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RP-HPLC Method Development and Validation for Simultaneous Estimation of Losartan Potassium and Amlodipine Besilate in Bulk & Marketed Formulation

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Abstract

A new, precise, rapid, accurate RP-HPLC method was developed for the Simultaneous Estimation of Losartan Potassium and Amlodipine Besilate in tablet Dosage form. After optimization the good chromatographic separation was achieved by Isocratic mode with a mixture of Acetonitrile: Phosphate Buffer pH 6 in the ratio of 50:50 as the mobile phase with Waters C18 (150 x 4.6 mm I.D) 3.5 µm, column as stationary phase at flow rate of 0.8 mL/min and detection wavelength of 230 nm. The retention times for Losartan and Amlodipine found to be 2.203 min and 3.283 min respectively. The linearity of this method was found in the concentration range of 40 µg/mL to 140 µg/mL for Losartan and 4 µg/mL to 14 µg/mL for Amlodipine. The correlation coefficient R² value is found to be 0.997 for Losartan and 0.996 for Amlodipine. The LOD and LOQ for Losartan were found to be 0.09 µg/mL and 0.27 µg/mL respectively. The LOD and LOQ for Amlodipine were found to be 0.07 µg/mL and 0.23 µg/mL respectively. This method was found to be good percentage recovery for Losartan and Amlodipine were found to be 100.86 and 99.81 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage form. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, specificity and Robustness.

Keywords: UV spectrophotometer, Losartan Potassium, Amlodipine Besilate, High performance liquid chromatography

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Introduction

Losartan potassium chemically known as Lorzaar, Lorzaprex. IUPAC name: 2-Butyl-4-chloro-1-[p-(o-lH-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol, mono potassium salt. Mechanism of action of Losartan potassium is non-peptide angiotensin II receptor antagonist which blocks the action of angiotensin II that lead to decrease sympathetic activity, decrease the renal absorption of water and decrease the peripheral resistance that lead to decrease blood pressure. It is an antagonist of angiotensin type 1 receptor with antihypertensive activity. Amlodipine besilate IUPAC name: 2-[(2-Aminoethoxy) methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxy carbonyl-6-methyl-1, 4-dihydropyridine benzenesulfonate. The main mechanism is it is a calcium channel blocker. It specifically blocks the calcium influx. So smooth muscle undergoes relaxation that lead to decrease in the peripheral resistance resulting in decrease in blood pressure. It is effective in the treatment of angina pectoris and hypertension [1-4]. Several analytical methods [5: 18] for the determination of Losartan potassium and Amlodipine besylate by HPTLC, RP-HPLC, SPECTROPHOTOMETRY, UV SPECTROPHOTOMETRY, LC-MS/MS.

Experimental

Losartan potassium and Amlodipine besilate bulk drug purchased from sigma Aldrich. Amlodipine and Losartan tablets Obtained from local pharmacy. HPLC grade Acetonitrile, HPLC grade water, HPLC grade methanol purchased from Merck. Potassium Dihydrogen ortho phosphate/AR grade and Triethylamine/AR grade purchased from Rankem. UV –Visible Spectrophotometer from analytical Technologies Ltd. HPLC from Cyberlab (Salo Terrace, Millbury, USA), Ultra-
sonicator from Citizen, Digital Ultrasonic Cleaner, pH meter from Elico, Electronic balance from Shimadzu, Syringe from Hamilton, HPLC Column Waters C18(150x4.6 ID) 3.5µm.

Preparation of mobile phase
Preparation of Phosphate buffer pH 6
Place 50 mL of 0.2M Potassium dihydrogen phosphate in a 200 mL volumetric flask, add 5.6 mL of 0.2M Sodium hydroxide, and then add water to the volume.

Preparation of standard stock solution
Weighed accurately 50 mg of Losartan and 5 mg of Amlodipine in 50 mL of volumetric flask and made up the volume with mobile phase. From above stock solution 80 µg/mL of Losartan and 8 µg/mL of Amlodipine is prepared by diluting 0.8 mL to 10 mL with mobile phase. This solution is used for recording chromatogram.

