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Research Article

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

PARASVIRANI[1,2]*, RAJANIT SOJITRA[2], BHADRESH SAVAJ[2], HASUMATIRAJ[2], VINEET JAIN[2].

[1]Research Scholar2014, Gujarat TechnologicalUniversity,Gujarat, [2]QualityAssuranceDepartment, ShreeDhanvantaryPharmacyCollege, Kim, Surat

Email: Parasvirani@gmail.com,

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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 of any of the two drugs) were selectedfor measurement of absorptivity. Both the drugs show linearity in a concentration range of 05-30 µg/ml at theirrespective λmax and at the isoabsorptive point. Accuracy, precision and recovery studies were done by QC samplescovering 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 0.365µg/ml and 0.0622µg/ml for Irbesartan and atorvastatin, respectively. The limit of quantification was 1.108µg/ml and 0.188µg/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 [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 type1 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)

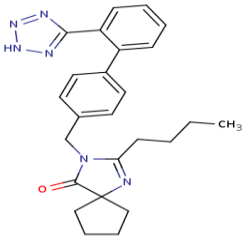

Fig.1: Structure of Irbesartan[3]

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.

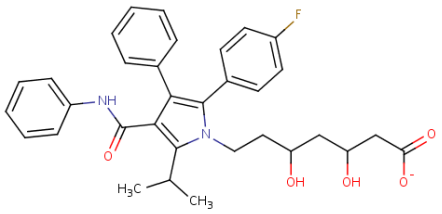

Fig.2: Structureofatorvastatin[5]

Atorvastatin iswhiteoralmostwhite,crystallinepowder.Solubilityisgiveninpractically insoluble in water, soluble in methanol, slightlysolublein methylenechloride.

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 atorvastati[6,7]

The review of literature regarding quantitative analysis of Irbesartan and atorvastatinrevealed that no attempt was made to develop analytical methods for Irbesartan and atorvastatin. Some spectrometric methods and chromatographic methods have been reported for theestimation 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 atorvastatinin Synthetic mixture.[8,9]

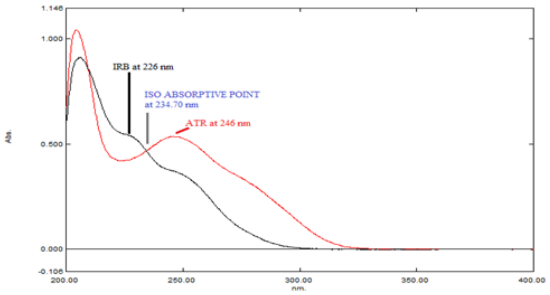

Fig. 3: Overlainzero orderspectraofIRB andATR in methanol (1:1)

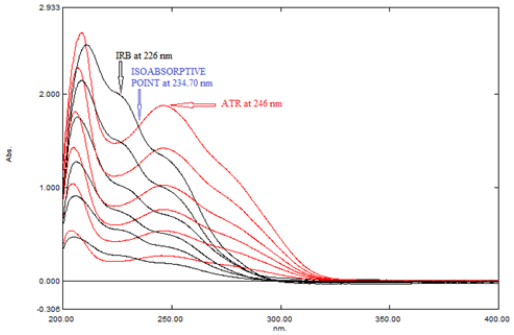

Fig. 4: Linearityzero orderspectraofIRB andATR in methanol (1:1)

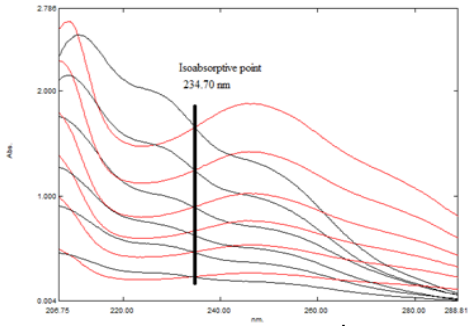

Figure 5: Iso absorptive point at 234.70nm in zero orderspectra (1:1)

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 (Borosillicte)

Standard solutionofIrbesartan (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 226.00nm and 246nm. Plotte 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 λ_{max} of the both drugs. Therefore, it can be easy to measure the response of the both drugs in the combined mixture. The λ_{max} 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 (λ_1) and 226.00 nm (λ_2) were selected for the estimation of both the drugs by absorbance ratio method.

