A Validated Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Aliskiren Hemifumarate and Amlodipine Besylate in Pharmaceutical Dosage Form

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The present study describes the stability indicating RP-HPLC method for simultaneous estimation of aliskiren hemifumarate and amlodipine besylate in pharmaceutical dosage forms. The proposed RP-HPLC method was developed by using waters 2695 separation module equipped with PDA detector and chromatographic separation was carried on C-8 Inertsil ODS (150 × 4.6 mm, 5 μ) column at a flow rate of 1 mL/min and the run time is 10 min. The mobile phase consisted of phosphate buffer and acetonitrile in the ratio of 40: 60% v/v and pH was adjusted to 3 with orthophosphoric acid and eluents were scanned using PDA detector at 237 nm. The retention time of aliskiren and amlodipine was found to be 3.98 and 5.14 min, respectively. A linearity response was observed in the concentration range of 30–225 μg/mL for aliskiren and 2–15 μg/mL for amlodipine, respectively. Limit of detection and limit of quantification for aliskiren are 0.161 μg/mL and 0.489 μg/mL and for amlodipine are 0.133 μg/mL and 0.404 μg/mL, respectively. The stability indicating method was developed by subjecting the drugs to stress conditions such as acid and base hydrolysis, oxidation, and photo- and thermal degradation and the degraded products formed were resolved successfully from the samples.

1. Introduction

Aliskiren is a novel antihypertensive agent and is the first orally active rennin inhibitor indicated for the treatment of hypertension [1–3]. Chemically, aliskiren is (2S, 4S, 5S, 7S)-N-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy) phenyl]-octanamide [4] (Figure 1(a)). Renin is secreted by the kidney, which cleaves the angiotensinogen to form angiotensin I and is then converted into angiotensin II by angiotensinogen converting enzyme. Aliskiren inhibits the catalytic activity of rennin system and inhibits the generation of angiotensin I and angiotensin II [5–7].

Amlodipine is a member of 1, 4-dihydropyridine class of calcium antagonist approved for the treatment of heart diseases like hypertension and angina pectoris. It is a long acting calcium channel blocker that inhibits the influx of calcium ions into the vascular smooth muscle and cardiac muscle [8, 9]. Chemically amlodipine is 3-ethyl-5-methyl-2-[2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydropyridine-6-methyl-3,5-dicarboxylate [10] (Figure 1(b)).

Through literature survey reveals that there are few analytical methods such as RP-HPLC [11, 12] and UV methods [13, 14] are reported for simultaneous estimation of aliskiren and amlodipine in pharmaceutical dosage forms. But so far there is no stability indicating method reported. Therefore the present investigation was carried out to develop new simple, precise, rapid, and cost-effective stability indicating RP-HPLC method for the simultaneous estimation of aliskiren and amlodipine in pharmaceutical dosage form.
The proposed method was used successfully to separate the degraded products from the samples.

2. Experimental

2.1. Reagents and Chemicals. Aliskiren and amlodipine standards were provided from Spectrum Research Laboratory, Hyderabad, and commercial tablet dosage form TEKEMLO was purchased from local market. The HPLC grade acetonitrile and water were purchased from Merck and analytical grade potassium dihydrogen phosphate was purchased from RANKEM. Analytical grade triethylamine, orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were purchased from S.D. Fine Chemicals.

2.2. HPLC Instrument. The chromatographic separation was carried out by waters 2695 HPLC system separation module (Waters Corporation, Milford, USA) equipped with PDA detector and autosampler. The Empower 2 software was used for signal monitoring and processing. UV chamber has been used for photolytic degradation and hot air oven was employed for thermal degradation.

2.3. Chromatographic Conditions. The chromatographic separation of analytes was carried out using waters 2695 RP-HPLC system with C-8 Inertsil ODS (150 × 4.6 mm, 5μ) column. The mobile phase consists of phosphate buffer and acetonitrile in the ration of 40:60% v/v and pH was adjusted to 3 with orthophosphoric acid solution that was used to separate the analytes and column temperature was maintained at 30°C. The analytes were detected at 237 nm using PDA detector. The run time was set at 10 min at a flow rate of 1 mL/min.

