Method Development and Validation of Irbesartan by RP-HPLC Method

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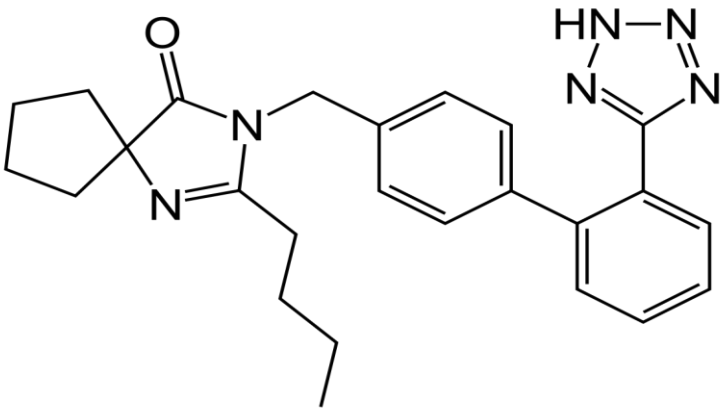
ABSTRACT

This study includes development of RP-HPLC method for estimation of Irbesartan. The developed method was validated as per ICH guidelines in terms of specificity, accuracy, linearity, LOD, LOQ, ruggedness and robustness. The inter-day and intra-day precision results were good enough to indicate that the proposed method was precise and reproducible. The assay experiment showed that the contents of Irbesartan estimated in the tablet dosage were free from interference of excipients, which indicate that developed method was specific. Recovery of standard added drugs was found to be 97.2-99.9% for Irbesartan indicating that the proposed method was accurate. A good linear relationship was observed for standard drug of Irbesartan and tablet form of Irbesartan in the concentration ranges of 60 – 100 µ/ml . The correlation coefficient for Irbesartan was found to be 0.998 µ/ml. After performing analysis by different analyst; it was found that the RP-HPLC method for the determination of Irbesartan was found to be rugged. % RSD for robustness was well within the limits ensuring that the proposed method was robust. The LOD was 0.710 µ/ml for standard drug of Irbesartan. For standard drug of Irbesartan the LOQ were found to be 0.116 µ/ml This demonstrated that the developed RP-HPLC method was simple, linear, precise, accurate, robust and rugged, could be conveniently adopted for the routine quality control analysis of Irbesartan from it's pharmaceutical dosage form and bulk drug.

KEYWORDS - Irbesartan, RP-HPLC.

INTRODUCTION

Irbesartan is used alone or in combination with other medication to treat high blood pressure. It also used to treat kidney disease caused by diabetes in patients with type 2 diabetes (condition in which the body does not use insulin normally and therefore cannot control the amount of sugar in the blood) and high blood pressure.(1) Irbesartan is in a class of medication called angiotensin II receptor antagonists. It works by blocking the action of certain natural substances that tighten the blood vessels, allowing the blood to flow more smoothly and the heart to pump more efficiently.(2)



Irbesartan is a nonpeptide tetrazole derivative and an angiotrnsin II antagonist that selectively block the binding of angiotensin II to the AT_1 receptor. In the rennin angiotensin system, aniotensin I is converted by angiotensin – converting enzyme (ACE) to form angiotensin II.[3-9]

MATERIAL AND METHOD [10,11]

List of Instruments

Equipment	Company
MaXia 220lectronic balance	Shinko Denshi Co Ltd, Japan
UV 150-02, Visible double beam spectrophotometer	Shimadzu corporation, japan
Digital pH Meter	Global, Ltd. Model No. – PGB 100
sonicattor	Wenser
HPLC Binary Gradient System Model No. - 3000 series HPLC pump – P – 3000- M Reciprocating Column- Cosmosil C-18 (4.6 ID × 250 mm , Particle size 5µ) Detector – UV 3000	Analytical Technoligies Ltd
Analytical Balance Model No. – PGB 100	Wenser

Table 1: List of apparatus/ instruments used.

List of Chemicals

Sr. No.	Reagents and Chemicals	Make	Details
1.	Water	In House Production	HPLC grade
2.	Methanol	Merck	HPLC grade

Table 2: List of chemical used.

