AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

New Stability Indicating Validated RP-HPLC Method for Simultaneous Estimation of Irbesartan and Atorvastatin in Combined Tablet Dosage Forms

Gandla Kumara Swamy[1]*, J.V.L.N.Seshagiri Rao[3], JM Rajendra Kumar[2]

1. Research scholar, Department of Pharmaceutical Analysis, Jawaharlal Nehru Technological University Kakinada, Kakinada - 533003-Andhra Pradesh, India.

2. Mylan Laboratories Limited, Plot no 31, 32, 33&34-A, Anrich Industrial Estate, Bollaram, Medak (Dist) 502325, India.

3. Srinivasarao College Of Pharmacy, PM Palem, Visakhapatnam-500041. A.P.India.

ABSTRACT

The present study is a simple, precise, accurate RP-HPLC method was developed and validated for the simultaneous determination of Irbesartan and Atorvastatin in combined tablet dosage forms. The proposed method was developed by HPLC Waters 2695 Separation Module with PDA detector connected to Empower-2 software using Inertsil C_{18} ODS (4.6 x 250mm, 5mm) with an injection volume of 10 µl was injected and eluted with a mobile phase composition of acetonitrile : potassium dihydrogen phosphate (pH 4.5 adjusting with orthophosparic acid) (70:30), which is pumped at a flow rate of 1.0 ml/min and detected by PDA detector at 254 nm. The column temperature was maintained at 25 ^{0}C and total run time was 10 mins. The retention time of Irbesartan and Atorvastatin were found to be 2.8 min. and 4.8 min respectively. Linearity was observed in the concentration range of 60-300 and 10-50 µg/ml for Irbesartan and Atorvastatin respectively with correlation coefficient 0.999 for both the drugs. Percent recoveries obtained for both the drugs were 98.0-101.50%, respectively. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The method developed IRB be used for the routine analysis of Irbesartan and Atorvastatin from their combined dosage form.

Keywords: Irbesartan and Atorvastatin; Isocratic Elution; RP- HPLC Method; Forced degradation studies; PDA Detector; Tablet dosage forms.

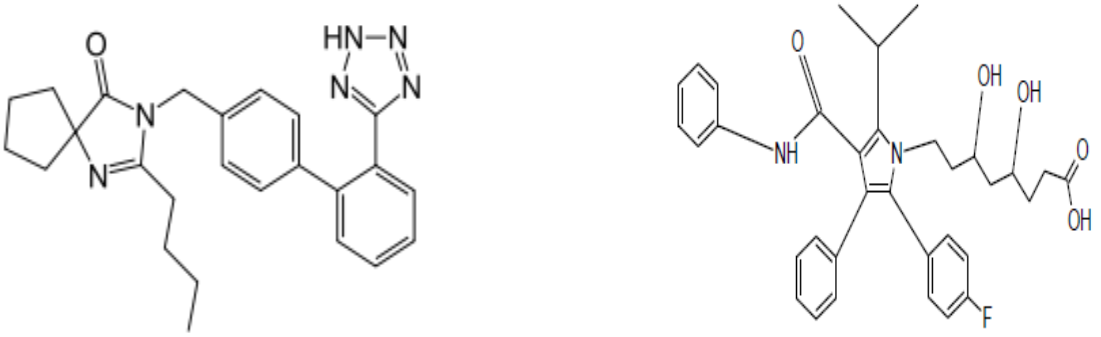
*Corresponding Author Email:kumaraswamy.gandla@gmail.com

Received 21 May 2015, Accepted 24 June 2015

Please cite this article as: GandlaKS*et al*., New Stability Indicating Validated RP-HPLC Method for Simultaneous Estimation of Irbesartan and Atorvastatin in Combined Tablet Dosage Forms. American Journal of PharmTech Research 2015.

