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Stability Indicating RP-HPLC Method Development for the Estimation of Irbesartan in Pharmaceutical Dosage Form

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ABSTRACT

Stability indicating RP-HPLC method was developed and validated for the determination of Irbesartan in bulk and dosage form. A COSMOSIL packed C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing Acetonitrile: water (50:50). The flow rate was 1 ml/min and effluents were monitored at the wavelength of 246 nm. The retention time for Irbesartan was 3.56 min. The method was validated as per ICH guidelines for linearity, accuracy, precision, specificity, the limit of detection, the limit of quantification, and robustness. Limit of detection (LOD) and limit of quantification (LOQ) were found 0.0026 µg/ml and 0.0086µg/ml respectively and recovery of Irbesartan from bulk and dosage forms was found from 99.18% to 99.55%. As the separation of the degradants using this mobile phase is quite good, isolation of the degradants with preparative techniques can also be achieved using this mobile phase. The drug was prone to degrade more in acidic, alkaline, oxidative, and thermal conditions. So this method can be economically very useful in both the research and industrial aspect.

INTRODUCTION:

Angiotensin antagonists represent the first major innovation in the management of essential hypertension as first-line treatment. Angiotensin II receptor antagonists have been developed to specifically and selectively block the AT1 receptor of the rennin angiotensin system by displacing angiotensin II from it. Irbesartan is a highly selective, non-peptide angiotensin-II receptor antagonists (ARA-II).

Irbesartan is a synthetic, nonpeptide antagonist of angiotensin-II with chemical name 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. Irbesartan is used mainly for the treatment of hypertension. Irbesartan pronounced is an angiotensin II receptor antagonist. A literature survey revealed that numerous methods for estimating Irbesartan have been reported in pharmaceutical formulations.

The present study involves the development of the RP-HPLC method using a simple mobile phase which is sensitive and rapid for quantification of Irbesartan in bulk and tablet dosage forms as well as subsequent validation of the developed method according to ICH guidelines.

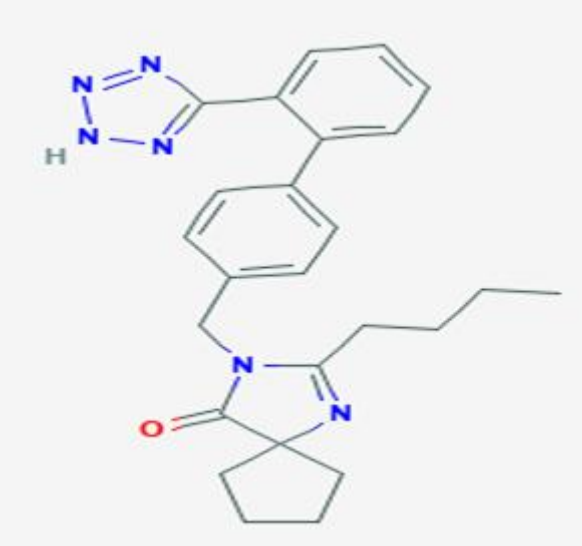

Figure No. 1: Chemical Structure of Irbesartan

MATERIALS AND METHODS:

Chemicals: The gift samples of Irbesartan (pure drug) were procured from Macleod Pharma Ltd., Gujarat. The required solvents like HPLC grade methanol, water were purchased from

Merck. (Merck specialties Pvt ltd India) HPLC grade water was prepared using Millipore System. All other reagents were of AR grade.

Instruments used:

Table No. 1: Instruments used

Sr. No.	Instrument	Make/ Model
1.	HPLC	1120 Compact LC
2.	UV Spectroscopy	Shimadzu-1700 UV/VIS
3.	FT-IR	Shimadzu
4.	Analytical Balance	LC/GC
5.	Ultrasonic Bath	Lifecare

Selection of Mobile phase:

Irbesartan was injected into the column with different mobile phases of different ratios with different flow rates till sharp peaks, without any interference peaks containing spectra were obtained. Different mobile phases were containing one or the combinations of two of the following: Acetonitrile, Distilled water (all reagents were of HPLC grade).

