Simultaneous estimation of Aliskiren hemifumarate and Hydrochlorothiazide in combined Tablet Formulation by Simultaneous equation, Absorbance ratio and First derivative Spectroscopic Methods

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ABSTRACT

Three simple, sensitive, precise and accurate UV-spectroscopic methods namely simultaneous equation, absorbance ratio and first derivative (zero crossing) spectroscopic methods were developed and validated for simultaneous determination of aliskiren hemifumarate and hydrochlorothiazide in tablet dosage form. Simultaneous equation method was based on the measurement of absorbance at 271 and 280 nm for both the drugs. In absorbance ratio method 255 and 271 nm was used for the quantification of aliskiren hemifumarate and hydrochlorothiazide. First derivative method was involved in the conversion of UV-spectra in to first derivative spectra and measurement of first derivative signal at 241 and 280.2 nm for aliskiren hemifumarate and hydrochlorothiazide, respectively using 2 nm as wavelength interval (Δλ) and 1 as scaling factor. Methods were validated as per ICH guidelines including parameters viz., specificity, linearity and range, precision, accuracy, limit of detection and quantification. All the methods were found to be linear in the concentration range of 6-300 µg/ml for aliskiren hemifumarate and 0.5-25 µg/ml for hydrochlorothiazide. Results of validation studies follows ICH guideline acceptable limits. Methods were compared based on the assay results obtained using one-way ANOVA followed by Bonferroni multiple comparison tests (95% confidence level) as appropriate using computer based fitting program (Prism, Graphpad version 5, Graphpad Software Inc). Results of statistical analysis revealed that there was no significant difference between simultaneous equation, absorbance ratio and first derivative method. Developed methods were simple, rapid, highly sensitive and cost effective as compared to existing methods and can be useful for simultaneous estimation of aliskiren hemifumarate and hydrochlorothiazide in commercial tablet formulation for routine quality control.

INTRODUCTION

Aliskiren hemifumarate (ALI) is chemically described as (2S,4S,5S,7S)-N- (2-methylpropyl) 5amino-4-hydroxy-2,7diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)- phenyl]-octamide hemifumarate, a renin inhibitor, widely 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 Figure 1. Hypertension is one of the most common and powerful risk factors for cardiovascular diseases. Blood pressure control is prerequisite for the management of cardiovascular diseases and complications. More than one medication is required for the effective control of blood pressure of the cardiovascular patient.

ALI is the first representative of a new class of non peptide, low molecular weight; orally active transition state renin inhibitor shows effective control of blood pressure and cardiovascular diseases when combined with HCT, a thiazide diuretic (Martindale, 2009; The Merck Index, 2001; Indian Pharmacopoeia, 2007; Sen et al., 2015).
Literature survey revealed various analytical methods for the determination of ALI along with HCT in laboratory prepared mixture and combined tablet dosage form using UV-spectrophotometry (Ezzeldin et al., 2013; Ezzeldin et al., 2013; Patel et al., 2014), HPLC (Sangoi et al., 2011; Karvelis et al., 2014; Belal et al., 2013; Dimitrios et al., 2014; Swamy et al., 2012), MEKC (Sangoi et al., 2011) and electrophoresis (Salim et al., 2014).

There was scope for more sensitive, reliable and statistically proven alternative methods for the determination of ALI and HCT in combined tablet dosage form using simple UV-spectroscopic methods compared to existing methods. Therefore, aim of the present work was to develop and validate some simpler, sensitive, precise, accurate and cost effective UV-spectroscopic methods as compared to existing methods for the determination of ALI and HCT in commercial tablet formulation (Rasilez HCT®).

The advantages of proposed methods are as follows; methods describe 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; all the developed methods were validated as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

ALI reference standard was provided as gift sample by Jubilant Life Sciences Ltd., Noida, Uttar Pradesh, India and HCT was obtained from Glenmark Pharmaceuticals Ltd., Mumbai, Maharashtra, India. Rasilez HCT® tablets containing 300 mg of ALI along with 25 mg of HCT were procured from commercial sources. Methanol used was of AR grade and procured from Loba Chemie Pvt. Ltd., Mumbai, India.

