DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR IRBESARTAN AND ATORVASTATIN BY SIMULTANEOUS EQUATION SPECTROSCOPIC METHOD

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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 simultaneous equation Method. In this spectroscopic method, 226.00 nm and 246.00 nm wavelengths were selected for measurement of absorptivity. Both the drugs show linearity in a concentration range of 05-30 μg/ml at their respective λmax. 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 0.033 μg/ml and 0.125 μg/ml for Irbesartan and atorvastatin, respectively. The limit of quantification was 0.1008 μg/ml and 0.3792 μg/ml for Irbesartan and atorvastatin, respectively. Recovery of Irbesartan and atorvastatin were found to be 99.75% and 99.52% 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, Simultaneous equation method, analysis method.

INTRODUCTION:
Irbesartan, an angiotensin II receptor antagonist1 is used mainly for the treatment of hypertension. It is an orally active nonpeptidetetrazole 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-butyl-3-\{4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl]methyl\}-1,3-diazaspiro[4.4]non-1-en-4-one.2

![Figure:1 Structure of Irbesartan](image)

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.

Atorvastatin is used as lipid-lowering agents used in hyperlipidaemia condition. Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase.4 As HMG-CoA reductase is responsible
for converting HMG-CoA to mevalonate in the cholesterol biosynthesis pathway, this results in a subsequent
decrease in hepatic cholesterol levels and decreases blood cholesterol level.

Figure 2: Structure of atorvastatin

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. (6, 7)

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. (8, 9)

MATERIALS AND METHODOLOGY:

- Atorvastatin and Irbesartan were obtained as gift samples from S Kant pharmaceuticals and CTX life
  science Surat. Synthetic Mixture contain 20mg of Atorvastatin and 160mg 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 (Borosillicate)

- 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.

Preparation of standard mixture solution

From the stock solution of IRB take 1.6ml and from stock solution of ATR take 0.2ml and transferred in to 10ml
volumetric flask and diluted up to mark with methanol to give a solution having strength of IRB was 16 μg/ml
and ATR was 2 μg/ml.

Preparation of test solution

From the stock solution of IRB take 1.6ml and from stock solution of ATR take 0.2ml and transferred in to 10ml
volumetric flask and diluted up to mark with methanol to give a solution having strength of IRB was 16 μg/ml
and ATR was 2 μg/ml.
Calibration curves for Irbesartan

Pipette out 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of the stock solution of Irbesartan and atorvastatin (100µg/ml) into a series of 10ml volumetric flasks and the volume was adjusted to mark with methanol and measured absorbance at 226.00nm and 246nm. Plot the graph of absorbance versus respective concentration of Irbesartan and atorvastatin. Linearity range of IRB and ATR was found with correlation co-efficient.

**DEVELOPMENT AND VALIDATION OF SPECTROSCOPIC SIMULTANEOUS EQUATION METHOD**

**SELECTION OF WAVELENGTH AND METHOD DEVELOPMENT FOR DETERMINATION OF IRBESARTAN AND ATORVASTATIN**

The standard solution of IRB and ATR were scanned separately between 200-400nm, and IRB show edabsorbance maxima at 226.00nm and ATR at 246.00nm. (figure 3)

![Figure 3 Overlap zero orders spectra of IRB and ATR (8:1) ratios, respectively](image)

**VALIDATION PARAMETERS**

1. **Linearity and Range**

The Zero order (fig.3) showed linear absorbance at 226.00 nm for IRB(05-30 µg/ml) and 246.00nm for ATR (5-30 µg/ml) with correlation coefficient ($r^2$) of 0.9994 and 0.9993 for IRB and ATR, respectively. This method obeyed beer's law with correlation coefficient ($r^2$) of 0.9994 and 0.9993 for IRB and ATR, respectively. (Table 1)

Correlation coefficient ($r^2$) for calibration curve of IRB and ATR was found to be 0.9994 and 0.9993, respectively (figure 4 and 5)

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

\[ y = 0.0983x - 0.2385 \text{ for IRB} \]

\[ y = 0.0642x - 0.0695 \text{ for ATR} \]

**Table 1**: Calibration data for IRB and ATR at 226.00 nm and 246.00 nm respectively. *n=6*
2. Precision

I. Intraday precision

The precision of the developed method was assessed by analyzing combined standard solution containing three different concentrations 05, 15, 30 μg/ml for IRB and 05, 15, 30 μg/ml ATR. Three replicate (n=3) each on same day. Intraday precision data presented in Table 2

These %RSD value was found to be less than ±2.0 indicated that the method is precise.

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>IRB Abs.* ± % RSD</th>
<th>ATR Abs.* ± % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>±% RSD IRB</td>
<td>±% RSD ATR</td>
</tr>
<tr>
<td>IRB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>0.372±0.45</td>
<td>0.266±0.57</td>
</tr>
<tr>
<td>15</td>
<td>1.211±0.21</td>
<td>0.884±0.92</td>
</tr>
<tr>
<td>30</td>
<td>2.763±0.52</td>
<td>1.877±0.23</td>
</tr>
<tr>
<td>ATR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

II. Interday precision

The precision of the developed method was assessed by analyzing combined standard solution containing three different concentrations 05, 15, 30 μg/ml for IRB and 05, 15, 30 μg/ml ATR triplicate (n=3) per day for consecutive 3 days for inter-day precision. Interday precision data presented in Table 3

These %RSD value was found to be less than ±2.0 indicated that the method is precise.
Table 3 Inter-day precision data for estimation of IRB and ATR*(n=3)

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>IRB Abs.* ±% RSD</th>
<th>ATR Abs.* ±% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>±% RSD IRB</td>
<td>±% RSD ATR</td>
</tr>
<tr>
<td>05</td>
<td>0.377±0.55</td>
<td>0.270±0.56</td>
</tr>
<tr>
<td>15</td>
<td>1.215±0.25</td>
<td>0.887±0.17</td>
</tr>
<tr>
<td>30</td>
<td>2.786±0.85</td>
<td>1.881±0.36</td>
</tr>
</tbody>
</table>

3. Accuracy

Accuracy of the method was determined by recovery study from synthetic mixture at three levels (80%, 100%, 120%) of standard addition. The recovery values are tabulated in Table 4 and 5. The percent recovery for IRB and ATR by this method was found in the range of 100.07% to 100.43% and 99.21% to 100.55%, respectively. The value of % RSD within the limit indicated that the method is accurate and percent recovery shows that there is no interference from the excipients.