2.4. Preparation of Standard Stock Solution. Standard stock solutions of aliskiren and amlodipine were prepared separately by dissolving 50 mg of aliskiren and 10 mg of amlodipine in 10 mL volumetric flasks with water:acetonitrile (50:50% v/v) as diluent and sonicated for 5 min. From the above solution transfer 0.3 mL of aliskiren and 0.1 mL of amlodipine separately into 10 mL volumetric flasks and make up the volume with diluent to get 150 μg/mL of aliskiren and 10 μg/mL of amlodipine standard stock solution.

2.5. Preparation of Sample Solution. Five tablets (TAKEML tablets: 150 mg aliskiren and 10 mg amlodipine) were weighed and the average weight of each tablet was calculated; then the weight equivalent to 5 tablets was transferred into a 250 mL volumetric flask; 60 mL of diluent was added and sonicated for 25 min; further the volume was made up with diluent and filtered. From the filtered solution 0.5 mL was pipetted out into a 10 mL volumetric flask and made up to 10 mL with diluent.
2.6. Forced Degradation Studies. Forced degradation studies of the drug formulation were carried out by treating the drug samples under stress induced conditions like acid and base hydrolysis, oxidation, and photo- and thermal degradation and interference of the degraded products was investigated. These studies help to know the inherent stability characteristics of the active molecules in drug product and the possible degradation products [15].

2.6.1. Acid Degradation Studies. To 1mL stock solution of aliskiren and amlodipine, 1mL of 2N hydrochloric acid was added and refluxed for 30 mins at 60°C. The resultant solution was diluted to obtain 150μg/mL and 10μg/mL solution and 10μL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

2.6.2. Alkali Degradation Studies. To 1mL stock solution of aliskiren and amlodipine, 1mL of 2N sodium hydroxide was added and refluxed for 30 min at 60°C. The sample solution was prepared to obtain the concentration of 150μg/mL and 10μg/mL solution and 10μL was injected into the system and the chromatograms were recorded to assess the stability of sample.

2.6.3. Oxidation. To 1mL stock solution of aliskiren and amlodipine, 1mL of 20% hydrogen peroxide (H₂O₂) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the sample solution was prepared to obtain the concentration of 150μg/mL and 10μg/mL solution and 10μL was injected into the system and the chromatograms were recorded to assess the stability of sample.

2.6.4. Photostability Studies. The photochemical stability of the drug was also studied by exposing the 150μg/mL and 10μg/mL solution to UV light by keeping the beaker in UV chamber for 7 days or 200 watt hours/m² in photostability chamber. For HPLC study, the sample solution was prepared to obtain the concentration of 150μg/mL and 10μg/mL solution and 10μL was injected into the system and the chromatograms were recorded to assess the stability of sample.

2.6.5. Dry Heat Degradation Studies. The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the sample solution was prepared to obtain the concentration of 150μg/mL and 10μg/mL solution and 10μL was injected into the system and the chromatograms were recorded to assess the stability of sample.

Table 1: Optimized chromatographic conditions.

<table>
<thead>
<tr>
<th>S. number</th>
<th>Parameter</th>
<th>Optimized condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Column</td>
<td>Inertsil ODS C-8 (150 × 4.6 mm, 5μ) column</td>
</tr>
<tr>
<td>2</td>
<td>Mobile phase</td>
<td>Phosphate buffer pH 3 and acetonitrile in the ration of 40:60% v/v</td>
</tr>
<tr>
<td>3</td>
<td>Flow rate</td>
<td>1mL/min</td>
</tr>
<tr>
<td>4</td>
<td>Detector</td>
<td>PDA detector at 237 nm</td>
</tr>
<tr>
<td>5</td>
<td>Injection volume</td>
<td>10μL</td>
</tr>
<tr>
<td>6</td>
<td>Temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>7</td>
<td>Retention time</td>
<td>Aliskiren 3.98 min and amlodipine 5.14 min</td>
</tr>
</tbody>
</table>

