

**Original Research Article****Validated method for estimation of irbesartan in bulk and dosage form by high performance liquid chromatography technique**N. S. Kulkarni^{1,3*}, D. S. Rathore¹, N. S. Ranpise², S. N. Dhole³¹NIMS institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India²Sinhgad college of Pharmacy, Vadgaon (Bk), Pune, Maharashtra, India³PES, Modern college of Pharmacy (For Ladies), Moshi, Pune, Maharashtra, India**ARTICLE INFO:****Article history:**

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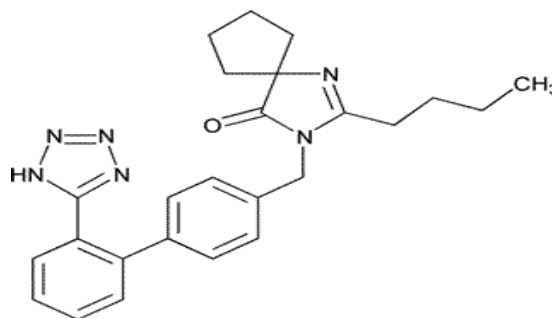
Keywords:Linearity,
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Regression.**ABSTRACT**

A simple, specific, accurate and precise new high performance liquid chromatographic method was developed for the estimation of irbesartan in bulk and its developed dosage form. The mobile phase containing acetonitrile: Phosphate buffer pH 3.5 in proportion of 50:50 v/v was employed with flow rate of 1.0 ml/min and eluting medium was monitored at 240 nm. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantification and robustness for irbesartan. A linear response was observed in the range of 5-40 µg/ml. Linear regression of absorbance on concentration gave equation $y = 101.9x + 195.3$ with a regression co-efficient $r^2 = 0.993$. The method was validated for different parameters as per the ICH guidelines. The degradation studies were carried out by using the developed method. Thus the method was found to be useful for the determination of irbesartan in bulk as well as for dosage forms.

Introduction

Irbesartan is an angiotensin II, the principal pressor agent of the renin-angiotensin system, is responsible for effects such as vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal

reabsorption of sodium. The chemically irbesartan is 2-butyl-3-({4-[2-(2H-1,2,3,4-tetrazol-5yl) phenyl]phenyl} methyl)-1,3-diazaspiro[4.4]non-1-en-4-one with molecular weight of 428 and practically insoluble in water[1].

**Structure of irbesartan**

It is mandatory requirement from regulatory authorities to show the proper qualification of its degradation

pathways and characterization of known degraded product that are present. Degradation can arise during the

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shelf life/ storage as of the drug substances and their acceptance based on studies or known safety data need to be studied. In the investigation an ideal stability indicating chromatographic method which estimates the drug and able to resolve the drug from its degradation products. Hence an attempt has been made to develop an accurate, rapid and reproducible method for the determination of irbesartan in presence of its degradation products for its content analysis in pharmaceutical dosage form as per ICH guidelines [2,3]. The objective of the study is to develop an accurate, rapid and reproducible method for the determination of irbesartan in presence of its degradation products for its content analysis in pharmaceutical dosage form as per ICH guidelines.

Materials and Methods

Instrument: The liquid chromatographic system consist of Cyberlab MAO1527, USA with binary double reciprocating high pressure gradient mixer with UV/VIS detector. Analysis was performed using column C-18 (250 mm length x 4.6 mm internal diameter and 5 µm particle size).

Reagents and materials

Irbesartan was obtained as gift samples from Que Pharmaceutical Pvt. Ltd, Wadhvan, Gujrat. HPLC grades water, HPLC grades Acetonitrile were purchased from Merck, Research lab, Molychem, India. All chemicals were of analytical grade.

Preparation of stock solution

Irbesartan (50 mg) was accurately weighed and transferred to a volumetric flask of 25 ml capacity. Volume was made upto 25 ml with HPLC grade methanol. Ten ml of this solution was diluted to 100 ml with mobile phase containing acetonitrile: phosphate buffer pH 3.5 (50:50 v/v) to obtain concentration of 200 µg/ml. Further dilutions

Chromatographic Conditions

The mobile phase consisting were filtered through membrane filter, degassed and pumped through solvent reservoir in 50:50 proportion into the column with flow rate of 1.0 ml/min and wavelength of 240 nm set for the determination of irbesartan. The volume of injection loop was 20 µl prior to injection of the drug solution the column was equilibrated with mobile phase for 30 min. The column and the HPLC system were kept in 22°C with pressure of 8 psi.

