SIMULTANEOUS ESTIMATION OF IRBESARTAN AND ATORVASTATIN BY FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD IN THEIR SYNTHETIC MIXTURE USE IN HYPERTENSION CONDITION

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical first derivative spectrophotometric method for the simultaneous determination of Irbesartan (IRB) and Atorvastatin (ATR) in synthetic mixture. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra was obtained in methanol and the determinations were made at 225.20 nm (ZCP of Atorvastatin) for Irbesartan and 308.15 nm (ZCP of Irbesartan) for Atorvastatin. The linearity was obtained in the concentration range of succinate 5-30 μg/ml for Irbesartan and 5-30 μg/ml for Atorvastatin succinate. The mean recovery was 99.25 and 99.65% for Irbesartan and Atorvastatin succinate, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of Irbesartan and Atorvastatin in synthetic mixture. The results of analysis have been validated statistically and by recovery studies. 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, First order derivative, spectroscopy

INTRODUCTION

Irbesartan, an angiotensin II receptor antagonist [1], 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-buty1-3-{4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1,3-diazaspiro[4.4]non-1-ene-4-one [2].

Fig. 1. Structure of Irbesartan [3]

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

Atorvastatin is used as a lipid-lowering agent used in hyperlipidaemia condition. Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase [3]. 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.

Fig. 2. Structure of Atorvastatin [5]

Atorvastatin is white or almost white, crystalline powder. Solubility is given 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 spectrophotometric 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(D120/2L, TRANS-O-SONIC)
- Class ‘A’ volumetric glassware were used (Borosilicate)

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

**Preparation of test solution**

From the stock solution of IRB take 3.2ml and from stock solution of ATR take 0.4ml and transferred in to 10ml volumetric flask and diluted up to mark with methanol to give a solution having strength of IRB was 32μg/ml and ATR was 4 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 225.20nm and 308.15nm. Plot the graph of absorbance versus respective concentration of Irbesartan and atorvastatin. Linearity range of IRB and ATR was found with correlation co-efficient.

**First Order Derivative Spectrophotometric 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 λmax of the both drugs. Therefore, it can be easy to measure the response of the both drugs in the combined mixture. The λmax of the Irbesartan and Atorvastatin was found to be 226.00 nm and 246.00 nm respectively in methanol.

The synthetic mixture of Irbesartan and Atorvastatin is present in 8:1 ratios respectively. The absorption spectra of pure drug and their mixture were recorded between 200-400 nm using Distilled Water as solvent and proceed to first derivative spectra. The IRB was showed the ZP at 308.15nm and ATR showed the ZP at 225.20nm. On the basis these IRB can be quantified by measuring the absorbance at 225.20nm and at ATR can be quantified by measuring the absorbance at 308.15nm.

**RESULT AND DISCUSSION**

**Validation Parameters[10]**

**Linearity and Range**

The first-derivative spectra (Fig. 3) showed linear absorbance at 225.20 nm (ZP of IRB) for IRB (1-6μg/ml) and 308.15 nm (ZP of ATR) for ATR (25-150μg/ml) with correlation coefficient (r²) of 0.9996 and 0.9996 for IRB and ATR, respectively.

This method obeys Beer’s law in the concentration range 1-6μg/ml and 25-150μg/ml for IRB and ATR, respectively. (Table 1)

**Correlation coefficient (r²) for calibration curve of IRB and ATR was found to be 0.9996 and 0.9996 respectively (figure and table 7).**

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

\[ y = -0.0008x - 0.0003 \text{ for IRB} \] (1)

\[ y = -0.0011x + 0.0003 \text{ for ATR} \] (2)

![Fig. 3: Overlain zero orderspectra of IRB and ATR in methanol (1:1)](image)

![Fig. 4: Overlain first orderspectra of IRB and ATR in 8:1 ratios, respectively with the combination solution (8:1)](image)

![Fig. 5: Overlain linear first orderspectra of IRB (Pink) and ATR (Blue) in 8:1 ratios](image)
From the combinations solution of IRB and ATR, the dilution was made in a ratio of 8:1, and absorbance was recorded (Table 1) and correlation coefficient ($r^2$) of 0.9938 (figure 6) and 0.9994 (figure 6) for IRB and ATR, respectively.

