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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF IRBESARTAN IN BULK AND TABLET DOSAGE FORM

Yogeesh C. S.*, Sowmya H. G. and Jose Gnana Babu C.

*Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathinagara, K M Doddi, Maddur Taluk, Mandya District, Karnataka, India-571422.

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***Corresponding Author**
Yogeesh C. S.
Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathinagara, K M Doddi, Maddur Taluk, Mandya District, Karnataka, India-571422.

ABSTRACT

A simple, sensitive, precise and accurate HPLC method has been developed and validated for determination of Irbesartan in bulk drug and in pharmaceutical dosage form in the presence of degradation products. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, Phenomenex Gemini 5µ C18 (2) 100A (250 x 4.60mm, 5 µ) column. Hamilton syringe (705 NR, 50 µL) was used for injecting sample and standard solution. Data was compiled using Spinchrom software. Mobile phase consists of mixture of Phosphate buffer: Acetonitrile (70:30 v/v pH 6.0) at a flow rate of 1.0 ml /min. The linear regression analysis data for the calibration curve showed a good linear relationship with regression coefficient 0.9996. The detection was carried out at a wavelength of 224nm. **Results:** The linearity of the method were excellent over range 1-5µg/ml, the linear regression equation was Y=74061x + 1333.3. The Irbesartan was subjected to stress conditions of hydrolysis (acid, base), photolysis and thermal degradation. Degradation was observed for Irbesartan in acid, base, heat and UV. The degradation products were well resolved from the main peak. The percentage recovery of Irbesartan was from (98.0 to 102.0%.) in the pharmaceutical dosage form. **Conclusion:** The results demonstrated that the method would have a great value when applied in quality control and stability studies. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, specificity and robustness according to the ICH guidelines. The forced degradation studies prove the stability indicating power of the method.

KEYWORDS: Irbesartan, HPLC, phosphate buffer**:** Acetonitrile (70:30), ICH guidelines and forced degradation studies.

INTRODUCTION

Irbesartan is an angiotensin II receptor antagonist used mainly for treatment of Hypertension. It was developed by Sanofi research. It is a reasonable initial treatment for high blood pressure. It is an orally active nonpeptidetetrazole derivative and selectively inhibits angiotensin II receptor. Angiotensin II receptor type 1 antagonist has been widely used in treatment of disease like hypertension, heart failure, myocardial infarction and diabetic nephropathy.[1]

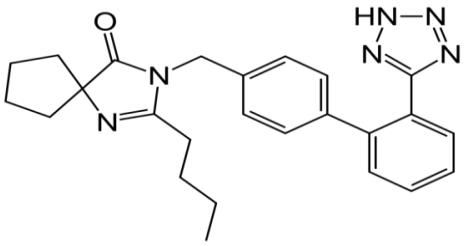

Figure1: Chemical structure of Irbesartan.

Irbesartan is chemically 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, It has molecular formula $C_{25}H_{28}N_6O$ and molecular weight is 428.53g/mol. Irbesartan is practically insoluble in water, sparingly soluble in methanol and slightly soluble in methylene chloride. The melting point of Irbesartan is 180-181˚C.[2]

Literature Survey revealed that the drug has been estimated by RP-HPLC[3-7], HPTLC[8-10] and UV-Spectrophotometric[11-17]; Liquid chromatographic method has been reported so far.

The present study describes a simple, precise and accurate analytical method for the estimation of Irbesartan in bulk and pharmaceutical dosage forms. The above method was developed and validated according to the ICH guidelines.

MATERIALS AND METHODS

Material and reagents: The Irbesartan was obtained as a gift sample from the pharmaceutical industry and tablet Irbesartan were obtained from Pharmacy store. Hydrochloric acid, sodium hydroxide pellets, potassium dihydrogen phosphates and Acetonitrile from were obtained Bharathi College of pharmacy, Bharathinagara, KM Doddi,

Maddur Taluk, Mandya District, India. All chemicals used are of HPLC grade. Distilled water was used throughout the experiment.