RESULT AND DISCUSSION

Validation Parameters[10]

Linearity andRange

Different concentrations of Irbesartan (5- 30µg/ml) and Atorvastatin (5- 30µg/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 234.70nm (λ_1) and 226.00nm (λ_2) 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.

Correlationcoefficient(r^2)forcalibrationcurveofIRBandATRwasfound to be 0.9994 and 0.9995,respectively.

Theregression line equation forIRB and ATR are as following,

y = 0.0645x - 0.0849 for IRB at 226.00nm_____ (1)
y = 0.0641x - 0.0795 for ATR at 246.00nm_____ (2)
y = 0.0572x - 0.0915 for IRB at 234.70nm_____ (3)
y = 0.0561x - 0.0721 for ATR at 234.70nm_____ (4)

- **Absorption ratio equation**

$$C_x = \{(Q_M-Q_y)/ (Q_x-Q_y)\}* (A_1/ax_1)$$

$$C_y = \{(Q_M-Q_x)/ (Q_y-Q_x)\}* (A_1/ay_1)$$

Where, C_x = Concentration of IRB
C_y = Concentration of ATR

A_1 = Absorbance of test at λ_1 (iso absorptive point)

A_2 = Absorbance of test at λ_2 (λmax of IRB)

$Q_M = A_2/A_1$

$Q_x = ax_2/ax_1$

$Q_y = ay_2/ay_1$

ax_1 = Absorptivity of x drug at λ_1

ax_2 = Absorptivity of x drug at λ_2

ay_1 = Absorptivity of y drug at λ_1

ay_2 = Absorptivity of y drug at λ_2

Table 1: Absorbance forIRB andATRat 226.00nmand 234.70nm, respectively. *(n=6)

IRB			ATR		
Conc. µg/ml	**Mean Abs.* At 234.70nm**	**Mean Abs.* At 226.00nm**	**Conc. µg/ml**	**Mean Abs.* At 234.70nm**	**Mean Abs.* At 226.00nm**
05	0.2308±0.0022	0.2711±0.0023	05	0.2351±0.0014	0.2061±0.0020
10	0.4646±0.0017	0.5460±0.0029	10	0.4665±0.0033	0.4246±0.0017
15	0.7561±0.0020	0.8438±0.0025	15	0.7763±0.0015	0.7061±0.0027
20	1.0236±0.0019	1.2121±0.0020	20	1.0361±0.0017	0.9250±0.0026
25	1.3216±0.0033	1.5225±0.0030	25	1.2958±0.0020	1.1991±0.0031
30	1.6650±0.0016	1.8683±0.0026	30	1.6513±0.0017	1.4838±0.0019

Table 2: Average of absorptivities at 228.60 and 226.00 nm

at 234.70nm		at 226.00nm	
ax_1	0.0475	**ax_2**	0.0428
ay_1	0.0468	**ay_2**	0.0555

