A VALIDATED REVERSE PHASE HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF IRBESARTAN AND AMLODIPINE IN PHARMACEUTICAL DOSAGE FORM

G.Kumara Swamy\(^1\)*, JMR Kumar \(^2\), J.V.L.N.Seshagiri Rao\(^3\).

\(^1\)Research scholar, Department of Pharmaceutical Analysis, Jawaharlal Nehru Technological University Kakinada, Kakinada - 533 003-Andhra Pradesh, India.

\(^2\)Mylan Laboratories Limited, Plot no 31, 32, 33&34-A, Anrich Industrial Estate, Bollaram, Medak(Dist) 502325, India.

\(^3\)Srinivasarao College Of Pharmacy, Pothinamallayapalem, Madhurawada, Visakhapatnam-500041.A.P.India.

ABSTRACT

A simple, accurate and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous estimation of Irbesartan and Amlodipine in bulk and Pharmaceutical dosage forms. Chromatographic separation was achieved isocratically on a Waters C\(_{18}\) column (Xbridge -150×4.6 mm, 5 μ particle size) using a mobile phase Methanol and Potassium dihyrogen orthophosphate (adjusted to pH 3.4 with orthophosphoric acid) in the ratio of 60:40 v/v , delivered at a flow rate of 1.0 ml / min and wavelength of detection at 250 nm. The retention times of Irbesartan and Amlodipine were 5.80 min and 3.12min respectively. The developed method was validated according to ICH guidelines. The method with high percent recovery and short retention time of Irbesartan and Amlodipine were found to be simple, rapid and reproducible. The proposed method can be used for determination of these drugs in combined dosage forms.

KEYWORDS: Irbesartan and Amlodipine; RP-HPLC; PDA detection; Tablet dosage form.

INTRODUCTION

Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs\(^1\). It involves separating, identifying, and determining the relative amounts of...
components in a sample matrix. The RP-HPLC is a method of choice for assay that involves sophisticated equipment. The aim to develop a simple, rapid, specific and sensitive RP-HPLC method for the determination of Irbesartan and Amlodipine in pharmaceutical dosage form (tablets). The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation. The survey of literature reveals that few analytical methods are available for the drugs like Irbesartan and Amlodipine, but the methods for Simultaneous estimation of these drugs were still emerging.

**Irbesartan**: (2-butyl-3-[[4-[2-(2h-tetrazol-5-yl) phenyl] methyl]-1,3-diazaspiro[4.4]non-1-ene 4-one) is an angiotensin II (fig.01) receptor antagonist use and mainly for the treatment of hypertension[1-4].

**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 trans membrane 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. These two drugs are used in the treatment of hypertension alone or on combination. Because of their synergistic potential as antihypertensive; both drugs are combined in a single dosage form and are available in market. Irbesartan and Amlodipine were officially available in various pharmacopeias like IP[5] (Indian pharmacopeia), B.P[6] (British pharmacopeia), USP[7] (United pharmacopeia) EP[8] (European pharmacopeia). JP[9] (Japanese pharmacopeia). Literature survey[10-12] reveals that some methods have already been developed for the estimation of these drugs like HPLC[13-14] and HPTLC[15] Methods for individual estimation or the simultaneous estimation of the drugs etc. there are few methods were reported for the pharmacokinetic study and pharmacological study of IRB and AMD some methods were reported for the estimation of IRB and AMD in bulk individual estimation.

The aim of the present work was to develop and validate accurate, fast and reliable Simultaneous RP-HPLC method with UV detection for the simultaneous determination of Irbesartan and Amlodipine in bulk and in tablet dosage forms. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, short elution time (less than 10 min) IRB and AMD good precision (R. S. D. less than 2%) and high recovery (greater than 98%).
Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH)\cite{16-17} for the simultaneous determination of IRB and AMD in bulk and in tablet dosage form.

**Fig.01 Chemical structure of Irbesartan.**

**Fig.02. Chemical structure of Amlodipine.**

**Reagents and Chemicals**
IRB API and AMD API (Note:IRB is Irbesartan and AMD is Amlodipine) were obtained as gift samples from Dr. Reddy’s laboratories limited, Hyderabad. The branded formulations (tablets) (Irovel-H tablets containing 100 mg of Irbesartan and 10 mg of Amlodipine) were procured from the local market. Methanol, Water, Potassium dihyrogen ortho phosphate and orthophosphoric acid used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.

