A NEW VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF IRBESARTAN AND ATORVASTATIN IN COMBINED TABLET DOSAGE FORMS

Lalitha Repudi[1]*, M.Lakshmi Surekha[2]

Abstract:
The combination of Irbesartan and Atorvastatin is prescribed for the treatment of hypertension. To develop andvalidate HPLC Method for simultaneous estimation of Irbesartan and Atorvastatin Pharmaceutical formulations. Thechromatographic separation was performed on Shimadzu HPLC instrument on a Intersil C_{18} column (250 × 4.6 mm, 5 µm particle size) using A mixture of potassium dihydrogen phosphate (pH 3.5) and acetonitrile in the ratio of 30:70 v/v was used as the mobile phase and pumped at a flow rate of 1mL/min. The detector wavelength was set at 254 nm and flow rate of 1 mL/min. The developed method was validated according to ICH Q2R1 guideline. The linearity was established over a concentration range of 20-50 ng and 60-300 µg/mL with correlation coefficient r2 = 0.9995 and 0.9997 for Irbesartan and Atorvastatin, respectively. The Rt of Irbesartan and Atorvastatin were found to be 4.01±0.03 and 7.03±0.03 respectively. Recovery of drug was achieved in the range of 99.72–99.89% and 99.89–101.07% for Irbesartan and Atorvastatin, respectively by developed method. The LOD and LOQ of Irbesartan and Atorvastatin were found to be 316.92 and 2629 µg/mL, 104.58 and 722.1 µg/mL respectively. The developed HPLC method was applied for simultaneous estimation of two drugs in their synthetic mixture and results were found to be in good agreement with the labeled claim.The developed HPLC method was found to be accurate, precise, specific and sensitive. It can be applied for routine analysis (assay) of tablets containing combination of Irbesartan and Atorvastatin.

Keywords: Atorvastatin, Irbesartan, Reverse phase -High Performance Liquid Chromatography (RP-HPLC), PDA Detection; Simultaneous estimation Forced degradation.

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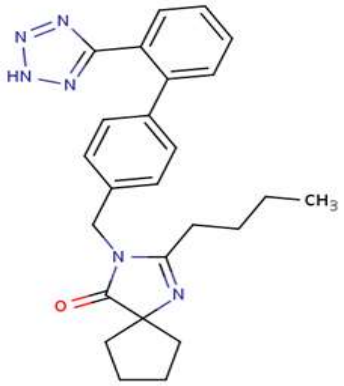
INTRODUCTION

1. Irbesartan

Irbesartan[1-3]-butyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl]-1,3diazaspiro [4.4] non-1-en-4-one, is a non-peptidic angiotensin receptor blocker (ARB) mainly used for the treatment of hypertension. It exerts its antihypertensive by selectively blocking AT1 receptors that are abundantly present in vascular smooth muscle and inhibiting the strong vasoconstrictor action of angiotensin II.

A combination therapy(Table 1) employing Irbesartan and Atorvastatin is found to be useful in the treatment of Antihypertensive. A "Rovelito" combination tablet is a novel fixed-dose combination medicine of Irbesartan.(Fig.1).

Chemical Structure



Chemical name: a 2-butyl-3-[p-(o-1Htetrazol-5-ylphenyl) benzyl]- 1,3- diazaspiro[4.4]non-1-en-4-one
Molecular formula: $C_{25}H_{28}N_6O$

Molecular weight: 428.5

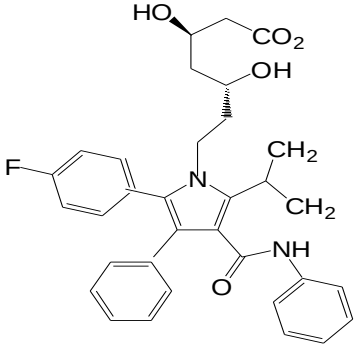
Appearance: White to yellow crystalline powder
Solubility: slightly soluble in alcohol and methylene chloride and practically insoluble in water.

2. Atorvastatin

Atorvastatin[1-3] is a selective, competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol (fig 2.).

Chemically Atorvastatin is (3*R*, 5*R*)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3, 5 dihydroxy heptanoic acid.

Chemical Structure:



Chemical name: (3*R*, 5*R*)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5- propan-2-ylpyrrol-1-yl]-3, 5 dihydroxy heptanoic acid.

Molecular formula: $C_{33}H_{35}FN_2O_5$

Molecular weight: 558.6398 g/mol

Appearance: White to yellow crystalline powder
Solubility: Very slightly soluble in distilled water, phosphate buffer, and acetonitrile; slightly soluble in ethanol; and freely soluble in acetonitrile

A few analytical methods have been reported for the determination of Irbesartan and Atorvastatin in bulk drug and pharmaceutical dosage forms, alone and in combination with other drugs[4-17] No method has been reported for their simultaneous estimation in combined dosage form. Hence it was thought of interest to develop and validate HPLC[18-22] method.

Table 1 Formulation containing Irbesartan and Atorvastatin

Brand Name	Formulation	Strength		Manufacturer
Rovelito	Tablets	Irbesartan	Atorvastatin	1. Hanmi Pharmaceutical, Japan. 2. Sanofi. Pharmaceuticals,India.
		150 mg	10mg	

EXPERIMENTAL AND RESULTS

MATERIALS AND METHODS

Instrumentation

The chromatographic system consisted of Waters -2695 chromatograph equipped with an Intersil C_{18} column (250 × 4.6 mm; 5µ), LC-20AD pumps and an SPD-20A Photo diode Array (PDA) detector. Samples were injected into the system through a Rheodyne 7725 injection valve via a 20 µL loop. The output signal was monitored and integrated by Empower-2 software. Waters C_{18} column (250 x 4.6 mm; 5µ) was used for this method. Solubility of the compound was enhanced by sonication on an ultra sonicator (PCI Analytics PCI81). All the weighings in the experiments were done with Shimadzu balance (model AX200). PVDF membrane filters were purchased from Merck Millipore.

Drugs and chemicals

Reference samples of Irbesartan (purity 99.7%) and Atorvastatin (purity 99.9%) were obtained from AET Laboratories (Hyderabad, India) as gift samples. The commercial tablet formulation, "Rovelito" (Hanmi Pharmaceuticals, (Korea, Japan) & Sanofi india Ltd, India) was purchased from local market. Potasium dihydrogen phosphate and orthophsparic acid were purchased from Qualigens Chemicals Limited. HPLC grade acetonitrile was procured from Merck104 Limited. HPLC grade water was prepared by using Millipore Milli-Q system.

Preparation of buffer

About 1.17 g of Potassium dihydrogen orthophosphate was accurately weighed and taken into 250 ml volumetric flask.