Preparation of Marketed formulation
Twenty tablets were weighed accurately and powdered. A quantity of powder equivalent to 50mg of Losartan Potassium and 5mg of Amlodipine Besilate in 50 mL volumetric flask and make up mark with mobile phase. From above solution Pipette 0.8 mL of the clear solution in to 10 mL volumetric flask and make up volume with mobile phase. The resulting solution is used to record the chromatogram.

Optimisation of the Method
Preparation of samples for Assay
Preparation of Standard solution
About 100 mg of Losartan Potassium and 10 mg of Amlodipine Besilate were weighed into a 100 mL volumetric flask, to this 25mL of mobile phase was added, sonicated and the volume was made up with the mobile phase.

Preparation of Sample solution
Sample name: ZILOS AM
Manufacturer name: FDC
Twenty tablets were weighed accurately and powdered. A quantity of powder equivalent to 50mg of Losartan Potassium and 5mg of Amlodipine Besilate in 50 mL volumetric flask and make up mark with mobile phase. From above solution Pipette 0.8 mL of the clear solution in to 10 mL volumetric flask and make up volume with mobile phase. The resulting solution is used to record the chromatogram.

System Suitability
To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 80 µg/mL of Losartan and 8 µg/mL of Amlodipine were injected six times and the chromatograms were recorded for the same.

System precision
The system precision was determined by analysing standard preparation of Losartan (80 µg/mL) and Amlodipine (8 µg/mL) for six times.

Method precision
Method precision was determined by injecting sample solutions of concentration Losartan (80 µg/mL) and Amlodipine (8 µg/mL) for six times are prepared separately. The chromatograms were recorded and the results were summarized.

Linearity and Range
Preparation of standard stock solution and working solution
Standard stock solutions of Losartan and Amlodipine (mg/mL) were prepared by dissolving 100 mg of Losartan and 10 mg of Amlodipine in 100 mL of mobile phase. After that filtered the solution using 0.45 micron syringe filter and Sonicated for 5 min further dilutions were prepared for Losartan in the range of 40-140 µg/ml and for Amlodipine in the range of 4-14 µg/ml.

Limit of Detection and Limit of Quantitation
LOD and LOQ is calculated from standard deviation of response from precision and slope from linearity
LOQ = 10 σ / S
LOD = 3.3 σ / S
Where
σ is standard deviation from response
S is slope from calibration curve

Specificity
Preparation of analytical solutions
Preparation of blank solution
Acetonitrile: Buffer pH 6 (50:50) v/v was taken as blank and the chromatogram was recorded.

Preparation of standard solution
Standard stock solutions of Losartan and Amlodipine (mg/mL) were prepared by dissolving 100 mg of Losartan and 10 mg of Amlodipine in 100 mL of mobile phase. The above solution is filtered by using 0.45-micron syringe filter and Sonicated for 5 min. Further dilution of 80 µg/mL of Losartan and 8 µg/mL was made by adding 0.8 mL of stock solution to 10 mL of mobile phase. The standard solution was injected and the chromatogram was recorded.

Accuracy
Accuracy of the method was determined by Recovery studies. To the formulation (pre analysed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug.

Preparation of spiking standard solution
About 100 mg of Losartan Potassium and 10 mg of Amlodipine Besilate were weighed into a 100 mL volumetric flask, to this 25mL of mobile phase was added, sonicated and the volume was made up with the mobile phase. 0.8 mL of this solution was diluted to 10 mL with the mobile phase. 20 µL of the solution was injected three times and chromatograms were recorded for the same.

Preparation of 80% sample solution
About 1 mL of spiking standard solution was taken to this 0.6 mL of test stock solution was added and the volume was made up to 10 mL with the mobile phase to get a concentration of 68 µg/mL of Losartan Potassium and 6.8 µg/mL of Amlodipine Besilate respectively. 20 µL of the solution was injected three times and chromatograms were recorded and results of the chromatogram obtained.

Preparation of 100% sample solution
About 1 mL of spiking standard solution was taken to this 0.8 µL of test stock solution was added and the volume was made up to 10 mL with the mobile phase to get a concentration of 88 µg/mL of Losartan Potassium and 8.8 µg/mL of Amlodipine Besilate respectively. 20 µL of the solution was injected three times and chromatograms were recorded and results of the chromatogram obtained.