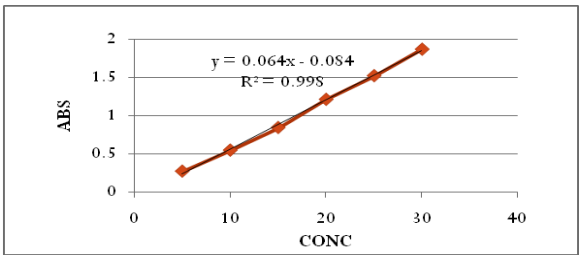

Fig. 6: Calibration graph of Irbesartan at 226.00 nm

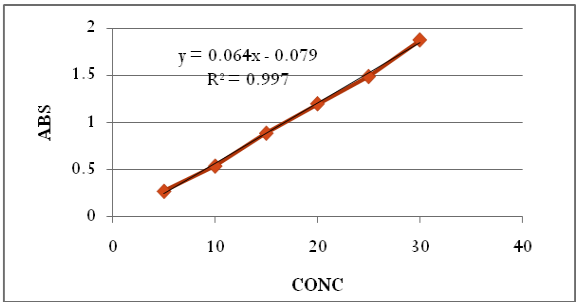

Fig.7: Calibration graph of Atorvastatin at 246.00 nm

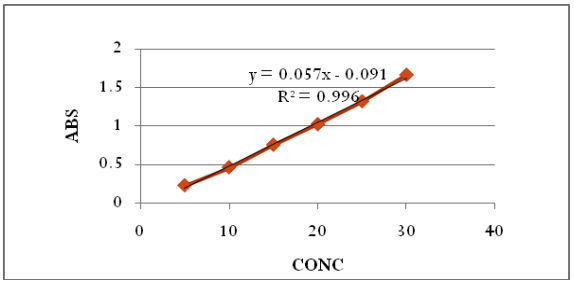

Fig. 8: Calibration graph of Irbesartan at 234.70 nm

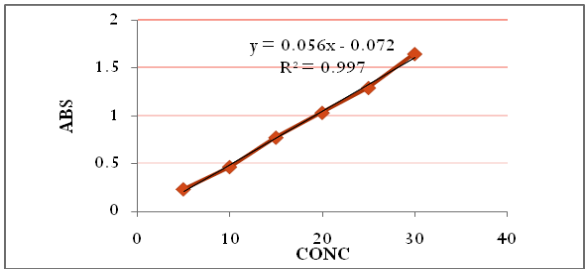

Fig. 9: Calibration graph of Atorvastatin at 234.70 nm

Precision

Intraday precision

Mixed solutions of IRB and ATR containing 5, 15 and 30 µg/ml and 05, 15 and 30 µg/ml respectively series were analyzed three times on the same day using developed spectroscopic method and %RSD was calculated. The%RSD wasfoundto be 0.32– 0.75% forIRBand 0.41 -0.56% for ATR. These%RSDvaluewasfoundtobelessthan±2.0indicatedthatthemetho d is precise.(Table 6.19)

Table 3: Intraday precision data for estimation ofIRB andATR*(n=3)

Conc. (µg/ml)		**Mean Abs.* ±SD IRB**	**% RSD**	**Mean Abs.*±SD ATR**	**% RSD**
IRB	ATR				
5	5	0.4613±0.00035	0.75	0.4416±0.00025	0.56
15	15	1.4122±0.00060	0.38	1.3183±0.00064	0.48
30	30	2.7513±0.00091	0.32	2.6456±0.00010	0.41

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 wasfoundto be 0.31 – 0.82% forIRBand 0.32 – 0.71% for ATR. These% RSD valuewasfoundtobelessthan±2. 0indicatedthatthemethod is precise. (Table 6.20)

Table 4: Interday precision data for estimation ofIRB andATR*(n=3)

Conc. (µg/ml)		Mean Abs. *±SD IRB	% RSD	Mean Abs.*±SD ATR	% RSD
IRB	ATR				
5	5	0.4852±0.00040	0.82	0.4712±0.00030	0.64
15	15	1.4477±0.00055	0.31	1.3621±0.00096	0.71
30	30	2.7513±0.00094	0.34	2.6617±0.00085	0.32

Accuracy

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 %.

% recoveryforIRBandATRbythismethodwasfoundintherange of99.80 to 101.71% and 98.61 to 101.113%, respectively (Table 6.21 and 6.22)

Thevalueof%RSDwithinthelimitindicatedthatthemethodisaccuratea nd percentagerecoveryshows that thereis no interferencefrom the excepients.

