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ORIGINAL RESEARCH ARTICLE

Method Development, Validation and Stability Study of Irbesartan in Bulk and Pharmaceutical Dosage Form by UV-Spectrophotometric Method

Kishanta Kumar Pradhan[*1], Uma Shankar Mishra[1], Subasini Pattnaik[2],Debananda Mishra[1], Ghanshyam Panigrahi[1], Kanhu Charana Sahu[1,]

[1]Department of pharmaceutical Analysis and Quality assurance,Royal College of Pharmacy and Health Sciences, Andhapasara Road, Berhampur,Odisha.
[2]PG Department of Zoology, Berhampur University, Bhanja Bihar, Berhampur,Odisha.

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ABSTRACT

A simple, precise, accurate and stability indicating UV-method have been developed and validated for estimation of Irbesartan. Many experiments were conducted by taking combination of drugs & also by single drug but no suitable validation with stability studies were reported. Irbesartan has the absorbance maxima at 246nm.Method A involves method development and validation, showed sharp peak at 246nm.Method B involves forced degradation study. All the methods utilize methanol as solvent. Linearity for the detector response was observed in the concentration range of 5-45 µg/ml. Validation experiments were performed to demonstrate System suitability, Specificity, Precision, Linearity, Accuracy Precision, robustness, ruggedness. Furthermore stability studies of Irbesartan were carried out under acidic, alkali, neutral, oxidation &photolytic degradation as per stability indicating assay methods. The results of analysis have been validated and recovery studies were carried out by adding specific drug amount (80%, 100%, and 120%) and shows recovery studies in the range (99.335-100.583) %. The proposed method can be successfully applied for method development, validation and stability study of Irbesartan.

Key Words: Irbesartan, Beer's law, Analytical method validation, Forced degradation.

INTRODUCTION

Irbesartan (IRBE), chemically known as (2-butyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl]-1, 3-diazaspiro [4.4] non-1-en-4-one), is an Anti-hypertensive drug (Angiotensin-II̅ receptor antagonist) and prevents binding of Angiotensin-II̅ to AT_1 receptor. This is used for treatment of hypertension & Diabetic nephropathy with an elevated serum creatinine & proteinuria (>300mg/day) in patients with type-2 diabetes & hypertension [1,2,3,4] .

UV Spectrophotometric method was developed and validated as per ICH guidelines[5]. Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380nm). Irbesartan is official in British Pharmacopoeia' which recommends UV spectrophotometry for its analysis.

The API is subjected to a number of forced degradation conditions to include acidic, basic, and oxidative conditions. Forced degradation should be one of the activities performed early in the development process to ensure that the method is discriminating before a lot of time, effort and money have been expended. Depending on the API, not every stress agent may effect a degradation, but each agent has to be evaluated to determine whether degradation results.

Literature survey reveals availability of UV, HPLC, and HPTLC methods for estimation of Irbesartan, Hydrochlorothiazide with many drugs combiningly other than this single drug with stability study. However no method has been reported till date for method validation and stability study of Irbesartan by uv-spectrometric

***Corresponding Author:** Kishant Kumar Pradhan, **Email:** kishantakumar@gmail.com,**Phone No:** +91-9583208861

method. Present work deals with the method development, validation and stability study of IRBE by various UV- Spectrophotometric methods.

MATERIALS AND METHODS

Apparatus

Digital balance, Ultrasonicator, a double-beam UV-Visible spectrophotometer, 1700 pharmaspec with spectral band width of 2nm, wavelength accuracy ± 0.5nm and a pair of 1-cm matched quartz cells was used to measure absorbance of the resulting solutions.

Solvent used: Methanol was used as a solvent.

Stock solution: Standard stock solution of IRBE (100µg/ml) was prepared and from this required concentrations were used for analysis.

Absorption Maxima Method

For the selection of analytical wavelength, 20 µg/ml solution of IRBE was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 to 200nm.From the spectra of drug (**Fig 1**) λ_{max} of IRBE ,246nm was selected for the analysis.

