HCT in tablet formulation was developed and validated as per ICH guidelines. The method uses simple reagents and sample preparation procedures.

**Conclusion:**

Quantification was achieved with PDA detection at 280 nm. Validation parameters of the proposed method such as specificity, linearity, accuracy, precision and robustness were evaluated according to ICH guidelines.

**Results:**

Linear concentration range was between 1.2-240 µg/ml for ALI and 0.1-20 µg/ml for HCT and correlation coefficient was found to be 0.9995 and 0.9998, respectively. The limit of detection and limit of quantification for ALI was found to be 0.3376 and 1.0230 µg/ml and for HCT 0.0288 and 0.0873 µg/ml, respectively. The results of precision (% RSD<2) studies showed good reproducibility. Recovery study was performed at 50, 100 and 150 % level to check the interferences between analytes and formulation excipients and % recovery was found to be 99.49±0.9868 for ALI and 99.87±0.8556 for HCT. The percentage assay was found to be 100.36±0.9201 and 99.40±0.7624 for ALI and HCT, respectively.

**Conclusion:**

A simple, rapid, cost effective and highly sensitive RP-HPLC method as compared to existing methods for the determination of ALI and HCT in tablet formulation was developed and validated as per ICH guidelines. The method uses simple reagents and sample preparation procedures were minimal. Thus, the proposed method can be applied for routine quality control determination of ALI and HCT in tablet formulation.

**Keywords:** Aliskiren hemifumarate, Hydrochlorothiazide, RP-HPLC, Tablet formulation.

**INTRODUCTION**

Aliskiren hemifumarate (ALI) is chemically described as (2S,4S,5S,7S)-N-(2-methylpropyl) 5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octamide hemifumarate, a rennin inhibitor used for the treatment of essential hypertension. Hydrochlorothiazide (HCT), chemically 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonyl-1,1-dioxide, is a thiazide diuretic used in the management of hypertension. Chemical structures of both the drugs are shown in fig. 1.

**Fig. 1:** Chemical structures of ALI (aliskiren hemifumarate) and HCT (hydrochlorothiazide)

Hypertension is one of the most common and powerful risk factors for cardiovascular diseases. Blood pressure control is pre requisite for the management of cardiovascular diseases and complications. More than one medication is recommended for control of blood pressure of the cardiovascular patients. ALI is the first representative of a new class of non peptid, low molecular weight, orally active transition state rennin inhibitor shows the effective control of blood pressure and cardiovascular diseases when combined with HCT, a thiazide diuretic [1-3].

**ABSTRACT**

**Objective:** Development and validation of quantitave simultaneous determination of aliskiren hemifumarate (ALI) and hydrochlorothiazide (HCT) in combined tablet formulation by RP-HPLC.

**Methods:** The separation of components were achieved on Enable C18 column (250×4.6 mm, 5 µm) with a mobile phase consisting of 0.2 %v/v triethylamine in water (pH 6 was adjusted with orthophosphoric acid): methanol (10:90 %v/v) at a flow rate of 1 ml/min was employed. Quantification was achieved with PDA detection at 280 nm. Validation parameters of the proposed method such as specificity, linearity, accuracy, precision and robustness were evaluated according to ICH guidelines.

**Results:** Linear concentration range was between 1.2-240 µg/ml for ALI and 0.1-20 µg/ml for HCT and correlation coefficient was found to be 0.9995 and 0.9998, respectively. The limit of detection and limit of quantification for ALI was found to be 0.3376 and 1.0230 µg/ml and for HCT 0.0288 and 0.0873 µg/ml, respectively. The results of precision (% RSD<2) studies showed good reproducibility. Recovery study was performed at 50, 100 and 150 % level to check the interferences between analytes and formulation excipients and % recovery was found to be 99.49±0.9868 for ALI and 99.87±0.8556 for HCT. The percentage assay was found to be 100.36±0.9201 and 99.40±0.7624 for ALI and HCT, respectively.