Apparatus

Shimadzu double beam UV-visible spectrophotometer (UV-1800, UV Probe, Shimadzu Corporation, Kyoto, Japan) along with matched quartz cell of 1 cm path length was used throughout the experiment. Highly sensitive electronic balance Adventurer Pro AVG264C, Ohaus Corporation, Pine Brook, NJ, USA was used for weighing purpose.

Preparation of Standard Solution

Stock solution of ALI and HCT were prepared by weighing accurately 11.05 mg of ALI (11.05 mg of ALI is equivalent to 10 mg of aliskiren) and 10 mg of HCT standard drug which was then transferred to a 10 ml volumetric flask separately and diluted to 10 ml with methanol to get the concentration of the drugs 1000 µg/ml. Further dilutions were made to get desired concentration with methanol.

Procedure

Simultaneous Equation and Absorbance Ratio Method

Standard stock solutions of ALI and HCT were further diluted separately with methanol to get the drug solutions containing 60 µg/ml of ALI and 5 µg/ml of HCT, respectively. Both the solutions were scanned in the UV region (200 - 400 nm) and spectra were recorded. Based on the spectral pattern, SE (simultaneous equation) and AR (absorbance ratio) method (Beckett and Stenlake, 2005) was selected for the estimation of both the drugs. From the overlain spectra (Figure 2), 271 nm (λmax of HCT) and 280 nm (λmax of ALI) were selected for SE; 255 nm (isobestic point) and 271 nm (λmax of HCT) were selected, which showed good linearity and hence used for simultaneous estimation. Different concentrations of ALI (6-300 µg/ml) and HCT (0.5-25 µg/ml) were prepared from respective stock solutions. The absorbances were noted at 271 and 280 nm for SE; 255 and 271 nm for AR method. The absorptivity values were calculated for ALI and HCT at their respective wavelengths by using following formula:

Absorptivity = absorbance / concentration (gm/100 ml)

Absorptivity value of individual solution was calculated and average absorptivity value (Table 1) at specific wavelength of particular drug was used for calculating concentration of drugs.

Fig. 1: Chemical structures of ALI (aliskiren hemifumarate) and HCT (hydrochlorothiazide).
**First Derivative (zero crossing) Method**

The normal UV-spectra of ALI and HCT were converted into first and second derivative spectra. Based on the spectral pattern and zero crossing points, first DR (derivative spectroscopic) method was selected for the study. First derivative spectra showed typical zero-crossing points at 280.20 nm for ALI and 241 nm for HCT using 2 nm as wavelength interval (Δλ) and 1 as scaling factor. From the overlain spectra, 241 nm and 280.20 nm were selected for further studies (Figure 3). Calibration curve was plotted for both ALI and HCT in the range of 6 to 300 µg/ml and 0.5 to 25 µg/ml, respectively. Results were subjected to regression analysis by least square method to calculate the values of slope, intercept and correlation coefficient.

**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. Flask was vortexed after adding 30 ml of methanol and shaken for 10 minutes and volume was made up to the mark with methanol. Contents were filtered through whatman filter paper no 41 and suitable aliquots were prepared to get desired concentrations (eg. ALI 120 µg/ml and HCT 10 µg/ml).

**Analysis of Sample Solution**

**Simultaneous Equation Method**

After scanning the sample solution (formulation) between 200 to 400 nm, absorbances were noted at 271 and 280 nm. The unknown concentration of drugs in sample solution was estimated by solving SE using following formula [Sen et al., 2015]:

\[ C_x = \frac{A_x a_{1x} - A_y a_{2x}}{a_{1x} a_{1y} - a_{1x} a_{2y}} \]

\[ C_y = \frac{A_y a_{1y} - A_y a_{1y}}{a_{1x} a_{1x} - a_{1y} a_{1y}} \]

Where, \( C_x \) and \( C_y \) are the concentrations of ALI and HCT, \( ax_1 \) and \( ax_2 \) are absorptivities of ALI at 271 and 280 nm, respectively. \( ay_1 \) and \( ay_2 \) are absorptivities of HCT at 271 and 280 nm, respectively. \( A_1 \) and \( A_2 \) are the absorbances of sample solution at 271 and 280 nm.

