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Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR IRBESARTAN AND ATORVASTATIN BY SIMULTANEOUS EQUTION 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 usingsimultaneous eqution Method.In this spectroscopic method, 226.00 nm and 246.00 nm wavelengths were selectedfor measurement of absorptivity. Both the drugs show linearity in a concentration range of 05-30 µg/ml at theirrespective λmax. 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 0.033µg/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 [1].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 type1 antagonists have been widely used in treatment of diseases like hypertension, heart

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.

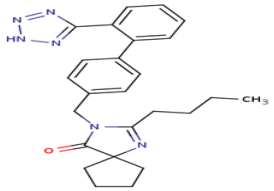

Fig.:1 Structure of Irbesartan[3]

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.

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

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)

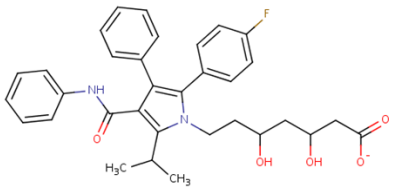

Fig. 2: Structureofatorvastatin(5)

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 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)

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 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 2µg/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 2µg/ml.

alibration 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. Linearityrange of IRB and ATR was found with correlation co-efficient.

DEVELOPMENT AND VALIDATION OF SPECTROSCOPIC SIMULTANEOUS EQUATION METHOD

SELECTION OFWAVELENGTHAND METHOD DEVELOPMENT FORDETERMINATION OF IRBESARTAN AND ATORVASTATIN

ThestandardsolutionofIRBandATRwerescannedseparatelybetween200-400nm, and IRBshowedabsorbancemaximaat226.00nmandATRat246.00nm.(figure 3)

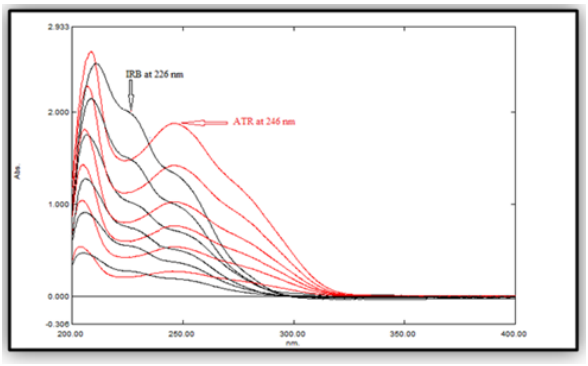

Fig.3 Overlainzero orderspectra ofIRB andATR(8:1) ratios, respectively

Table 1Calibrationdata forIRB andATRat226.00 nmand246.00 nmrespectively. *(n=6)

Sr. No	Concentration (µg/ml)		Absorbance* (226.00nm)±SD IRB	Absorbance* (246.00nm)±SD ATR
	IRB	ATR		
1	05	05	0.3708±0.0023	0.2672±0.0015
2	10	10	0.7460±0.0020	0.5674±0.0017
3	15	15	1.2171±0.0013	0.8872±0.0018
4	20	20	1.6972±0.0015	1.1974±0.0012
5	25	25	2.2225±0.0013	1.5232±0.0022
6	30	30	2.7653±0.0025	1.8772±0.0016

VALIDATION PARAMETERS(10)

Linearity andRange

TheZero order(fig.3)showedlinearabsorbanceat 226.00 nm for IRB(05-30 µg/ml)and 246.00 nmforATR(5-30 µg/ml)withcorrelationcoefficient(r^2)of0.9994and0.9993forIRB and ATR, respectively.

Thismethodobeyedbeer'slawintheconcentrationrange05 -30µg/mland5 -30 µg/ml forIRB and ATR, respectively. (Table 1)

Correlationcoefficient(r^2)forcalibrationcurveofIRBandATRwasfound to be 0.9994 and 0.9993,respectively(figure4and 5)

Theregression line equation forIRB and ATR are as following,

y = 0.0983x - 0.2385 for IRB _____ (1)

y = 0.0642x - 0.0695 for ATR _____ (2)