**Conclusion:** A simple, rapid, cost effective and highly sensitive RP-HPLC method as compared to existing methods for the determination of ALI and HCT in tablet formulation was developed and validated as per ICH guidelines. The method uses simple reagents and sample preparation procedures were minimal. Thus, the proposed method can be applied for routine quality control determination of ALI and HCT in tablet formulation.

**Keywords:** Aliskiren hemifumarate, Hydrochlorothiazide, RP-HPLC, Tablet formulation.

**MATERIALS AND METHODS**

**Chemicals and reagents**

AlI hemifumarate reference standard was provided by Jubilant Life Sciences Ltd., Noida, Uttar Pradesh, India and HCT was obtained from Glenmark Pharmaceuticals Ltd., Mumbai, Maharashtra, India. Rasiliz HCT tablets containing 300 mg of ALI along with 25 mg of HCT were purchased from commercial sources. Methanol and triethylamine used were of HPLC grade, 0.2% triethylamine in water (pH 6 with orthophosphoric acid) and methanol as mobile phase, which can be afforded by all the laboratories; fumaric acid was well separated from both the analytes; method describes standard and sample preparation procedure based on the form of analytes under investigation, i.e. aliskiren (13.26 mg of aliskiren hemifumarate is equivalent to 12 mg of aliskiren); wide concentration range with high sensitivity. Moreover, the method can be used for the analysis of ALI and HCT in biological fluids or in pharmacokinetics studies.

**Apparatus and chromatographic condition**

Shimadzu UFLC Prominece (Shimadzu, Kyoto, Japan) equipped with LC-20AD binary pump, SPD-M20A PDA detector and LC
solution as software was used for the analysis. Separation was achieved on Enable C18 column (250×4.6 mm i.d., 5 µm particle size, 120 Å). To protect the analytical column a guard column (C18, 120 Å) was used. Isocratic Shimadzu LC system was operated at 25 °C using a mobile phase consisting of 0.2% triethylamine in water (pH 6 was adjusted using 5 %v/v orthophosphoric acid) and methanol (10:90 %v/v) at a flow rate of 1 ml/min and eluents were monitored at 280 nm using PDA detector with 20 µl injection volume.

Preparation of standard solution
The stock solution of HCT was prepared by weighing accurately 10 mg of HCT standard drug which was then transferred to a 10 ml volumetric flask and diluted to 10 ml with mobile phase to get the concentration of the drug 1000 µg/ml. The mixed standard stock solution of ALI and HCT was prepared by weighing accurately, 13.26 mg (1.326 mg of aliskiren hemifumarate is equivalent to 12 mg of aliskiren) of ALI was mixed with 1 ml of HCT standard solution (1000 µg/ml) in a 10 ml volumetric flask and diluted to 10 ml with mobile phase to get the concentration of the drugs 1200 and 100 µg/ml respectively.

Validation of chromatographic method
Analytical method development and validation plays a major role in the different stages of discovery, development and manufacturing of pharmaceuticals. The developed method was validated in accordance with the International Conference on Harmonization [21] guidelines for validation of analytical procedures.

Specificity
In order to determine the specificity of the method, a placebo solution (in-house mixture of all the tablet excipients) was prepared as described in the Handbook of Pharmaceutical Excipients and was analyzed to evaluate the interference among excipients and drugs peak.

Linearity and range
Linearity and range of the method was checked by analyzing mixed standard solutions containing ALI, 120, 60, 60, 120, 180 and 240 µg/ml and HCT, 5 mg (1000 µg/ml) in a 10 ml volumetric flask and shaken for 10 minutes and volume was made up to the mark with the mobile phase. Contents were filtered through 0.45 µm membrane filter and suitable aliquots were prepared to get desired concentrations (ALI 120 µg/ml and HCT 10 µg/ml).

Preparation of sample solution
Twenty tablets of Rasilez HCT (300 mg of ALI and 25 mg of HCT) were accurately weighed and average weight was calculated. All the tablets were crushed to fine powder and quantity equivalent to 60 mg of ALI and 5 mg of HCT were weighed and transferred to a 50 ml volumetric flask. Flasks was vortexed after adding 30 ml of mobile phase and shaken for 10 minutes and volume was made up to the mark with the mobile phase. Contents were filtered through 0.45 µm membrane filter and suitable aliquots were prepared to get desired concentrations (ALI 120 µg/ml and HCT 10 µg/ml).

