METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TELMISARTAN AND AMLODIPINE BESYLATE IN TABLET DOSAGE FORM BY RP-HPLC

*Pathuri Jnana Nagarjuna¹, Sree Vidya Parvataneni²

¹,² KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, A.P

ABSTRACT

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Telmisartan and Amlodipine besylate in combination. The separation was carried out using a mobile phase consisting of acetonitrile: water: triethyelamine (68:31.8:0.2 v/v) with pH 4 adjust by using ortho-phosphoric acid. The column used was C-18 column (Inertsil ODS-3 250*4.6 mm ) with flow rate of 1 ml / min using PDA detection at 240 nm. The described method was linear over a concentration range of 4-60 μg/ml and 10-150 μg/ml for the assay of Amlodipine and Telmisartan respectively. The retention times of Amlodipine and Telmisartan were found to be 2.3 and 2.7 mins respectively. Results of analysis were validated statistically and by recovery studies. The limit of detection (LOD) and the limit of quantification (LOQ) for Amlodipine and Telmisartan were found to be 0.0046 μg/ml and 0.0018 μg/ml, 0.014 and 0.0056 μg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Amlodipine and Telmisartan bulk drug and in its pharmaceutical dosage form.

Keywords: Amlodipine, Telmisartan, RP-HPLC, Pharmaceutical dosage form.

INTRODUCTION

Amlodipine besylate 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester benzene sulphonate is an anti-hypertensive drug of Calcium Channel Blocker class of drug.[1-5] It inhibits the influx of
extracellular calcium across the myocardial and vascular smooth muscle cell membranes. The decrease in extracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased afterload. Amlodipine occupies the plasma membrane dihydropyridine receptor and causes competitive blockade of the voltage-operated slow calcium channel. Amlodipine is slowly and almost completely absorbed from the gastrointestinal tract and the peak plasma concentration is seen 6-12 h after drug administration. \[1\]

Amlodipine has large volume of distribution and is 97.5% bound to plasma protein. Amlodipine is extensively (about 90%) converted to inactive metabolites via hepatic metabolism. However, metabolites have no significant pharmacological activity. Elimination half-life is relatively longer and ranges from 35 to 45 hours, permitting once daily dosage. About 60% of the drug is excreted through urine, 20 to 25% in faeces and less than 10% is recovered unchanged. Amlodipine is used in the treatment of Hypertension, Chronic stable angina, Vasospastic Angina (Prinzmetal's or Variant Angina), Angiographically Documented coronary artery disease. As it is contraindicated in some cases consumption of Amlodipine has to be avoided in patients during Pregnancy. \[2\][3]

**Fig. 1: Chemical structure of Amlodipine**

Telmisartan 2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl]methyl]phenyl]benzoic acid is an anti hypertensive drug of angiotensin II receptor blockers class of drug.\[1-4\] It appears as a white solid and initial dose of 40 mg once daily.
This may be increased, if necessary to a maximum dose of 80 mg once daily. Telmisartan is a non-peptide angiotensin II receptor antagonist. Angiotensin II is formed from angiotensin I in a reaction catalysed by angiotensin converting enzyme. Angiotensin II is the principle pressor agent of the rennin angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium. Telmisartan blocks the vasoconstriction and aldosterone-secreting effects of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland, leading to a reduction in arterial blood pressure. Telmisartan is rapidly absorbed from the gastrointestinal tract; the absolute oral bioavailability is dose dependant and is about 42% following a 40 mg dose and 58% following a 160 mg dose. Peak plasma concentrations of Telmisartan are reached about 0.5 to 1 hr. after an oral dose. Telmisartan is over 99% bound to plasma proteins mainly albumin and α1-acid glycoprotein.\[^4\][^5]\n
Telmisartan is metabolized by conjugation to form a pharmacologically inactive acylglucuronide. The terminal elimination half life of Telmisartan is about 24 hours. It is excreted almost entirely in the faeces via bile mainly as unchanged drug; only minute amounts were found in the urine. In case of over doses it may cause hypotension, dizziness and tachycardia; bradycardia could occur from parasympathetic (vagal) stimulation.\[^6\]

Fig. 2: Chemical structure of Telmisartan

MATERIALS AND METHODS

Chemicals and Reagents

Samples of Amlodipine and Telmisartan confirmed by spectral characterization and SOR (specific optical rotation) were obtained from Process Research Department of Emergent
Laboratories Ltd, Hyderabad, India. HPLC-grade Acetonitrile was procured from Rankem. ACS Grade Triethylamine and Ortho-phosphoric acid (OPA) from Sigma-Aldrich, India.

**Instrumentation**

Chromatographic system consisted of a Shimatzu HPLC equipped with auto sampler Photodiode array detector. The data recorded using LC Solutions software. The separation was carried out using a mobile phase consisting of acetonitrile: water: triethylamine (68:31.8: 0.2 v/v) with pH 4 adjusted with ortho phosphoric acid. The column used was C-18 column. (Inertsil ODS-3 250*4.6 mm) with flow rate of 1 ml / min using PDA detection at 240 nm.