**Instrumentation**
Chromatographic separation was performed on a “WATERS-2695” chromatographic system equipped with a LC-20AT pump; variable wavelength programmable PDA detector, SPD-20A and Rheodyne injector (7725i) with 20μl fixed loop.
Chromatographic conditions
Hypersil Xbridge, (C18, 150 x 4.6 mm, 5μ) was the column used for separation. Mobile phase consisting of Potassium dihydrogen ortho phosphate pH 3.4 adjusted with ortho phosphoric acid : Methanol in the ratio of 60:40 v/v was delivered at a flow rate of 1.0 ml/min with detection at 250 nm. The mobile phase was filtered through a 0.45μ nylon filter and sonicated for 15 min. Analysis was performed at ambient temperature.

OPTIMIZED METHOD
Buffer
Weighed accurately about 1.34gm of Potassium dihydrogen ortho phosphate in a 1000ml of volumetric flask added about 900ml of milli-Q water added and degtas to sonicate and finally make up the volume with water. Finally PH is adjusted to 3.4 with OPA

Mobile phase
Buffer and Methanol was taken in the ratio 60:40.

Chromatographic conditions
Flow rate : 1ml/min
Column : Xbridge,( C18, 150 x 4.6 mm, 5μ)
Detector wave length : 250nm
Column temperature : 30°C
Injection volume : 10μL
Run time : 10 min
Diluent : Methanol

Method development
In order to obtain sharp peak and base line separation of the components, the author has carried out a number of trails by varying the commonly used solvents, their compositions and flow rate. In order to effect ideal separation of the drug under isocratic conditions, mixtures of commonly used solvents like water, methanol and Acetonitrile with or without different buffers in different combinations were tested as mobile phases on C8 stationary phase. A mixture of Potassium dihydrogen ortho phosphate pH 3.4 adjusted with ortho phosphoric acid ) in the ratio of 60:40 /v/v was proved to be the most suitable of all the combinations since the chromatographic peaks obtained with this mobile phase were better defined and resolved and almost free from tailing. A mobile phase flow rate of 1.0 ml/min. was found to be suitable in the studied range of 0.5 – 1.5 ml/min. the optimized chromatogram is shown in figure 3.
System Suitability Testing

The system suitability parameters such as Theoretical plates, tailing Factor and resolution were performed to verify the system is adequate for the analysis to be performed. The results are performed in Table 1.

Table 1. System suitability studies

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(a)Irbesartan±S.D</th>
<th>(b)Amlodipine±S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time((t'_R)) (<em>a &amp; b</em>)</td>
<td>5.848±0.51</td>
<td>3.156±0.026</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>3692</td>
<td>3264</td>
</tr>
<tr>
<td>Asymmetry Factor</td>
<td>1.05</td>
<td>1.04</td>
</tr>
<tr>
<td>HETP (cm)</td>
<td>24.61</td>
<td>21.76</td>
</tr>
<tr>
<td>Resolution(<em>a &amp; b</em>)</td>
<td>Between Irbesartan and Amlodipine 3.513</td>
<td></td>
</tr>
</tbody>
</table>

*a &b Average of five values with S.D of Retention time

Preparation of Solutions

Preparation of Standard stock solution

Accurately Weighed and transferred 100mg of Irbesartan and 10mg of Amlodipine working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents.(standard stock Irbesartan(10000µg/ml) Amlodipine (1000µg/ml).

METHOD VALIDATION

As per ICH guidelines, the method validation parameters checked were specificity, linearity, precision, accuracy, limit of detection, limit of quantitation and robustness
Specificity
A blank solution (mobile phase) was injected and the chromatogram showed no inferring peaks at retention time of the two drugs. The chromatogram of Irbesartan and Amlodipine extracted from the tablet were compared with those acquired from Irbesartan and Amlodipine standards, correlation was good (in terms of $t_R$ and area) indicates specificity of method. Common tablet excipients like starch, lactose, magnesium stearate were dispersed in dichloromethane, filtered and injected. There was no interference found.