Chromatographic Conditions:

A reverse phase C-18 column was equilibrated with the mobile phase. The mobile phase flow rate was maintained at 1ml/min and eluents were monitored at 246 nm. The samples were injected using a 20 µl fixed loop.

All determinations were performed at ambient temperature for a run time of 6 min.

Preparation of Mobile phase:

The mobile phase was prepared by mixing 500 ml of Acetonitrile along with 500 ml of water to get the proportion of 50:50 v/v. The mobile phase was sonicated on an ultrasonic bath for 15 minutes and filtered through a 0.45µ membrane filter.

Preparation of Irbesartan Stock Solution (1000 µg/ml):

About 5 mg of Irbesartan was weighed accurately and was taken in a 50 ml volumetric flask. It was dissolved in the mobile phase.

Preparation of Irbesartan Working Stock Solution (100µg/ml):

From the above-prepared stock solution of Irbesartan 5 ml was pipette out into a 50 ml volumetric flask and the volume was made to up to the mark with the mobile phase.

Calibration Curve:

From Irbesartan standard stock solution (100µg/ml), working solution 1 ml, 2 ml, 3ml….to 9 ml were taken in nine separate 10 ml volumetric flask to produce solutions of concentration range 10 µg/ml to 90 µg/ml of Irbesartan. Then each prepared solution was filtered and sonicated.

Method Validation:

Validation of the method was performed using parameters like Accuracy, Precision, Linearity and Range, Robustness, Ruggedness, LOD, LOQ, Specificity, and System suitability.

a) System Suitability Parameters

Six replicate injections of system suitability solutions (working standard solution) were injected. The retention time, areas, theoretical plates, peak asymmetry, and resolution were calculated for standard solutions.

b) Linearity

The linearity of test solutions for the assay method was prepared from the Irbesartan stock solution at nine concentration levels from 10 to 90 µg/ml of the assay analyte concentration (10, 20, 30, 40, 50, 60, 70, 80, 90 µg/ml). The calibration curve with concentration versus peak area was plotted by injecting the above-prepared solutions and therefore the obtained data were subjected to multivariate analysis using the smallest amount squares method.

c) Method Precision (Repeatability)

In this study, six replicates injections of the standard solutions of Irbesartan were prepared and analyzed using the proposed method. The repeatability data were expressed in terms of % RSD & was found to be less than 2% and measure the peak areas and retention times.

d) Accuracy (Recovery Study)

A recovery study was carried out by applying the method to drug content present in tablet dosage form to which a known amount of Irbesartan was added at 80%, 100%, 120% levels.

The techniques include the addition of standard drug solution to a pre-analysed sample solution. The recovery study was performed three times at each level.

e) Robustness

The robustness of the study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions. The factors that were chosen for this study were the flow rate (± 1 ml/min.) i.e. (0.9 mL/min. & 1.1 ml/min.), mobile phase composition Acetonitrile and Water (50:50 % v/v) & temperature 25ºC.

And another factor was chosen for this study were the wavelength variation (λmax) i.e. (240 nm & 250 nm).

f) Ruggedness

The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts.

g) Limit of Detection and Limit of Quantification

The detection limit (LOD) and quantification limit (LOQ) were determined separately using the equations (1) and (2), respectively, based on the standard deviation of the y-intercept and the calibration curve slope.

$$LOD = 3.3\ \sigma/S \quad \ldots\ldots\ldots (1)$$

$$LOQ = 10\ \sigma/S \quad \ldots\ldots\ldots (2)$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve the slope S could also be estimated from the calibration curve of the analyte.

Forced degradation studies:

The specificity of the method can be demonstrated through force degradation studies conducted on the sample using acid, alkaline, oxidative, thermal, UV, and photolytic degradations. The sample was exposed to these conditions and the API peak was studied for peak purity, which will indicate the method effectively separated from the degradation products.

Degradation in Neutral Condition:

About 100 mg of Irbesartan was accurately weighed and taken into three sets of three different 100 ml volumetric flasks and dissolved in a minimum volume of methanol. Then the volumes were made up to the mark with water and refluxed in round bottom flasks for 1 hr, 2 hr, and 6 hr. From these samples, different solutions were prepared and 20 µl of the sample solutions were injected into the HPLC system.