Preparation of working Standard Solutions

The Prepared stock solution was further diluted with methanol to get working standard solution of 5ppm, 10ppm……. 45ppm to construct Beer's law plot for, Irbesartan. The absorbance of each solution was measured at 246 nm against methanol as blank. (**Table-1**)

Preparation of calibration curve

The standard graph/calibration curve for Irbesartan was plotted by taking concentration of drug on X-axis and absorbance on Y-axis. (**Fig 2**)

Preparation of Sample Solutions

For analysis of commercial formulations, 1tablet was weighed and powdered and powder equivalent to 10mg of Irbesartan were transferred into 100ml volumetric flask and dissolved in methanol to get 100 µg/ml solutions. Then the solution was sonicated for 15 min and filtered and further dilutions were made with methanol to get the concentrations within the linearity range of respective drugs and measured the absorbance at 246 nm for solution against methanol. Here 2ml were taken and made up to 10ml. The drug content in each tablet was estimated by using the standard graph.

Method A- Method Validation:

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of IRBE. Beer-Lambert's concentration range was found to be 5-45 µg/ml for below methods.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100%, and 120%) of the bulk sample of Irbesartan to the previously analyzed solution of formulation of concentration 20 µg/ml.Percentage recovery for IRBE, by all the methods, was found in the range of (99.335-100.583) %**.** Shown in (**Table 3**)

Precision

The precision of the proposed method was ascertained by actual determination of eight replicates of fixed concentration of the drug within the beer's range and finding out the absorbances by taking a fixed concentration of 20µg/ml by the proposed method. From these absorbances mean, Standard deviation, %R.S.D was calculated, shown in (**Table 4**).

Intra Day Assay

The assay procedure was carried out in the same day in the duration of 2 hours to 3 hours at fixed concentration and the results were compared. Shown in (Table-4a)

Inter Day Assay

The assay procedure was carried out for 3days with freshly prepared solution from stock solution at fixed concentration at 24hour interval**.** Shown in (Table-4b)

Ruggedness

To determine the ruggedness the same procedure was carried out by another analyst and the result is compared with the same previous procedure. Shown in (**Table 5**)

Robustness

This procedure was carried out by changing the solvent system composition in different ratio (9:1, 8:2). Then results were compared. Shown in (**Table 6**)

Method B- Forced Degradation Study:

Acid degradation

First in a 10ml volumetric flask, accurately weighed 10mg of bulk drug was dissolved with, few drops of Methanol and volume was made by 0.1N HCl. Then this solution was refluxed for 5-6 hours. Then in each hour interval withdrawn of sample was done. So, from that solution 0.2ml was taken in a 10 ml volumetric flask & the volume was made with Methanol, which gives a concentration of 20µg/ml. Then absorbance was measured by scanning the prepared Solution in

U.V Spectrophotometer. Shown in (**Table 7 & Fig 3**)

Alkali Degradation

First in a 10ml volumetric flask, accurately weighed 10mg of bulk drug was dissolved with, few drops of Methanol and volume was made by o.1N NaOH. Then this solution was refluxed for 5-6 hours at 60^0c. Then in each hour interval withdrawn of sample was done. So, from that solution 0.2ml was taken in a 10 ml volumetric flask & the volume was made with Methanol, which gives a concentration of 20µg/ml. Then absorbance was measured by scanning the prepared Solution in U.V Spectrophotometer. Shown in (**Table 8 & Fig 4**)

Neutral Degradation

Accurately weighed 10 mg of bulk drug was taken in 10 ml volumetric flask. Then little amount of methanol was added for dissolving of the drug. The volume was adjusted up to the mark with double distilled water. Then that solution was refluxed for 5 hours. From that 0.2ml of solution was taken out and volume was made with methanol. The absorbance was measured at each hour interval by withdrawing the required amount of sample solution. Then scanning with UV-spectrophotometer. Shown in (**Table 9 & Fig 5**)

Thermal degradation

A specific amount of bulk drug was taken in a cleaned Petridis and the Petridis along with bulk drug was put it into the oven for 8 hours. From that in each 6 hours interval accurately 10mg of bulk drug was weighed and was transferred to 10 ml volumetric flask and the volume was adjusted with methanol, which gives a concentration of 1000µg/ml. After that required concentration of (20 µg/ml) was made and absorbance was measured by UV spectrophotometer. Shown in (**Table 10 & Fig 6**)

Photolytic degradation

In this process 150mg of bulk drug was weighed & was put into the Petridis. Then placed that under direct sun light for three days. In each 6 hours interval 10mg was weighed and was transferred in to 10ml volumetric flask, then dissolved and made the volume with methanol, which gives a concentration of 1000 µg/ml solution. From that 20µg/ml was prepared by using solvent methanol. Then absorbance was measured by U.V spectrophotometer. Shown in (**Table 11 & Fig 7**)