Instrumentation

Chromatographic separation was performed on a Shimadzu LC-20AT HPLC system comprising a variable wavelength programmable UV/ VIS detector SPD-20A (VP- series), Shimadzu LC-20AT (VP series) pump and Phenomenex Gemini 5µ C18 (2) 100A (250 x 4.60mm, 5 µ) column. Hamilton syringe (705 NR, 50 µL) was used for injecting sample and standard solution. Data was compiled using Spinchrom software.

CHROMATOGRAPHIC CONDITIONS

Table 1: HPLC method development parameters.

HPLC method development parameters	
Column	C18, 150 X 4.6 mm, 5µ
Flow rate	1.0 ml / min
Wavelength	224 nm
Column temperature	30°C
Injection volume	50 µl
Run time	10 minutes
Diluents	Mobile phase
Elution	Isocratic

PREPARATION OF SOLUTIONS

Mobile phase preparation

Buffer Preparation

Weigh accurately about 6.8 gm of KH_2PO_4 and dissolve with 500ml of HPLC Grade water than make up to 1000 ml with HPLC grade water then adjust the pH: 6.0 with ortho phosphoric acid or sodium hydroxide.

Mobile phase

Then add 70 volumes of buffer, 30volumes of Acetonitrile and sonicate for 15 min and filtered through a 0.45 µ membrane filter.

Preparation of stock and standard solutions

All solutions were prepared on a weight basis and solution concentrations were also measured on weight basis to avoid the use of an internal standard. Standard solution of Irbesartan was prepared by dissolving the drug in the diluents and diluting them to the desired concentration. Diluents were composed of Phosphate buffer and Acetonitrile in the ratio (70:

30 v/v). Approximately 100 mg of Irbesartan was accurately weighed, transferred in a 100 ml volumetric flask, add 30 ml of diluents and sonicate to dissolve and dilute to volume with diluents. Transfer 10 ml of standard stock solution into 100 ml volumetric flask and dilute to volume with diluents. And an appropriate concentration of sample was prepared at the time of analysis. 50µl of these solutions were injected in triplicate into HPLC system and the peak areas were recorded.

Preparation of sample solution

Crush to powder 20 tablets of Irbesartan weigh and transfer the tablet powder equal to 100 mg of Irbesartan into 100 ml volumetric flask add 30 ml of diluents, sonicate to dissolve for 10 minutes and dilute to volume with diluents. Further filter the solution through 0.45µ filter. And an appropriate concentration of sample (was prepared at the time of analysis. 50µl of these solutions were injected in triplicate into HPLC system and preceded as said for the standard respectively.

Assay

Dilute to 10 ml of standard stock solution, into 100 ml and make up to volume with diluents. Repeat the same procedure for three preparations.

System suitability requirements from stock and standard solutions

a) Tailing factor: NMT 2.0

b) Theoretical Plates: NLT 2000

Procedure for forced degradation study

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light and enables recommendation of storage conditions, retest periods, and shelf life to be established. The two main aspects of drug product that plays an important role in shelf-life determination are assay of the active drug and the degradation products generated during stability studies.

Acidic degradation

5 mg drug were dissolved in the diluents A&B. Forcibly degrade the sample by using 0.1 N HCl at room temperature. Collect 10 ml of the sample after 48 hours.

Alkaline degradation

5 mg drug were dissolved in the diluents A&B. Forcibly degrade the sample using 0.1N NaOH at room temperature. Collect 10 ml of the sample after 48 hours.

Thermal degradation

10 mg drug were forcibly degrading the sample exposed to heat under over 75˚C of temperature. The working solution was prepared using the diluents A&B. Collect the sample after 10th day.

Photo degradation

10 mg of drug is exposed to the short wavelength (224 nm) UV light for 48 h. Then the working solution was prepared by using diluents A&B. Forcibly degrades the sample under UV.

Method validation

The method is validated according to the ICH guidelines.