Degradation in Acidic Condition:

About 100 mg of Irbesartan was accurately weighed and taken into three sets of three different 100 ml volumetric flasks and dissolved in a minimum volume of methanol. Then the volumes were made up to the mark with 0.1 M, 0.5 M, and 1 M HCl and refluxed in round bottom flasks for 1 hr, 2 hr, and 6 hr. From these samples, different solutions were prepared and 20 µl of the sample solutions were injected into the HPLC system.

Degradation in Basic Condition:

About 100 mg of Irbesartan was accurately weighed and taken into three sets of three different 100 ml volumetric flasks and dissolved in a minimum volume of methanol. Then the volumes were made up to the mark with 0.1M, 0.5M, and 1 M NaOH and refluxed in round bottom flasks for 1 hr, 2 hr, and 6 hr. From these samples, different solutions were prepared and 20 µl of the sample solutions were injected into the HPLC system.

Oxidation Degradation:

About 100 mg of Irbesartan was accurately weighed and taken into three sets of three different 100 ml volumetric flasks and dissolved in a minimum volume of methanol. Then the volumes were made up to the mark with 1 % w/v H_2O_2, 3 w/v % H_2O_2 and 6 % w/v H_2O_2 and refluxed in round bottom flasks for 1 hr, 2 hr, and 6 hr. From these samples, different solutions were prepared and 20 µl of the sample solutions were injected into the HPLC system.

Photolytic Degradation:

About 100 mg of Irbesartan was taken in a clean Petri dish and exposed to daylight. Sampling was done at 12 hr, 24 hr, and 72 hr intervals. From these samples, different solutions were prepared and 20 µl of the sample solutions were injected into the HPLC.

UV-Degradation:

About 100 mg of Irbesartan was taken in a clean Petri dish and subjected to UV illumination of 1.2×106 lux hours. Sampling 12 hrs, 24 hrs, and 72 hrs, and various solutions were injected into the HPLC system from these samples.

Thermal Degradation:

About 100 mg of Irbesartan was taken in three separate clean Petri dishes and subjected to dry heat at 70ºC. Sampling was done at intervals of 10 days, 20 days, and 30 days. The solutions were prepared and injected 20 µl of the samples into the HPLC system.

RESULTS AND DISCUSSION:

The objective of the present work was to develop and validate a stability-indicating RP-HPLC method for the determination of Irbesartan in bulk and dosage forms. The method was found to be simple and the accuracy, precision, intra-day precision, inter-day precision, repeatability, and the assay was performed and the results were tabulated below. The retention time for Irbesartan was 3.56 min. The method was validated for linearity, accuracy, precision, specificity, the limit of detection, the limit of quantification, and robustness. The limit of detection and limit of quantification were found 0.0026 µg/ml and 0.0086 µg/ml respectively and recovery of Irbesartan from bulk and dosage forms was found 99.55 %. With this study, the degradation pattern was also studied and results are shown in the corresponding Tables and the Figures are also given.