![Figure 4. Chromatogram of Blank formulation](image)

**Figure 4. Chromatogram of Blank formulation**

**Linearity and range**
Aliquots of standard stock solutions of Irbesartan and Amlodipine were taken in 10 ml volumetric flasks and diluted with dichloromethane to get final concentrations in range of 25-150 $\mu$g/ml for IRB and 2.5-15 $\mu$g/ml for AMD. Triplicate injections were made five times for each concentration for each drug separately and chromatographed under the conditions as described above. The plots of peak area versus respective concentrations of Irbesartan and Amlodipine were found to be linear in the concentration range of 25-150 $\mu$g/ml for IRB and 2.5-15 $\mu$g/ml for AMD respectively. The linear regression equations of the lines are:

- For Irbesartan: $y = 16293x + 26244$, ($r^2 = 0.999$)
- For Amlodipine: $y = 12686x + 2924$, ($r^2 = 0.999$)

**Precision**
Precision study was performed to find out intra-day and inter-day variations. The percent relative standard deviation for intra-day precision was 0.431% for Irbesartan and 0.423% for Amlodipine and inter-day precision standard deviation was 0.9709 %w/v and %RSD
0.9761 w/v for Irbesartan and 0.4492% w/v and %RSD was 0.4522 for Amlodipine. Both the values were well within the limit of 2% as per ICH guidelines.

**Accuracy**

The accuracy was determined by recovery studies. The recovery studies were performed by standard addition method, at 50%, 100%, 150% level. Percent recovered was calculated using regression equation. For both the drugs, recovery was performed in same way and in triplicate. The percentage recovery were calculated and presented in Table 2.

**Table 02. Recovery studies of Irbesartan and Amlodipine.**

<table>
<thead>
<tr>
<th>Name of Drug</th>
<th>Amount present (mg)</th>
<th>Amount added (% Level)</th>
<th>% Recovery ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irbesartan</td>
<td>100</td>
<td>50</td>
<td>100.057±0.579</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>98.515±0.50</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>150</td>
<td>100.334±1.21</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>10</td>
<td>50</td>
<td>99.602±0.410</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>100</td>
<td>101.19±0.388</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>150</td>
<td>100.46±0.474</td>
</tr>
</tbody>
</table>

**Limit of detection and limit of quantitation**

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value.

\[
\text{LOD} = 3.3 \times \text{Standard deviation of y intercept (}\sigma\text{)} \\
\quad \text{Slope of calibration curve (S)}
\]

*Irbesartan* - 5.315 μg/ml

*Amlodipine* - 0.7606 μg/ml

The LOQ is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy.

\[
\text{LOQ} = 10 \times \text{Standard deviation of y intercept (}\sigma\text{)} \\
\quad \text{Slope of calibration curve (S)}
\]

*Irbesartan* – 16.10 μg/ml

*Amlodipine* - 2.304 μg/ml

**Robustness**

Robustness of the method was determined by making slight deliberate changes in chromatographic conditions like 1% change in ratio of mobile phase constituents, ± 1nm change in detection wavelength and 0.05% change in flow rate. It was observed that there
were no marked changes in the chromatogram. It suggests that the developed method is robust.

RESULTS AND DISCUSSION
The proposed method was found to be simple and sensitive with linearity in the concentration range of 25-150 μg/ml for IRB and 2.5-15 μg/ml for AMD. The method was found to be accurate and precise as indicated by results of recovery studies and %RSD not more than 2%. LOD and LOQ for Irbesartan and Amlodipine were found to be 16.10μg/ml and 2.304μg/ml respectively and LOD for Irbesartan and Amlodipine were found to be 5.135μg/ml and .07606μg/ml respectively. The proposed method was found to be specific as there is no interference from common tablet excipients like lactose, starch etc.

Calibration curve
Accurately measured volumes of working standard solution of IRB and AMD were transferred into a series of 10ml volumetric flasks and diluted appropriately with mobile phase. 20ml of each solution was injected under operating chromatographic conditions described above. Calibration Curves were obtained by plotting the response (area of drug peak) versus concentration of drug. Regression equations were calculated. The method was found linear over a concentration range of 25-150 μg/ml for IRB and 2.5-15 μg/ml for AMD.