**Preparation of stock, working standard solutions, and sample solution:**

A stock solution of Amlodipine and Telmisartan was prepared, by taking 10 mg of standard Amlodipine besylate and 20 mg of Telmisartan was weighed accurately and transferred to two separate 100 ml volumetric flasks. Both the drugs were dissolved in 50 ml of mobile phase with shaking and then volume was made up to the mark with mobile phase to get 200 μg/ml and 100 μg/ml of standard stock solution of Telmisartan and Amlodipine besylate respectively. These stock solutions were filtered through 0.2 μm Nylon 66 (N66) 47mm membrane filter paper. For each drug, appropriate aliquots were pipetted out from the standard stock solution into a series of 10 mL volumetric flasks to get a concentration of 4, 8, 20, 25, 40, 50 and 60 μg/ml of Amlodipine, 10, 20, 50, 62.5, 100, 125 and 150 μg/ml of Telmisartan.

**Development and validation of HPLC Method**

Present study was conducted to obtain a new, affordable, costeffective and convenient method for HPLC determination of Amlodipine and Telmisartan in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996, and Global Quality Guidelines–2002. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, and robustness.

**System suitability**

The system suitability was assessed by six replicate analysis of Amlodipine and Telmisartan at a 100% level to verify the resolution and reproducibility of the chromatographic system adequate for the analysis to be done. This method was evaluated by analyzing the repeatability of retention time, peak area for both Amlodipine and Telmisartan tailing factor,
theoretical plates (Tangent) of the column and resolution between the peaks of Amlodipine and Telmisartan.

**Selectivity**[^12]
Selectivity was determined in the presence of common excipients used in the tablet formulation. Sample containing 100% Amlodipine and Telmisartan was injected first. Then the samples mixed with three different placebo formulations were injected to find out the selectivity of the method.

**Linearity**[^13]
Linearity of the method was determined by constructing calibration curves. Standard solutions of Amlodipine and Telmisartan at different concentrations level were used for this purpose. Before injection of the solutions, the column was equilibrated for at least 30 min with the mobile phase. Each measurement was carried out in six replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentrations of Amlodipine and Telmisartan to obtain the calibration curves. The five concentrations of the standard were subjected to regression analysis to calculate calibration equation and correlation coefficients.

**Accuracy**[^14]
The accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by means of recovery experiments, by addition of active drug to placebo formulations. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay.

**Precision**[^15]
The precision of the method was determined by repeatability (intraday) and intermediate precision (inter-day) study. Repeatability was determined by performing four repeated analysis of the standard solutions of Amlodipine (40 μg/ml) and Telmisartan (100 μg/ml) on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis of previous standard solutions on three different days (inter-day) in the same laboratory. The relative standard deviation (% RSD) was determined in order to assess the precision of the method.
Robustness\textsuperscript{[16]}

The robustness of the method was assessed by altering the some experimental conditions such as, by changing the flow rate from 0.9 to 1.1 ml/min, amount of acetonitrile (67%, 68%, 69%) the temperature of the column (26 °C to 28 °C) and PH of the mobile phase.

RESULTS AND DISCUSSION

The developed method has been validated as per ICH guidelines\textsuperscript{10}. Every 20 μL of the working standard solution of Amlodipine in the mass concentration range of 4-60 μg/ml, and that for Telmisartan in the mass concentration range of 10-150 μg/ml were injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of Amlodipine and Telmisartan were obtained by plotting the peak area ratio versus the applied concentrations of Amlodipine and Telmisartan. The linear regression coefficients were found to be 0.9998 and 0.9991 for Amlodipine and Telmisartan respectively.

Repeatability of the method was checked by injecting replicate injections of the solution 40 μg/ml and 100 μg/ml of Amlodipine and Telmisartan respectively and the RSD was found to be 0.32% and 0.37%. Variability of the method was studied by analyzing the solution on the same day (intra-day precision) and on three different days (inter-day precision). The results obtained for intra-day precision (RSDs) were 0.34 % & 0.36 % respectively, at \( n = 6 \), for both Amlodipine and Telmisartan. The inter-day precisions (RSDs) were 0.41 % and 0.4 %, respectively, at \( n = 6 \), for both Amlodipine and Telmisartan.

Accuracy of the method was tested by carrying out recovery studies at different spiked levels. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated, results are shown in Table 1.

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 μg/mL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Amlodipine and Telmisartan from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.
Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 $\sigma$/S and 10 $\sigma$/S, respectively as per ICH guidelines, where $\sigma$ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The results of validation parameters and System suitability parameters were shown in Table 2.