Preparation of calibration curve

The standard solutions of 5 to 40 µg/ml of irbesartan were prepared from the stock solution. These solutions were injected into HPLC column of chromatographic system. The plot of AUC versus concentration of irbesartan and coefficient of regression was determined [4, 5, 6].

Method validation

The developed method was validated for linearity, precision, robustness, limit of detection and limit of quantification [7,8].

Linearity

It is a measure of how well a calibration plot of response vs concentration approximates a straight line. A series of concentrations were injected and the linearity range was established. The experiments were repeated in triplicate.

Precision

Solution with 20 µg/ml concentration were injected repeatedly (n=3) by averaging the peak areas and determination of SD and RSD of all injections. Intermediate precision (intra and inter day) was performed by measuring absorbance of standard solution at three different time during single day and on three consecutive days respectively.

Robustness

To validate the robustness of the developed method, small change in system parameters was carried out and its effect on resultant retention time (t_R) was determined. Variations in mobile phase composition ($\pm 2\%$) and flow rate (+ 1.5 and -0.5 ml/min) has been done. The results were compared with the earlier results.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD is defined as the lowest concentration of an analyte in a sample that can be estimated. It was determined by first examining the noise of the instrument by injecting the mobile phase in triplicates and finding values with the highest and lowest peak areas at a range covering the retention time of the drug. The difference in the areas gave the noise of the instrument. Peak areas having three times the noise give an estimate of the LOD. The LOD is calculated by using the following formula,

$$\text{LOD} = \frac{3\sigma}{S}$$

Where, σ - standard deviation

S- slope of the calibration curve

LOQ is the smallest concentration of analyte, which gives a response that can be accurately quantified. Noise of the instrument was determined as given above and the peak area having ten times the value of noise give an estimate of LOQ.

The LOQ is calculated by using the following formula,

$$\text{LOQ} = \frac{10 \sigma}{S}$$

Where, σ - standard deviation

S- Slope of the calibration curve

Forced Degradation Study of irbesartan

Forced degradation study of irbesartan in bulk was carried out under the conditions of acid hydrolysis, alkaline hydrolysis, oxidation study.

Acid hydrolysis

To 10 ml of stock solution (200 $\mu\text{g/ml}$), 10 ml of 0.1 M HCl was added to obtain the working standard of drug. The solution was refluxed for 2 h at 70°C, the solution was neutralized. One ml of the resultant solution was diluted with mobile phase upto 10 ml. A 20 μl of resulting solution was injected into HPLC, under optimized chromatographic conditions.

Alkaline degradation

To 10 ml of stock solution (200 $\mu\text{g/ml}$), 10 ml of each 0.1 M NaOH was added to obtain the working standard of drug. The solution was refluxed for 2 h at 70°C, the solution was neutralized. One ml of the resultant solution was diluted with mobile phase upto 10 ml. A 20 μl of resulting solution was injected into HPLC, under optimized chromatographic conditions.

Oxidation studies

Hydrogen Peroxide (3 %) solution for oxidation studies was prepared in water. To 10 ml of stock solution (200 $\mu\text{g/ml}$), Ten ml of 3 % H_2O_2 Solution was added. The solution was refluxed for 2 h at 70°C. One ml of the resultant solution was diluted with mobile phase upto 10 ml. A 20 μl of resulting solutions was injected into HPLC, under optimized chromatographic conditions.

Formulation of dosage form

The inclusion complex of irbesartan: β -cyclodextrin (β -CD) with soluplus by freeze drying was prepared as per the method described by Kulkarni N. S. *et al.* [9]. The freeze dried inclusion complex equivalent to 75 mg of inclusion complex were passed through the sieve no 66 # respectively and weighed. The aerosil, lactose and talc were added to the inclusion complex 80 mg, 30 mg and 5 mg respectively. The powders were filled into hard gelatin capsule (size 3) shell and evaluated for drug content by developed HPLC method.