RESULTS AND DISCUSSION
The limit of detection and limit of quantification of ALI and HCT was calculated using the following equation as per ICH guidelines.

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S} \\
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where \( \sigma \) is the standard deviation of the response, \( S \) is the slope of the calibration curve.

Robustness
The robustness of an analytical procedure is referred to its ability to remain unaffected by small and deliberate variations in the method parameters. The method should be robust enough with respect to all critical parameters so as to allow routine laboratory use. Robustness of the method was checked on the basis of slight alteration in the wavelength of detection (±2 nm), mobile phase composition (±2 %), flow rate (±0.1 ml/min), buffer pH (±0.5 units) and buffer strength (± 0.1%).

Stability of the solution
Stability of the solutions were checked by observing any changes in the chromatographic pattern compared with freshly prepared solutions by keeping the solutions at room temperature and analyzing at a frequent interval.

Precision
The precision of the method was checked by carrying out repeatability, intra-day and inter-day precision. To check the repeatability of the method a standard mixed solution was injected 6 times and %RSD was calculated. Intra-day precision was carried out by analyzing three replicate injections at two different concentration levels on the same day within the linearity range. Inter-day precision was studied by comparing the results on two different days analyzing three replicate injections at three different concentration levels within the linearity range.

Accuracy
Recovery studies were carried out by the addition of standard drug to pre-analyzed sample solution at three different levels: 50, 100 and 150% to check the accuracy of the method. The resulting solutions were reanalyzed and % recovery was calculated. The result of the accuracy study was assessed based on the percentage of standard ALI and HCT recovered from the formulation.

Robustness
The robustness of an analytical procedure is referred to its ability to remain unaffected by small and deliberate variations in the method parameters. The method should be robust enough with respect to all critical parameters so as to allow routine laboratory use. Robustness of the method was checked on the basis of slight alteration in the wavelength of detection (±2 nm), mobile phase composition (±2 %), flow rate (±0.1 ml/min), buffer pH (±0.5 units) and buffer strength (± 0.1%).

Stability of the solution
Stability of the solutions were checked by observing any changes in the chromatographic pattern compared with freshly prepared solutions by keeping the solutions at room temperature and analyzing at a frequent interval.

System suitability test
System suitability tests were performed to confirm that the instrument was in appropriate condition for the analysis to be performed. Six replicates of the standard solution was injected and chromatograms were recorded to confirm the suitability of the chromatogram.

RESULTS AND DISCUSSION
The objective of the present study was to develop and validate a simple, sensitive and cost effective RP-HPLC method for simultaneous estimation of ALI and HCT in tablet dosage form. Moreover, the other objective of the developed chromatographic method was to obtain better resolution between ALI, HCT and FA (fumaric acid). The chromatographic conditions were optimized to achieve the best resolution and peak shape for both the drugs. The separation of components were achieved on Enable C18 column with a mobile phase consisting of 0.2% triethylamine in water (pH 6 was adjusted with orthophosphoric acid) and methanol (10:90 %v/v) at a flow rate of 1 ml/min was employed with PDA detection at 280 nm. The retention time of ALI and HCT was found to be 5.3±0.0264 and 2.82±0.0027 min, respectively, are shown in fig. 2.

![Fig. 2: RP-HPLC chromatogram of ALI (120 µg/ml) and HCT (10 µg/ml)](image-url)
No interfering peaks were found within the stipulated run time. The developed method was validated as per ICH guidelines. Linearity of the method was established by plotting standard calibration curves between peak area vs concentration. ALI and HCT were found to be linear in the concentration range of 1.2-240 µg/ml and 0.1-20 µg/ml, respectively. As both the analytes under investigation obey Beer’s law in a wide concentration range, developed method can be applied for formulation as well as bio-analysis. Results were subjected to regression analysis by the least squares method to calculate the values of slope, intercept and correlation coefficient. Results of precision studies expressed in % RSD follows ICH guideline acceptable limits, which indicates good repeatability and low inter-day variability, indicating an excellent precision of the developed method. The results of recovery studies ranged from 98-101 % for both the drugs showing the accuracy of the method. This indicates that there is no interference from tablet excipients. The values of LOD and LOQ were found to be 0.3376 and 10.230 µg/ml for ALI, 0.0288 and 0.0873 µg/ml for HCT, respectively (table 1), proves the sensitivity of the developed method.