Table 1: Calibration data for IRB and ATR at 225.20 nm and 308.15 nm, respectively. *$n=6$

| Sr. No | Concentration (µg/ml) | Absorbance* (225.20 nm) ±SD | Absorbance* (308.15 nm) ±SD |
|--------|-----------------------|----------------------------|-----------------------------|
|        | IRB                   | ATR                        | IRB                         |
| 1      | 05                    | 05                         | -0.00265 ± 0.00058          | -0.00412 ± 0.00315 |
| 2      | 10                    | 10                         | -0.00612 ± 0.00063          | -0.00936 ± 0.00339 |
| 3      | 15                    | 15                         | -0.01185 ± 0.00095          | -0.01358 ± 0.00316 |
| 4      | 20                    | 20                         | -0.01735 ± 0.00065          | -0.01975 ± 0.00456 |
| 5      | 25                    | 25                         | -0.02246 ± 0.00086          | -0.02156 ± 0.00490 |
| 6      | 30                    | 30                         | -0.02932 ± 0.00092          | -0.02574 ± 0.00413 |

The %R.S.D was found to be 0.39 - 0.65% for IRB and 0.34 - 0.68% for ATR.

These %R.S.D values were found to be less than 1.0, indicating that the method is precise.

Fig. 6 Calibration curve for IRB at 225.20 nm

Fig. 7 Calibration curve for ATR at 308.15 nm

Table 2: Intraday precision data for estimation of IRB and ATR* ($n=3$)

| Conc. (µg/ml) | Abs. (IRB)* Avg. ± SD (225.20 nm) | % RSD | Abs. (ATR)* Avg. ± SD (308.15 nm) | % RSD |
|---------------|----------------------------------|-------|----------------------------------|-------|
| IRB           |                                  |       |                                  |       |
| 5             | -0.00374                         | -0.65 | -0.0205                          | -0.68 |
| 15            | -0.01258                         | -0.43 | -0.01073                         | -0.5  |
| 30            | -0.02505                         | -0.39 | -0.0293                          | -0.34 |

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

| Conc. (µg/ml) | Abs.* (IRB) Avg. ± SD (225.20 nm) | % RSD | Abs. (ATR)* Avg. ± SD (308.15 nm) | % RSD |
|---------------|----------------------------------|-------|----------------------------------|-------|
| IRB           |                                  |       |                                  |       |
| 5             | -0.0041 ± 0.00035                | 0.84  | -0.0023 ± 0.00020                | 0.89  |
| 15            | -0.0135 ± 0.00010                | 0.72  | -0.0117 ± 0.00051                | 0.49  |
| 30            | -0.0248 ± 0.00162                | 0.41  | -0.0302 ± 0.00011                | 0.38  |

Accuracy

Accuracy of the method was determined by recovery study from synthetic mixture at three levels (80%, 100%, and 120%) of standard addition. The % recovery values are tabulated in Table 4 and 5. Percent recovery for IRB and ATR by this method was found in the range of 98.95 to 101.56% and 99.16 to 100.5%, respectively. The value of %RSD with the limit indicated that the method is accurate and the percentage recovery shows that there is no interference from the excipients.

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Table 4: Recovery data ofIRB *(n=3)

| Conc. of IRB from formulation (µg/ml) | Amount of Std.IRB added (µg/ml) | Total amount of IRB (µg/ml) | Total amount of IRB found (µg/ml) | % Recovery* (n=3) | % RSD IRB |
|-------------------------------------|---------------------------------|-----------------------------|---------------------------------|------------------|----------|
| 16                                  | 12.8                            | 28.8                        | 28.5 ± 0.25                     | 98.95            | 0.32     |
| 16                                  | 16                              | 32                          | 32.5 ± 0.57                     | 101.56           | 0.46     |
| 16                                  | 19.2                            | 35.2                        | 35.3 ± 0.42                     | 100.28           | 0.33     |