Preparation of 120% sample solution
About 1 mL of spiking standard solution was taken to this 0.9 mL of test stock solution was added and the volume was made up to 10 mL with the mobile phase to get a concentration of 108 µg/mL of Losartan and 10.8 µg/mL of Amlodipine respectively. 20 µL of the solution was injected three times and chromatograms were recorded and results of the chromatogram obtained.

Robustness
The Robustness of the method was determined under different conditions including change in flow rate, wave length the chromatograms were recorded for changes in flow rate and the results of the chromatograms. Chromatograms for variations in wavelength were observed. The results obtained by deliberate variation in method parameters are summarized.
Results and Discussion
The peak area for Losartan and Amlodipine were 3311.412 and 151.699. The % assay of Losartan and Amlodipine were 98.96 and 98.54. So the both drugs % assay found to be within the limits.

System Suitability
System suitability parameters obtained for Losartan were theoretical plates 3215, tailing factor 1.476, % RSD for retention time and peak areas were 0.07 and 0.03 respectively and for Amlodipine were theoretical plates 4227, tailing factor 1.391, % RSD for retention time and peak areas were 0.34 and 0.29 respectively.

System precision
The chromatograms were recorded and the results were summarized. % RSD of retention time and peak areas obtained for losartan were 0.07 and 0.03 respectively and for amlodipine were 1.33 and 0.33 respectively.

Limit of Detection and Limit of Quantitation
The LOD for this method was found to be 0.09 µg/mL for Losartan and 0.07 µg/mL for Amlodipine respectively. The LOQ for this method was found to be 0.27 µg/mL for Losartan and 0.23 µg/mL for Amlodipine respectively.

Specificity
The retention times for Losartan and amlodipine were found to be 2.210 and 3.218 min respectively.

Mobile phase
Acetonitrile: Buffer PH 6 (50:50) v/v

Column
waters C18 (150x4.6 ID) 3.5 µm

Flow rate
0.8 mL/min

Column temperature
Room temperature (20-25°C)

Sample temperature
Room temperature (20-25°C)

Wavelength
230 nm

Injection volume
20 µL

Run time
5 min

Retention time
2.2 min for Losartan and 3.2 min for Amlodipine

Table 1: Optimisation Results

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Volume from standard stock transferred in mL</th>
<th>Volume made up in mL (with mobile phase)</th>
<th>Conc. obtained (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Losartan</td>
<td>Amlodipine</td>
</tr>
<tr>
<td>Preparation 1</td>
<td>0.4</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Preparation 2</td>
<td>0.6</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>Preparation 3</td>
<td>0.8</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>Preparation 4</td>
<td>1.0</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Preparation 5</td>
<td>1.2</td>
<td>10</td>
<td>120</td>
</tr>
<tr>
<td>Preparation 6</td>
<td>1.4</td>
<td>10</td>
<td>140</td>
</tr>
</tbody>
</table>

Table 2: Result Of Linearity Of Losartan Potassium And Amlodipine Besilate

<table>
<thead>
<tr>
<th>Conc</th>
<th>Amount present(µg/mL)</th>
<th>Amount added(µg/mL)</th>
<th>Amount found(µg/mL)*</th>
<th>%Recovery *</th>
<th>%mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>60</td>
<td>8</td>
<td>68.52</td>
<td>100.79</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>80</td>
<td>8</td>
<td>88.31</td>
<td>100.35</td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>100</td>
<td>8</td>
<td>109.57</td>
<td>101.45</td>
<td>100.86</td>
</tr>
</tbody>
</table>

Table 3: Results For Recovery Studies Of Losartan Potassium
### Table 4: Results for Recovery Studies of Amlodipine Besilate

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Amount present (µg/mL)</th>
<th>Amount added (µg/mL)</th>
<th>Amount found (µg/mL)*</th>
<th>%Recovery *</th>
<th>%mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>6</td>
<td>0.8</td>
<td>6.79</td>
<td>99.82</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>8</td>
<td>0.8</td>
<td>8.77</td>
<td>99.65</td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>10</td>
<td>0.8</td>
<td>10.80</td>
<td>99.97</td>
<td>99.81</td>
</tr>
</tbody>
</table>