Oxidation with H_2O_2

10mg of bulk drug was weighed accurately, 2-3 drop of Methanol was added to make the drug soluble then the volume was made up by 3% H_2O_2 & keeps it in room temp. For 12 hours. In each 6 hours interval the specified amount of sample was taken & the required concentration (20µg/ml) was prepared & it was scanned by U.V spectrophotometer. Shown in (**Table 12 & Fig 8**)

RESULTS:

Fig 1: A Typical UV Chromatogram Showing Irbesartan at 246nm

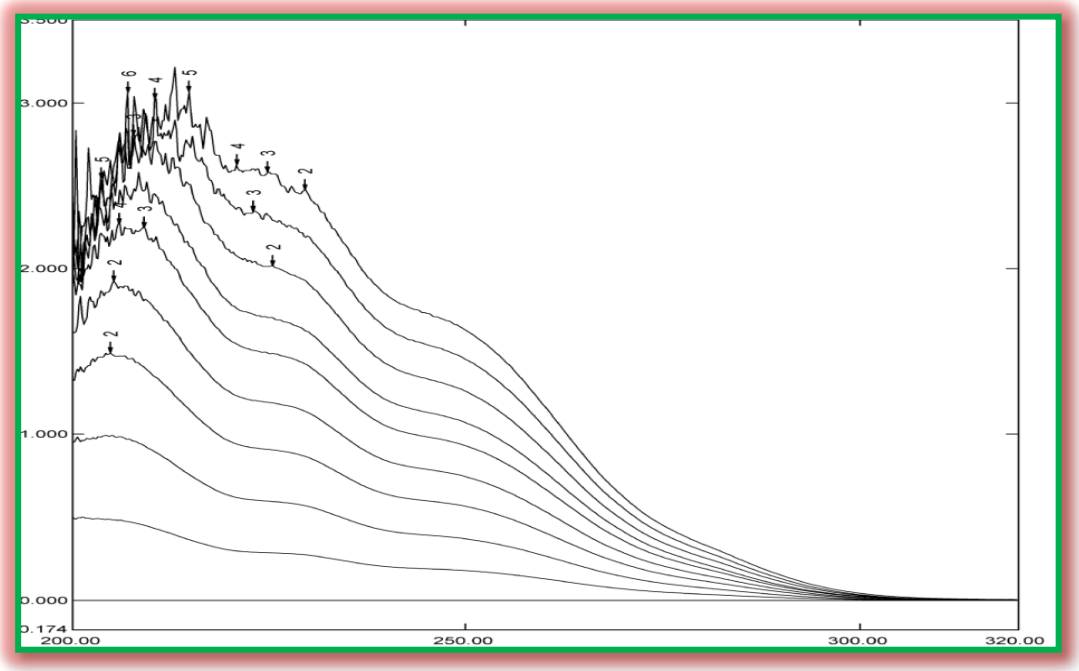

Table-1: Linearity of Irbesartan

S.No	Optical Characters	Values
1	Absorbance Maxima	246nm
2	Beer's Limit	5-45 µg/ml
3	% R.S.D	1.103003
4	Regression equation (Y*)	0.038X+0.003
5	Slope (a)	0.038
6	Intercept (b)	0.003
7	Correlation Coefficient (R^2)	0.999