Table 5: Recovery data ofATR*(n=3)

| Conc. of ATR from formulation (µg/ml) | Amount of Std.ATR added (µg/ml) | Total amount of ATR (µg/ml) | Total amount of ATR found (µg/ml) | % Recovery* (n=3) | % RSD ATR |
|-------------------------------------|---------------------------------|-----------------------------|---------------------------------|------------------|----------|
| 2                                   | 1.6                             | 3.6                         | 3.57 ± 0.078                    | 99.16            | 0.77     |
| 2                                   | 2                               | 4                           | 4.02 ± 0.018                    | 100.5            | 0.57     |
| 2                                   | 2.4                             | 4.4                         | 4.37 ± 0.025                    | 99.31            | 0.48     |

Limit of detection and quantitation

The LODfor IRB and ATR was confirmed to be 10.290 µg/ml and 9.630 µg/ml, respectively.

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

| Conc. (µg/ml) | ATR | Abs.* (IRB) | % | Abs.* (ATR) | % R |
|---------------|-----|-------------|---|-------------|----|
| 5             | 5   | -0.0037 ± 0.0082 | 1.178 | 5.29 | 4.63 |
| LOD (µg/ml)   |     |             |   |             |    |
| LOQ (µg/ml)   |     |             |   |             |    |

Robustness and Ruggedness

The obtained Ruggedness and Robustness results are presented in Table 7.

The % R.S.D was found to be 0.22-0.94% for IRB and 0.33-0.86% for ATR.

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

| Conc. (PPM) | Irbesartan (Mean Abs.* ±% RSD) | Atorvastatin (Mean Abs.* ±% RSD) |
|-------------|---------------------------------|---------------------------------|
| 2           | -0.0041 ± 0.84                  | -0.0023 ± 0.65                  |
| 3           | -0.0136 ± 0.73                  | -0.0024 ± 0.61                  |
| 4           | -0.0255 ± 0.49                  | -0.0024 ± 0.61                  |

APPLICATION OF THE PROPOSED METHOD FOR ANALYSIS OF IRB AND ATR SYNTHETIC MIXTURE

A first order derivative spectrum of the samples containing 32 mg of IRB and 4 mg of ATR was recorded and the absorbance at 225.20 nm and 308.15 nm were noted. The estimation of IRB and ATR was done using the corresponding calibration graph.

The results from the analysis of synthetic mixture containing Irbesartan (32 mg) and Atorvastatin (4 mg) in combination are presented in Table 8.

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

| Concentration (µg/ml) | % Recovery (Mean ± SD) |
|-----------------------|------------------------|
| 2                     | -0.0042 ± 0.65         |
| 3                     | -0.0133 ± 0.75         |
| 4                     | -0.0255 ± 0.49         |
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Table 9: Summary of validation parameters

| Sr. No. | Formulation (synthetic mixture) | Absorbance* (225.20nm) | %Assay IRB ±SD | Absorbance* (308.15nm) | %Assay ATR ±SD |
|---------|-------------------------------|--------------------------|----------------|--------------------------|----------------|
| 1       | IRB                           | -0.0265                  | 99.25 ± 0.71   | -0.00213                 | 99.21 ± 0.21   |
| 2       | ATR                           | -0.0264                  |                | -0.00212                 |                |
| 3       |                               | -0.0265                  |                | -0.00215                 |                |

First-derivative UV Spectrometry

| PARAMETERS                      | Irbesartan | Atorvastatin |
|---------------------------------|------------|--------------|
| Concentration range [µg/ml]     | 5 - 30     | 5 - 30       |
| Regression equation             | y = -0.0008x - 0.0003 | y = -0.0011x + 0.0033 |
| Correlation coefficient (r^2)   | 0.9984     | 0.9938       |
| Accuracy (% Recovery) (n=3)     | 100.26     | 99.65        |
| Intra-day Precision (%RSD) (n=3)| 0.39-0.65  | 0.34-0.68    |
| Inter-day Precision (%RSD) (n=3)| 0.41-0.94  | 0.38-0.89    |
| LOD [µg/ml]                     | 3.396      | 3.178        |
| LOQ [µg/ml]                     | 10.290     | 9.630        |
| Ruggedness and Robustness       | 0.22-0.94  | 0.33-0.86    |
| % Assay                         | 99.25      | 99.21        |

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