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

Conc. ofIRB from formulation (µg/ml)	Amount of Std.IRB added (µg/ml)	Total amount of IRB (µg/ml)	Total amount ofIRB found (µg/ml)* Mean± SD	% Recovery	% RSD
16	12.8	28.8	28.81±0.064	100.03	0.22
16	16.0	32.0	32.55±0.068	101.71	0.21
16	14.2	35.2	35.13±0.104	99.80	0.28

Table 6: Recovery data ofATR*(n=3)

Conc. ofATR from formulation (µg/ml)	Amount of Std.ATR added (µg/ml)	Total amount of ATR (µg/ml)	Total amount ofATR found (µg/ml)* Mean± SD	% Recovery	% RSD
2	1.6	3.6	3.55±0.064	98.61	0.90
2	2.0	4.0	4.01±0.030	100.75	0.75
2	2.4	4.4	4.46±0.035	101.13	0.78

Limit of detection and quantitation

TheLODforIRBandATRwasconformedtobe 0.365µg/mland 0.0622µg/ml, respectively. TheLOQforIRBandATRwasconformedtobe 1.108 µg/mland 0.188µg/ml, respectively. TheobtainedLODandLOQresults arepresentedin Table 6.23.

Table 7:LOD andLOQ dataof IRB andATR *(n=10)

	IRB (µg/ml) *	ATR(µg/ml) *
LOD	0.365	0.0622
LOQ	1.108	0.188

Robustness andRuggedness

Theobtained Ruggedness andRobustness results arepresented in table6.24. The% RSD was foundto be for 0.17 – 0.52%IRB and0.24 – 0.59% for ATR. These%RSDvaluewasfoundtobelessthan±2.0indicatedthatthemethod is robust and rugged.

No significant changes in the spectrums were observed, proving that the developed method is ruggedand robust.

Table 8: RobustnessandRuggedness dataof IRB andATR*(n=3)

Conc. (PPM)	Irbesartan (Mean abs. ±% RSD)			
	Instrument 1	**Instrument 2**	**Stoke – 1**	**Stoke – 2**
2	0.4621±0.42	0.4623±0.52	0.4626±0.42	0.4629±0.45
3	0.9166±0.39	0.9169±0.38	0.9162±0.38	0.9159±0.31
4	1.4107±0.22	1.4109±0.22	1.4110±0.26	1.4149±0.17
Atorvastatin (Mean abs. ±% RSD)				
20	0.4412±0.59	0.4423±0.45	0.4417±0.28	0.4414±0.52
30	0.8829±0.24	0.8834±0.43	0.8821±0.35	0.8819±0.43
40	1.3222±0.35	1.3231±0.24	1.3233±0.36	1.3230±0.46

Stock-1:- 10 mg dissolve in 100 ml Methanol

Stock-2:- 20 mg dissolve in 100 ml Methanol

Application oftheproposed method for analysisof IRBand ATRin formulation

Azeroorderspectrum ofthe test solutionwasrecordedand Measure the absorbance at 234.70nm (λ_1) and 226nm (λ_2) forestimationofATR andIRB.The concentrations of IRB and ATR in formulation were determinedusing the absorption ratio equation. The%assayvaluesare givenin Table 6.25.

Table 9: Analysisdata of formulation*(n=3)

Sr. No	Drug	Formulation (µg/ml)	%Assay* ± SD
1	**IRB**	32.0	101.60±0.054
2	**ATR**	4.0	99.18±0.023

Table 10: Summary ofvalidation parameters

PARAMETERS	Absorption Ratio Method	
	IRB	**ATR**
Concentration range(µg/ml)	5-30	5-30

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-dayPrecision (%RSD) (n=3)	0.32-0.75	0.41-0.56
Inter-dayprecision (%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.

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