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 absorbitivity. Both the drugs show linearity in a concentration range of 0.5-30 μg/ml at their respective λmax. Accuracy, precision and recovery studies were done by QC samples recovering 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 antagonist has been widely used in 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 the treatment of diseases like hypertension, heart failure, myocardial infarction and diabetic nephropathy. IUPAN name of Irbesartan is 2-butyl-3-{[(1R)-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl]-1,3-diazaspiro[4.4]non-1-ene-4-one.

Atorvastatin is used as a lipid-lowering agent used in hyperlipidaemia condition. Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase. 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.

Atorvastatin is white or almost white, crystalline powder. Solubility is given in practically insoluble in water, sparingly 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.

MATERIALS AND METHODOLOGY

Atorvastatin and Irbesartan were obtained as gift samples from S Kant Pharmaceuticals and CTX life science Surat. Synthetic Mixture contains 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-0-SONIC) and a Class ‘A’ volumetric glassware were used (Borosillicte).

### 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 100μg/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 100μg/ml.

#### Preparation of standard mixture solution

From the stock solution of IRB take 1.6 ml and from stock solution of ATR take 0.2 ml 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 2μg/ml.

#### Preparation of test solution

From the stock solution of IRB take 1.6 ml and from stock solution of ATR take 0.2 ml 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 2μg/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 200-400 nm, and IRB showed absorbance maxima at 226.00 nm and ATR at 246.00 nm (figure 3).

**Fig.3 Overlay zero orderspectra ofIRB and ATR(8:1) ratios, respectively**

### Table 1 Calibration data for IRB and ATR at 226.00 nm and 246.00 nm respectively. *(n=6)*

| Sr. No | Concentration (μg/ml) | Absorbance* (226.00 nm) ±SD IRB | Absorbance* (246.00 nm) ±SD ATR |
|--------|-----------------------|---------------------------------|---------------------------------|
| 1      | IRB 0.050             | 0.3708±0.0023                   | 0.2672±0.0015                   |
| 2      | ATR 0.050             | 0.746±0.0020                    | 0.5674±0.0017                   |
| 3      | IRB 0.015             | 1.2171±0.0013                   | 0.8872±0.0018                   |
| 4      | ATR 0.015             | 1.6972±0.0015                   | 1.1974±0.0012                   |
| 5      | IRB 0.025             | 2.225±0.0013                    | 1.5252±0.0022                   |
| 6      | ATR 0.025             | 2.765±0.0025                    | 1.8772±0.0016                   |

### VALIDATION PARAMETERS *(10)*

#### Linearity and Range

The Zero order (figure 3) showed linear absorbance of IRB at 226.00 nm for IRB (0.05-30 μg/ml) and ATR at 246.00 nm for ATR (5-30 μg/ml) with correlation coefficient ($r^2$) of 0.9994 and 0.9993 for IRB and ATR, respectively.

This method obeys Beer’s law in the concentration range 0.05-30 μg/ml for IRB and ATR, respectively. (Table 1)

Correlation coefficient ($r^2$) for calibration curves 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.

\[ y = 0.0983x - 0.2385 \] for IRB \( \text{(1)} \)

\[ y = 0.0642x - 0.0695 \] for ATR \( \text{(2)} \)
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.

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.

Accuracy

Accuracy of the method was determined by recovery study from synthetic mixture at three level (80%, 100%, 120%) of standard addition. The recovery values are tabulated in Table 4 and 5.

Limit of detection and quantitation

The LOD for IRB and ATR was confirmed to be 0.03 μg/ml and 0.125 μg/ml.
The LOQ for IRB and ATR was found to be 0.1008 µg/ml and 0.379 µg/ml, respectively. The obtained LOD and LOQ results are presented in Table 6.

### Table 6 LOD and LOQ data of IRB and ATR *(n=10)*

| Conc. (µg/ml) | Avg.abs* ± SD (226.00nm) IRB | % RSD | Avg.abs* ± SD (246.00nm) ATR | % RSD |
|---------------|--------------------------------|-------|-------------------------------|-------|
| IRB | ATR |
| 5 | 5 | 0.371 ±0.0007 | 1.93 | 0.270 ±0.0006 | 0.45 |
| LOD (µg/ml) | 0.033 | | | 0.125 | |
| LOQ (µg/ml) | 0.1008 | | | 0.3792 | |

### 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)*

| Condition | Conc. (µg/ml) | Different Instrument | Different Stock Solution Preparation |
|-----------|---------------|----------------------|-------------------------------------|
| IRB | ATR |
| Mean (n=3) | 0.376±0.32 | 0.374±0.47 | 0.376±0.12 | 0.373±0.82 |
| ± % RSD | 0.1215±0.56 | 0.1216±0.22 | 0.1215±0.42 | 0.1216±0.56 |
| Atorvastatin | 0.271±0.54 | 0.269±0.43 | 0.272±0.42 | 0.270±0.11 |
| Mean (n=3) | 0.885±0.66 | 0.882±0.33 | 0.894±0.15 | 0.885±0.33 |
| ± %RSD | 1.879±0.16 | 1.879±0.13 | 1.882±0.52 | 1.884±0.74 |

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 10 ml volumetric flask and sonicated for 15 min. 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 0.1 ml in 10 ml volumetric flask and volumewasmadeupto markwith methanol to obtain final solution containing 16 µg/ml of IRB and 2 µg/ml of ATR. A zereorder spectrum of the resulting solution was recorded and processed to first derivative spectra. Aspectru moth samplesolution was recorded and the absorbance at 226.00nm and 246.00nm were noted. The concentrations of IRB and ATR in formulation were determined using the corresponding calibration graph.

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

| Sr. No | Drug | Formulation (µg/ml) | % Assay* ± SD | USP limit(%) |
|--------|------|---------------------|---------------|--------------|
| 1      | IRB  | 16.0                | 99.75 ± 0.22  | 98-102%      |
| 2      | ATR  | 2.0                 | 99.52 ± 0.56  | 98-102%      |

### SUMMARY OF VALIDATION PARAMETER

#### Table 9 Summary of validation parameters

| SR. NO. | PARAMETER | Irbesartan | Atorvastatin |
|---------|-----------|------------|--------------|
| 1       | Wave length Max. | 226.00 nm  | 246.00 nm    |
| 2       | Linearity (µg/ml) (n=6) | 5 to 30 µg/ml | 5 to 30 µg/ml |
| 3       | Regression equation | y = 0.0983x - 0.2385 | y = 0.0642x - 0.0695 |
| 4       | Correlation coefficient (r²) | 0.9994 | 0.9993 |
| 5       | Accuracy(% Recovery) (n=3) | 100.26 | 100.13 |
| 6       | Precision | | |
| Intra-day (% RSD)(n=3) | 0.21-0.52 | 0.23-0.92 |
| Inter-day (% RSD)(n=3) | 0.25-0.85 | 0.17-0.56 |
| 7       | LOD (µg/ml) (n=10) | 0.033 | 0.125 |
| 8       | LOQ (µg/ml) (n=10) | 0.1008 | 0.3792 |
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.

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