Stability Study

The prepared formulation was kept in the stability chamber maintained at 40°C and 75 % RH for period of six months. The samples were withdrawn at 0, 1, 2, 3 and 6 months and evaluated for drug content by using developed HPLC method.

Drug content during stability

Ten capsules were accurately weighed. Powder equivalent to 75 mg of drug was placed in a 100 ml volumetric flask, methanol was added and sonicated. The suspension was sonicated in a sonicator water bath for 15 min. These were then allowed to stand and volume was made with HPLC grade methanol. The suspension was filtered through whatman filter paper (0.22 μ) and 10 ml of the filtrate was transferred to volumetric flask of 100 ml. Volume was made up with mobile phase and solution was analyzed by HPLC.

Result and Discussion

Optimization of mobile phase was performed based on symmetric peak and peak area obtained. Different proportion of phase acetonitrile with phosphate buffer pH 3.5 were tried but the well resolved and symmetrical peak was obtained in proportion of 50:50 v/v ratio. The retention time (t_R) for irbesartan was found to be 1.50 min (Figure 1). The number of theoretical plate was found to be 11218, which suggests efficient performance of the column. Standard curve for irbesartan was obtained by plotting AUC verses concentration in $\mu\text{g/ml}$. Linear relationship between AUC and concentration was found in the range of 5 – 40 $\mu\text{g/ml}$ ($y = 101.9x + 195.3$, $r = 0.993$) where x – concentration of irbesartan and y – respective peak area.

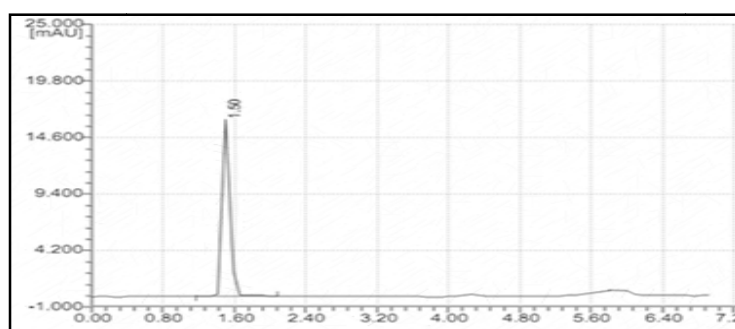


Figure 1: HPLC chromatogram of irbesartan in mobile phase

Method validation

Linearity

The linear relationship between AUC and concentration was found in the range of 5-40 µg/ml ($y = 101.9x + 195.3$, $r = 0.993$). It can be concluded that the method was found to be linear.

Precision

The intra and inter day study for the method was done and low value of RSD for irbesartan revealed that the proposed method is precise (table 1).

Table 1: Precision data of irbesartan (Intraday and Inter day)

Conc in µg/ml	AUC	AUC	AUC	Average	SD	RSD
20 (Intra day)	2103	2105	2101	2103	2.0	0.0009
20 after 1 day	2110	2105	2108	2107	2.51	0.001
20 after 2 day	2112	2107	2108	2109	2.64	0.001

Robustness

Influence of small change in chromatographic conditions such as change in flow rate, change in percentage of acetonitrile in mobile phase and column temperature

were studied to determine robustness of the method. The results showed that there is no significant change in retention time (% RSD < 2%) of irbesartan as summarized in table 2.

Table 2: Variation in parameter and its effect on retention time

Factor	Level	Retention Time (t_R)
Flow rate	0.5 ml/min	1.53
	1.5 ml/min	1.45
Change in Acetonitrile	48 %	1.48
	52 %	1.51

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD of irbesartan was found 0.05 µg/ml and LOQ of irbesartan was found 0.16µg/ml.

Forced Degradation of Irbesartan

The results of forced degradation studies are given in table 3 and figure 2 to 4. Significant degradation was observed under acidic and basic conditional and additional peaks of degradation products were observed. No additional peak was observed in case of oxidation studies but significant change in retention time has been observed.