Fig 2: Linearity Graph of Irbesartan

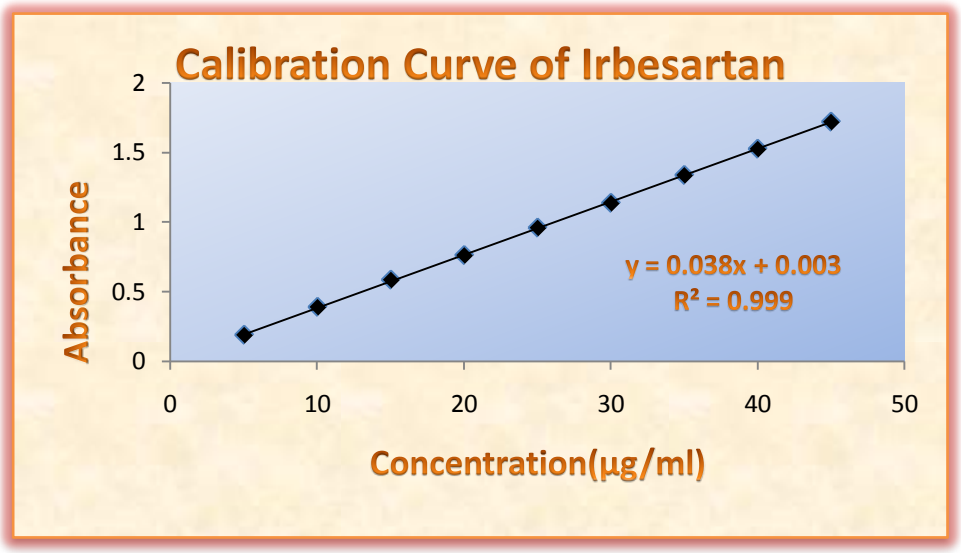

Table 2: Optical Characteristics of Irbesartan

Concentration	Absorbance
5	0.188
10	0.389
15	0.585
20	0.762
25	0.96
30	1.138
35	1.339
40	1.528
45	1.723

Table 3: Accuracy Readings of Irbesartan

	No. of preparations			Statistical Analysis		
	Formulation	Pure Drug	% Recovery	Mean	St. Dev	%RSD
S1 : 80 %	20	16	100.03			
S2 : 80 %	20	16	99.04	99.37	0.571577	0.575201
S3 : 80 %	20	16	99.04			
S4 : 100 %	20	20	101.04			
S5 : 100 %	20	20	100.04	100.51	0.502693	0.500142
S6 : 100 %	20	20	100.45			
S7 : 120 %	20	24	98.04			
S8 : 120 %	20	24	99.78	99.29333	1.09441	1.102199
S9 : 120 %	20	24	100.06			

Table 4: Precision Results Showing Repeatability of Irbesartan

Concentrations (µg/ml)	Absorbance	Calculated Amount	Statistical Analysis
20	0.762	19.97368	
20	0.765	20.05263	Mean=20.0307
20	0.769	20.15789	St. Dev=0.083908
20	0.76	19.92105	
20	0.766	20.07895	%RSD=0.418899
20	0.763	20	

Table 4(a): Intra Day Assay of Irbesartan

Conc.(mcg/ml)	Absorbance1	Absorbance 2	Absorbance 3	Statistical Analysis
20	0.764	0.763	0.762	
20	0.769	0.763	0.765	Mean =20.06579
20	0.763	0.769	0.769	
20	0.768	0.768	0.76	Std Dev =0.046417
20	0.771	0.762	0.766	%RSD =0.231323
20	0.77	0.764	0.763	
Mean	0.7675	0.764833	0.764167	
Calc.Amt.	20.11842	20.04825	20.0307	

Table 4(b): Inter Day Assay of Irbesartan:

Sl.No.	Concentration	Day 1	Day 2	Day 3	Statistical Analysis
1	20	0.764	0.765	0.762	
2	20	0.769	0.768	0.765	
3	20	0.763	0.76	0.769	Mean =20.0614
4	20	0.768	0.759	0.76	Std Dev =0.049427
5	20	0.771	0.769	0.766	
6	20	0.77	0.765	0.763	%RSD =0.24638
	Mean	0.7675	0.764333	0.764167	
	Calc. Amt.	20.11842	20.03509	20.0307	

Table 5: Results Showing Ruggedness of Irbesartan

Analyst-1				Analyst-2			
Conc. (mcg/ml)	Abs.	Calc. Amt	Statistical Analysis	Conc. (mcg/ml)	Abs.	Calc. Amt.	Statistical Analysis
20	0.762	19.97368		20	0.765	20.05263	
20	0.765	20.05263	Mean=20.0307	20	0.768	20.13158	Mean=20.0350
20	0.769	20.15789	S.D=0.08390	20	0.76	19.92105	S.D=0.10743
20	0.76	19.92105		20	0.759	19.89474	
20	0.766	20.07895	%RSD=0.4188	20	0.769	20.15789	%RSD=0.5362
20	0.763	20		20	0.765	20.05263	

Table 6: Results Showing Robustness of Irbesartan at Different Solvent Composition

(92:08)				(88:12)			
Conc. (mcg/ml)	Abs.	Calc. Amt	Statistical Analysis	Conc. (mcg/ml)	Abs.	Calc. Amt.	Statistical Analysis
20	0.765	20.05263		20	0.769	20.157	
20	0.768	20.13158	Mean=20.0350	20	0.768	20.131	Mean=20.05702
20	0.76	19.92105	S.D=0.10743	20	0.765	20.052	S.D=0.073339
20	0.759	19.89474		20	0.764	20.026	
20	0.769	20.15789	%RSD=0.53622	20	0.762	19.973	%RSD=0.365652
20	0.765	20.05263		20	0.763	20	