Specificity

Specificity of the HPLC method was checked for interference of impurities, degradants or excipients in the analysis of sample solution and was determined by injecting a volume of 20µl sample solution and the chromatogram was recorded. There is no interference of impurities, excipients on the peak of Irbesartan, indicating the high specificity of method.

Linearity and Range

Calibration curve was plotted for different concentrations of working standards prepared from standard drug solution of pure drug, shown in Fig-3 and showed linearity over a concentration range of 1-5 µg/ml shown in Table-2, along with regression parameters in Table-3. Each calibration was injected six times. The calibration curve was performed in six replicates.

Precision

The precision of the analytical method was determined by intraday and Interday precision. The sample solution was prepared as per the test method. In intraday precision, the same concentration of sample solution was injected 6 times in the same day and in Interday precision, injecting six solutions of same concentration for six different days in a week. The results of precision were tabulated in table-4. The average and standard deviation of mean

area were taken and %RSD was calculated and reported. %RSD values were within the limits and the method was found to be precise.

Accuracy

The accuracy of the method was determined by recovery studies by the determination of % mean recovery of the drug at three different levels (50%, 100% and 150%). At each level, three determinations were performed. A known amount of standard pure drug was added to preanalyzed tablet powder and the sample was then analyzed by developed method. Results of recovery studies were reported in table-5. The observed data were within the range, which indicates good recovery values.

Ruggedness

Ruggedness was determined between different analysts. The value of %RSD was found to be <2, showed ruggedness of developed analytical method. The values were shown in Table-6.

Limit of detection and Limit of quantitation

The LOD and LOQ of the present method were calculated based on standard deviation of the response and slope of linearity curve. LOD and LOQ values of Irbesartan were shown in Table-2.

Table: 2 Characteristic parameter of Irbesartan for the proposed RP- HPLC method.

Parameters	**RP-HPLC**
Calibration range (µg / ml)	1-5
Detection wavelength	224nm
Mobile phase (Buffer: Acetonitrile)	(70-30v/v, p^H 6.0)
Retention time	2.576
Regression equation (Y*)	Y=74061x + 1333.3
Slope (b)	74061
Intercept (a)	1333.3
Correlation coefficient(r^2)	0.999
Intraday Precision (% RSD**) Interday Precision (% RSD**)	1.1 1.0
Limit of detection (µg / ml)	0.105
Limit of quantitation (µg / ml)	0.324

*Y= b X + a, where X is the concentration of Irbesartan in µg / ml and Y is the absorbance at the respective λmax. **Average of six determinations.

Validation of analytical method

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical parameters.

Table: 3 Linearity results of Irbesartan by RP-HPLC method.

Concentration (µg / ml)	Avg. area
1	74461
2	153922
3	223383
4	294844
5	372305

**Average of five determinations.

Table: 4 precision results of Irbesartan by RP-HPLC method.

Sl. No.	INTRA-DAY STUDIES		INTER-DAY STUDIES	
	NAME	AREA	NAME	(Area)
1	Injection-1	224668	Injection-1	218099
2	Injection-2	222287	Injection-2	216656
3	Injection-3	223383	Injection-3	218571
4	Injection-4	227962	Injection-4	217903
5	Injection-5	222207	Injection-5	222735
Mean		224101.5	**Mean**	219012.7
Std. Dev		2378.1	**Std. Dev**	2103.5
%RSD.		1.1	**%RSD.**	1.0

**Average of five determinations.

Table: 5 Accuracy Results of Irbesartan by RP-HPLC method.

Drug name	Levels	Amount added in (µg/ml)	Amount recovery in (µg/ml)	%Recovery ± standard deviation	%RSD
Irbesartan	50%	1.5	1.50	100.2±212.6	0.2
	100%	3	3.0	100.0±1677.1	0.7
	150%	4.5	4.51	100.4±593.4	0.1

**Average of Six determinations.

Table 6: Results of Ruggedness of Irbesartan.