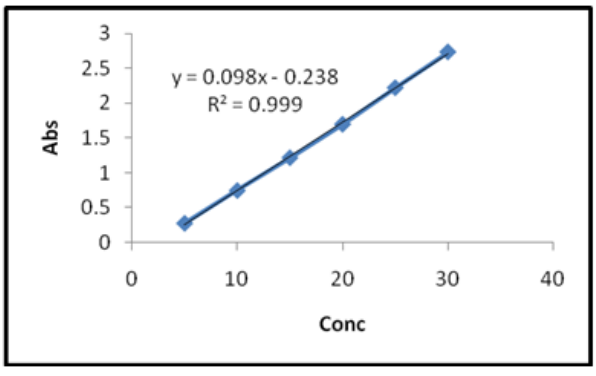

Fig.4CalibrationcurveforIRB at 226.00 nm

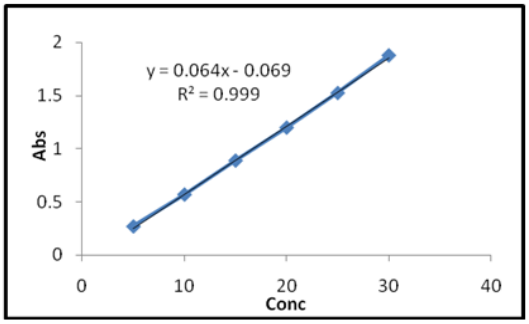

Fig. 5CalibrationcurveforATR at 246.00 nm

Precision

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% RSDvaluewasfoundtobelessthan±2.0indicatedthatthemethod is precise.

Table 2 Intraday precision data for estimation ofIRB andATR*(n=3)

Conc. (µg/ml)		IRB Abs.* ± % RSD ±% RSD Abs. ±% RSD IRB	ATR Abs.*± % RSD
IRB	ATR		
05	05	0.372±0.45	0.266±0.57
15	15	1.211±0.21	0.884±0.92
30	30	2.763±0.52	1.877±0.23

Interdayprecision

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%RSDvaluewasfoundtobelessthan±2.0indicatedthatthemethod is precise.

Table 3Interdayprecision data for estimation ofIRB andATR*(n=3)

Conc. (µg/ml)		IRB Abs.* ±% RSD ±% RSD Abs.	ATR Abs.*±%RSD
IRB	ATR	±% RSD IRB	
05	05	0.377±0.55	0.270±0.56
15	15	1.215±0.25	0.887±0.17
30	30	2.786±0.85	1.881±0.36

Accuracy

Accuracyofthemethodwasdeterminedbyrecoverystudyfromsynthetic mixture at threelevel (80%, 100%, 120%)of standard addition. The% recoveryvalues aretabulated in Table 4and 5

PercentagerecoveryforIRBandATRbythismethodwasfoundinthera nge of100.07 to 100.43% and99.21 to 100.55%, respectively,

Thevalueof%RSDwithinthelimitindicatedthatthemethodisaccurate and percentagerecoveryshows that thereis no interferencefrom theexcepients.

Table 4Recovery 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 (n=3)	% RSD IRB
8	6.4	14.4	12.81±0.022	100.07%	0.32%
8	8.0	16.6	16.07±0.013	100.43%	0.68%
8	9.6	17.6	19.22±0.045	100.10%	0.28%

Table 5Recovery 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 (n=3)	% RSD ATR
1	0.8	1.8	1.81±0.021	100.55%	0.84%
1	1.0	2.0	2.00±0.036	100.50%	0.22%
1	1.2	2.2	2.19±0.20	99.21%	0.35%

Limit of detection and quantitation

TheLODforIRBandATRwasconformedtobe0.033µg/mland0.125µg

/ml, respectively.

TheLOQforIRBandATRwasconformedtobe0.1008µg/mland0.379 µg/ml, respectively.

TheobtainedLODandLOQresults arepresentedin Table 6

Table 6LOD andLOQ dataofIRB andATR *(n=10)

Conc. (µg/ml) IRB	Conc. (µg/ml) ATR	Avg.abs* ± SD (226.00nm) IRB	% RSD	Avg.abs*±SD (246.00nm) ATR	% RSD
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	
		UV-2450	UV-1800	Stock-1*	Stock-2*
Irbesartan	05	0.376±0.32	0.374±0.47	0.376±0.12	0.373±0.82
Mean (n=3)	15	1.215±0.56	1.216±0.22	1.215±0.42	1.216±0.56
± % RSD	30	2.763±0.23	2.765±0.84	2.764±0.21	2.763±0.32
Atorvastatin	05	0.271±0.54	0.269±0.43	0.272±0.42	0.270±0.11
Mean(n=3)	15	0.885±0.66	0.882±0.33	0.884±0.15	0.885±0.33
± %RSD	30	1.879±0.16	1.878±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 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 volumewasmadeuptomarkwithmethanol toobtainfinalsolutioncontaining16µg/mlofIRB and 2µg/ml ofATR. Azeroorder spectrum ofthe resulting solutionwasrecordedandprocessedtofirstderivativespectra.Aspectru mofthesamplesolution wasrecordedandtheabsorbanceat 226.00nmand246.00nmwerenoted forestimationofIRB andATR,respectively.The concentrations of IRB and ATR in formulation were determinedusing thecorresponding calibrationgraph.

Table 8 Analysisdata ofcommercial 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 (r^2)	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

9	Robustness and Ruggedness (%RSD)	0.12-0.84	0.11-0.73
10	Assay	99.75±0.22	99.52 ±0.56

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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