1.17 g of Potassium dihydrogen orthophosphate was transferred into a beaker containing 250 mL of water and mixed. The pH of the solution was

adjusted to 3.5 with orthophospharic acid. The solution was then filtered through a 0.45µ membrane filter and sonicated.

Preparation of diluent

A mixture of the buffer and acetonitrile in the ratio of 50:50 v/v was used as the diluent.

Preparation of mobile phase

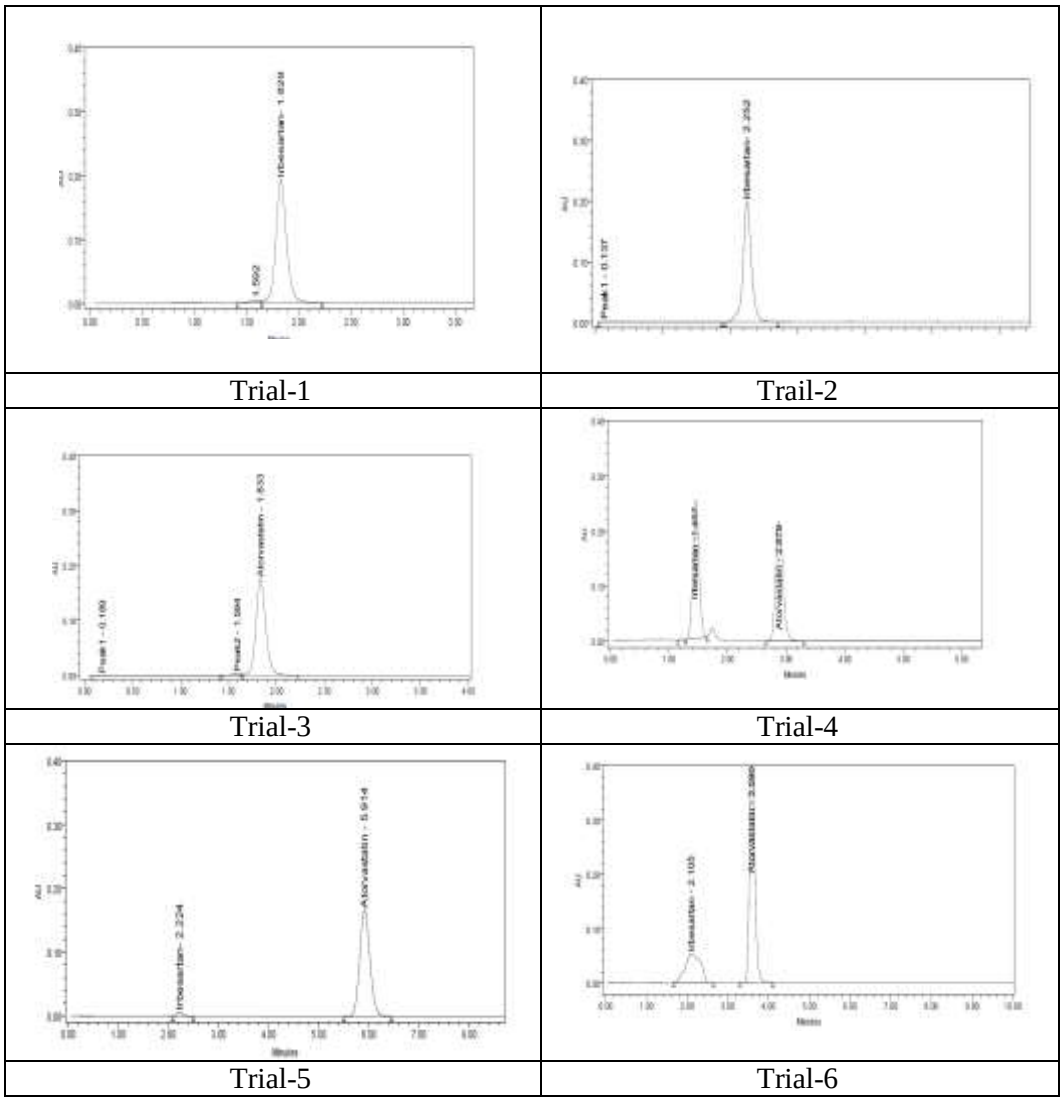
The above buffer (pH 3.5) was mixed thoroughly with acetonitrile in the ratio of 30:70 v/v. This solution was used as the mobile phase.

Preparation of mixed working standard solution of Irbesartan and Atorvastatin

10 mg of each Irbesartan and Atorvastatin were accurately weighed and transferred into three separate 10 mL volumetric flasks. About 5 mL of acetonirile was added in to each flask and sonicated. The volumes were made up to with further quantity of Acetonitrile and mixed well. A quantity of 1.0 mL of each of the above drug solutions was transferred into a 10 mL volumetric flask and the volume was made up with the diluent to get concentrations of 100 and 100 µg/mL of Irbesartan and Atorvastatin respectively.

Optimization of chromatographic conditions and method development

A mixture of potassium dihydrogen phosphate (pH 3.5) and acetonitrile in the ratio of 30:70 v/v was used as the mobile phase and pumped at a flow rate of 1mL/min. The detector wavelength was set at 254 nm. The injection volume was 10 µL. The chromatography was carried out at 26°C. Prior to injection of the drug solution, the column was equilibrated for at least 20 min by pumping the mobile phase through it. Typical chromatograms of the blank solution and mixed working standard solution of the combination of the drugs are shown in Fig. 1 and 2 respectively and shown the data on Table 2.



Trial-1 | Trail-2

Trial-3 | Trial-4

Trial-5 | Trial-6

No. of Trial	Mobile phase	Observation
1.	Acetonitrile: water (50: 50% v/v)	Irbesartan eluted but another one merged
2.	methanol: water (50: 50% v/ v)	Peak response not good
3.	Acetonitrile: phosphate buffer pH 3.2 (55: 45% v/ v)	Peaks are merged and shape not good
4.	Acetonitrile: phosphate buffer pH 3.5 (55: 45% v/ v)	Both drugs were eluted with good resolution but lower plate count
5.	Acetonitrile: phosphate buffer pH 3.5 (60: 40% v/ v)	One drug response was not good
6.	Acetonitrile: phosphate buffer pH 3.5 (65: 35% v/ v)	Peak merged and more tailing
7.	Acetonitrile: phosphate buffer pH 3.5 (70: 30% v/ v)	All three drugs were eluted and optimized condition

Table 2. Optimized Chromatographic Conditions

Parameter	Value
Column	Intersil C_8 (250 x 4.6 mm; 5µ)
Mobile Phase	Phosphate buffer :acetonitrile (30:70)v/v
Flow Rate	1.0 mL /min
Run time	10 min
Column Temperature	26±1 ˚C
Volume of Injection	10 µL
Detection wave Length	254 nm
Retention time	Irbesartan 2.46 min and Atorvastatin 5.08 min.