Parameter	Analyst I	Analyst II
Mean area**	224101.5	219012.7
Std. Dev	2378.1	2103.5
%RSD.	1.1	1.0

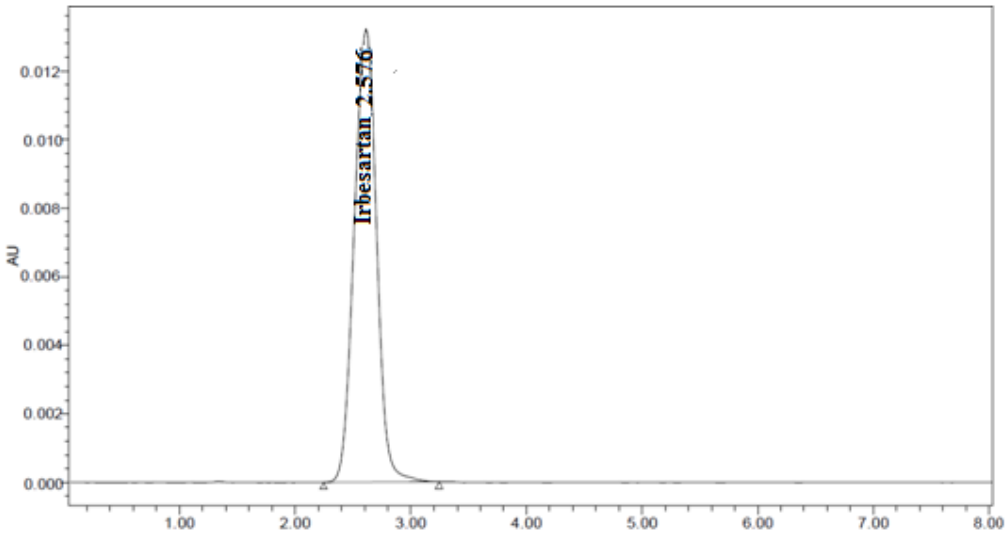

Fig 2: Chromatogram of Irbesartan.

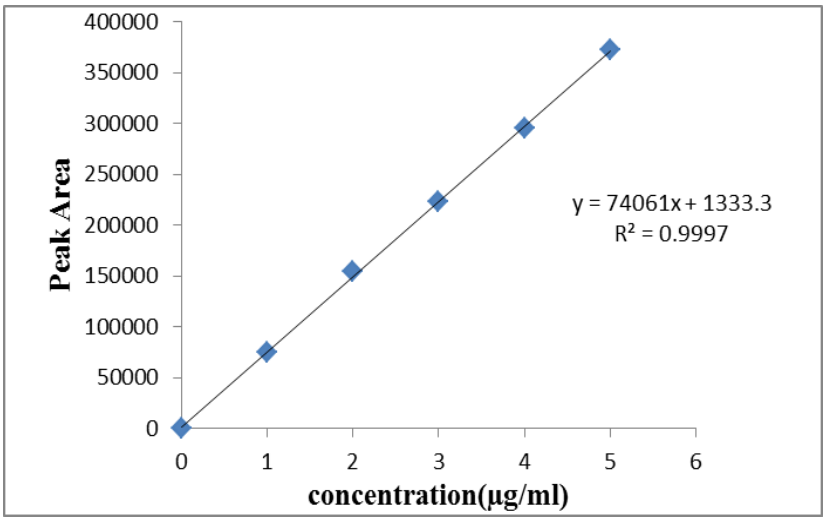

Figure 3: linearity of Irbesartan.

CONCLUSION

From the above it can be concluded that all validation parameters (precision, accuracy, linearity, LOD, LOQ and Ruggedness) met the predetermined acceptance criteria as mentioned in ICH guidelines. The developed HPLC method is simple, rapid, accurate, precise and shown good linearity. Hence it can be applied for routine analysis of Irbesartan in bulk and its dosage forms.