INTRODUCTION

Irbesartan is an Antihypertensive Agents. The chemical name for 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, and with a molecular weight of 428.5294.Atorvastatin is a Atorvastatin calcium (ATR) is a synthetic hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor that has been used as a lipid lowering agent4. Chemically, ATR [Fig.. 1(b)] is [R-(R*, R*)]-2-(4-flurophenyl)-B, B—dihydroxy-5-(1-methylethyl)-3-phenyl-4- [(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid5. ATR is a competitive inhibitor of HMG-CoA reductase. This enzyme catalyzes the reduction of 3-hydroxy-3-methylgultaryl-coenzyme-A to mevalonate, which is the rate-determining step in the hepatic cholesterol synthesis. Because cholesterol synthesis decreases, hepatic cells increase the number of LDL receptors on the surface of the cells, which in turn increase the amount of LDL uptake by the hepatic cells, and decrease the amount of LDL in the blood. The empirical formula is $C_{25}H_{38}O_5$. The chemical structures of Irbesartan and Atorvastatin are shown in Fig. 1a & b.



(a) (b)

Fig. 1: Chemical structures of (a) Irbesartan and b) Atorvastatin

MATERIALS AND METHOD

Reagents and chemicals

Acetonitrile HPLC grade was procured from E.Merck Ltd, Mumbai. Acetonitrile, Acetonitrile. Fine chemicals, Hyderabad. Water HPLC grade was prepared using Millipore purification system. Irbesartan and Atorvastatin reference standards procured from AET Laboratories, Hyderabad.

Instrumentation

The HPLC system consists of water 2695 having photodiode array detector system, which was connected with the help of Empower -2 software for data integration and processing. Inertsil ODS (250 X 4.6 mm) 5µ column was used for the analysis.

Preparation of phosphate buffer and mobile phase

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.

The mobile phase was prepared above buffer and acetonitrile in a ratio of 30:70 v/v was used as the mobile phase. Mobile phase was used as diluents for preparing the working solution of the drug.

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.

Preparation of the sample solution:

The tablet powder equivalent to 100 mg of Irbesartan and Atorvastatin were weighed and taken into a 100mL volumetric flask. To this 25 mL of diluents was added and sonicated for 15min to dissolve the drugs then made up the volume to required volume with the diluents. From this solution 10 ml was taken into a 100 mL flask and made up to final volume with diluents to get concentration 100 µg/ml concentration of Irbesartan and atorvastatin and filtered through 0.45µ filters under vacuum filtration. From this stock solution further dilutions were made for the validation of the method developed.

HPLC conditions

The contents of the mobile phase were Acetonitrile and Phosphate buffer the ratio of 70:30. These were filtered through 0.45µ membrane filter and degassed by sonication before use. The flow rate of mobile phase was optimized to 1 ml / min. The run time was set at 8 min and column temperature was maintained at 25^0 C temperature . The volume of injection was 20 µl, and the eluent was detected at 254 nm. Each of standard and test preparations was injected into the column and the responses recorded (Fig. 5 and Fig. 6).

Method

The RP-HPLC Method of Irbesartan and Atorvastatin were achieved by isocratic elution technique with PDA Detector. Irbesartan and Atorvastatin were determined at 254 nm respectively with the concentration range of 10-50 -60-300 µg/ml for both Irbesartan and Atorvastatin respectively. fig.03 & 04. For analysis of tablet formulation the tablet powder equivalent to 25 mg was taken, dissolved in 25 ml volumetric flask and made up to 25 ml with Acetonitrile. The solution was sonicated for 15min, centrifuged at 100 rpm for 15 min and filtered through Whatmann filter paper No.41. From clear solution, further dilutions were made to get 10 µg/ml of Irbesartan and Atorvastatin.

For recovery studies, to the reanalyzed formulation, solutions of raw material containing different concentrations were added and the amount of drug recovered was calculated. The procedure was repeated as per the analysis of formulation. The amount of drug recovered was calculated by using slope and intercept values from the calibration graph. Finally the method was validated as per ICH guide lines for precision, accuracy, specificity, linearity, reproducibility, limit of detection and limit of quantification.