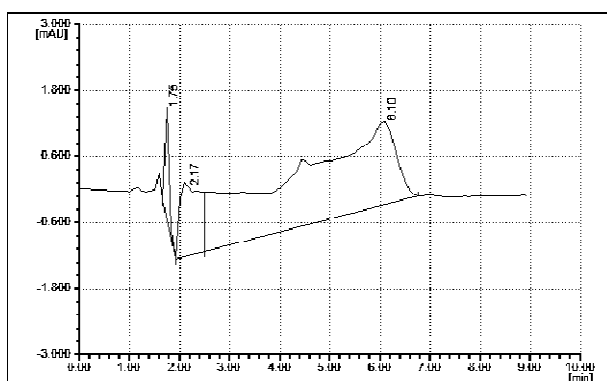


Figure 2: HPLC chromatogram of irbesartan degradation product in 0.1 M HCl

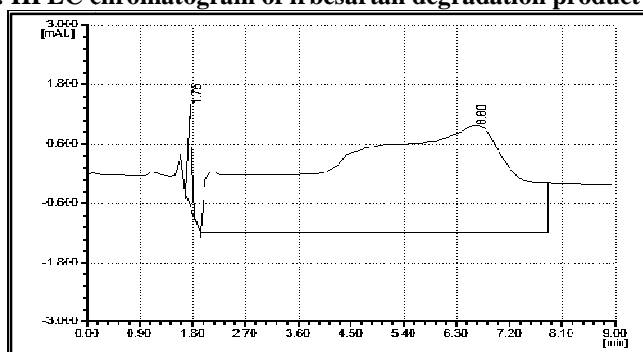


Figure 2: HPLC chromatogram of irbesartan degradation product in 0.1 M NaOH

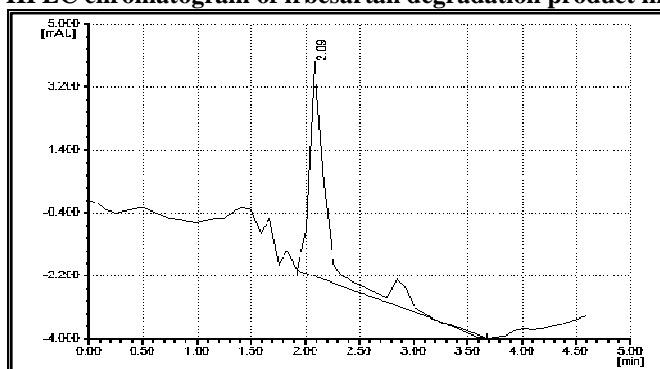


Figure 3: HPLC chromatogram of irbesartan degradation product in 3 % H₂O₂

Table 3: % degradation of irbesartan in acidic, basic and hydrolysis conditions

Stress Degradation	Amount of irbesartan Degraded	Amount of irbesartan Recovered	Retention time of degraded product
0.1 M HCL	67.26	32.73	1.75, 2.17, 6.10
0.1 M NaOH	67.13	32.86	1.75, 6.60
3 % H ₂ O ₂	77.94	22.05	2.09

Stability study of formulation

No significant change was observed in capsules properties with respect to appearance, drug content studies at condition of 40°C/75% RH for a period of six months. Absence of co eluting peak during the stability analysis along with the irbesartan peak indicates that the

developed HPLC method is specific for irbesartan and it is concluded that irbesartan does not undergo degradation at accelerated stability conditions when formulated as capsule dosage form containing irbesartan: β -cyclodextrin with soluplus. Hence according to ICH guidelines, the formulation was found to be stable.

Table 4: Drug content of Freeze dried irbesartan: β -CD: soluplus Capsule during stability

Sampling interval	Drug content
	40°C 75% RH
0 day	96.25 ± 0.34
1 months	97.29 ± 0.21
2 months	99.03 ± 0.43
3 months	96.29 ± 0.34
6 months	98.23 ± 0.65

Conclusion

Proposed HPLC method describes a new quantitation method for irbesartan. The method was found to be simple, sensitive

and accurate. The proposed method can be used for routine analysis of irbesartan in bulk and for its developed formulation as well as accelerated testing in bulk and pharmaceutical dosage forms.

Conflict of interest: We declare that we have no conflict of interest.

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