Table 2: System suitability parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Aliskiren</th>
<th>Amlodipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>3.98</td>
<td>5.14</td>
</tr>
<tr>
<td>USP plate count</td>
<td>3545</td>
<td>4743</td>
</tr>
<tr>
<td>USP tailing</td>
<td>1.33</td>
<td>1.25</td>
</tr>
</tbody>
</table>

3. Results and Discussions

3.1. Method Development. A series of trials was conducted with different columns like Inertsil ODS and agilent XDB C-18 and C-8 columns with different mobile phases to develop a suitable RP-HPLC method for estimation of aliskiren hemifumarate and amlodipine besylate in tablet dosage form, and finally a typical chromatogram was obtained with phosphate buffer and acetonitrile in the ration of 40:60% v/v and pH was adjusted to 3 with orthophosphoric acid at a flow rate of 1mL/min. The chromatographic separation was performed on C-8 Inertsil ODS (150 × 4.6 mm, 5μ) by injecting 10μL and analytes were detected with PDA detector at 237 nm. The retention time of aliskiren and amlodipine was found to be 3.98 min and 5.14 min, respectively. Forced degradation studies were also carried using the developed method and the degraded compounds were effectively resolved from the aliskiren and amlodipine in tablet dosage form. The optimized conditions were given in Table 1.

3.2. Method Validation. The validation was performed with above developed RP-HPLC method for simultaneous estimation of aliskiren and amlodipine according to ICH guidelines. Various parameters were evaluated such as system suitability, precision, accuracy, linearity, robustness, LOD, and LOQ [16].

3.2.1. System Suitability. System suitability was performed to verify the acceptability of the resolution and repeatability of the system. System suitability was performed by injecting six replicate injections of the standard solution (100%) and parameters such as peak area, USP tailing, theoretical plates, retention time, and peak asymmetry were evaluated. The % RSD was determined and reported within the limits. The results were shown in Table 2.
3.2.2. Accuracy. The accuracy of the proposed method was evaluated by calculating the recovery studies of the test drug at three different concentration levels (50%, 100%, and 150%) by standard addition method. A known amount of aliskiren and amlodipine was added to prequantified sample solution and three replicates of each concentration were injected in developed chromatographic conditions. The mean percentage recovery of aliskiren and amlodipine was varied between 99.99 and 101.7% indicating that the developed method was found to be accurate. The % recovery results were shown in Table 3.

3.2.3. Precision. The precision of an analytical procedure may be defined as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The method precision and system precision studies were carried out by injecting six replicates \( (n = 6) \) of both standard and test solutions with the same concentration. The % RSD was calculated from the chromatograms and results obtained were within the limits of 2% and proposed method was found to be precise. The precision data was given in Table 4.

3.2.4. Linearity. The linearity of the method was determined at different concentration levels ranging from 30 to 225 \( \mu \)g/mL of aliskiren and from 2 to 15 \( \mu \)g/mL of amlodipine. All the concentrations were prepared and injected into the system. The linearity curve was constructed by plotting peak area versus concentration of the analyte. From the results obtained the proposed method was found to be linear. The regression coefficient \( (r^2) \) was found to be 0.9990 for both aliskiren and amlodipine.

3.2.5. LOD and LOQ. In the present study the LOD and LOQ of aliskiren and amlodipine were evaluated based on the standard calibration curve method. Limit of detection is performed to know the lowest concentration level of the analytes that gives measurable response. The LOD was found to be 0.1614 \( \mu \)g/mL and 0.1336 \( \mu \)g/mL and LOQ was 0.4890 \( \mu \)g/mL and 0.4049 \( \mu \)g/mL for aliskiren and amlodipine, respectively.