Fig 3: Acid Degradation Spectrum

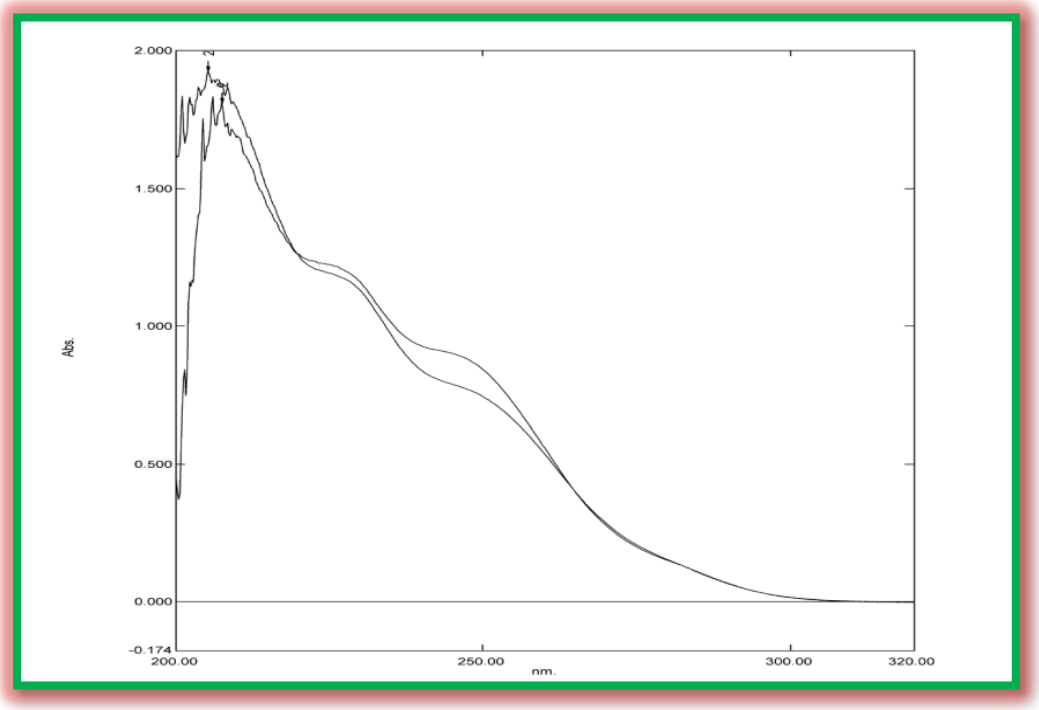

Table 7: Acid degradation

Sl.No.	Name	Absorbance	Concentration	% Degradation
1	Drug	0.762	20	0
2	Degradation 1hr	0.621	16.26	18.70
3	Degradation 2hr	0.595	15.578	22.11
4	Degradation 3hr	0.553	14.47	27.65
5	Degradation 4hr	0.485	13.02	34.90
6	Degradation 5hr	0.420	10.97	45.15