RESULTS AND DISCUSSION

A simple, selective, rapid and precise validated RP-HPLC Method for Simultaneous Estimation of Irbesartan and Atorvastatin in bulk material and in pharmaceutical formulation has been developed and validated. The correlation coefficient was found to be 0.9997&0.9998 for Irbesartan and Atorvastatin respectively. Figure 03 & figure 04. In this method the % purity of Irbesartan and Atorvastatin were found to be 101.25 ± 1.074 and 100.19 ± 1.031 respectively. The recovery studies range is.99.98-100.01% and 99.94 – 100.03 % for Irbesartan and Atorvastatin, respectively. The Intraday and Inter day analysis carried out for precision. The ruggedness study was performed. The % purity were found to be 100.25 ± 1.0054 and 101.49 ± 1.9305 for Irbesartan and Atorvastatin, respectively. The recovery studies range is 99.98-100.01% and 99.94 – 100.03 %. Table 1.The Intraday and Inter day analysis carried out for precision. The ruggedness study was performed. The method was validated for statistical analysis.

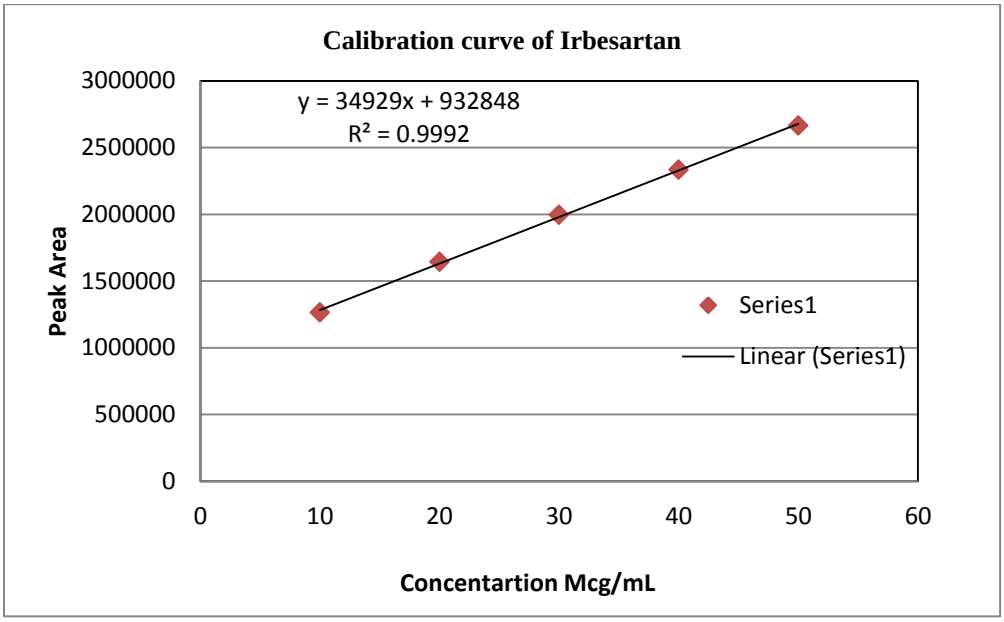


Fig. 3: Calibration graph of Irbesartan

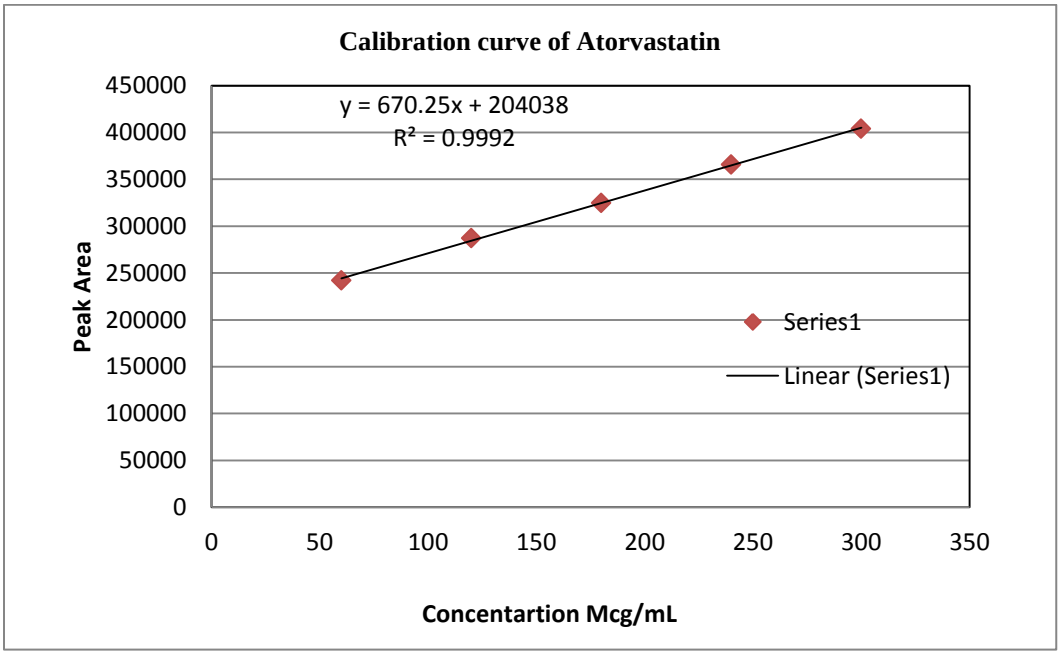


Fig. 4: Calibration graph of Atorvastatin

Table 1: Recovery Studies

a) Irbesartan:

% Concentration (at specification 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	99.60	100.32	99.17	100.18
100%		2247349	243951	10		9.96	10.02	99.6	100.2		
150%		3327592	366388	15		14.98	15.01	98.3	100.01		

b) Accuracy Values for Atorvastatin:

%Concentration (at specification Level)	Peak Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	121880	5	5.01	100.32%	100.18%
100%	243951	10	10.02	100.2%	
150%	366388	15	15.01	100.01%	

Degradation study

In a order to the determine whether the analytical methods were stable Irbesartan and Atorvastatin dosage forms are stressed on the different conditions to applied degradation studies. The guidelines are expressed in ICH Q2A, Q3B, Q2B all the required for development & for the validation of stability study. The degradation of a sample was prepared by the transfer the individual tablet powder was equivalent to the weight of each tablet was transfer into 100 ml flask & it was treated under the acidic, alkaline, thermal, oxidizing and photolytic conditions. When degradation was complete the solution were left to equilibrate to the room temp & dil. with mobile phase to furnish the solutions of a concentration equivalent to a 30 µg/mL of Irbesartan and Atorvastatin. The specific degradative conditions are described below.