Table 1: Recovery studies of Amlodipine and Telmisartan.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. Of Std. (μg/mL)</th>
<th>Conc. Of solution (μg/mL)</th>
<th>Amount found (μg/mL)</th>
<th>% Recovery</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine</td>
<td>10</td>
<td>10</td>
<td>19.90</td>
<td>99.50</td>
<td>0.324</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>20</td>
<td>30.14</td>
<td>100.46</td>
<td>0.687</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>40.87</td>
<td>102.17</td>
<td>0.652</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>25</td>
<td>25</td>
<td>49.69</td>
<td>100.53</td>
<td>0.517</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>50</td>
<td>74.82</td>
<td>99.38</td>
<td>0.674</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>75</td>
<td>99.83</td>
<td>99.83</td>
<td>0.285</td>
</tr>
</tbody>
</table>

Table 2: System suitability of parameters

<table>
<thead>
<tr>
<th>Validation parameters</th>
<th>Amlodipine</th>
<th>Telmisartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (μg/ml)</td>
<td>4-60</td>
<td>10-150</td>
</tr>
<tr>
<td>Limit of Detection (μg/ml)</td>
<td>0.0046</td>
<td>0.0018</td>
</tr>
<tr>
<td>Limit of Quantification (μg/ml)</td>
<td>0.014</td>
<td>0.0056</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>2.31 ± 4.72</td>
<td>2.78 ± 5.68</td>
</tr>
<tr>
<td>Resolution factor</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Capacity factor</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Theoretical plate</td>
<td>3009</td>
<td>4000</td>
</tr>
<tr>
<td>Intraday (%RSD)</td>
<td>0.34</td>
<td>0.36</td>
</tr>
<tr>
<td>Inter day (%RSD)</td>
<td>0.41</td>
<td>0.4</td>
</tr>
<tr>
<td>Repeatability (%RSD)</td>
<td>0.32</td>
<td>0.37</td>
</tr>
<tr>
<td>Accuracy</td>
<td>99.5-102.17</td>
<td>99.38-100.53</td>
</tr>
<tr>
<td>Peak purity index</td>
<td>1.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>Regression coefficient (r)</td>
<td>0.999</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Analysis of Formulation: Twenty tablets of Amlodipine and Telmisartan in combination were weighed, their average weight was determined, and finally they were crushed to a fine powder. The tablet powder equivalent to 10 mg of Amlodipine and 20 mg of Telmisartan was weighed and transferred to a 100 mL volumetric flask, first dissolved in 50 mL of mobile phase, and then the volume was made up to the mark with the mobile phase. The content was ultrasonicated for 30 min for complete dissolution. The solution was then what Mann’s filter paper No-41. The selection of the mixed sample solution for analysis was carried out by the optimization of various dilutions of the tablet dosage form, considering the label claim. The mixed sample solution of 10 μg/mL of Amlodipine and 25 μg/mL of Telmisartan, which was falling in the Beer’s-Lamberts range, showed good results and was selected for the entire
analysis. The results of tablet analysis \((n = 6)\) were found to be 98.81 and 100.16 for Amlodipine and Telmisartan respectively.

From the typical chromatogram of Amlodipine and Telmisartan (Fig. 6), it was found that the retention time of Amlodipine was 2.3 min and Telmisartan was 2.7 min, which were well-resolved peaks with a resolution factor of 2.7 for Telmisartan. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and the RT chromatograms were shown in Fig 4 & 5. The results analysis was shown in Table – 3.

**Table 3: Analysis of formulation**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Labeled amount (mg)</th>
<th>Amount taken (mg)</th>
<th>Amount found for assay (μg/mL)</th>
<th>% Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine</td>
<td>5</td>
<td>10</td>
<td>9.96</td>
<td>99.69</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>40</td>
<td>20</td>
<td>19.40</td>
<td>99.26</td>
</tr>
</tbody>
</table>

![Fig. 3: Overlain spectrum of Amlodipine and Telmisartan](image)

![Fig. 4: Chromatogram of Amlodipine](image)
CONCLUSION
The developed method was validated in terms of accuracy, repeatability, and precision. A good linear relationship was observed for Amlodipine and Telmisartan in the concentration ranges of 4-60 μg/ml and 10-150 μg/ml respectively. The correlation coefficient for Amlodipine was found to be 0.9998 and that for Telmisartan was 0.9991. The inter-day and intra-day precision results were good enough to indicate that the proposed method was precise and reproducible. The assay experiment showed that the contents of Amlodipine and Telmisartan estimated in the tablet dosage form were free from the interference of excipients. This demonstrated that the developed HPLC method was simple, linear, precise, and accurate, and could be conveniently adopted for the routine quality control analysis of Amlodipine and Telmisartan simultaneously, from its pharmaceutical formulations and bulk drug.
ACKNOWLEDGEMENT
We thank Emergent Laboratories Pvt. Ltd, Hyderabad, India for providing the facilities to carry our research work.

REFERENCES


