowest amount of analyte that was reproducibly quantified. These two parameters were calculated using the
formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations, LOD=3.3 × s/s and LOQ=10 × s/s, where s = standard deviation, S= slope of the calibration curve.

**Assay of Azilsartan and Amlodipine in Tablet dosage forms (Zacras =brand name):** Assay of marketed (Zacras =brand name) product was carried out by using the proposed developed method. Sample solutions were prepared and injected into RP-HPLC system. The sample solution was scanned at 254 nm. The % drug estimated was found to be 99.86. The chromatogram showed two single peaks of Azilsartan and Amlodipine was observed with retention times of 3.53 and 2.33 min (Figure 3)

![Precision chromatogram for Azilsartan and Amlodipine](image)

**Table No.1 Accuracy studies**

<table>
<thead>
<tr>
<th>% Concentration (at specification Level)</th>
<th>Area</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>90441.3</td>
<td>5</td>
<td>4.99</td>
<td>99.9%</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>180387.3</td>
<td>10</td>
<td>9.96</td>
<td>99.6%</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>274315.8</td>
<td>15</td>
<td>15.1</td>
<td>101.0%</td>
<td>100.2%</td>
</tr>
</tbody>
</table>
LINEARITY

Preparation of stock solution
Accurately weigh and transfer 10 mg of Amlodipine and 40 mg of Azilsartan working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)
Further pipette 1.0 ml of the above Amlodipine stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – I (2.5 ppm of Amlodipine & 10 ppm of Azilsartan):
0.25 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (5 ppm of Amlodipine & 20 ppm of Azilsartan)
0.5 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (10 ppm of Amlodipine & 40 ppm of Azilsartan)
1.0 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (15 ppm of Amlodipine & 60 ppm of Azilsartan)
1.5 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (20 ppm of Amlodipine & 80 ppm of Azilsartan)
2.0 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Procedure
Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.
Table No. 2. Linearity Results: (for Amlodipine)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Linearity Level</th>
<th>Concentration</th>
<th>Area Amlodipine</th>
<th>Area Azilsartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>2.5ppm</td>
<td>88076</td>
<td>1240446</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>5ppm</td>
<td>134961</td>
<td>1883086</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>10ppm</td>
<td>179342</td>
<td>2495768</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>15ppm</td>
<td>226635</td>
<td>3253808</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>20ppm</td>
<td>272235</td>
<td>3820923</td>
</tr>
</tbody>
</table>

**Correlation Coefficient**

- Amlodipine: 0.999
- Azilsartan: 0.999

**Acceptance Criteria:** Correlation coefficient should be not less than 0.999

**LIMIT OF DETECTION:** (for Amlodipine)

**Preparation of 8µg/ml solution:**

Accurately weigh and transfer 10 mg of Amlodipine working standard into a 10mL clean dry volumetric flask, add about 7mL of Diluent, and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 1.0ml of the above Amlodipine stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 0.8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation of 0.04µg/ml solution**

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Pipette 0.5mL of solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

**Calculation of S/N Ratio**

Average Baseline Noise obtained from Blank: 52µV

Signal Obtained from LOD solution: 151 µV

\[
S/N = \frac{151}{52} = 2.90
\]

**Acceptance Criteria**

S/N Ratio value shall be 3 for LOD solution.
LIMIT OF QUANTIFICATION

Preparation of 8µg/ml solution
Accurately weigh and transfer 10 mg of Amlodipine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)
Further pipette 1.0ml of the above Amlodipine stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 0.8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.13µg/ml solution
Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Pipette 1.6 mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio
Average Baseline Noise obtained from Blank : 52 µV
Signal Obtained from LOQ solution : 512µV
S/N = 512/52 = 9.84

Acceptance Criteria
S/N Ratio value shall be 10 for LOQ solution.

LIMIT OF DETECTION: (for Azilsartan)

Preparation of 64µg/ml solution:
Accurately weigh and transfer 20mg of Azilsartan working standard into a 25mL clean dry volumetric flask add about 20mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)
Further pipette 0.8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Preparation of 0.02µg/ml solution)
Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 0.4ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio
Average Baseline Noise obtained from Blank : 52 µV
Signal Obtained from LOD solution : 161 µV
S/N = 161/52 = 3.09

Acceptance Criteria
S/N Ratio value shall be 3 for LOD solution.

LIMIT OF QUANTIFICATION
Preparation of 64µg/ml solution
Accurately weigh and transfer 20mg of Azilsartan working standard into a 25mL clean dry volumetric flask add about 20mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)
Further pipette 0.8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.08µg/ml solution)
Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Pipette 1.4mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.
Calculation of S/N Ratio

Average Baseline Noise obtained from Blank: 52 µV
Signal Obtained from LOQ solution: 528µV
S/N = 528/52 = 10.1

ROBUSTNESS

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a). The flow rate was varied at 0.8 ml/min to 1.2ml/min.