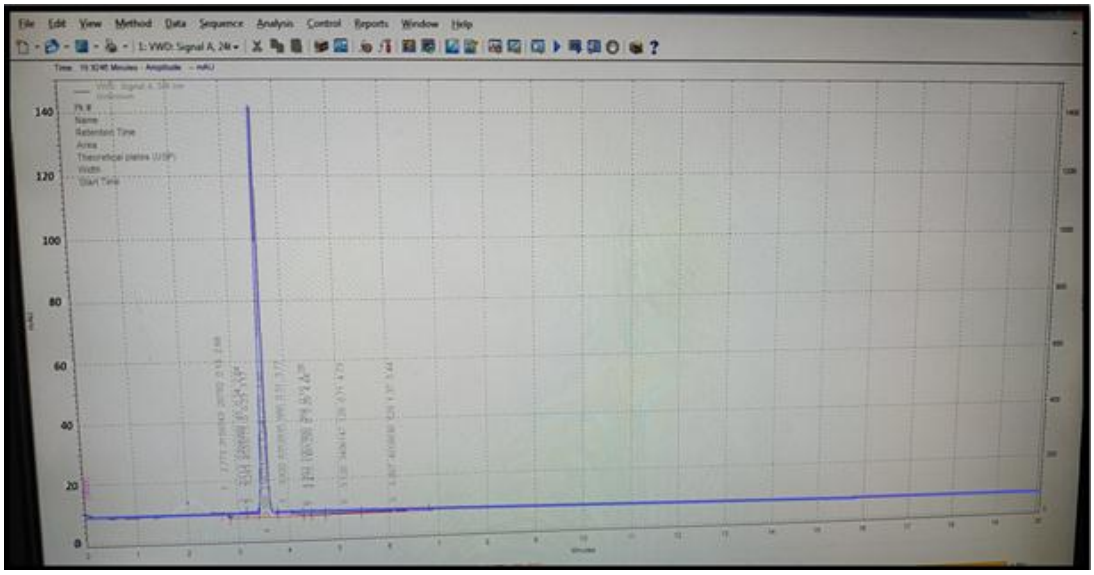

Figure No. 2: Final chromatogram of Irbesartan

RP-HPLC Analysis of Irbesartan: The various concentrations of Irbesartan (10-90 µg/ml) were subjected to HPLC analysis and the resultant chromatogram as given below.

Table No. 2: Calibration Table of Irbesartan

Sr. No.	Conc. of Irbesartan (ppm)	Peak Area
1.	0	000
2.	10	2147978
3.	20	3860751
4.	30	6122184
5.	40	8182322
6.	50	9923702
7.	60	12032957
8.	70	13514189
9.	80	15601600
10.	90	18058778
Slope ± S.D.		196633 ± 17.084
Intercept		95939 (Y-intercept)
Correlation Coefficient		0.9988

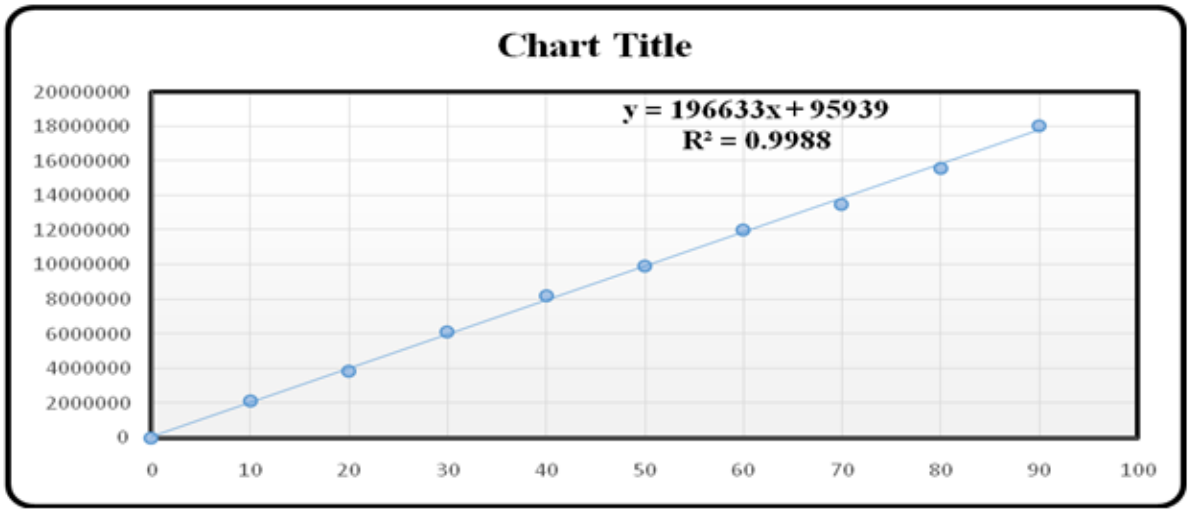

Figure No. 3: Calibration Curve of Irbesartan

Validation Parameters:

Table No. 3: Accuracy Data of the Method for Irbesartan

Level of Recovery	Amount present (mg)	Standard amount (mg)	Amount of drug Recovered (mg)	Peak Area at 246 nm	% Recovery	% RSD
80 %	5	4	3.96	12801366	99.025%	0.3726 %
	5	4	3.99	13201600	99.75%	
	5	4	3.98	13314189	99.52%	
100%	5	5	4.91	36607510	98.22%	0.3099 %
	5	5	4.94	35881892	98.82%	
	5	5	4.93	36091990	98.62%	
120%	5	6	5.94	8282322	99.01%	0.1714 %
	5	6	5.96	8423702	99.35%	
	5	6	5.95	8560136	99.18%	

Table No. 4: Precision Data Showing Repeatability of the Method for Irbesartan

Injection No.	Conc. (µg/ml)	Peak Area of IRB	Conc. Found (µg/ml)	% Purity	Acceptance Criteria
1.	20	5595860	19.93	99.65 %	The % RSD of peak area of Irbesartan should not be more than 2.0
2.	20	5544465	19.96	99.80 %	
3.	20	5540811	19.93	99.65 %	
4.	20	5598809	19.94	99.70 %	
5.	20	5575879	19.98	99.90 %	
6.	20	5588607	19.92	99.60 %	
Mean (n=6)		99.72 %			
SD		0.0011			
% RSD		0.1128%			

Table No. 5: Intraday Precision Data of the Method for Irbesartan

Injection No.	Conc. (µg/ml)	Peak Area of IRB	Conc. Found (µg/ml)	% Purity	Acceptance Criteria
1.	20	5595860	19.93	99.65 %	The % RSD of peak area of Irbesartan should not be more than 2.0
2.	20	5544465	19.96	99.80 %	
3.	20	5540811	19.93	99.65 %	
4.	20	5598809	19.94	99.70 %	
5.	20	5575879	19.98	99.90 %	
6.	20	5588607	19.92	99.60 %	
Mean (n=6)		99.72 %			
SD		0.0011			
% RSD		0.1128%			

Table No. 6: Interday Precision Data of the Method for Irbesartan

Parameters	Day-1	Day-2	Day-3
Conc.(µg/ml)	20	20	20
Peak area	5574071	5362838	5061095
Conc. Found (µg/ml)	19.944	19.912	19.846
% Purity	99.72 %	99.56 %	99.20 %
SD	0.0011	0.0052	0.0028
% RSD	0.1128 %	0.5237 %	0.2851 %

Table No. 7: Robustness Data of the Method for Irbesartan

Factor	Level	Retention Time	Tailing Factor
A. Flow Rate (ml/min.)			
0.9	-1	3.48	1.47
1	0	3.56	1.5
1.1	+1	3.72	1.61
Mean ± SD (n=3)		3.586 ± 0.122	1.526 ± 0.073
B. Wavelength Variation (λmax)			
240 nm	-6	3.56	1.61
246 nm	0	3.84	1.65
250 nm	+ 4	4.01	1.72
Mean ± SD (n=3)		3.803 ± 0.2272	1.656 ± 0.055

Table No. 8: Summary Result of Irbesartan

Sr. No.	Parameter	Result	Acceptance Criteria
1.	System Suitability		
	1. Theoretical Plates	5069.44	1) Not less than 3000
	2. Tailing factor	1.25	2) Not more than 2.0
	3. Retention time	3.56	-
	4. % RSD	0.8184	3) Not more than 2.0
2.	Specificity	Specific	Specific
3.	Method precision (% RSD)	0.1128 %	Not more than 2.0 %
4.	Linearity	10-100 (µg/ml)	
	Correlation Coefficient (r^2)	0.9988	Not less than 0.990
5.	Accuracy (% Recovery)		
	80 %	99.43 %	97- 103 %
	100 %	98.55 %	
	120 %	99.18 %	
6.	Robustness	All the system suitability parameters are within the limits.	