**Absorbance Ratio Method**

The unknown concentration of drugs in sample solution was estimated by AR method using following formula:

\[ C_x = \frac{Qm - Qy}{Qx - Qy} \times \frac{a_{1x}}{a_{1x}} \]

\[ C_y = \frac{Qm - Qx}{Qy - Qx} \times \frac{a_{1y}}{a_{1y}} \]

Where, \( ax_1 \) and \( ax_2 \) are absorptivities of ALI at 255 and 271 nm, respectively. \( ay_1 \) and \( ay_2 \) are absorptivities of HCT at 255 and 271 nm, respectively. \( A_1 \) and \( A_2 \) are the absorbances of sample solution at 255 and 271 nm. \( C_x \) and \( C_y \) are the concentrations of ALI and HCT, respectively in sample solution.

\[ Qm = \frac{a^2}{A_1} \]

\[ Qx = \frac{ax^2}{ax_1} \]

\[ Qy = \frac{ay^2}{ay_1} \]

**First Derivative (zero crossing) Method**

Sample solution was scanned in the UV region (200-400 nm) and spectrum was recorded and converted into their 1st derivative spectra and amplitude was measured at 241 and 280.20 nm. The unknown concentration of drugs in sample solution was estimated by using regression equation.

**Validation of Spectroscopic Methods**

The developed methods were validated in accordance with “International Conference on Harmonization” guidelines [ICH, 2005] for validation of analytical procedures.

**Specificity**

To check the interference between tablet excipients used in the marketed formulation and drug substance, specificity study was performed. Tablet excipients were mixed in proportion (as per marketed formulation) and diluted using methanol and filtered through whatman filter paper no 41. All the solutions (Placebo and standard) were scanned in the UV region and compared with standard spectra to evaluate the interference between excipients and drugs.

**Linearity and Range**

Linearity and range of all the three methods were checked by analyzing all the standard solutions separately, containing ALI (6, 12, 60, 120, 180, 240 and 300 µg/ml) and HCT (0.5, 1, 5, 10, 15, 20 and 25 µg/ml) in methanol and absorbances were measured at 271 and 280 nm for SE method; 255 and 271 nm for AR method; 241 and 280.20 nm for 1st DR method. Calibration graphs were plotted using absorbances of standard drug solutions versus concentration for SE and AR method; 1st derivative signal of standard drug solutions versus concentration for DR method. Regression analysis was performed by least squares method to calculate the values of slope, intercept and correlation coefficient.

**Precision**

Precision of the methods were checked by carrying out repeatability, intra-day and inter-day precision. Repeatability of the methods were checked by analyzing sample solutions (ALI 60 & 120 µg/ml; HCT 5 & 10 µg/ml) six times by measuring the absorbances of both the drug solutions at 271 and 280 nm for SE method; 255 and 271 nm for AR method; 241 and 280.20 nm for 1st DR method, respectively and % RSD was calculated. Intra-day precision was carried out by analyzing sample solutions (ALI 60 & 120 µg/ml; HCT 5 & 10 µg/ml) in triplicate at two different concentration levels for three times on the same day within the linearity range and % RSD was calculated. Inter-day precision was determined by repeated analysis of sample solutions (ALI 60 & 120 µg/ml; HCT 5 & 10 µg/ml) in triplicate at two different concentration levels within the linearity range on three different days and percentage RSD was calculated.

**Accuracy**

In order to ensure the suitability and reliability of the projected methods, recovery studies were carried out by standard
Fig. 2: Overlaid UV spectra of ALI (60 μg/ml) and HCT (5 μg/ml).

Fig. 3: Overlaid 1st derivative UV-spectra of ALI (60 μg/ml) and HCT (5 μg/ml).