Fig 4: Alkali Degradation Spectrum

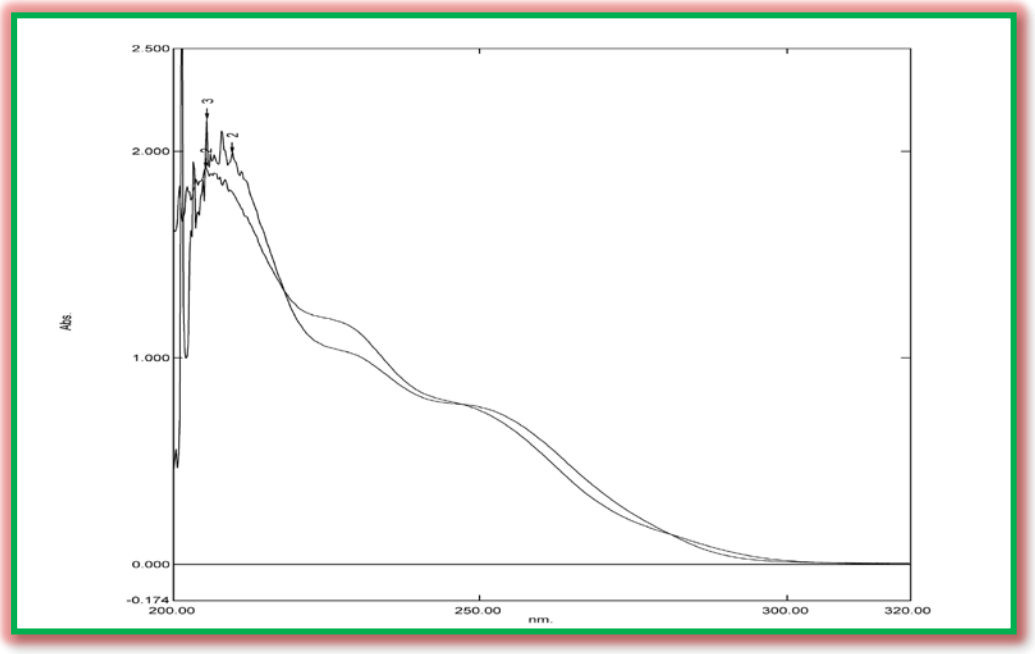

Table 8: Alkali degradation

Sl.No.	Name	Absorbance	Concentration	%Degradation
1	Drug	0.762	20	0
2	Degradation 1hr	0.721	18.89	5.55
3	Degradation 2hr	0.547	14.31	28.45
4	Degradation 3hr	0.484	12.65	36.75
5	Degradation 4hr	0.420	10.97	45.15
6	Degradation 5hr	0.412	10.76	46.20

Fig 5: Neutral Degradation Spectrum

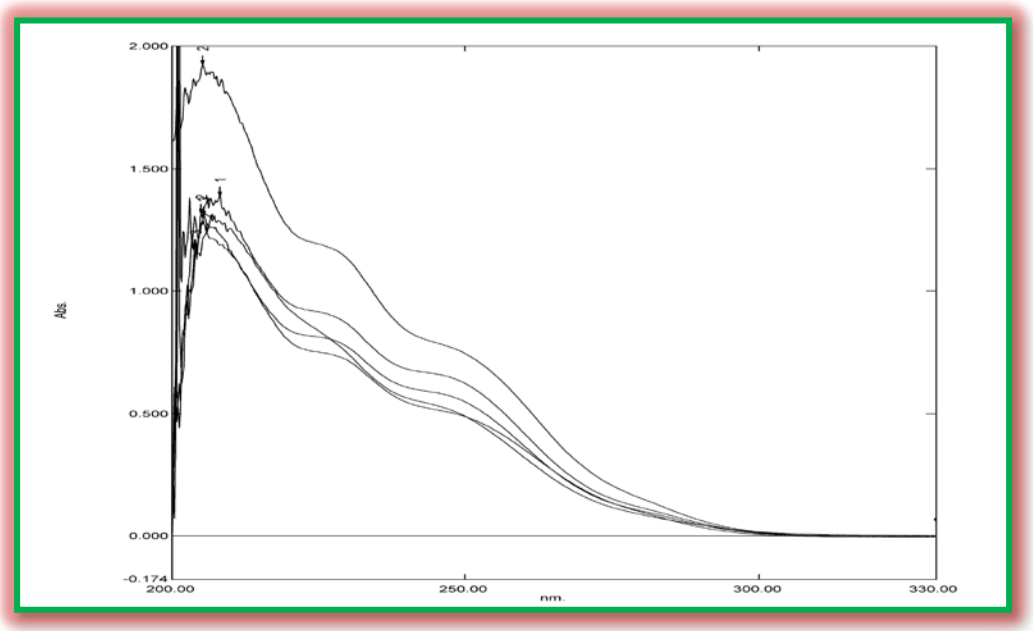

Table 9: Neutral degradation

Sl.No.	Name	Absorbance	Concentration	%Degradation
1	Drug	0.762	20	0
2	Degradation 1hr	0.601	15.73	21.35
3	Degradation 2hr	0.567	14.84	25.80
4	Degradation 3hr	0.553	14.47	27.65
5	Degradation 4hr	0.482	12.60	37.0
6	Degradation 5hr	0.458	11.97	40.15