The proposed method was checked through all the parameters described earlier under robustness studies, but no significant changes found in retention time, peak area or symmetry of the peaks. Solution stability was performed at room temperature and it was found to be stable up to two days. System suitability tests were performed and the results shows that the parameters tested were within the acceptable range (%RSD<2) and are presented in table 2, which suggests that the method is suitable for an intended use.

The proposed method was successfully used for the quantitative determination of ALI and HCT in commercial formulation (Rasilez HCT tablet: 300 mg of ALI and 25 mg of HCT). Six replicate determinations were carried out and experimental values are presented in table 3. The percentage assay result obtained ranged from 98-101. Therefore, the proposed method can be successfully applied for the quantitative analysis of ALI and HCT in tablet formulation.

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise, cost effective and highly sensitive as compared to existing methods. The method uses simple reagents and sample preparation procedures were minimal. Therefore, the proposed method can be used successfully for routine analysis of ALI and HCT in tablet dosage form without any interference from the excipients used in the tablet formulation.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmacy, Sumandeep Vidyapeeth University, Piparia, Vadodara, Gujarat, India for providing all the necessary facilities to carry out the project work.

Table 1: Summary of validation parameters for the proposed method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ALI</th>
<th>HCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/ml)</td>
<td>1.2-240</td>
<td>0.1-20</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9995</td>
<td>0.9998</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 5276x+1763</td>
<td>y = 64274x+4229</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td>0.1917</td>
<td>0.6728</td>
</tr>
<tr>
<td>Repeatability of injection (n=6)</td>
<td>0.0942</td>
<td>0.9319</td>
</tr>
<tr>
<td>Inter-day (n=3)</td>
<td>0.1220</td>
<td>1.2343</td>
</tr>
<tr>
<td>Accuracy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Recovery (n=3)</td>
<td>99.49±0.9868*</td>
<td>99.87±0.8556*</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.9899</td>
<td>0.7689</td>
</tr>
<tr>
<td>Specificity</td>
<td>No interference</td>
<td></td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.3376</td>
<td>0.0288</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>1.0230</td>
<td>0.0873</td>
</tr>
</tbody>
</table>

n = Number of determination, *SD=Standard deviation

Table 2: System suitability parameters

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Retention time* (%) RSD&lt;2**</th>
<th>Peak area reproducibility* (%) RSD&lt;2**</th>
<th>Resolution*</th>
<th>Theoretical plates*</th>
<th>Tailing factor*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALI</td>
<td>5.34±0.0480*</td>
<td>68258.57±634.53*</td>
<td>9.53±0.1132</td>
<td>4877±36.20*</td>
<td>0.85±0.0155*</td>
</tr>
<tr>
<td>HCT</td>
<td>2.81±0.0353*</td>
<td>66369.57±5370.09*</td>
<td>4.40±0.0549</td>
<td>3552±43.58*</td>
<td>0.84±0.0099*</td>
</tr>
</tbody>
</table>

*±SD (n=6), **%RSD

Table 3: Analysis of formulation

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Labeled amount (mg/tablet)</th>
<th>Amount found (%)*</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALI</td>
<td>300</td>
<td>100.36±0.9201</td>
<td>0.9097</td>
</tr>
<tr>
<td>HCT</td>
<td>25</td>
<td>99.40±0.7624</td>
<td>0.7589</td>
</tr>
</tbody>
</table>

*±SD (n=6)

CONFLICT OF INTERESTS

Declared None

REFERENCES

3. Sangoi MS, Sangoi MW, Oliveira PR, Todeschini V, Rolim CMB. Rapid simultaneous determination of aliskiren and hydrochlorothiazide from their pharmaceutical formulations.