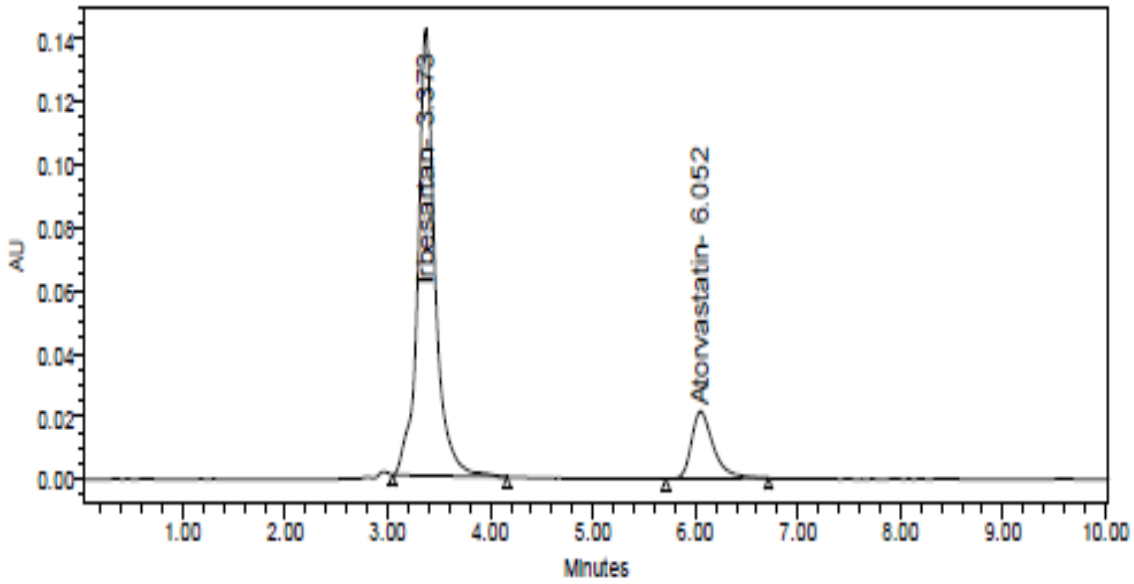


Fig. 5: Typical Chromatograms for Recovery Studies

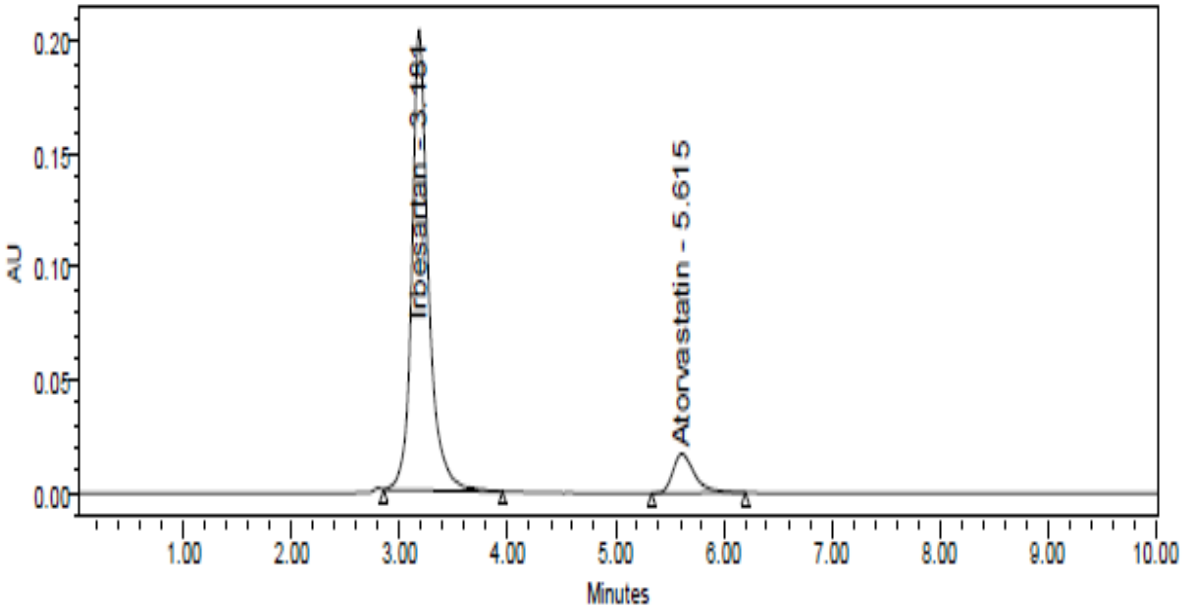


Fig. 6: Typical chromatogram of Irbesartan and Atorvastatin

Acid degradation study:

The Acid degradation was done by sample was treated with 3 ml of 1N hydrochloric acid and kept for 10 hrs at 60º C. After 10hrs the solution was neutralized with 3 ml of 1N sodium hydroxide, made the volume up to the mark with mobile phase and analyzed using HPLC.

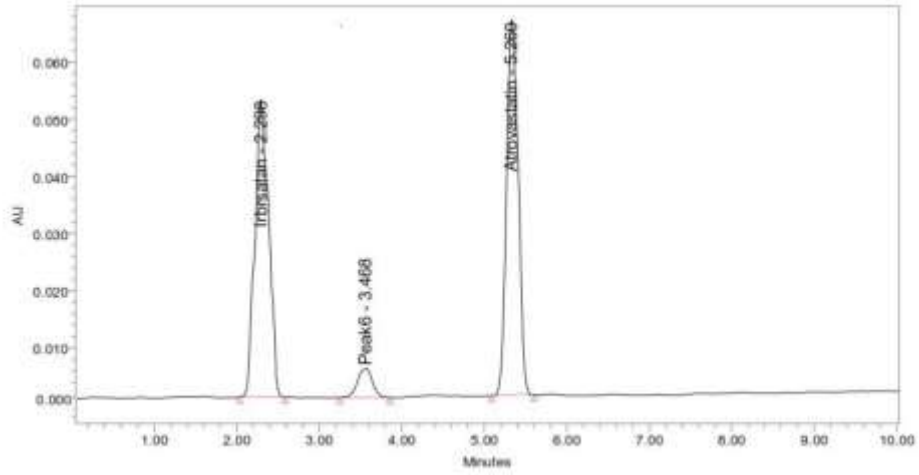


Fig. 7: Chromatogram showing effect of acidic degradation

Alkaline degradation:

The Alkaline degradation was done by sample was treated with 3 ml of 1N sodium hydroxide and kept the sample for 10 hr. After 10 hr solution was neutralized to add 3 ml of 1N hydrochloric acid, made the volume up to the mark with irrelevant media and analyzed using HPLC. (Fig. 8).

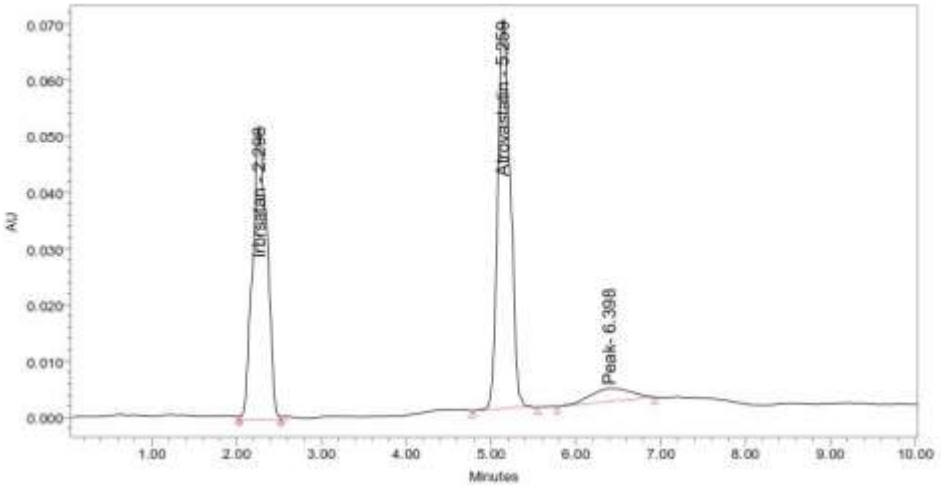


Fig. 8: Chromatogram showing effect of Alkali degradation

Oxidative degradation:

The oxidative degradation was done by sample was mixed with 3mL of 30 %v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume upto the mark with mobile phase and analyzed using HPLC. (Fig. 9.

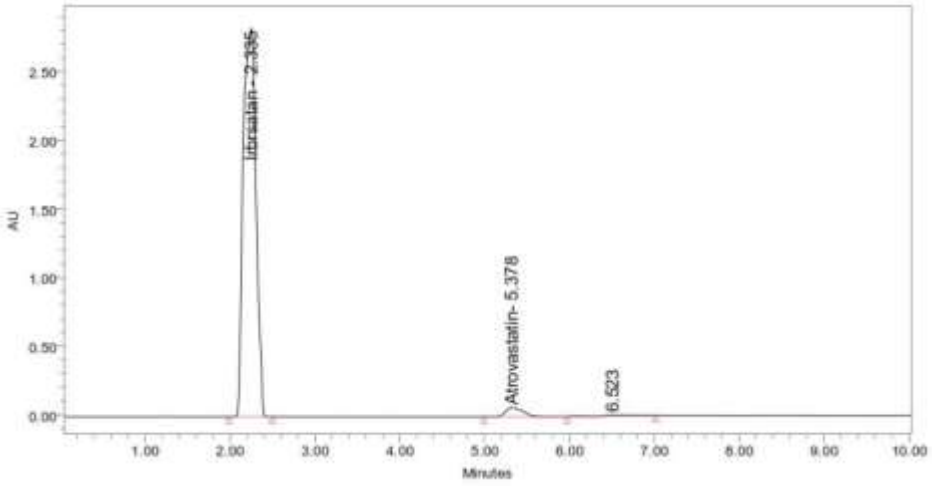


Fig. 9: Chromatogram showing effect of oxidative degradation

Photolytic degradation:

The photolytic degradation was done by exposing of drug content under the UV light for 15 mins to 7 days. The drug degradation observed in the above specific photolytic degradation condition (Fig. 10).