Linearity
The method was linear in the range of 25-150 μg/ml for IRB and 2.5-15 μg/ml for AMD Standards. Linear regression data was given in Figure 5 &6 and Table 4&5.

![Calibration graph of Amlodipine](image-url)
Table No. 4. Calibration Data of Amlodipine

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2.5</td>
<td>36111</td>
</tr>
<tr>
<td>2.</td>
<td>5</td>
<td>69239</td>
</tr>
<tr>
<td>3.</td>
<td>10</td>
<td>130341</td>
</tr>
<tr>
<td>4.</td>
<td>12.5</td>
<td>159035</td>
</tr>
<tr>
<td>5.</td>
<td>15</td>
<td>193692</td>
</tr>
</tbody>
</table>

Fig No. 6. Calibration graph of Irbesartan.

Table No. 5. Calibration graph values of Irbesartan.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td>25</td>
<td>454398</td>
</tr>
<tr>
<td>7.</td>
<td>50</td>
<td>854012</td>
</tr>
<tr>
<td>8.</td>
<td>100</td>
<td>1666961</td>
</tr>
<tr>
<td>9.</td>
<td>125</td>
<td>2041119</td>
</tr>
<tr>
<td>10.</td>
<td>150</td>
<td>2472988</td>
</tr>
</tbody>
</table>

**Precision**

The precision of the method was demonstrated by inter day and intraday variation studies. In the Intraday studies, solutions of standard and sample were repeated thrice in a day and percent relative standard deviation (%RSD) for response factor was calculated. The intraday %RSD of IRB and AMD were found to be 0.49 and 0.4522 respectively. In the interday variation studies, injections of standard and sample solutions were made on three consecutive days and %RSD was calculated. The interday %RSD for IRB and AMD were found to be 0.62 and 0.75 respectively. From the data obtained the developed RP-HPLC method was found to be precise.
Accuracy
The accuracy of the method was determined by recovery experiments. Known concentration of Working standard was added to the fixed concentration of the pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated. Percent recovery was within the range of 98.51 to 100.33 ± 1.2%w/v for IRB and 99.60 to 100.46±0.47%w/v for AMD which indicates that the method was accurate.

Procedure for analysis of tablets
Twenty tablets were weighed and powdered into uniform size in a mortar. From this the average weight of a tablet was calculated. An accurately weighed portion from this powder equivalent to 100 mg of IRB and 10 mg of AMD was transferred to a 100mL volumetric flask containing 20 ml of the methanol. The contents of the flask were sonicated for about 20 min for complete solubility of the drug and the volume was made up to 100 ml with mobile phase. Then the mixture was filtered through 0.45μ membrane filter. From the above solution a two ml of aliquot was taken into a separate 10 ml volumetric flask and made up to the volume with mobile phase and mixed well. The above solution (20 μl) was then injected eight times into the column. The mean peak areas of the drugs were calculated and the drug content in the formulation was calculated by the regression equation of the method. The results of analysis shows that the amount of drug was in good agreement with the label claim of formulation.

Robustness
Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase ratio, pH of buffer, flow rate. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust.

Solution stability
In order to demonstrate the stability of both standard and sample solutions, the solutions were analyzed over a period of 12 hours at room temperature. The results show that, the retention time and peak area of IRB and AMD remained unchanged (%RSD less than 0.2) and no significant degradation within the indicated period was observed. This indicates that both
solutions were stable for at least 12 hours, which was sufficient to complete the analytical procedure.

CONCLUSION
The validated isocratic RP-HPLC method has been developed for the simultaneous determination of IRB and AMD in tablet dosage form. The proposed method is simple, rapid, accurate, precise, and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in a short period of time. Therefore, it is suitable for the routine analysis of IRB and AMD in pharmaceutical dosage form. The simultaneous estimation method allows for application in laboratories that lack sophisticated analytical instruments such as GC-MS that is complicated, costly and time consuming rather than a simple RP-HPLC method. Hence the proposed method could be useful for the national quality control laboratories in developing countries.

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