SIMULTANEOUS ESTIMATION OF IRBESARTAN AND ATORVASTATIN BY Q ABSORPTION RATIO METHOD IN THEIR SYNTHETIC MIXTURE USE IN CARDIAC CONDITION

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ABSTRACT

A simple, accurate and precise spectroscopic method was developed for simultaneous estimation of Irbesartan and atorvastatin in synthetic mixture using Q absorption Ratio Method. In this spectroscopic method, 234.7 nm (as an iso-absorptive point) and 226 nm wavelengths (λmax or any of the two drugs) were selected for measurement of absorbivity. Both the drugs show linearity in a concentration range of 05-30 μg/ml at their respective λmax and at the isoabsorptive point. Accuracy, precision and recovery studies were done by QC samples covering lower, medium and high concentrations of the linearity range. The relative standard deviation for accuracy, precision studies were found to be within the acceptance range (<2%). The limit of determination was 0.365 μg/ml and 0.0622 μg/ml for Irbesartan and atorvastatin, respectively. The limit of quantification was 1.108 μg/ml and 0.188 μg/ml for Irbesartan and atorvastatin, respectively. Recovery of Irbesartan and atorvastatin were found to be 100.51% and 100.16% respectively confirming the accuracy of the proposed method. The proposed method is recommended for routine analysis since they are rapid, simple, accurate and also sensitive and specific by no heating and no organic solvent extraction.

Keywords: Irbesartan, atorvastatin, simultaneous estimation, Q absorption ratio method, Q value analysis method.

INTRODUCTION

Irbesartan, an angiotensin II receptor antagonist is used mainly for the treatment of hypertension. It is an orally active nonpeptide tetrazole derivative and selectively inhibits angiotensin II receptor type 2. Angiotensin II receptor type 1 antagonists have been widely used in treatment of diseases like hypertension, heart failure, myocardial infarction and diabetic nephropathy. IUPAN name of Irbesartan is 2-buty1-1H-[1,2,3,4-tetrazol-5-yl]phenyl[phenylmethyl]-1,3-diazaspiro[4,4]non-1-ene-1. Fig. 1: Structure of Irbesartan

Irbesartan is white or almost white, crystalline powder. Solubility is given in practically insoluble in water, sparingly soluble in methanol, slightly soluble in methylene chloride.

Fig.2: Structure ofatorvastatin

Atorvastatin is white or almost white, crystalline powder. Solubility is given in practically insoluble in water, soluble in methanol, slightly soluble in methylene chloride.

Hypertension frequently coexists with hyperlipidaemia and both are considered to be major risk factors for developing cardiac disease ultimately resulting in adverse cardiac events. This clustering of risk factors is potentially due to a common mechanism. Further, patient compliance with the management of hypertension is generally better than patient compliance with hyperlipidaemia. It would therefore be advantageous for patients to have a single therapy which treats both of these conditions with help of fixed dose combination of irbesartan and atorvastatin.

The review of literature regarding quantitative analysis of irbesartan and atorvastatin revealed that no attempt was made to develop analytical methods for irbesartan and atorvastatin. Some spectrometric methods and chromatographic methods have been reported for the estimation of the individual drugs. The focus of the present study was to develop and validate a rapid, stable, specific, and economic spectroscopic method for the estimation of irbesartan and atorvastatin in Synthetic mixture.
Accurately weighed quantity of Atorvastatin 10 mg was transferred to 100 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 100 μg/ml.

Preparation of standard mixture solution
From the stock solution of IRB take 3.2 ml and from stock solution of ATR take 0.4 ml and transferred in to 10 ml volumetric flask and diluted up to mark with methanol to give a solution having strength of IRB was 32 μg/ml and ATR was 4 μg/ml.

Preparation of test solution
From the stock solution of IRB take 3.2 ml and from stock solution of ATR take 0.4 ml and transferred in to 10 ml volumetric flask and diluted up to mark with methanol to give a solution having strength of IRB was 32 μg/ml and ATR was 4 μg/ml.

Calibration curves for Irbesartan
Pipe out 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of the stock solution of Irbesartan and atorvastatin (100 μg/ml) into a series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol and measured absorbance at 226.00 nm and 246.00 nm. Plot the graph of absorbance versus respective concentration of Irbesartan and atorvastatin. Linearity range of IRB and ATR was found with correlation co-efficient.

Q Absorption Ratio Method
Development of Method
Different solutions were prepared in the different solvents according to the solubility of the drugs. It was found that methanol showing good overlay and distinct λabs of the both drugs. Therefore, it can be easy to measure the response of the both drugs in the combined mixture. The λabs of the Irbesartan and Atorvastatin was found to be 226.00 nm and 246.00 nm respectively in methanol.

The overlain derivative spectra (zero order) of IRB and ATR at different concentrations revealed that different concentration of IRB and ATR possess iso-absorptive point at 234.70 nm. Considering above facts, wavelength 234.70 nm (λ1) and 226.00 nm (λ2) were selected for the estimation of both the drugs by absorbance ratio method.