Forced Degradation Studies of Irbesartan:

A. Acidic Degradation of Irbesartan-

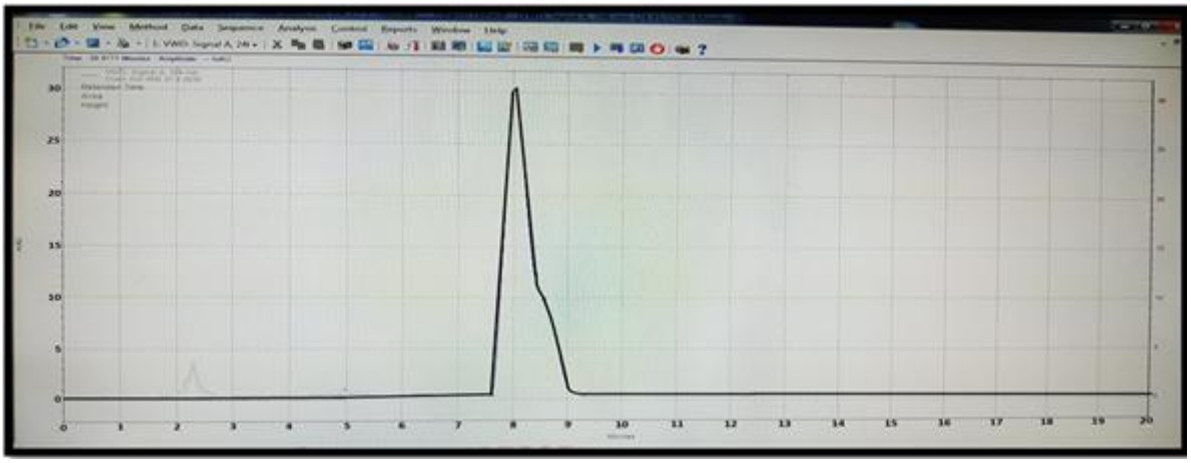

Figure No. 4: Representative Chromatogram of Acidic Degradation of Irbesartan

B. Basic Degradation of Irbesartan-

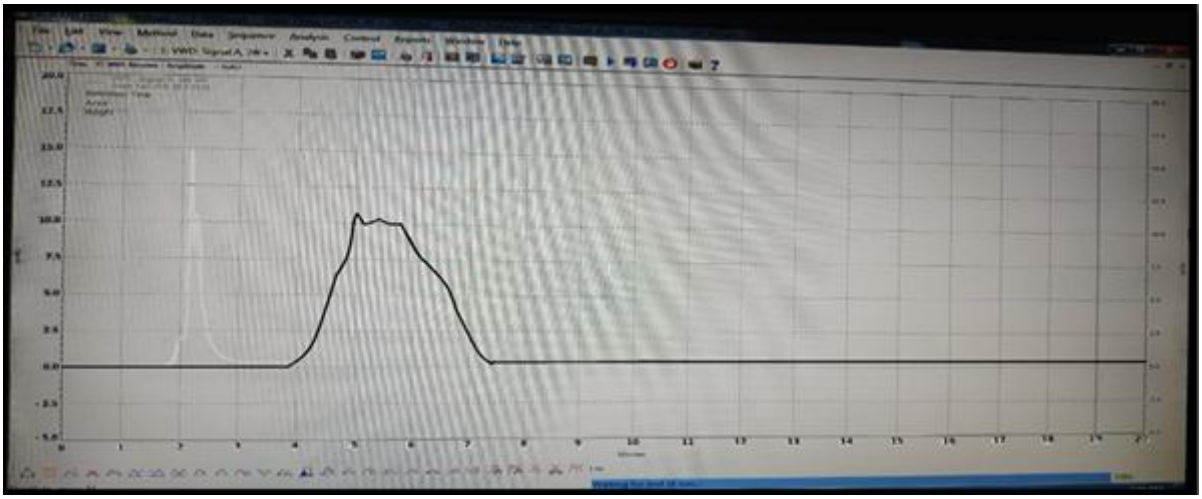

Figure No. 5: Representative Chromatogram of Basic Degradation of Irbesartan