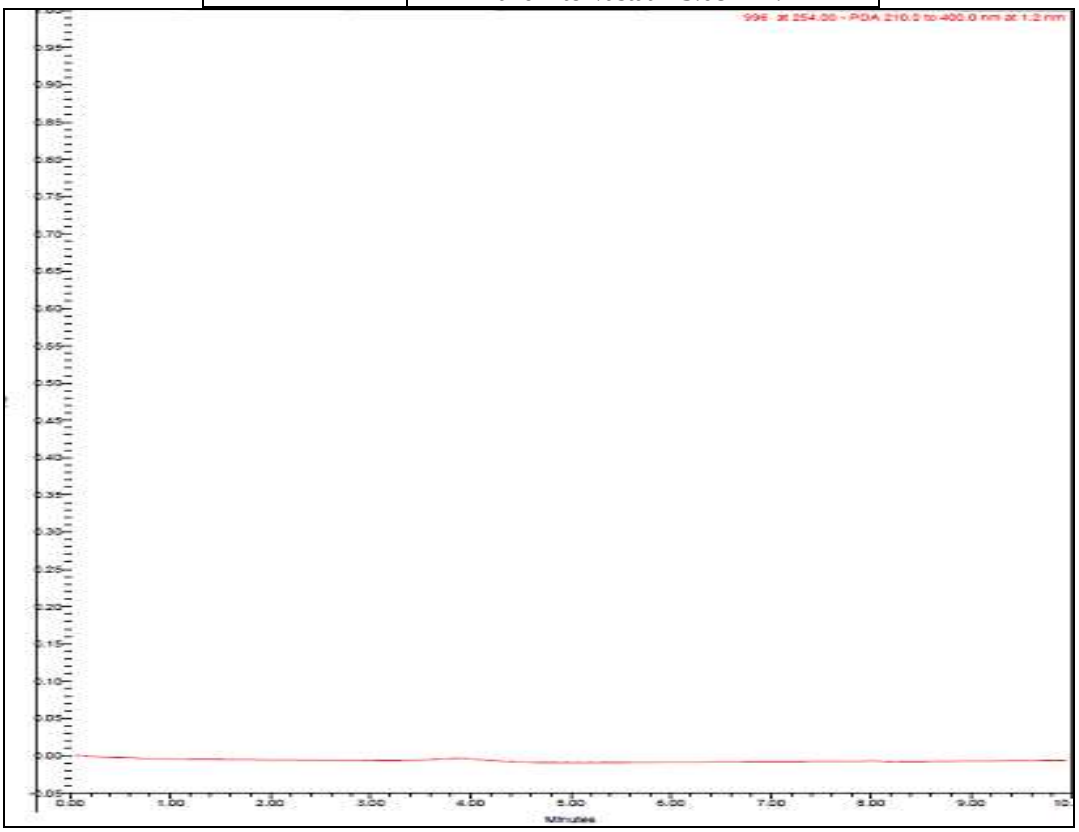


Fig 1. Chromatogram of the Blank solution

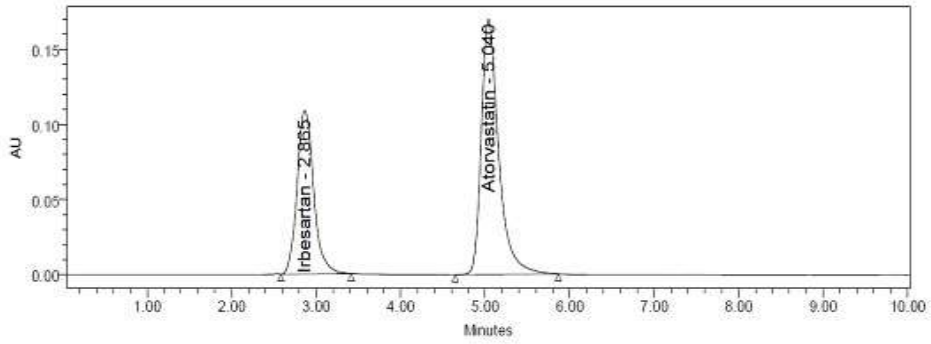


Fig.2.Chromatogram showing separation of Irbesartan and Atorvastatin from the mixed working standard solution.

Validation Of The Proposed Method

The method was validated in compliance with ICH guidelines. The parameters determined for validation are specificity, precision, accuracy, robustness, Linearity, Forced Degradation, system suitability and stability of analytical solution.

Linearity and range

Mixed standard solutions of Irbesartan and Atorvastatin were prepared at different concentration levels including the working concentration mentioned in the experimental condition were prepared. 10 µL of each concentration were injected into the HPLC system (n=3). The response was read at 254 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. Linearity data Irbesartan and Atorvastatin are given in the Tables 3. respectively. Linearity plots for Irbesartan and Atorvastatin are depicted in Fig. 3. and 4. respectively.

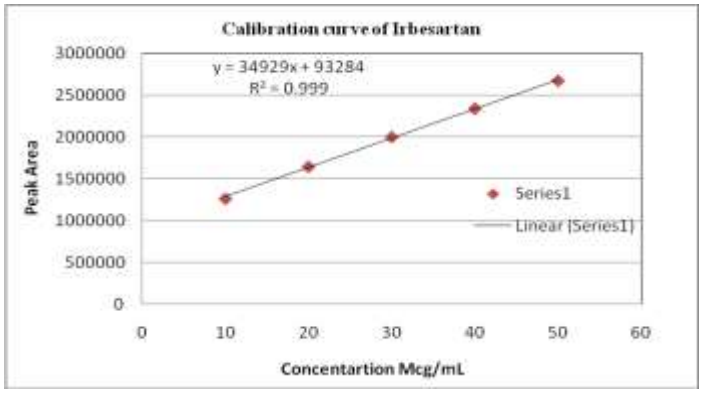


Fig 3. Linearity plot of Irbesartan

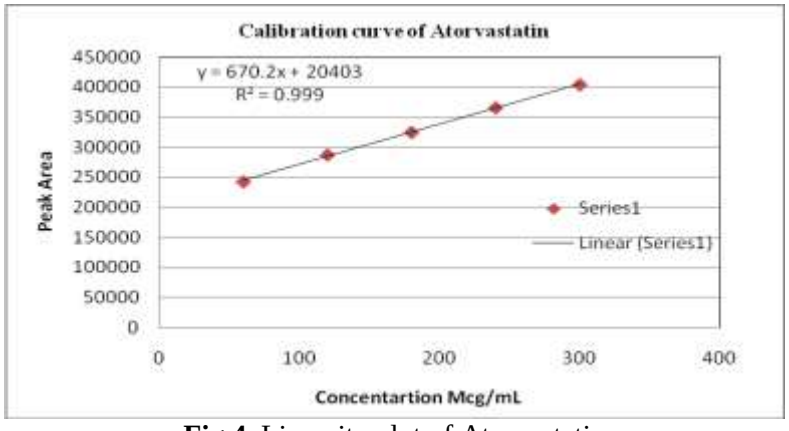


Fig 4. Linearity plot of Atorvastatin

Table 3. Linearity data results

S.NO	Concentration (µg/mL)		Peak Area	
	Irbesartan	Atorvastatin	Irbesartan	Atorvastatin
1.	10	60	1263683	242157
2.	20	120	1644323	287062
3.	30	180	1996498	324652.
4.	40	240	2333501	365570
5.	50	300	2665523	403979