RESULT AND DISCUSSION
Validation Parameters

Linearity and Range
Different concentrations of Irbesartan (5 to 30 μg/ml) and Atorvastatin (5 to 30 μg/ml) were prepared from respective stock solutions. The absorbances were noted at 226.00 and 246.00 nm. It was noted that at the wavelengths 234.70 and 246.00 nm good linearity was observed and hence these wavelengths were fixed for their simultaneous estimation.

Measure the absorbance at 234.70 nm (λ1) and 226.00 nm (λ2) for both drugs. The absorptivities were calculated for Irbesartan and Atorvastatin at the selected wavelengths and average of absorptivities given in table 6.17.

The calibration curve of both drugs shown in figure 6.9 and 6.10.

Correlation coefficient (r) for calibration curve of IRB and ATR was found to be 0.9994 and 0.9995, respectively.

The regression line equation for IRB and ATR are as following:

\[ y = 0.0645x - 0.0049 \text{ for IRB at 226.00 nm} \]
\[ y = 0.0641x - 0.0795 \text{ for ATR at 246.00 nm} \]
\[ y = 0.0572x - 0.0915 \text{ for IRB at 234.70 nm} \]
\[ y = 0.0561x - 0.0721 \text{ for ATR at 234.70 nm} \]

Absorption ratio equation

\[ C_1 = \frac{(Q_1 - Q_2)}{(Q_2 - Q_1)} \times \left( \frac{A_1}{A_2} \right) \]
\[ C_2 = \frac{(Q_2 - Q_1)}{(Q_1 - Q_2)} \times \left( \frac{A_1}{A_2} \right) \]

Where, \( C_1 \) = Concentration of IRB
\( C_2 \) = Concentration of ATR

MATERIALS AND METHODOLOGY

- Atorvastatin and Irbesartan were obtained as gift samples from S Kant pharmaceuticals and CTX life science Surat. Synthetic Mixture contain 20 mg of Atorvastatin and 160 mg of Irbesartan.
- A double beam UV/Visible spectrophotometer (Shimadzu model 2450, Japan) with spectral width of 2 nm, 1 cm quartz cells was used to measure absorbance of all the solutions.
- Spectra were automatically obtained by UV-Probe system software.
- An analytical balance (Sartorius CD2250, Gottingen, Germany) was used for weighing the samples.
- Sonicator (D120/2H, TRANS-O-SONIC)
- Class ‘A’ volumetric glassware were used (Borosilicite)

Standard solution of Irbesartan (IRB)
Preparation of stock solution of IRB
Accurately weighed quantity of Irbesartan 10 mg was transferred to 100 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 100 μg/ml.

Preparation of stock solution of ATR

Accurately weighed quantity of Atorvastatin 10 mg was transferred to 100 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 100 μg/ml.
Mixed solutions of IRB and ATR containing 5, 15 and 30 μg/ml respectively series were analyzed three times on the same day using developed spectroscopic method and %RSD was calculated. Thes\%RSD found to be 0.31% for IRBand 0.41% for ATR. Thes\%RSD value was found to be less than 0.56%. *(n=6)

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>Mean Abs.* ±SD IRB</th>
<th>% RSD</th>
<th>Mean Abs.* ±SD ATR</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.461±0.00035</td>
<td>0.75</td>
<td>0.442±0.00025</td>
<td>0.56</td>
</tr>
<tr>
<td>15</td>
<td>1.412±0.00060</td>
<td>0.38</td>
<td>1.318±0.00064</td>
<td>0.48</td>
</tr>
<tr>
<td>30</td>
<td>2.751±0.00091</td>
<td>0.32</td>
<td>2.645±0.00010</td>
<td>0.41</td>
</tr>
</tbody>
</table>

**Table 3: Intraday precision data for estimation of IRB and ATR**(n=3)

**Interday precision**

Mixed solutions of IRB and ATR containing 5, 15 and 30 μg/ml and 5, 15 and 30 μg/ml respectively series were analyzed three times on the different day using developed spectroscopic method and %RSD was calculated. The%RSD value was found to be 0.31 – 0.82% for IRBand 0.32 – 0.71% for ATR. These%RSD values were found to be less than 2.0% indicated that the method is precise. *(Table 6.20)*
The developed UV spectroscopic method was checked for the accuracy. It was determined by calculating the recovery of IRB and ATR from formulation solution by standard addition method in the combined mixture solution. The spiking was done at three levels: 80%, 100%, and 120%.

Table 4: Interday precision data for estimation of IRB and ATR* (n=3)

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>Mean Abs. *±SD</th>
<th>% RSD</th>
<th>Mean Abs. *±SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRB</td>
<td></td>
<td></td>
<td>ATR</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.485±0.0040</td>
<td>0.82</td>
<td>0.4712±0.00030</td>
<td>0.64</td>
</tr>
<tr>
<td>15</td>
<td>1.4477±0.0055</td>
<td>0.31</td>
<td>1.3621±0.00096</td>
<td>0.71</td>
</tr>
<tr>
<td>30</td>
<td>2.7513±0.00094</td>
<td>0.34</td>
<td>2.6617±0.0085</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Accuracy

The value of % RSD with the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients.