Table 1: Absorptivity values for SE and AR method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SE</th>
<th>AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelengths (nm)</td>
<td>ALI</td>
<td>HCT</td>
</tr>
<tr>
<td>255 nm</td>
<td>15.11</td>
<td>15.11</td>
</tr>
<tr>
<td>271 nm</td>
<td>35.28</td>
<td>49.62</td>
</tr>
<tr>
<td>280 nm</td>
<td>268.20</td>
<td>271 nm</td>
</tr>
</tbody>
</table>

(n = 6) Average of six determinations.

Table 2: Summary of validation parameters for the proposed methods.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SE</th>
<th>HCT</th>
<th>AR</th>
<th>HCT</th>
<th>DR</th>
<th>HCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelengths (nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linearity range (μg/ml)</td>
<td>271 nm</td>
<td>280 nm</td>
<td>280 nm</td>
<td>ALI: 6-300; HCT: 0.5-25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9995</td>
<td>0.9998</td>
<td>0.9995</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9992</td>
</tr>
<tr>
<td>Regression equation intercept</td>
<td>0.0006</td>
<td>0.0036</td>
<td>0.0007</td>
<td>0.0006</td>
<td>0.0045</td>
<td>0.0069</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0036</td>
<td>0.0091</td>
<td>0.0592</td>
<td>0.0268</td>
<td>0.0016</td>
<td>0.0036</td>
</tr>
<tr>
<td>LOD (μg/ml)</td>
<td>1.26</td>
<td>0.64</td>
<td>0.05</td>
<td>0.05</td>
<td>1.44</td>
<td>1.26</td>
</tr>
<tr>
<td>LOQ (μg/ml)</td>
<td>3.82</td>
<td>1.94</td>
<td>0.14</td>
<td>0.16</td>
<td>4.35</td>
<td>3.82</td>
</tr>
<tr>
<td>Specificity</td>
<td>No interference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability of measurement (n=6)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-day (n=3)*</td>
<td>0.61</td>
<td>0.45</td>
<td>0.72</td>
<td>0.72</td>
<td>0.61</td>
<td>0.61</td>
</tr>
<tr>
<td>Inter-day (n=3)*</td>
<td>0.75</td>
<td>0.65</td>
<td>0.55</td>
<td>0.85</td>
<td>0.80</td>
<td>0.73</td>
</tr>
</tbody>
</table>

*n = number of determinations, % RSD (Percentage relative standard deviation).
addition method. To an equivalent quantity of pre-analyzed sample solution (Formulation, ALI: 60, 90 and 120 µg/ml; HCT: 5, 7.5 and 10 µg/ml), a known concentration of standard ALI and HCT were added at 50, 100 and 150% level and the resulting solutions were reanalyzed by proposed methods and % recoveries were calculated. The results of accuracy studies were assessed based on the percentage of standard ALI and HCT recovered from the formulation by using following formula:-

% Recovery = (Amount of drug found after addition of standard drug - Amount of drug found before addition of standard drug) / (Amount of standard drug added) × 100

**LOD and LOQ**

Sensitivity of the proposed methods were determined in terms of LOD and LOQ. The limit of detection and limit of quantification of ALI and HCT were calculated using following equation as per ICH guidelines.

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

Where \( \sigma \) = The standard deviation of the response, \( S \) = The slope of the calibration curve

**Stability of the Solution**

Stability of the solutions were checked by observing any changes in terms of absorbance and spectral pattern compared to freshly prepared solutions by keeping the solutions at room temperature and analyzing at a frequent interval.

**RESULTS AND DISCUSSION**

Three simple, sensitive, precise and accurate UV-spectroscopic methods namely SE, AR and 1st DR spectroscopic methods were developed and validated for simultaneous estimation of ALI and HCT in tablet dosage form. SE method was based on the measurement of absorbance at 271 and 280 nm for both the drugs. In AR method 255 and 271 nm was used for the detection and quantification of ALI and HCT. 1st DR method was involved in the conversion of UV-spectra in to first derivative spectra and measurement of first derivative signal at 241 & 280.20 nm for ALI and HCT, respectively using 2 nm as wavelength interval (\( \Delta \lambda \)) and 1 as scaling factor. Comparative overlain spectra of placebo and drug solutions indicate that there was no interference between excipients and standard drugs (Figure 4 & 5). ALI and HCT were found to be linear in the concentration range of 6-300 µg/ml and 0.5-25 µg/ml, respectively for all the methods. Calibration graphs were plotted using absorbance of standard drug versus concentration for SE and AR method. 1st derivative signal of standard drug solution versus concentration was used to plot calibration curve for DR method. Regression analysis was performed by least square method to calculate the values of slope, intercept and correlation coefficient for ALI and HCT at respective wavelengths. Results of precision studies expressed in % RSD follows ICH guideline acceptable limits (<2), which shows good repeatability, low intra and inter-day variability, indicating an excellent precision of the developed methods (Table 2).

