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Research Article

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 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 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]

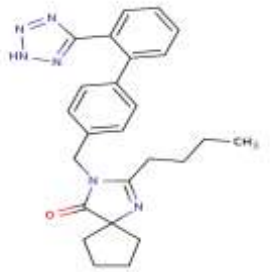

Fig.1 Structure of Irbesartan[3]

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.

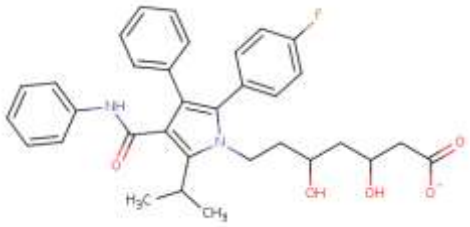

Fig. 2: Structureofatorvastatin[5]

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 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 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. Plotte 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 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.

Thesynthetic mixtureofIrbesartan andAtorvastatinispresentin 8:1ratios,respectively.Theabsorptionspectraofpuredrug andtheirmixturewere recorded between 200-400 nm using Distilled Water as solvent and proceed to first derivativespectra.TheIRB wasshowstheZCPat308.15nmandATRshowsthe ZCP at 225.20nm. On thebasis theseIRB can bequantified bymeasuringthe absorbanceat225.20nmandATRcanbequantifiedbymeasuringtheabs orbanceat 308.15nm.

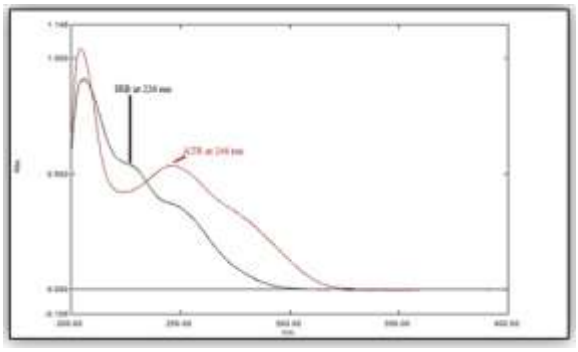

Fig.3: Overlainzero orderspectraofIRB andATR in methanol (1:1)

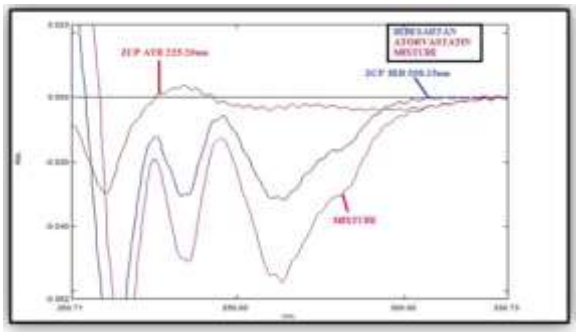

Fig. 4: Overlain firstorderspectra ofIRB andATR in8:1 ratios,respectivelywith thecombination solution (8:1)

RESULT AND DISCUSSION
Validation Parameters[10]
Linearity andRange

TheFirst-derivativespectra(fig.5)showedlinearabsorbanceat 225.20nm (ZCPofATR)forIRB (1-6µg/ml)and 308.15 nm(ZCPofIRB)forATR(25-150µg/ml)withcorrelationcoefficient(r^2)of0.9996and0.9996forIRB and ATR, respectively.

Thismethodobeyedbeer'slawintheconcentrationrange1-6µg/mland25-150µg/ml forIRB and ATR, respectively. (Table 1)

Correlationcoefficient(r^2)formcalibrationcurveofIRB andATRwasfound to be 0.9996 and 0.9996,respectively(figure6and 7)

Theregression line equation forIRB and ATR are as following,

y = -0.0008x - 0.0003for IRB _____ (1)

y = -0.0011x + 0.003 for ATR _____ (2)

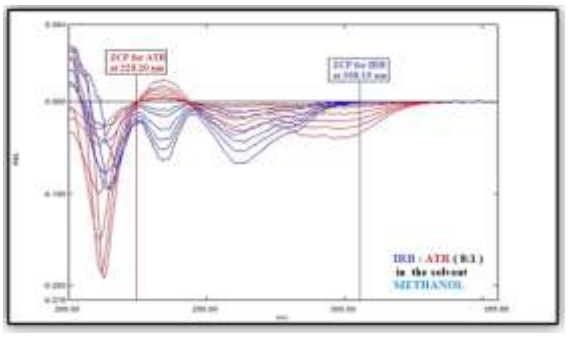

Fig. 5 Overlainlinearfirstorderspectra ofIRB (Pink) andATR(Blue) in8:1 ratios

FromthecombinationsolutionofIRB andATRthedilutionweremadeinratioof 8:1 andabsorbancewererecorded(Table1)andcorrelationcoefficient(r^2)of 0.9938 (figure6) and 0.9984(figure6) for IRB and ATR, respectively.