3.2.6. Robustness. Robustness of the proposed method has been evaluated by small deliberate changes in the system parameters such as flow rate, mobile phase composition, pH of the mobile phase, and temperature. It was found that none of the above parameters caused alteration in the peak area, retention time, and USP tailing by small changes like \( \pm 0.1 \) mL change in flow rate, \( \pm 5\% \) change in mobile phase, and \( \pm 5^\circ C \) change in temperature. The % RSD was found to be within the limits and the method was found to be robust. The robustness results were shown in Table 5.

3.3. Assay of Marketed Formulation. Analysis of marketed formulation (TAKEMLO tablets, 150 mg aliskiren and 10 mg of amlodipine, Novartis, Mumbai, India) was purchased from local market. Five tablets were weighed and average weight was calculated; weight equivalent to 5 tablets was transferred into a 250 mL volumetric flask, 60 mL of diluent was added and sonicated for 25 min, and further the volume was made up with diluent and filtered. From the filtered solution 0.5 mL was pipetted out into a 10 mL volumetric flask and made up to 10 mL with diluent. From the resulting solution 0.5 mL was injected into HPLC system and peak areas were recorded. The % assay of the marketed formulation was found to be 99.15% for aliskiren and 99.87% for amlodipine (Table 6).

3.4. Forced Degradation Studies. In the present study forced degradation studies were carried out to ensure the effective separation of aliskiren and amlodipine from degradation products. Degradation was observed by decreasing the peak

### Table 3: Percentage recovery results of aliskiren and amlodipine.

<table>
<thead>
<tr>
<th>Spiked level</th>
<th>Aliskiren</th>
<th>Amlodipine</th>
<th>Mean percentage recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>99.45</td>
<td>101.07</td>
<td>100.38</td>
<td>0.988</td>
</tr>
<tr>
<td></td>
<td>101.37</td>
<td>100.38</td>
<td></td>
<td>0.682</td>
</tr>
<tr>
<td></td>
<td>100.85</td>
<td>99.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>100.09</td>
<td>100.72</td>
<td>100.60</td>
<td>0.518</td>
</tr>
<tr>
<td></td>
<td>99.65</td>
<td>100.78</td>
<td></td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td>99.06</td>
<td>100.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>99.09</td>
<td>100.10</td>
<td>99.82</td>
<td>0.661</td>
</tr>
<tr>
<td></td>
<td>100.41</td>
<td>99.67</td>
<td></td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>99.77</td>
<td>99.71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Results of method precision for aliskiren and amlodipine.

<table>
<thead>
<tr>
<th>Sample ((n = 6))</th>
<th>Aliskiren</th>
<th>Amlodipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.52</td>
<td>98.93</td>
</tr>
<tr>
<td>2</td>
<td>100.25</td>
<td>99.07</td>
</tr>
<tr>
<td>3</td>
<td>100.32</td>
<td>100.00</td>
</tr>
<tr>
<td>4</td>
<td>99.78</td>
<td>102.25</td>
</tr>
<tr>
<td>5</td>
<td>99.12</td>
<td>99.06</td>
</tr>
<tr>
<td>6</td>
<td>99.29</td>
<td>100.28</td>
</tr>
<tr>
<td>AVRG</td>
<td>99.71</td>
<td>99.93</td>
</tr>
<tr>
<td>SD</td>
<td>0.495</td>
<td>1.265</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.497</td>
<td>1.265</td>
</tr>
</tbody>
</table>
**Table 5: Results of robustness.**

<table>
<thead>
<tr>
<th>S. number</th>
<th>Parameters</th>
<th>Aliskiren</th>
<th>Amlodipine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT USPC TF</td>
<td>RT USPC TF</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Flow rate 0.8 mL</td>
<td>4.38 3926 1.29</td>
<td>5.58 4912 1.18</td>
</tr>
<tr>
<td></td>
<td>Flow rate 1.2 mL</td>
<td>3.68 3581 1.23</td>
<td>4.68 4441 1.15</td>
</tr>
<tr>
<td>2</td>
<td>Temperature 25°C</td>
<td>3.83 3675 1.26</td>
<td>4.81 4821 1.18</td>
</tr>
<tr>
<td></td>
<td>Temperature 35°C</td>
<td>3.93 3923 1.29</td>
<td>4.81 4821 1.18</td>
</tr>
<tr>
<td>3</td>
<td>Mobile phase (−5%)</td>
<td>3.47 3205 1.17</td>
<td>4.36 3668 1.05</td>
</tr>
<tr>
<td></td>
<td>Mobile phase (+5%)</td>
<td>4.40 3625 1.21</td>
<td>5.61 4322 1.09</td>
</tr>
</tbody>
</table>