Table 5: Recovery data of IRB* (n=3)

<table>
<thead>
<tr>
<th>Conc. of IRB from formulation (μg/ml)</th>
<th>Amount of Std. IRB added (μg/ml)</th>
<th>Total amount of IRB (μg/ml)</th>
<th>Mean±SD</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>12.8</td>
<td>28.8</td>
<td>28.81±0.064</td>
<td>100.03</td>
<td>0.22</td>
</tr>
<tr>
<td>16</td>
<td>16.0</td>
<td>32.0</td>
<td>32.55±0.068</td>
<td>101.71</td>
<td>0.21</td>
</tr>
<tr>
<td>16</td>
<td>14.2</td>
<td>35.2</td>
<td>35.13±0.104</td>
<td>99.80</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table 6: Recovery data of ATR* (n=3)

<table>
<thead>
<tr>
<th>Conc. of ATR from formulation (μg/ml)</th>
<th>Amount of Std. ATR added (μg/ml)</th>
<th>Total amount of ATR (μg/ml)</th>
<th>Mean±SD</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.6</td>
<td>3.6</td>
<td>3.55±0.064</td>
<td>98.61</td>
<td>0.90</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>4.0</td>
<td>4.01±0.030</td>
<td>100.75</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>2.4</td>
<td>4.4</td>
<td>4.46±0.035</td>
<td>101.13</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Limit of detection and quantitation

The LOD for IRB and ATR was confirmed to be 0.365 μg/ml and 0.0622 μg/ml, respectively. The LOQ for IRB and ATR was confirmed to be 1.108 μg/ml and 0.188 μg/ml, respectively. The obtained LOD and LOQ results are represented in Table 6.3.

Robustness and ruggedness

The obtained ruggedness and Robustness results are represented in Table 6.4. The % RSD was found to be 0.17 - 0.52% for IRB and 0.24 - 0.59% for ATR. These % RSD values were found to be less than ±2.0 and indicated that the method is robust and rugged.

No significant changes in the spectra were observed, proving that the developed method is rugged and robust.

Table 7: LOD and LOQ data of IRB and ATR* (n=10)

<table>
<thead>
<tr>
<th>LOD IRB (μg/ml)</th>
<th>0.365</th>
<th>ATR (μg/ml)</th>
<th>0.0622</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ IRB</td>
<td>1.108</td>
<td>ATR</td>
<td>0.188</td>
</tr>
</tbody>
</table>

Table 8: Robustness and ruggedness data of IRB and ATR* (n=3)

<table>
<thead>
<tr>
<th>Conc. (PPM)</th>
<th>Irbesartan (Mean abs. ±% RSD)</th>
<th>Atorvastatin (Mean abs. ±% RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Instrument 1</td>
<td>Instrument 2</td>
</tr>
<tr>
<td>2</td>
<td>0.462±0.42</td>
<td>0.462±0.52</td>
</tr>
<tr>
<td>3</td>
<td>0.916±0.39</td>
<td>0.916±0.38</td>
</tr>
<tr>
<td>4</td>
<td>1.410±0.22</td>
<td>1.410±0.22</td>
</tr>
</tbody>
</table>

Application of the proposed method for analysis of IRB and ATR in formulation

A zero-order spectrum of the test solution was recorded and measured. The absorbance at 234.70nm (λ₁) and 226nm (λ₂) for irbesartan and ATR were measured. The concentrations of IRB and ATR in formulation were determined using the absorption ratio equation. The absorbance values are given in Table 6.25.

Table 9: Analysis data of formulation* (n=3)

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Drug</th>
<th>Formulation (μg/ml)</th>
<th>% Assay ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IRB</td>
<td>32.0</td>
<td>101.60±0.054</td>
</tr>
<tr>
<td>2</td>
<td>ATR</td>
<td>4.0</td>
<td>99.18±0.023</td>
</tr>
</tbody>
</table>

Table 10: Summary of validation parameters

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Absorption Ratio Method</th>
<th>IRB</th>
<th>ATR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range (μg/ml)</td>
<td>S-30</td>
<td>5-30</td>
<td></td>
</tr>
</tbody>
</table>
Regression equation

\[ y = 0.0645x - 0.0849 \]
\[ y = 0.0561x - 0.0721 \]

Correlation Coefficient \((r^2)\)

0.9982
0.9970

Accuracy(%Recovery) \((n=3)\)

100.51
100.16

Intra-day Precision (%RSD) \((n=3)\)

0.32-0.75
0.41-0.56

Inter-day precision (%RSD) \((n=3)\)

0.31-0.82
0.32-0.71

LOD(µg/ml)

0.365
0.0622

LOQ(µg/ml)

1.108
0.188

Ruggedness and Robustness(%RSD) \((n=3)\)

0.24-0.59
0.17-0.52

%Assay\((n=3)\)

101.60
99.18

CONCLUSION

A new, Q absorption ratio method has been developed for estimation of Irbesartan and Atorvastatin in synthetic mixture. The method was validated by employment of ICH(18) guidelines. The validation data is indicative of good precision and accuracy, and prove the reliability of the method.

REFERENCE

2. Irbesartan drug info in drugbank. (database available on internet): http://www.drugbank.ca/drugs/db01029