Fig 6: Thermolytic Degradation Spectrum

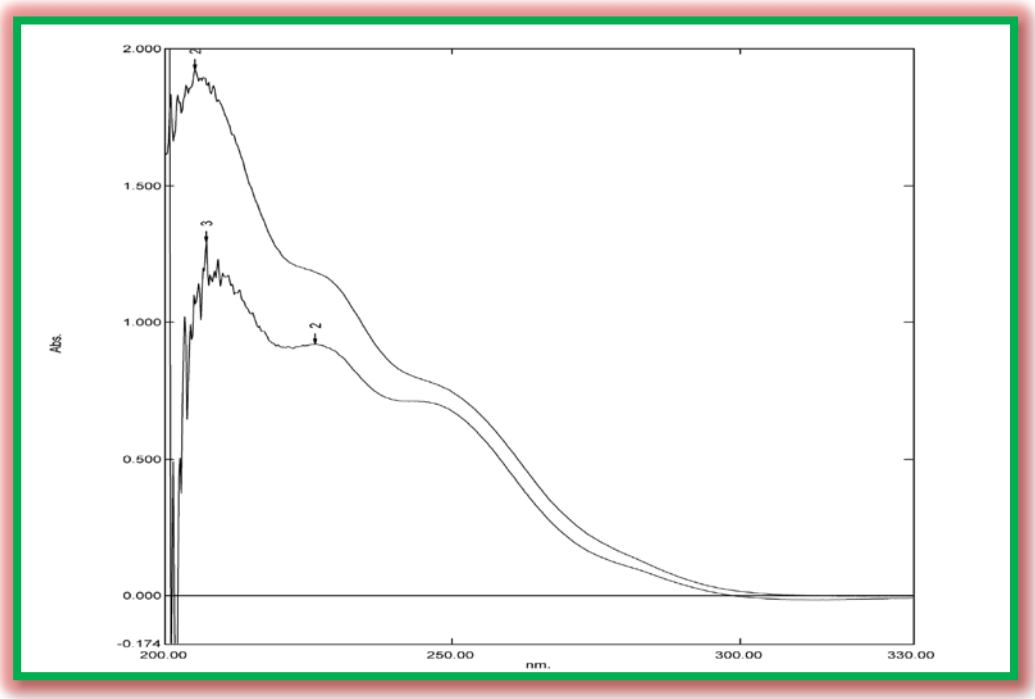

Table 10: Thermal Degradation

S.No.	Name	Absorbance	Concentration	%Degradation
1	Drug	0.762	20	0
2	Degradation1	0.602	15.76	21.20

Fig 7: Photolytic Degradation Spectrum

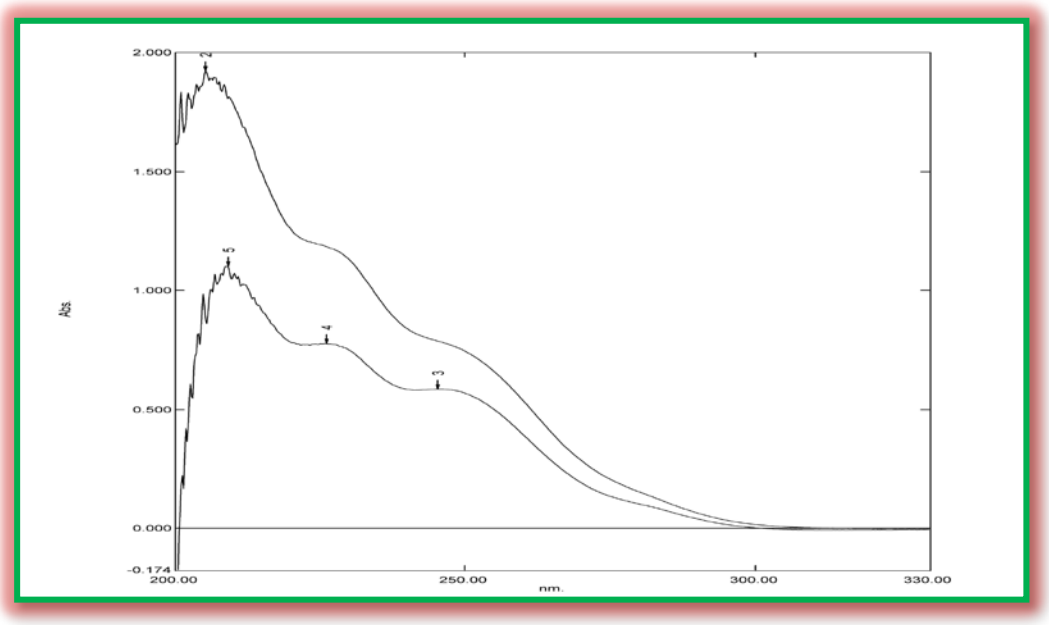

Table 11: Photolytic Degradation

S.No.	Name	Absorbance	Conc.(µg/ml)	%Degradation
1	Drug	0.762	20	0
2	Degradation 1	0.581	15.21	23.95
3	Degradation 2	0.462	12.07	39.65