C. Oxidative Degradation of Irbesartan-

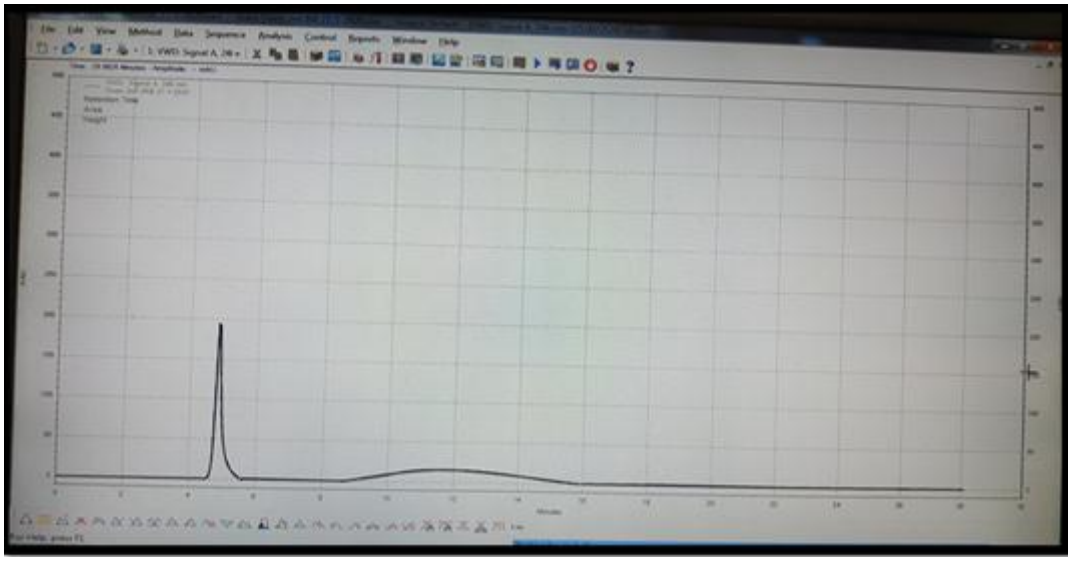

Figure No. 6: Representative Chromatogram of Oxidative Degradation of Irbesartan

D. Photolytic Degradation of Irbesartan-

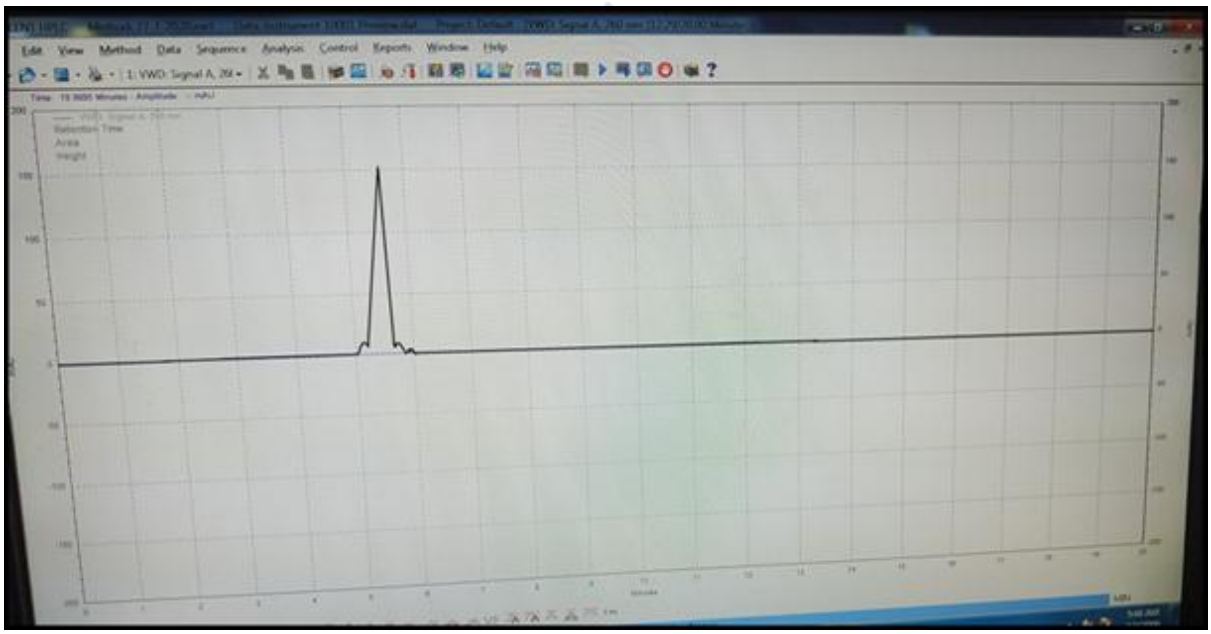

Figure No. 7: Representative chromatogram of Photolytic Degradation of Irbesartan