### Table 5: Results for Robustness of Losartan and Amlodipine

<table>
<thead>
<tr>
<th>Chromatographic changes</th>
<th>Retention time (min)</th>
<th>Tailing factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LP</td>
<td>AB</td>
</tr>
<tr>
<td>Flow rate (mL/min)</td>
<td>0.7</td>
<td>2.50</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>2.21</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>1.98</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>228</td>
<td>2.207</td>
</tr>
<tr>
<td></td>
<td>230</td>
<td>2.21</td>
</tr>
<tr>
<td></td>
<td>232</td>
<td>2.20</td>
</tr>
</tbody>
</table>

### Table 6: Results for Method Precision for Losartan and Amlodipine

<table>
<thead>
<tr>
<th>Injection</th>
<th>Losartan</th>
<th>Amlodipine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention times</td>
<td>Area</td>
</tr>
<tr>
<td>1</td>
<td>2.207</td>
<td>3310.025</td>
</tr>
<tr>
<td>2</td>
<td>2.207</td>
<td>3312.4</td>
</tr>
<tr>
<td>3</td>
<td>2.207</td>
<td>3312.508</td>
</tr>
<tr>
<td>4</td>
<td>2.207</td>
<td>3312.525</td>
</tr>
<tr>
<td>5</td>
<td>2.21</td>
<td>3312.600</td>
</tr>
<tr>
<td>6</td>
<td>2.21</td>
<td>3312.600</td>
</tr>
<tr>
<td>Average</td>
<td>2.208</td>
<td>3312.081</td>
</tr>
<tr>
<td>SD</td>
<td>0.0015</td>
<td>1.010</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.07</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Citation: Nalini Kanta Sahoo et al. (2019), RP-HPLC Method Development and Validation for Simultaneous Estimation of Losartan Potassium and Amlodipine Besilate in Bulk & Marketed Formulation. Adv Res Chem & App Sci. 1:1, 2-8
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<table>
<thead>
<tr>
<th>Injection</th>
<th>Losartan</th>
<th>Amlodipine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention times</td>
<td>Area</td>
</tr>
<tr>
<td>1</td>
<td>2.21</td>
<td>3310.082</td>
</tr>
<tr>
<td>2</td>
<td>2.207</td>
<td>3311.465</td>
</tr>
<tr>
<td>3</td>
<td>2.207</td>
<td>3312.425</td>
</tr>
<tr>
<td>4</td>
<td>2.207</td>
<td>3312.506</td>
</tr>
<tr>
<td>5</td>
<td>2.207</td>
<td>3311.633</td>
</tr>
<tr>
<td>6</td>
<td>2.210</td>
<td>3310.543</td>
</tr>
<tr>
<td>Average</td>
<td>2.208</td>
<td>3311.442</td>
</tr>
<tr>
<td>SD</td>
<td>0.0015</td>
<td>0.979</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.07</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 7: Results For System Precision For Losartan And Amlodipine

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Name</th>
<th>RT</th>
<th>Height</th>
<th>Area</th>
<th>TP</th>
<th>TF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Losartan</td>
<td>2.207</td>
<td>570.143</td>
<td>3311.465</td>
<td>3330</td>
<td>1.476</td>
</tr>
<tr>
<td>2</td>
<td>Amlodipine</td>
<td>3.210</td>
<td>22.090</td>
<td>151.668</td>
<td>4107</td>
<td>1.462</td>
</tr>
</tbody>
</table>

Table 8: Results for Losartan and Amlodipine in tablet formulation

**FIG 1: Structure Of Losartan Potassium**

**FIG 2: Structure Of Amlodipine Besilate**
Citation: Nalini Kanta Sahoo et al. (2019), RP-HPLC Method Development and Validation for Simultaneous Estimation of Losartan Potassium and Amlodipine Besilate in Bulk & Marketed Formulation. Adv Res Chem & App Sci. 1:1, 2-8

FIG 3: CHROMATOGRAM OF STANDARD DRUG OF LOSARTAN POTASSIUM AND AMLODIPINE BESILATE

FIG 4: Chromatogram Of Marketed Samples Of Losartan Potassium And Amlodipine Besilate

FIG 5: LINEARITY CURVE OF LOSARTAN POTASSIUM

\[ y = 35.428x + 382.88 \]
\[ R^2 = 0.9976 \]
Conclusion
From the above experimental results and parameters it was concluded that, the chromatographic method developed for the simultaneous estimation of Losartan and Amlodipine was found to be precise, accurate, sensitive and rapid and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

References