Table 1:Calibrationdata forIRB andATRat 225.20nmand308.15nm, 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

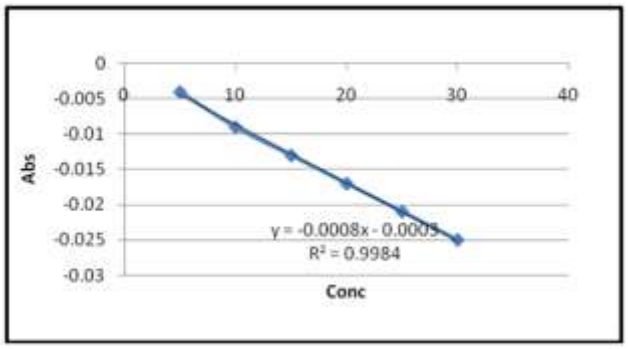

Fig.6 CalibrationcurveforIRB at 225.20nm

Precision

Intraday precision

ThedataforintradayprecisionforcombinedstandardsolutionofIRB and ATRis presented in Table 2

The%R.S.D wasfoundto be 0.39 - 0.65% forIRB and 0.34 -0.68% for ATR.

These%RSDvaluewasfoundtobelessthan±1.0indicatedthatthemethod is precise.

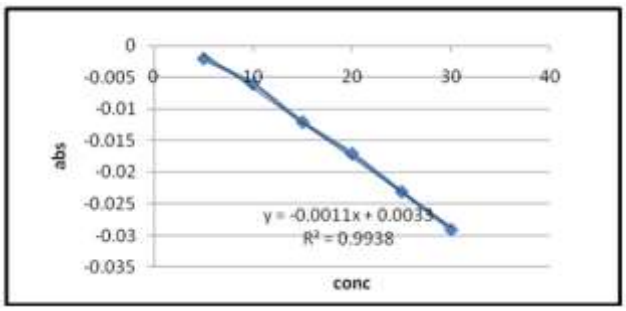

Fig.7CalibrationcurveforATR at 308.15nm

Table 2 Intraday precision data for estimation ofIRB andATR*(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

ThedataforinterdayprecisionforcombinedstandardsolutionofIRB and ATRis presented in Table 3

The% R.S.D was foundto be0.41-0.84% forIRB and0.38-0.89%for ATR.

These%RSDvaluewasfoundtobelessthan±1.0indicatedthatthemethod is precise.

Table 3 Interday precision data for estimation ofIRB andATR*(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

Accuracyofthemethodwasdeterminedbyrecoverystudyfromsynthetic mixture at threelevels (80%, 100%, and 120%) of standard addition.

The% recoveryvalues aretabulated in Table 4and 5

PercentagerecoveryforIRB andATRbythismethodwasfoundintherange of98.95 to 101.56% and99.16 to 100.5%, respectively,

Thevalueof%RSDwithinthelimitindicatedthatthemethodisaccurateand percentagerecoveryshows that thereis no interferencefrom the excipients.

Table 4: 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* (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 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
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

TheLODforIRB andATRwasconformedtobe3.396µg/mland3.178µg/ml, respectively.

TheLOQforIRB andATRwasconformedtobe10.290µg/mland9.630µg/m, respectively.

TheobtainedLODandLOQresults arepresentedin Table 6

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

Conc. (µg/ml) IRB	ATR	Abs.* (IRB) Avg. ± SD(225.20nm)	%	Abs.* (ATR) Avg.±SD(308.15nm)	% R
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 andRuggedness

Theobtained Ruggedness andRobustness results arepresented in table7

The% R.S.D was foundto be 0.22-0.94% forIRB and0.33-0.86% for ATR.

These%RSDvaluewasfoundtobelessthan±1.0indicatedthatthemethod is precise.

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

Table 7RobustnessandRuggedness dataof IRB andATR*(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 OFTHEPROPOSED METHOD FOR ANALYSISOF IRB AND ATRIN SYNTHETIC MIXTURE

Afirstorderderivativespectrumofthesamplesolutioncontaining32µg/ mlof IRB and4µg/mlofATRwasrecordedandtheabsorbanceat 225.20nmand 308.15nm werenotedforestimation of IRB and ATR, respectively.

TheconcentrationofIRB andATRinmixture wasdeterminedusingthe corresponding calibration graph.

Theresultsfromtheanalysisof synthetic mixturecontaining Irbesartan (32mg)and Atorvastatin (4mg) in combination arepresented in Table8.

Thepercentassayshowsthatthereisnointerferencefromexcipientsandt he proposedmethodcansuccessfully appliedtoanalysisofcommercial formulationcontainingIRB andATR.The%assayvaluesare tabulatedin Table 8

Table 8 Analysisdata ofcommercial 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 ofvalidation parameters

PARAMETERS	First-derivativeUV 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^2)	0.9984	0.9938
Accuracy(%Recovery) (n=3)	100.26	99.65
Intra-dayPrecision (%RSD) (n=3)	0.39-0.65	0.34-0.68
Inter-dayprecision (%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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