Precision

Repeatability and intermediate precision were assessed by analyzing 150 µg/mL Irbesartan and 100 µg/mL Atorvastatin on the same day and consecutive days, respectively. The results of repeatability and intermediate precision studies are depicted in the Tables 4 and 5 respectively.

Table 4. Repeatability

No.of Injections	Peak areas of Irbesartan	Peak areas of Atorvastatin
Injection-1	1304256	1407005
Injection-2	1321031	1396996
Injection-3	1329111	1408163
Injection-4	1373908	1419361
Injection-5	1317585	1446664
Average	1332430	1410344
Standard Deviation	1329720.4	1414756.9
%RSD	23796.9	17191.3

Table 5. Intermediate precision

No.of Injections	Peak areas of Irbesartan	Peak areas of Atorvastatin
Injection-1	1304256	1407005
Injection-2	1321031	1396996
Injection-3	1329111	1408163
Injection-4	1373908	1419361
Injection-5	1317585	1446664
Average	1332430	1410344
Standard Deviation	1329720.4	1414756.9
% RSD	23796.9	17191.3

Accuracy

The accuracy of the method was determined by spiking and analyzing in triplicate a known mixture of the drugs corresponding to 50 %, 100 % and 150 % levels of Irbesartan (60 µg/mL, 75 µg/mL and 90 µg/mL), Atorvastatin (50 µg/mL, 100 µg/mL, 150 µg/mL)

The percent recovery was calculated by noting the differences b

Table 6 Recovery data of Irbesartan and Atorvastatin

% Concentration Level		Peak Area		Amount Added (mg)		Amount Found (mg)		% Recovery (%)		Mean Recovery (%)	
IRB	ATN	IRB	ATN	IRB	ATN	IRB	ATN	IRB	ATN	IRB	ATN
50%		1120256	121880	5		4.98	5.01	99.6	100.32	99.17	100.18
100%		2247349	243951	10		9.96	10.02	99.6	100.2		
150%		3327592	366388	15		14.9	15.01	98.3	100.01		

Table 7. Recovery data of Irbesartan and Atorvastatin.

	Concentration level	Peak area	% Recovery	Mean % recovery
Atorvastatin	50	1250261	99.0%	98.2
	100	2504966	99.1%	
	150	3732011	98.5%	
Irbesartan	50	90441.3	99.9%	100.2
	100	180387.3	99.6%	
	150	274315.8	101.0%	

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were calculated using residual standard deviation of the response and the slope of the regression line. The LOD and LOQ of Irbesartan and Atorvastatin were found to be 316.92 and 2629 µg/mL, 104.58 and 722.1 µg/mL respectively.

Robustness study

The robustness of the method was determined as per ICH guidelines under a variety of conditions like change in flow rate and pH of buffer. The results obtained by deliberately variation in method parameters and data are summarized below.

Table 8. Robustness data- flow rate 0.9 mL/min

Drug	Retention time (min)	Tailing factor	Number of theoretical plates	Resolution
Irbesartan	3.2	1.1	2159	------
Atorvastatin	2.6	1.5	2110	3.9

Table 9. Robustness data- flow rate 1.1 mL/min

Drug	Retention time (min)	Tailing factor	Number of theoretical plates	Resolution
Irbesartan	4.64	1.1	2221	------
Atorvastatin	8.22	1.45	3450	6.35

Table 10. Robustness data- less organic composition

Drug	Retention time (min)	Tailing factor	Number of theoretical plates	Resolution
Irbesartan	2.67	1.1	2643	-----
Atorvastatin	5.27	1.5	2110	3.45

Table11. Robustness data- More organic composition

Drug	Retention time (min)	Tailing factor	Number of theoretical plates	Resolution
Irbesartan	2.63	1.1	2740	------
Atorvastatin	6.25	1.6	2300	1.5

Specificity and selectivity of the proposed method

Specificity is the extent to which the procedure applies to analyte(s) of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in formulation did not interfere with the drug peaks and thus the method is specific. The HPLC chromatograms recorded for the drug matrix did not show any interfering peak within retention time ranges. Fig. 1 and 2 shows the representative chromatograms obtained from the analysis of Irbesartan and Atorvastatin from blank solution, working standard solution and the formulation sample solution. The figures show that the selected drugs were clearly separated. Thus the proposed HPLC method is selective.

System suitability

For system suitability, six replicates of the working standard sample were injected and the parameters like plate number (N), HETP, peak symmetry and resolution of samples were calculated. These results are shown in table.12.

Table 12. System suitability parameters for the present method

S.No	Parameter	Results	
		Irbesartan	Atorvastatin
1.	Retention time (Min)	2.615	4.59

2.	Resolution	----------	6.06
3.	Number of Theoretical Plates	2271	3217.17
4.	Tailing Factor	1.2	1.4
5.	HETP	0.0660	0.0466

Determination of drugs from tablet dosage forms

Twenty tablets of "Rovelto" (Sanofi-aventis Pharmaceutical pvt, Ltd India.) were weighed and ground to a fine powder. From this, an amount equivalent to about 10 mg of Irbesartan and was transferred into a 100 mL volumetric flask and to it 60 mL of acetonitrile was added and sonicated for 20 min. The volume was made up with acetonitrile. A portion of this solution was filtered through a 0.22 µm membrane filter (discarding the first few mL of the filtrate). 5.0 mL of this filtrate was transferred into a 100 mL volumetric flask containing 50 mL of the diluent. The volume was made up with the diluent and mixed well to get final concentrations of 150 µg/mL, 20 µg/mL Irbesartan and Atorvastatin respectively. This solution was chromatographed six times. The mean peak areas of the drugs were calculated and the drug content in the formulation was calculated. A typical chromatogram obtained from the analysis of Rovelto tablet is shown in the Fig. 6.