4. Limit of detection and quantitation

The LOD for IRB and ATR was confirmed to be 0.033 µg/ml and 0.125 µg/ml, respectively. The LOQ for IRB and ATR was confirmed to be 0.1008 µg/ml and 0.379 µg/ml, respectively. The obtained LOD and LOQ results are presented in Table 6.
5. Robustness and Ruggedness

The obtained Ruggedness and Robustness results are presented in Table 6.3.8. The % R.S.D was found to be 0.12 – 0.84 % for IRB and 0.11 – 0.74 % for ATR. These %RSD value was found to be less than ± 2.0 indicated that the method is precise. No significant changes in the spectrums were observed, proving that the developed method is rugged and robust.

Table 7 Robustness and Ruggedness data of IRB and ATR *(n=3)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Conc. (μg/ml)</th>
<th>Different Instrument</th>
<th>Stock Solution Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UV-2450</td>
<td>UV-1800</td>
</tr>
<tr>
<td>Irbesartan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean(n=3) ± % RSD</td>
<td>05</td>
<td>0.376±0.32</td>
<td>0.374±0.47</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.215±0.56</td>
<td>1.216±0.22</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2.763±0.23</td>
<td>2.765±0.84</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean(n=3) ± % RSD</td>
<td>05</td>
<td>0.271±0.54</td>
<td>0.269±0.43</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.885±0.66</td>
<td>0.882±0.33</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.879±0.16</td>
<td>1.878±0.13</td>
</tr>
</tbody>
</table>

Stock-1 :- 10 mg dissolve in 100 ml Methanol
Stock-2 :- 50 mg dissolve in 250 ml Methanol

APPLICATION OF THE PROPOSED METHOD FOR ANALYSIS OF IRB AND ATR IN COMBINED CAPSULE DOSAGE FORM.

All the excipients were mixed in 10ml volumetric flask and sonicate for 15min. make up the volume with Distilled Water. The solution was filtered through Whatman filter paper No. 42. Finally the solution had concentration 1600μg/ml for IRB and 200μg/ml for ATR. from that pipette out 0.1ml in 10 ml volumetric flask and volume was made upto mark with methanol to obtain final solution containing16μg/ml of IRB and 2μg/ml of ATR. A zero order spectrum of the resulting solution was recorded and processed to first derivative spectra. As pectrum of the sample solution was recorded and the absorbance at 226.00nm and 246.00nm were noted for estimation of IRB and ATR, respectively. The concentrations of IRB and ATR in formulation were determined using the corresponding calibration graph.

Table 8 Analytical data of commercial formulation *(n=3)

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Drug</th>
<th>Formulation (μg/ml)</th>
<th>% Assay ± SD</th>
<th>USP limit(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IRB</td>
<td>16.0</td>
<td>99.75 ± 0.22</td>
<td>98-102%</td>
</tr>
<tr>
<td>2</td>
<td>ATR</td>
<td>2.0</td>
<td>99.52 ± 0.56</td>
<td>98-102%</td>
</tr>
</tbody>
</table>
**SUMMARY OF VALIDATION PARAMETER**

Table 9 Summary of validation parameters

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>PARAMETER</th>
<th>Irbesartan</th>
<th>Atorvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wave length Max.</td>
<td>226.00 nm</td>
<td>246.00 nm</td>
</tr>
<tr>
<td>2</td>
<td>Linearity (µg/ml) (n=6)</td>
<td>5 to 30 µg/ml</td>
<td>5 to 30 µg/ml</td>
</tr>
<tr>
<td>3</td>
<td>Regression equation</td>
<td>y = 0.0983x - 0.2385</td>
<td>y = 0.0642x - 0.0695</td>
</tr>
<tr>
<td>4</td>
<td>Correlation coefficient (r^2)</td>
<td>0.9994</td>
<td>0.9993</td>
</tr>
<tr>
<td>5</td>
<td>Accuracy(%Recovery) (n=3)</td>
<td>100.26</td>
<td>100.13</td>
</tr>
<tr>
<td>6</td>
<td>Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intra-day (%RSD)(n=3)</td>
<td>0.21-0.52</td>
<td>0.23-0.92</td>
</tr>
<tr>
<td></td>
<td>Inter-day (%RSD)(n=3)</td>
<td>0.25-0.85</td>
<td>0.17-0.56</td>
</tr>
<tr>
<td>7</td>
<td>LOD (µg/ml) (n=10)</td>
<td>0.033</td>
<td>0.125</td>
</tr>
<tr>
<td>8</td>
<td>LOQ (µg/ml) (n=10)</td>
<td>0.1008</td>
<td>0.3792</td>
</tr>
<tr>
<td>9</td>
<td>Robustness and Ruggedness (%RSD)</td>
<td>0.12-0.84</td>
<td>0.11-0.73</td>
</tr>
<tr>
<td>10</td>
<td>Assay</td>
<td>99.75±0.22</td>
<td>99.52 ±0.56</td>
</tr>
</tbody>
</table>

**CONCLUSION:**

A new, Simultaneous Equation 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:**