Standard solution 8ppm of Amlodipine & 64ppm of Azilsartan was prepared and analysed using the varied flow rates along with method flow rate.

The results are summarized

On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

Table No.3 System suitability results

<table>
<thead>
<tr>
<th>S.No</th>
<th>Flow Rate (ml/min)</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amlodipine</td>
<td>Azilsartan</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>5720.0</td>
<td>8965.0</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>4488.5</td>
<td>7617.1</td>
</tr>
<tr>
<td>3</td>
<td>1.2</td>
<td>3945.0</td>
<td>6755.0</td>
</tr>
</tbody>
</table>

* Results for actual flow (1.0 ml/min) have been considered from Assay standard.

b). The Organic composition in the Mobile phase was varied from 75% to 65%.

Standard solution 8 µg/ml of Amlodipine &64µg/ml of Azilsartan was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

The results are summarized

On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10%
Table No. 4. System suitability results

<table>
<thead>
<tr>
<th>S.No</th>
<th>Change in Organic Composition in the Mobile Phase</th>
<th>System Suitability Results</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP Plate Count</td>
<td>USP Tailing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amlodipine Azilsartan</td>
<td>Amlodipine Azilsartan</td>
</tr>
<tr>
<td>1</td>
<td>10% less</td>
<td>3764.0 7721.0</td>
<td>1.2 1.1</td>
</tr>
<tr>
<td>2</td>
<td>*Actual</td>
<td>4488.5 7617.1</td>
<td>1.2 1.1</td>
</tr>
<tr>
<td>3</td>
<td>10% more</td>
<td>3652.0 7460.0</td>
<td>1.2 1.1</td>
</tr>
</tbody>
</table>

* Results for actual Mobile phase composition (30:70 Buffer: Methanol) have been considered from Accuracy standard.

**Stability studies:** Forced degradation studies typically involved the exposure of samples of the drugs to the relevant stress conditions of acid, base, hydrolysis, oxidation, thermal, photo stability. Stability testing was established for estimating the allowed time span between sample collection and sample analysis. It is also important to evaluate an analytical method’s ability to measure drug products in the presence of its degradation products.

**Oxidation**

To 1 ml of stock solution of Azilsartan Medoxomil and Amlodipine, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 40μg/ml & 25μg/ml solution and 10 μl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Acid Degradation Studies**

To 1ml of stock solution of Azilsartan Medoxomil and Amlodipine, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c .The resultant solution was diluted to obtain 40μg/ml & 25μg/ml solution and 10 μl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Alkali Degradation Studies**

To 1 ml of stock solution Azilsartan Medoxomil and Amlodipine, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 40μg/ml & 25μg/ml solution and 10 μl were injected into the system and the chromatograms were recorded to assess the stability of sample.
Dry Heat Degradation Studies

The standard drug solution was placed in oven at 1050c for 6 hours to study dry heat degradation. For HPLC study, the resultant solution was diluted to 40μg/ml & 25μg/ml solution and 10μl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 100 μg/ml solution to UV Light by keeping the beaker in UV Chamber. For 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 40μg/ml & 25μg/ml solutions and 10 μl were injected into the system and the chromatograms were recorded to assess the stability of sample.

CONCLUSION

The present Research work, a simple, precise and accurate RP-HPLC method for the simultaneous determination of Azilsartan and Amlodipine. The method was validated according to ICH guidelines. From the chromatogram (Figure 3) good separation Azilsartan and Amlodipine were performed at retention time of Amlodipine (2.3) and Azilsartan (3.5) were observed with a correlation coefficient (r²) 0.999 for Azilsartan and Amlodipine (r²) 0.999. The limit of detection (LOD) was calculated and found to be 0.9μg/ml Azilsartan and 0.69 μg/ml Amlodipine. Limit of quantification (LOQ) was found to be 2.7 μg/ ml Azilsartan and 2.02 μg/ml Amlodipine. Intraday precision values %RSD values were found to be 1.0% for Azilsartan and 1.025% for Amlodiine and interday precision values 1.02% and 0.988% for Azilsartan and Amlodipine respectively. So the proposed method is more precise, accurate and robust. System suitability parameters were studied by injecting the working standard solution (20μg/mL) is tabulated in Table 2. The optimized RP-HPLC Method for simultaneous estimation of Azilsartan and Amlodipine with good resolution can be used for evaluating in the pharmaceutical companies and research laboratories for routine and biological sample analysis.

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