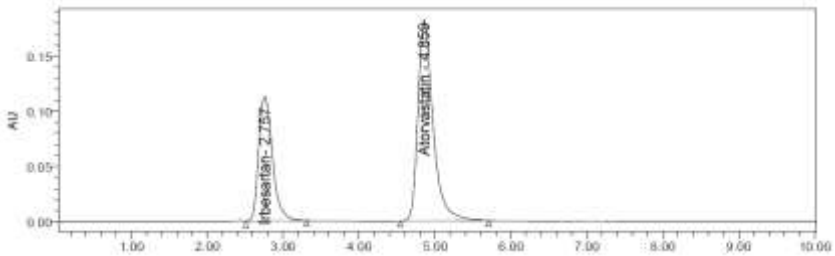


Fig. 5. Typical chromatogram obtained from analysis of Irbesartan and Atorvastatin from the formulation sample solution

Method suitability

The Marketed tablet formulation, "Rovelto" (Sanofi-aventis Pharmaceutical pvt. Ltd., India.) was estimated by the present method and the results are shown in Table 12. The values were found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of drugs in pharmaceutical dosage forms.

Table 13. Recovery of the drugs from the tablet dosage form "Rovelto"

Drug	Labelled amount (mg)	Amount recovered (mg) (n= 6)	% Recovery
Irbesartan	150	149	99.33
Atrvostatin	20	20.13	100.65

Degradation study

For studying the effect of different stress conditions on the stability of the drugs in the formulations 5 tablets (containing Irbesartan and Atorvastatin) were taken and made in to a fine powder. A quantity equivalent to the weight of one tablet was transferred into a 100 mL volumetric flask. The mobile phase was added to this and sonicated o effect complete solubility of the drugs. The contents of the flask were made up to the mark

From this stock solution, 1 mL was transfer into a 10 mL volumetric flask and subjected to intended stress condition. Separate samples were exposed to acidic, basic, thermal, oxidation and photolytic conditions.

When degradation was complete the solutions were brought to the room temperature and diluted with the mobile phase to get solutions of a concentration equivalent to a 30 µg/mL of Irbesartan and Atorvastatin. The specific degradative conditions are described below.

1. Treatment with hydrochloric acid.
2. Treatment with sodium hydroxide.
3. Treatment with hydrogen peroxide.
4. Thermal exposure.
5. Photolytic exposure.

Acid degradation*:*

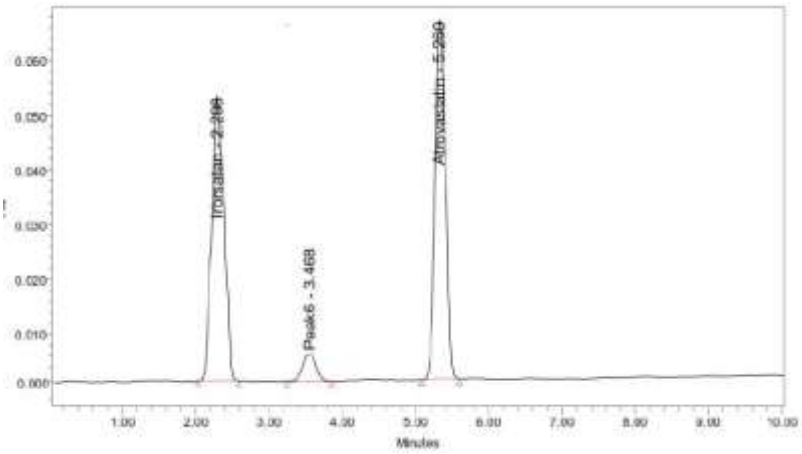


Fig. 6. Chromatogram showing effect of acidic degradation

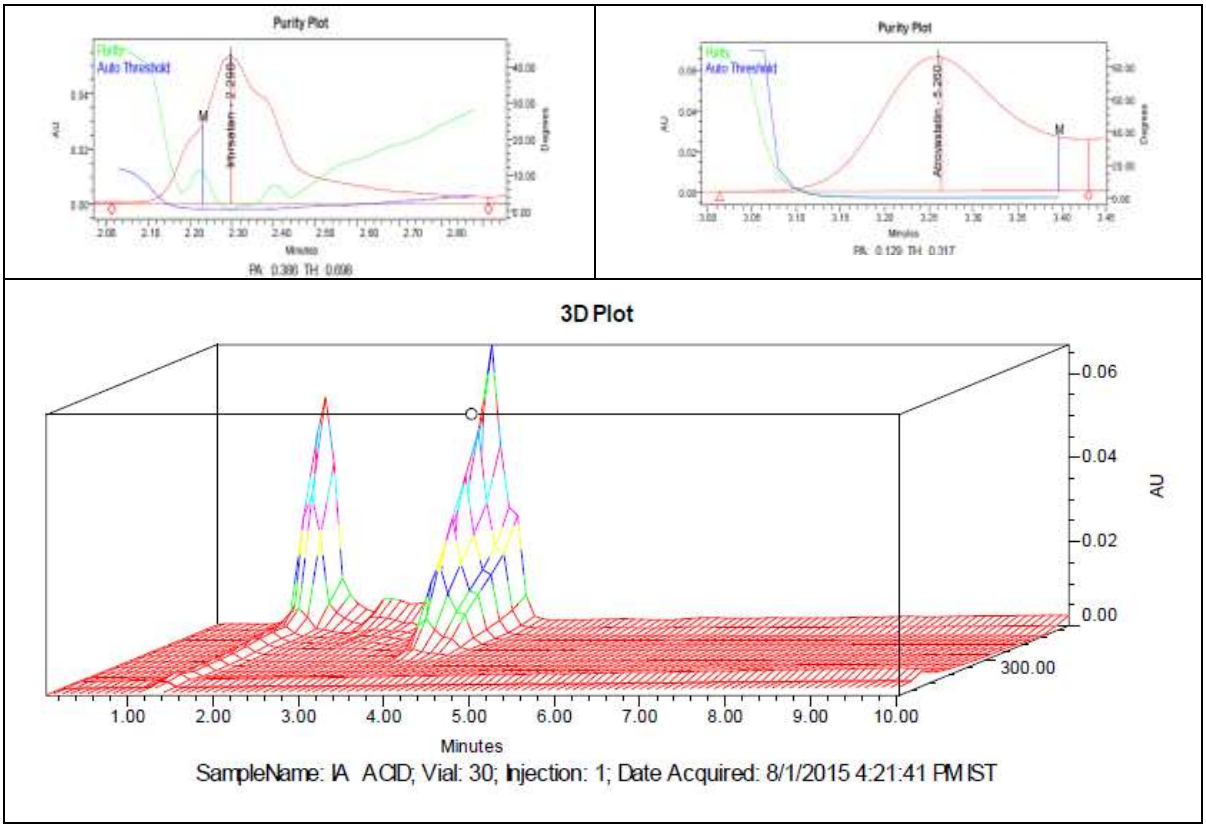


Figure 7. Purity Plots for Irbesartan and Atorvastatin

Alkaline degradation:

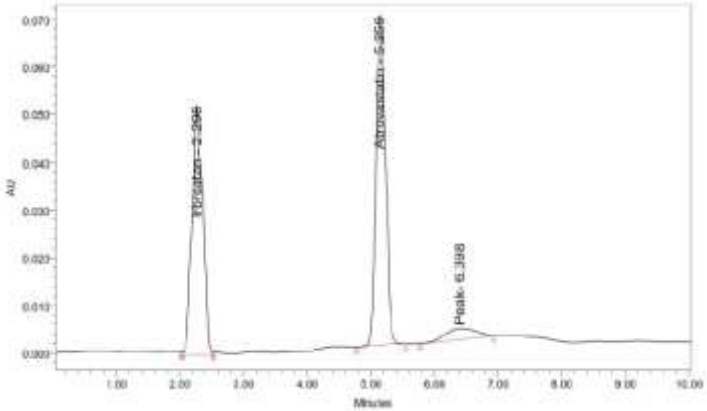


Fig. 8. Chromatogram showing effect of alkali degradation

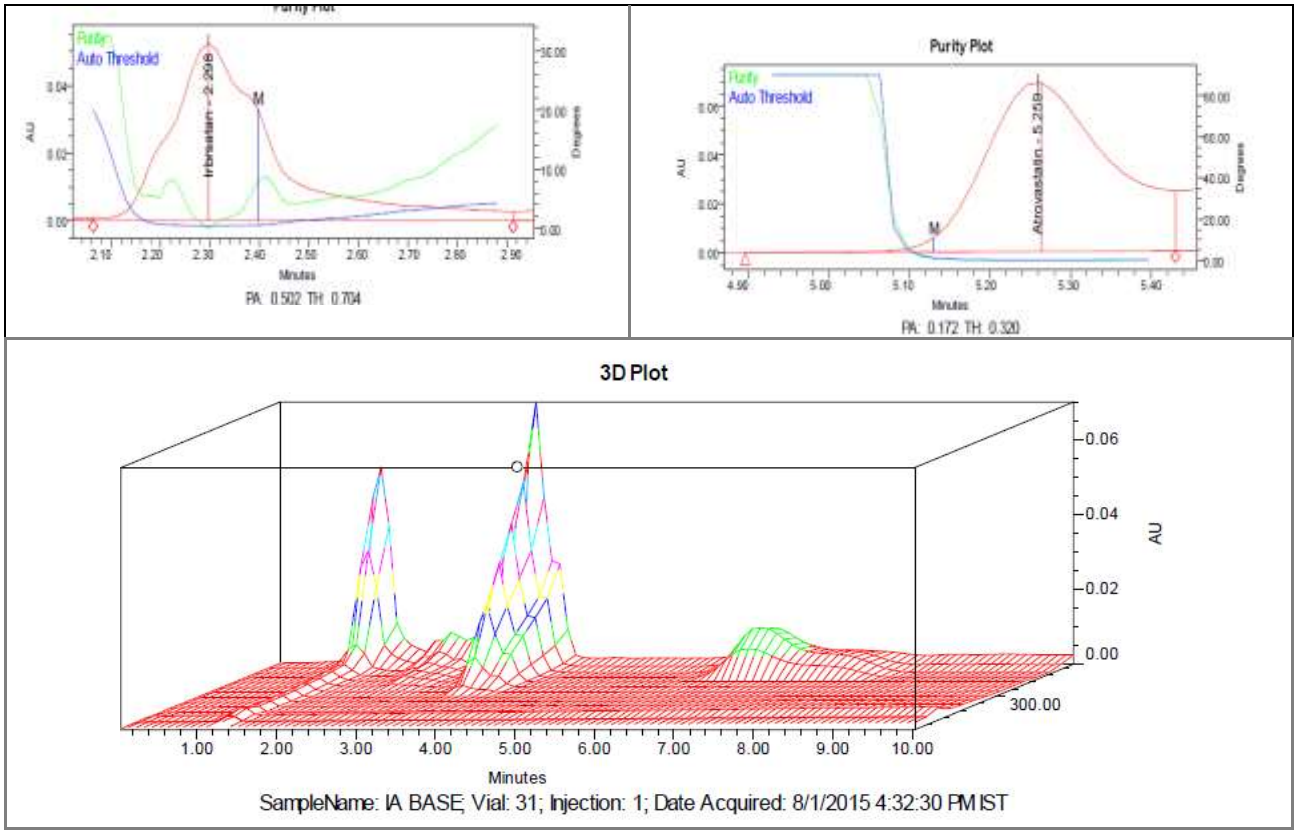


Fig.9. Purity Plots for Irbesartan and Atorvastatin

Oxidative degradation:

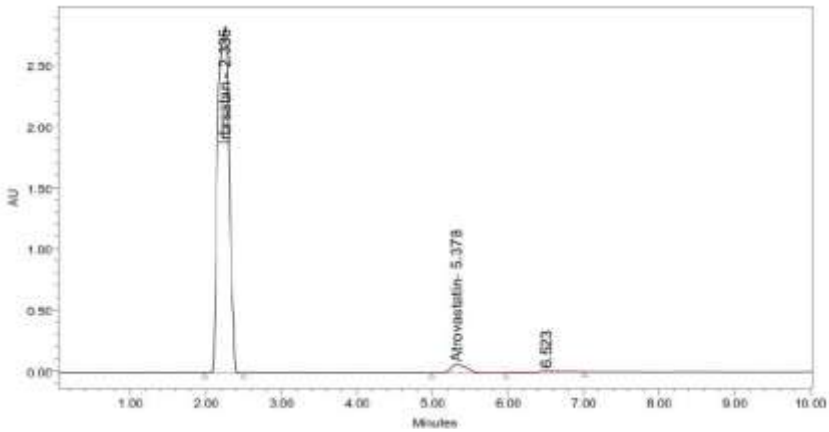
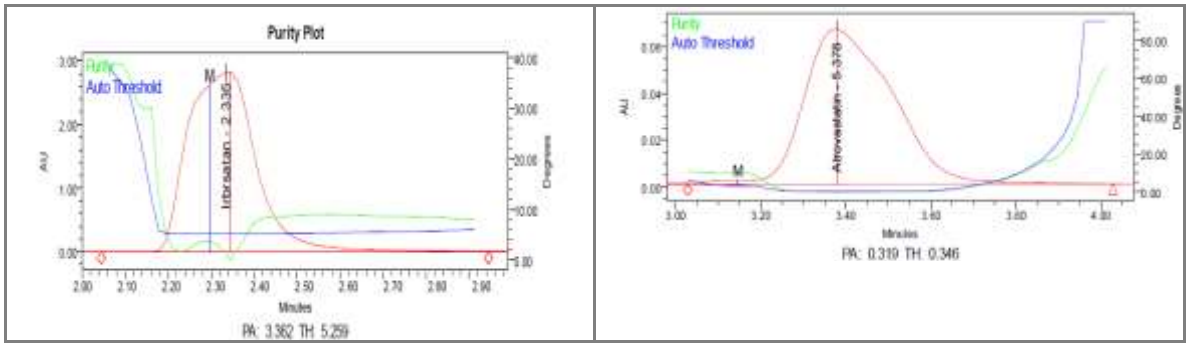