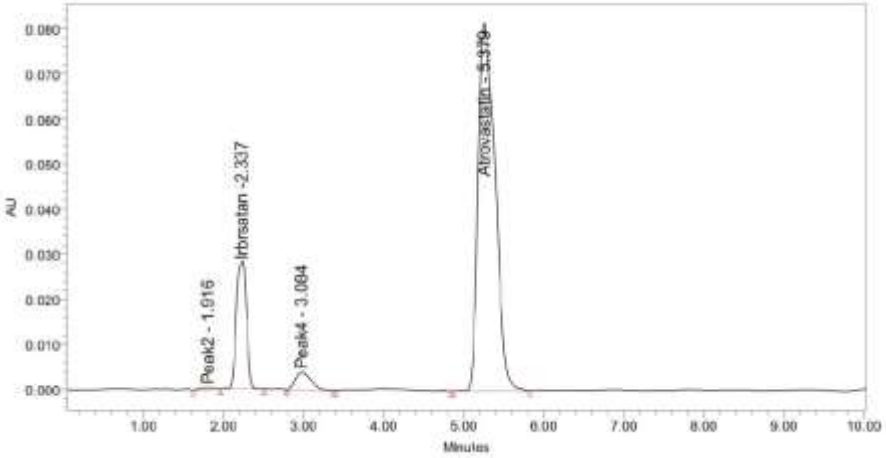


Fig. 10: Chromatogram showing effect of UV-light degradation

Thermal degradation:

The Thermal degradation is to be performing by the exposing the solid drug at the 80°C for 15 mins to 60 mins. Resultant chromatogram of thermal degradation study (Fig. 11) was indicates that the drug was found to be slightly stable under thermal condition.

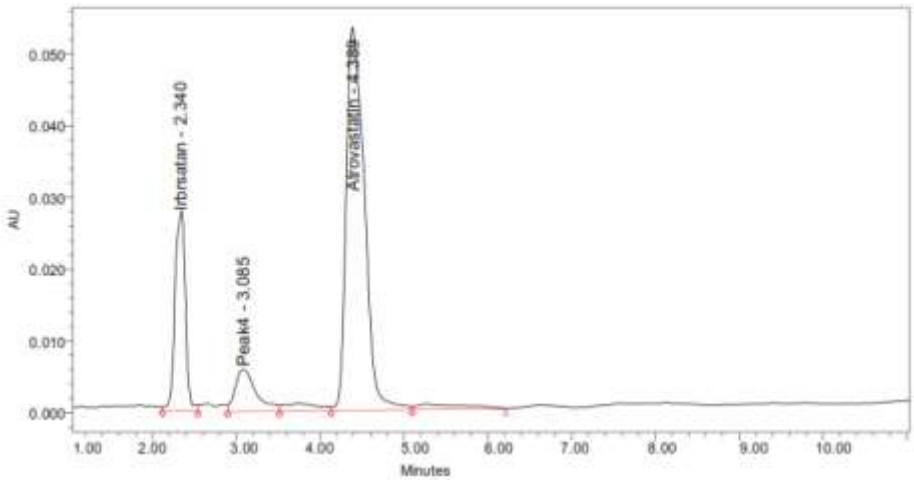


Fig. 11: Chromatogram showing effect of thermal degradation

Table 10: Peak purity results of Irbesartan and Atorvastatin

Stress Condition	Purity Angle		Purity Threshold	
	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	5.25	0.346
Photolytic Degradation	0.836	0.199	1.846	0.286
Thermal Degradation	0.948	0.388	1.486	0.375

CONCLUSION

The developed RP-HPLC method was validated and the statistical validation was performed with the simplicity and ease of operation ensures that the validated method IRB successfully used for routine Analysis of Irbesartan and Atorvastatin in bulk and tablet dosage formulation.

ACKNOWLEDGEMENTS

The authors would like to thankful Care College of Pharmacy, Warangal, for providing the necessary requirements for doing this Research work. And also thankful to AET laboratories, Hyderabad for proving standard drugs as a gift samples of Irbesartan and Atorvastatin.

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