

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

**PARASVIRANI<sup>1,2\*</sup>, RAJANIT SOJITRA<sup>2</sup>, BHADRESH SAVAJ<sup>2</sup>, HASUMATIRAJ<sup>2</sup>, VINEET JAIN<sup>2</sup>.**

<sup>1</sup>Research Scholar 2014, Gujarat Technological University, Gujarat, <sup>2</sup>Quality Assurance Department, Shree Dhanvantary Pharmacy College, Kim, Surat

Email: Parasvirani@gmail.com,

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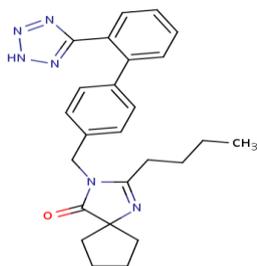
**ABSTRACT**

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 IAtorvastatin) 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-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]

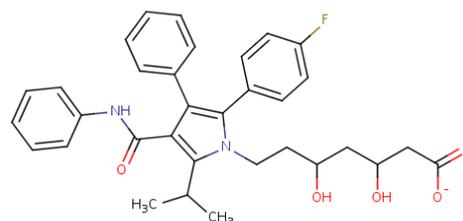


**Fig.1 Structure of Irbesartan[3]**

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.



**Fig. 2: Structure of atorvastatin[5]**

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 (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 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 32 µg/ml and ATR was 4 µg/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 32 µg/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  $\lambda_{max}$  of the both drugs. Therefore, it can be easy to measure the response of the both drugs in the combined mixture. The  $\lambda_{max}$  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 derivatives spectra. The IRB shows the ZCP at 308.15nm and ATR shows the ZCP at 225.20nm. On the basis these IRB can be quantified by measuring the absorbance at 225.20nm and ATR can be quantified by measuring the absorbance at 308.15nm.

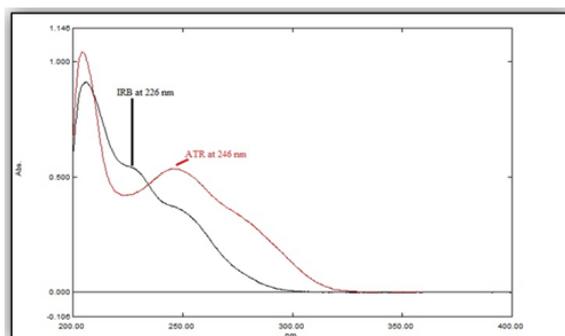


Fig.3: Overlaid zero order spectra of IRB and ATR in methanol (1:1)

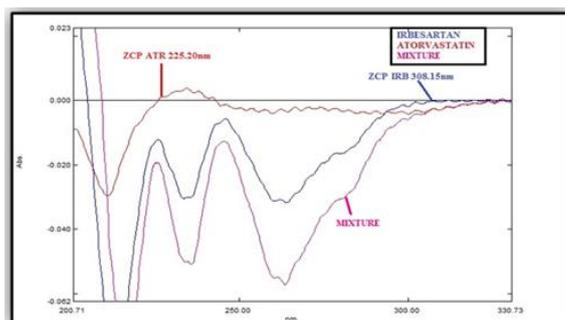


Fig. 4: Overlaid first order spectra of IRB and ATR in 8:1 ratios, respectively with the combination solution (8:1)

## RESULT AND DISCUSSION

### Validation Parameters [10]

#### Linearity and Range

The first-derivative spectra (Fig. 5) showed linear absorbance at 225.20 nm (ZCP of ATR) for IRB (1-6 µg/ml) and 308.15 nm (ZCP of IRB) for ATR (25-150 µg/ml) with correlation coefficient ( $r^2$ ) of 0.9996 and 0.9996 for IRB and ATR, respectively.

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

Correlation coefficient ( $r^2$ ) for calibration curve of IRB and ATR was found to be 0.9996 and 0.9996, respectively (Figure 6 and 7)

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

$$y = -0.0008x - 0.0003 \text{ for IRB} \quad (1)$$

$$y = -0.0011x + 0.003 \text{ for ATR} \quad (2)$$

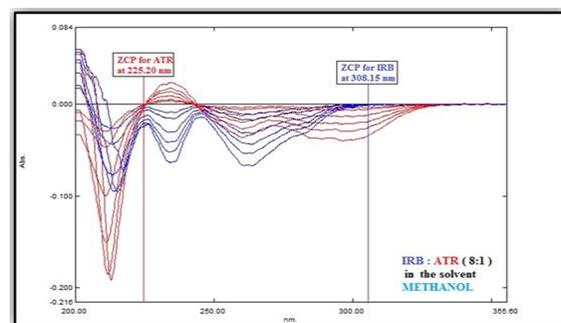
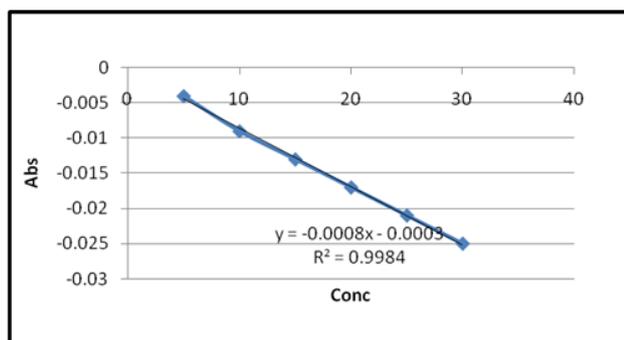


Fig. 5 Overlaid linear first order spectra of IRB (Pink) and ATR (Blue) in 8:1 ratios

From the combination solution of IRB and ATR the dilution was made in ratio of 8:1 and absorbance was recorded (Table 1) and correlation coefficient ( $r^2$ ) of 0.9938 (figure 6) and 0.9984 (figure 6) for IRB and ATR, respectively.