Fig 10. Chromatogram showing effect of oxidative degradation



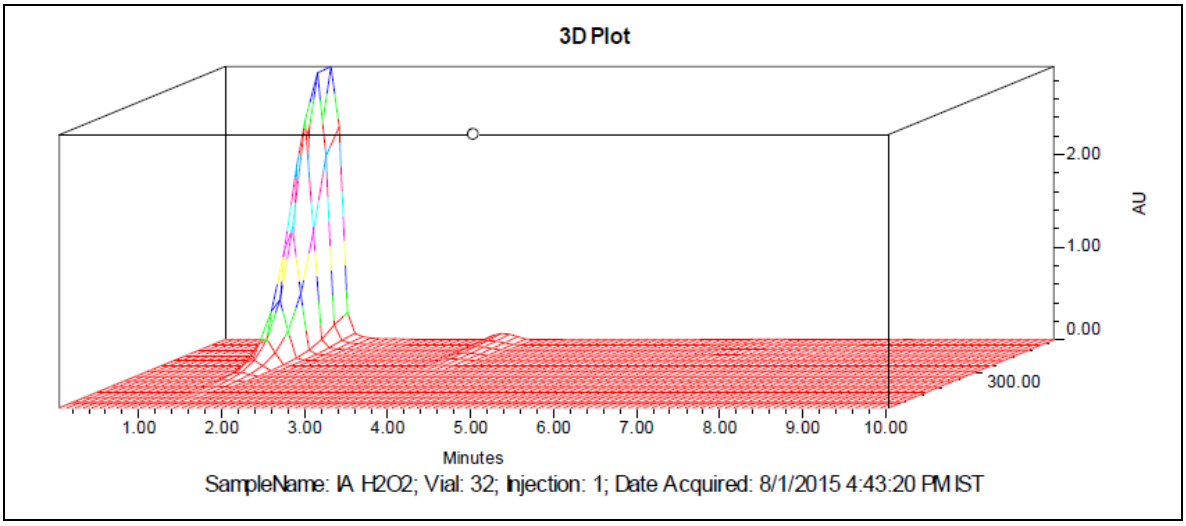


Fig. 11. Purity Plots of Irbesartan and Atorvastatin

Photolytic degradation:

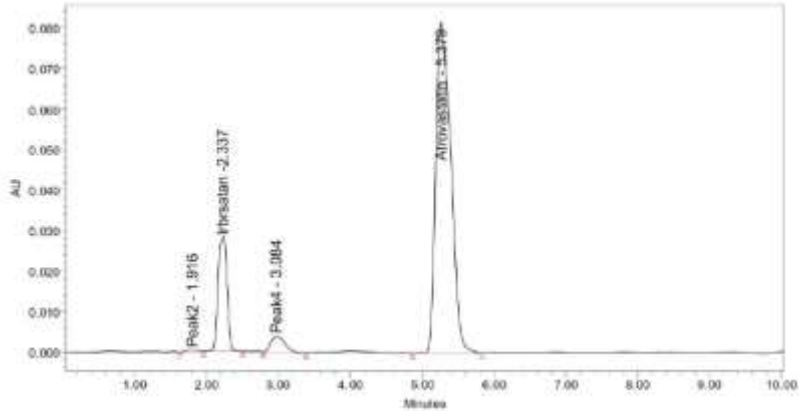


Fig.12. Chromatogram showing effect of UV-light degradation

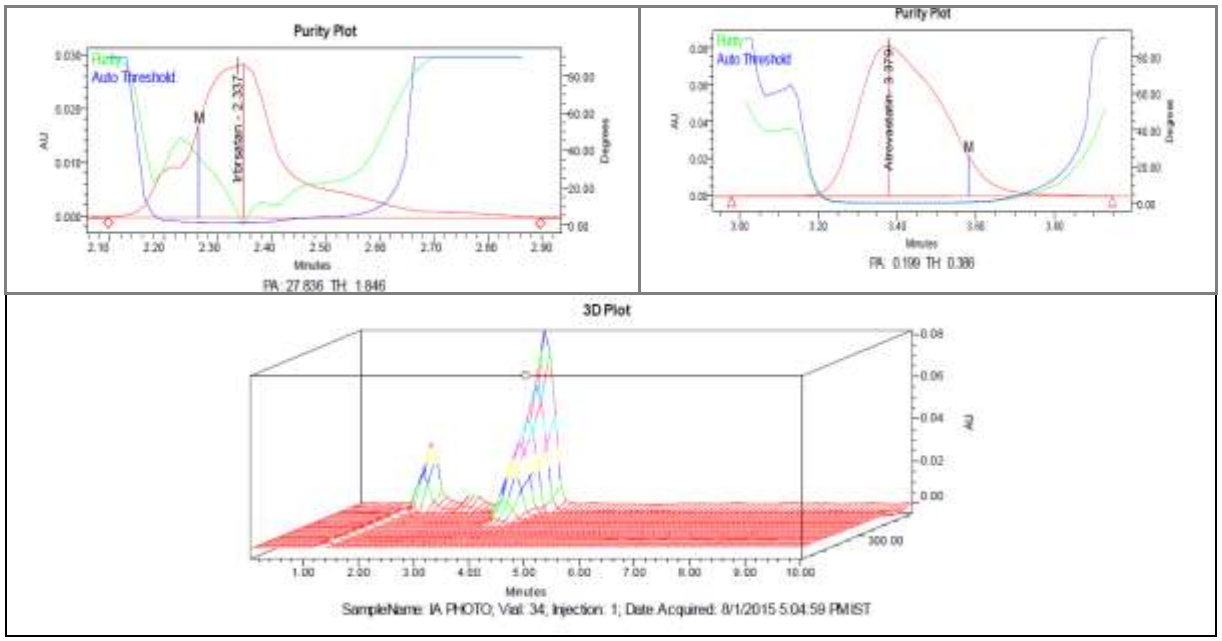


Fig.13. Purity Plots of UV-light degradation

Thermal degradation:

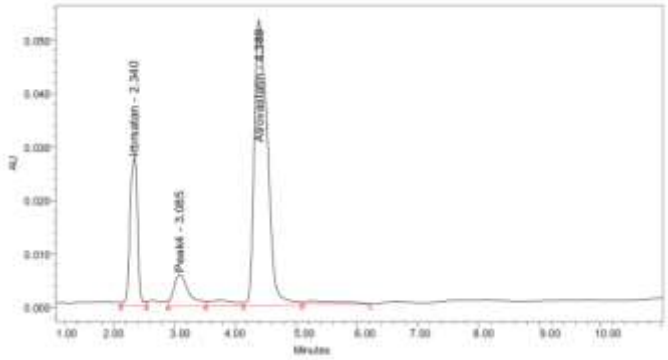


Fig. 14. Chromatogram showing effect of Thermal degradation

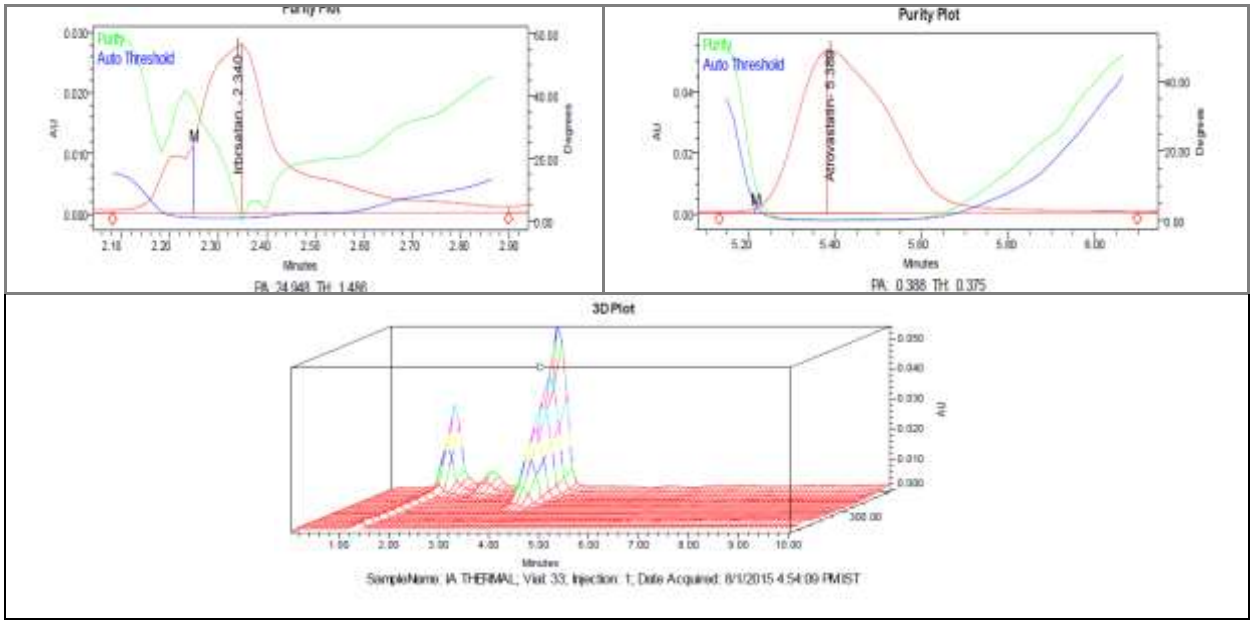


Fig.15. Purity Plots for Irbesartan and Atorvastatin in thermal degradation

Table13. Peak purity results of Irbesartan and Atorvastatin

Stress	**Purity Angle**		**Purity Threshold**	
Condition	Irbesartan	Atorvastatin	Irbesartan	Atorvastatin
Acid Degradation	0.386	0.129	0.698	0.317
Alkali Degradation	0.502	0.172	0.704	0.320
Oxidative Degradation	3.362	0.319	5.259	0.346
Photolytic Degradation	0.836	0.199	1.846	0.286
Thermal Degradation	0.948	0.388	1.486	0.375

Table 14. Percentage of degradation of Irbesartan and Atorvastatin

Drug Name		**Acid**	**Alkali**	**Oxidative**	**Photolytic**	**Thermal**
Irbesartan	Std. area	799605				
	Sample area	770831	748544	797136	749461	765381
	% of Degradation	14.1%	6.5%	13.7%	7.1%	0.88%
Atorvastatin	std Area	733171				
	Sample area	710491	746361	763587	795715	799520
	% of Degradation	6.9%	9.6%	14.6%	9.8%	4.4%
% Average of degradation		10.5%	8.05%	14.15%	8.45%	2.64%

SUMMARY OF THE RESULTS AND CONCLUSION

To optimize the mobile phase, various proportions of the buffer (pH 4.5) and acetonitrile were tested. The use of buffer and Acetonirile in the ratio of 30:70 v/v, pumped at a flow rate of 1.0 mL/min eluted the compounds with short retention times. The corresponding chromatograms showed good baseline stability, peak shape and peak resolution. By applying the present method, the retention times of Irbesartan and Atorvastatin were found to be 3.070 min and 4.5 min respectively. The linearity was obeyed in the concentration range of 10-50 and 60-300 µg/mL for Irbesartan and Atorvastatin respectively. The regression equations of the linearity plots constructed for Irbesartan and Atorvastatin were found to be y = 34929x + 92484 (R^2=0.9996) and y = 670.2x + 20403 (R^2=0.9996) respectively where y refers to the area of the peak and x refers to the concentration (µg/mL) of the drug. The correlation coefficients were greater than 0.99. The theoretical plate values obtained for Irbesartan and Atorvastatin 2271.50 and 3217.17 respectively. Since the number of theoretical plates for the three drugs was above 2000, it indicates that the column was efficient in separating all the three drugs. The limit of detection and limit of quantitation for Irbesartan and Atorvastatin were found to be 1.622 and 4.915 µg/mL, 3.245 and 9.833 µg/mL and 4.576 and 13.867 µg/mL respectively. These values reveal that the method is sensitive. The high percentage of recovery (100.10-100.12) indicates that the proposed method is accurate.

No interfering peaks were found in the chromatograms run for the formulation samples. This indicates that the excipients used in tablet formulations did not interfere with the estimation of the drugs by the proposed HPLC method.

The proposed method has several advantages over the earlier reported methods. The retention times of the drugs obtained in this method were shorter than the reported methods. The short run time shows the speed of analysis which enables more number of samples to be analyzed per unit time. The range of quantitation obtained in the current method is wider than some of the reported methods. The proposed RP-HPLC method is sensitive, robust, precise and accurate and can be used for routine quality control analysis for simultaneous determination of Irbesartan and Atorvastatin in their tablet dosage forms.

Acknowledgement:
The authors are very thankful to SuraPharma laboratory for providing standard drugs for our Research work and enourging and constant support for our entire research work.

Conflict of Interest: Authors does not have interest of conflict.

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