E. Thermal Degradation of Irbesartan-

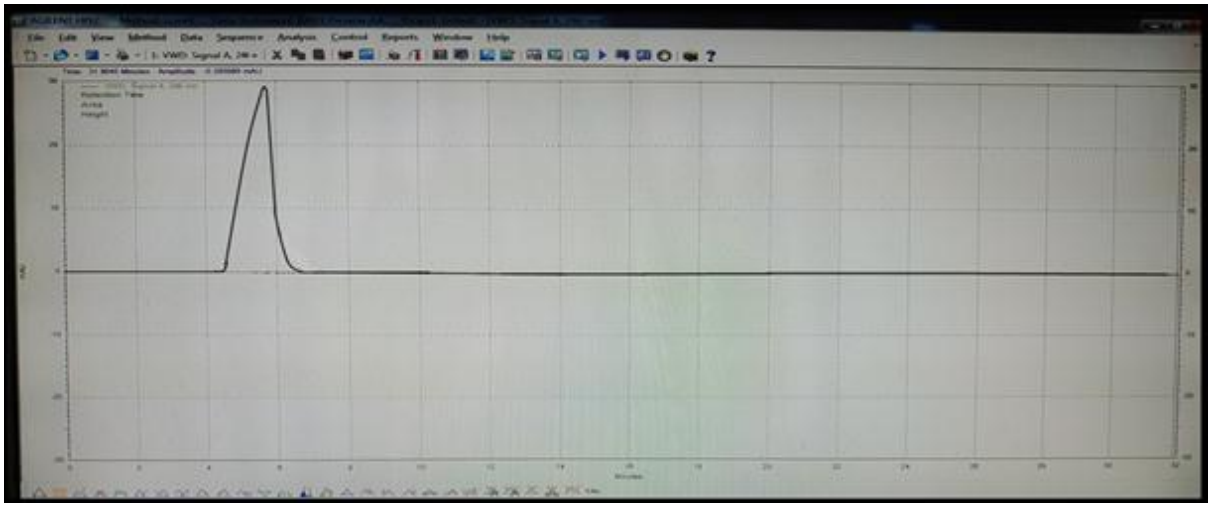

Figure No. 8: Representative chromatogram of Thermal Degradation of Irbesartan

Table No. 9: Overall Summary of Degradation Study

Conc. (µg/ml)	Stress Conditions	Degradation Time (Hrs)	IRBESARTAN		
			% Assay	% Degradation	Retention Time
20	Acid,1 M HCl at 80°C Reflux	6 Hours	84.7 %	15.3 %	7.610
20	Base, 1 M NaOH at 80°C Reflux	8 Hours	77.9 %	22.1 %	3.953
20	Neutral, In Water at 80°C Reflux	8 Hours	97 %	3 %	--
20	Oxidative, 10% V/V H_2O_2 at Room Temperature	12 Hours	78.2 %	21.8 %	4.652
20	Photolytic, Kept in Sunlight	24 Hours	96.87 %	3.13 %	5.051
20	Thermal, Kept inside Hot Air Oven at 80°C	48 Hours	90 %	10 %	4.283
20	UV Degradation, Kept inside UV Chamber	24 Hours	97.5 %	2.5 %	--

CONCLUSION:

In the presence of their respective degradants, the developed RP-HPLC stability-indicating assay method was found suitable for the drug analysis in its pure form since the resolution between the drugs with their corresponding degraders was better. The drug was prone to degrade more in acidic, alkaline, oxidative, and thermal conditions. The method was found to be quick, simple, reliable, sensible, economical, and accurate. The sensitivity and accuracy of the method were also ascertained by using internal standards. The results of stability studies of Irbesartan suggest that the drug is stable to Neutral, photolytic, and UV radiation degradations.

Therefore, the proposed method can be used for routine analysis of the estimation of Irbesartan in its bulk and dosage formulations.

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