The results of recovery studies ranged from 97-102% for both the drugs showing the accuracy of the proposed methods (Table 3). This indicates that there was no interference from tablet excipients. The values of LOD and LOQ were found to be very low which proves the sensitivity of the proposed methods (Table 2). Solution stability was performed at room temperature and it was found to be stable up to two days. The proposed methods were successfully applied 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 were found to be within 96 and 101 % for both the drugs and hence the developed methods can be used for the simultaneous determination of both the drugs in combined formulation (Table 4).

Statistical analysis was performed to assess the effect of all the developed methods based on assay results obtained. Statistical significance between all the methods were tested using one-way ANOVA followed by Bonferroni multiple comparison.
test (95% confidence level) as appropriate using computer based fitting program (Prism, Graphpad version 5, Graphpad software Inc). Significance level was set at p<0.05 for all the tests. Results of ANOVA are presented in Table 5. The results of assay revealed that there was no statistical significant difference between all the developed methods.

**CONCLUSION**

Different methods namely SE, AR and 1st DR spectroscopic methods were developed for simultaneous determination of ALI & HCT in combined tablet dosage form. All the developed methods were validated as per ICH guidelines. Proposed methods were found to be simple, sensitive, precise, accurate and cost effective. Developed methods possesses several advantages over existing methods are as follows: all the developed UV-spectrophotometric methods are very simple, requires little sample preparation procedure, wide concentration range with high sensitivity, 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). Statistical data reveals that there is no statistical significant difference between all the three methods. Therefore, all the methods can be used successfully for routine analysis of ALI and HCT in combined tablet dosage form.

**Table 3: Results of recovery studies.**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Level (%)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALI</td>
<td>50</td>
<td>99.71±0.47</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100.11±0.25</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>99.97±0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>HCT</td>
<td>50</td>
<td>97.51±0.24</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>98.23±0.26</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>98.68±0.26</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Mean ± SD (n = 3), SD (Standard deviation), % RSD (Percentage relative standard deviation).

**Table 4: Results of analysis of marketed formulation using different methods.**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Labeled Amount (mg/tab)</th>
<th>Amount Found (mg/tab)</th>
<th>Amount Found (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALI</td>
<td>300</td>
<td>296.87</td>
<td>98.96±0.82</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>295.29</td>
<td>98.43±0.18</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>295.72</td>
<td>98.43±0.12</td>
<td>1.14</td>
</tr>
<tr>
<td>HCT</td>
<td>25</td>
<td>24.70</td>
<td>97.88±1.20</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>24.47</td>
<td>97.88±1.21</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>24.48</td>
<td>97.93±1.12</td>
<td>1.20</td>
</tr>
</tbody>
</table>

*Mean ± SD (n = 6), SD (Standard deviation), % RSD (Percentage relative standard deviation).

**Table 5: Results of statistical comparison using one way ANOVA & Bonferroni multiple comparison test for SE, AR and DR spectroscopic method.**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Simultaneous Equation Method</th>
<th>Absorbance Ratio Method</th>
<th>First Derivative Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALI</td>
<td>98.96±0.82</td>
<td>98.43±0.18</td>
<td>98.58±0.34</td>
</tr>
<tr>
<td>HCT</td>
<td>98.81±1.20</td>
<td>97.88±1.12</td>
<td>97.93±1.12</td>
</tr>
</tbody>
</table>

All values are expressed in Mean ± SD (n=6).
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REFERENCES


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