PA: peak area; RT: retention time (min); USPC-USP plate count; TF: tailing factor.

**Table 6: Assay % of marketed formulation.**

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Drug</th>
<th>Dosage</th>
<th>Amount found</th>
<th>% assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAKEMLO</td>
<td>Aliskiren</td>
<td>150 mg</td>
<td>148.72 mg</td>
<td>99.15</td>
</tr>
<tr>
<td></td>
<td>Amlodipine</td>
<td>10 mg</td>
<td>9.98 mg</td>
<td>99.88</td>
</tr>
</tbody>
</table>

**Figure 2: Chromatograms of forced degradation studies.**

(a) Chromatogram of aliskiren and amlodipine without degradation

(b) Acid degradation

(c) Base degradation

(d) Peroxide degradation

(e) Thermal degradation

(f) Photodegradation
areas of the drug substances with same drug molecules of degraded peak areas. The percentage assay of degradation was calculated from the peak area obtained in degradation conditions and it was compared with assay of nondegraded conditions. Acidic and alkali degradation was carried out by treating the sample solution with 2N HCl and 2N NaOH solutions. From the chromatograms (Figure 2), it was found that both the molecules are susceptible to acidic and alkali degradation and percentage assay degradation in both acidic and alkali conditions was found to be within the limits. Oxidative degradation studies were performed by treating 20% H$_2$O$_2$ solution and keeping it at 60°C for 30 min. The results showed that there were no degradation products formed. For thermal stress studies the drug solutions were placed in oven at 105°C for 6 h and then injected into HPLC system and photostress testing was carried out by keeping the drug solutions in UV chamber for 7 days. In all the conditions the purity of angle is found to be less than that of purity of threshold which indicates that the developed method was stability indicating. The forced degradation studies were performed without intending to identify the degradation products but merely to show that they are not interfering with active molecules if any present. The results of stress studies were shown in Table 7.

### Table 7: Forced degradation studies of aliskiren and amlodipine.

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Aliskiren</th>
<th>Amlodipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>% degradation</td>
<td>Purity of angle</td>
<td>Purity of threshold</td>
</tr>
<tr>
<td>Acid degradation</td>
<td>9.7%</td>
<td>0.436</td>
</tr>
<tr>
<td>Alkali degradation</td>
<td>9.12%</td>
<td>0.513</td>
</tr>
<tr>
<td>Oxidative degradation</td>
<td>8.22%</td>
<td>0.429</td>
</tr>
<tr>
<td>Photolytic degradation</td>
<td>1.31%</td>
<td>0.305</td>
</tr>
<tr>
<td>Thermal degradation</td>
<td>8.05%</td>
<td>0.420</td>
</tr>
</tbody>
</table>

### 4. Conclusion

In the present study, a stability indicating RP-HPLC method has been developed and validated for simultaneous estimation of aliskiren and amlodipine in tablet dosage form. The validated method has been successfully used for stress testing analysis of aliskiren and amlodipine. The stress testing studies revealed that the method was successfully employed to resolve the degraded products from the sample. From the peak purity profile it was demonstrated that there was no interference of degradation products and the purity of angle was found to be less than the purity of threshold. The proposed method was proved to be selective, accurate, precise, and rapid and it can be used for the routine analysis of the aliskiren and amlodipine in the formulation.

### Conflict of Interests

The authors confirm that this paper has no conflict of interests.

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### References