REFERENCES

1. http://www.wikipedia.com/irbesartan.
2. https://www.medicinenet.com

3. Raja B, Himasri P, Ramadevi B, RP-HPLC Method for the estimation of Irbesartan and Hydrochlorothiazide in pharmaceutical dosage form. Int Res J Pharm Appl Sci., 2012; 2(3): 29-38.
4. Bhukya R, Elizebeth Y, Dhanalaxmi K, Nagarjunareddy D. Method development and pharmaceutical dosage form. Int J Pharm., 2013; 3(3): 521-26.
5. Nagamani M, Sahoo SK, Kavitha D, Mahapatra L. Validated RP-HPLC Method for simultaneous estimation of Irbesartan and Hydrochlorothiazide in tablet dosage form. Int J Pharm Res Health Sci., 2017; 5(5): 1419-23.
6. Dagariya RK, Jat RK. Method development and validation of Irbesartan, Chlorthalidone and Clinidipine in their combined tablet dosage form by high performance liquid chromatography. J Drug Del Ther., 2017; 7(4): 88-96.
7. Kalaisalvi P, Lalitha KG. Development and validation of RP-HPLC method for the simultaneous estimation of Chlorthalidone and Irbesartan in pharmaceutical dosage form. Pharmacophore, 2014; 5(2): 279-86.
8. Rosangluaia SP, Malarkodi V. Validated HPTLC method for simultaneous estimation of Irbesartan and Hydrochlorthiazide in a tablet dosage form. Der Pharma Chemica, 2011; 3(5): 310-7.
9. Khodke AS, Potale LV, Bothara KG, Damle MC. A validated stability indicating HPTLC method for simultaneous estimation of Irbesartan and Hydrochlorothiazide. Pharmaceutical methods., Oct 1, 2010; 1(1): 39-43.
10. Magesh AR, Dhanaraju MD. Method development and validation for the simultaneous estimation of Irbesartan and Hydrochlorothiazide in tablet formulation by HPTLC. Der pharma chemical, 2016; 8(20): 13-19.
11. Sridharan D, Thenmozhi A, Rajamanickam V, Sundaranandavalli S, Palanikumar B. Simultaneous estimation of Irbesartan and Hydrochlorothiazide in combined pharmaceutical dosage form by UV spectroscopy using multi component mode of analysis. Int J Chem Tech Res., 2010; 2(2): 876-9.
12. Piusha Shakya, Pushpendra Kumar Jain, Shrivastava, Asmita Gajbhiye. Simultaneous estimation of Irbesartan and Hydrochlorothiazide by UV spectroscopy. Int J Pharm Pharm Sci., 2015; 7(6): 389-91.
13. Lakshmi Banjare, Jay Kumar Chandra, Prabhat Patel. Method development and validation for estimation of Irbesartan in bulk drug and pharmaceutical dosage. J of Drug Delivery & Therapeuticals, 2013; 3(6): 87-90.

14. Syahputra Hafid, Muchisyam Masfria. Determination of simultaneous Irbesartan and Hydrochlorothiazide by Ultraviolet spectrophotometry with dual wavelength method. Asian J Pharm Res & Development, 2019; 7(3): 01-04.
15. Todkari VB, Mahale MV, Kane SR, Mohite SK, Magdum CS. Development and validation of simultaneous Spectrophotometric estimation of Irbesartan and Hydrochlorothiazide in tablet dosage form. J Pharm Res., 2012; 5(4): 2335-37.
16. Nissankararao S, Anikumar A, Bhimavarapu RD, Prasanna KV, Estimation of Irbesartan in bulk and dosage form by new simple UV spectrophotometry using hydrotropic technique. Pharma Analytica Acta, 2013; 4(8): 01-04.
17. Kumar JS, Annapurna MM. New spectrophometric methods for the simultaneous determination of Irbesartan and Hydrochlorothiazide in combined dosage forms. Pharm Methods, 2015; 6(3): 120-25.
18. ICH, Q2 (R1) Validation of analytical procedure: text and methodology, 2005.