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

Sr. No	Concentration (µg/ml)		Absorbance* (225.20nm)±SD IRB	Absorbance* (308.15nm)±SD ATR
	IRB	ATR		
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	-0.01795±0.00456
5	25	25	-0.02246±0.00086	-0.02156±0.00490
6	30	30	-0.02932±0.00092	-0.02574±0.00413



**Fig.6 Calibration curve for IRB at 225.20nm**

**Precision**

**Intraday precision**

The data for intraday precision for combined standard solution of IRB and ATR is presented in Table 2

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

Conc. (µg/ml)		Abs. (IRB)* Avg. ± SD(225.20nm)	% RSD	Abs. (ATR)* Avg.± SD(308.15nm)	% RSD
IRB	ATR				
5	5	-0.00374	-0.65	-0.00205	-0.68
15	15	-0.01258	-0.43	-0.01073	-0.5
30	30	-0.02505	-0.39	-0.0293	-0.34

**Interday precision**

The data for interday precision for combined standard solution of IRB and ATR is presented in Table 3

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

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

**Accuracy**

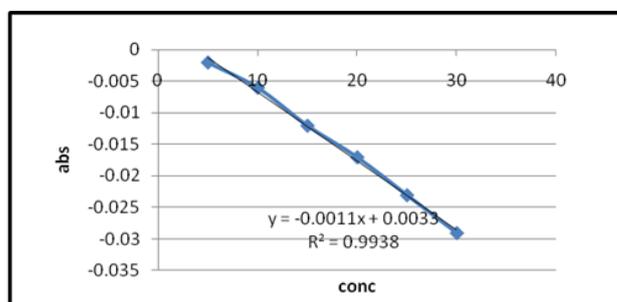
Accuracy of the method was determined by recovery study from synthetic mixture at three levels (80%, 100%, and 120%) of standard addition.

The recovery values are tabulated in Table 4 and 5

Percentage recovery for IRB

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

These %RSD values were found to be less than ±1.0 indicated that the method is precise.



**Fig.7 Calibration curve for ATR at 308.15nm**

The % R.S.D was found to be 0.41-0.84% for IRB and 0.38-0.89% for ATR.

These %RSD values were found to be less than ±1.0 indicated that the method is precise.

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 within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients.

Table 4: Recovery data of IRB \*(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) Mean*± SD	% 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 of ATR\*(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) Mean*± SD	% 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 LOD for IRB and ATR was conformed to be 3.396 µg/ml and 3.178 µg/ml, respectively.

The LOQ for IRB and ATR was conformed to be 10.290 µg/ml and 9.630 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)		Abs.* (IRB)	%	Abs.* (ATR)	% R
IRB	ATR	Avg. ± SD (225.20nm)		Avg. ± SD (308.15nm)	
5	5	-0.0037 ± 0.00082		-0.0023 ± 0.00101	
	LOD (µg/ml)	2.396		1.178	
	LOQ (µg/ml)	5.29		4.63	

**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.

These % RSD values were found to be less than ± 1.0, indicating that the method is precise.

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

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

Conc. (PPM)	Irbesartan (Mean Abs.* ± % RSD)				
	Instrument 1	Instrument 2	Stock - 1	Stock - 2	
2	-0.0041 ± 0.84	-0.0042 ± 0.94	-0.0042 ± 0.72	-0.0042 ± 0.75	
3	-0.0136 ± 0.73	-0.0145 ± 0.68	-0.0133 ± 0.75	-0.0136 ± 0.73	
4	-0.0255 ± 0.49	-0.0261 ± 0.22	-0.0253 ± 0.60	-0.0257 ± 0.22	
	Atorvastatin (Mean Abs.* ± % RSD)				
50	-0.0023 ± 0.65	-0.0024 ± 0.61	-0.0023 ± 0.65	-0.0023 ± 0.42	
75	-0.0115 ± 0.49	-0.0119 ± 0.84	-0.0115 ± 0.51	-0.0115 ± 0.86	
100	-0.0296 ± 0.51	-0.0302 ± 0.33	-0.0292 ± 0.34	-0.0294 ± 0.51	

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

A first order derivative spectrum of the sample solution containing 32 µg/ml of IRB and 4 µg/ml of ATR was recorded and the absorbance at 225.20 nm and 308.15 nm were noted for estimation of IRB and ATR, respectively.

The concentration of IRB and ATR in mixture was determined 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.

The percent assay shows that there is no interference from excipients and the proposed method can successfully be applied to analysis of commercial formulation containing IRB and ATR. The % assay values are tabulated in Table 8

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

Sr. No.	Formulation (synthetic mixture)		Absorbance* (225.20nm) IRB	%Assay IRB±SD	Absorbance* (308.15nm) ATR	%Assay ATR±SD
	IRB	ATR				
1			-0.0265		-0.00213	
2	32	4	-0.0264	99.25 ± 0.71	-0.00212	99.21 ± 0.21
3			-0.0265		-0.00215	

Table 9: Summary of validation parameters

PARAMETERS	First-derivative UV Spectrometry	
	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 <sup>2</sup> )	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)	0.41-0.84	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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