Fig 8: Oxidative Degradation Spectrum

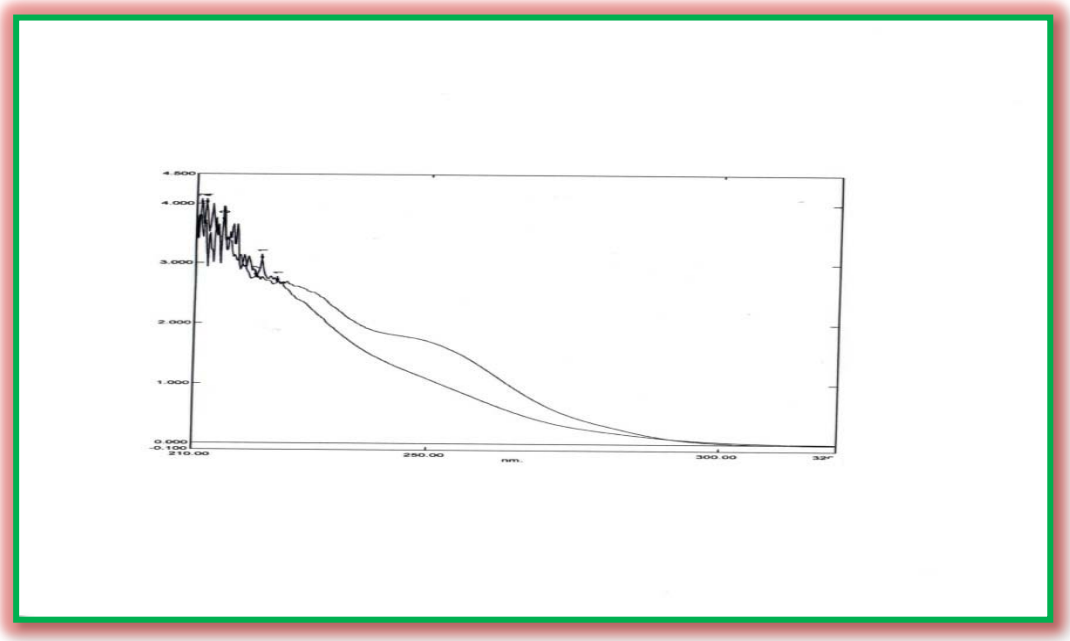

Table 12: Oxidative degradation

S.No.	Name	Absorbance	Conc.(µg/ml)	%Degradation
1	Drug	0.762	20	0
2	Degradation1	0.569	14.89	25.55
3	Degradation 2	0.489	12.78	36.10
4	Degradation 3	0.438	11.44	42.80
5	Degradation 4	0.382	9.97	50.15
6	Degradation 5	0.339	8.84	55.80

DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for method validation and stability study of Irbesartan in its pharmaceutical dosage form. Absorbance maxima of Irbesartan at 246nm. Linearity for detector response was observed in the concentration range of 5-45 µg/ml for all validated methods. Percent assay for IRBE by above validated methods, was found in the range of 99.43% to 100.36%.Standard deviation and coefficient of variance was found to be less than ±2.0 indicating the precision of the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Percent recovery for IRBE was found in the range of 99.335% to 100.583% values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of all the methods. For forced degradation study the absorbance's in all stressed condition was decreased for repeated times and percent degradation was found out. So, the drug IRBE undergoes degradation in all stressed condition. Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible and economical and can be employed for routine quality control of IRBE in its pharmaceutical dosage form.

CONCLUSION

The proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method is specific while estimating the commercial formulation without interference of excipients and the other additives. Hence, this method can be used for routine determination of Irbesartan in bulk sample and pharmaceutical formulation. The proposed method for stability study shows that there is appreciable degradation found in stress condition of Irbesartan.

The proposed UV-Spectrophotometric method has been evaluated over the linearity, accuracy, precision, specificity, LOD and LOQ and proved to be convenient and effective for the quality control and stability studies of Irbesartan. A new simple analytical method has been developed to be applied for the evaluation of the stability of Irbesartan to quantify Irbesartan and its degradation products in a solid premix dosage forms.

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