Sr. No.	Name	Specification	Manufacturer/ Supplier
1.	Irbesartan	Working standard	Macleods Pharmaceutical Ltd Sarigram Gujarat

Table 3: List of API used.

Preparation of mobile phase

Mixed a HPLC grade Methanol : Water with pH 3.0 (80:20) in volumetric flask. Filter through 0.45µ filter under vaccum filtration.

Diluent preparation: Use mobile phase as diluent.

Standard Stock Solution:

Procedure:

Accurately weighed quantity of Irbesartan 10 mg individually dissolved in 10 ml volumetric flask using mobile phase and solution was sonicated for 20 minutes and volume is make up to the mark to get 1000 µg/ml and filtered through 0.45µm membrane filter. 1ml from each solution taken and dissolved in 10ml volumetric flask seperately using mobile phase to get 100 µg/ml.

Preparations of working standard solution:

Procedure:

From the standard stock solution respectively Irbesartan solution taken and added in 10 ml volumetric flask and diluted up to the mark with mobile phase.

Preparation of Sample solution:

Procedure:

20 tablets were weighed and powdered, tablets powder equivalent to 10 mg of Irbesartan was transferred 100 ml volumetric flask, sufficient amount of mobile phase was added and dissolved by 20minutes ultrasonication. Then made the volume up to the mark with the mobile phase and filtered with 0.45µ filter paper. Pipette out respectively solution from above solution and diluted to 10 ml mobile phase and use for sample injection.

Selection of analytical wavelength:

Accurately weighed quantity of Irbesartan 10 mg dissolved in 100 ml volumetric flask using methanol and volume is make up to the mark to get 100 µg/ml. From this solution respectively solution was taken and added in 10 ml volumetric flask and diluted up to the mark using methanol. Solution was scanned using UV-Visible Spectrophotometer in the spectrum mode between the wavelength ranges of 400 nm to 200 nm. The wavelength selected was 209 nm.

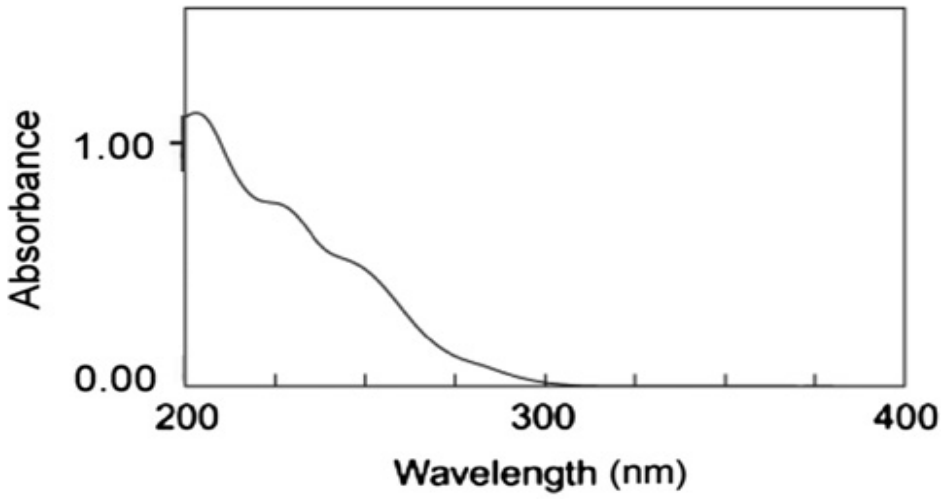


Fig. 1. Wavelength of Irbesartan

Optimized chromatographic condition:-

In the present study the validation of Irbesartan was achieved by using column Cosmosil C18, (250×4.6mm,5µ) with mobile phase consisting of mixture of methanol and Water (pH 3.0) in the ratio of 80:20 at a flow rate 1.0 ml/min with uv detection wavelength of 228nm at ambient temperature. The retention time for Irbesartan 3.257 min respectively.

RESULT AND DISCUSSION :

System Suitability:

HPLC system was optimized as per the chromatographic conditions. 20 µl of standard solutions of drugs were injected in triplicate into the chromatographic system. The chromatogram were recorded and measure the response for the major peak. system suitability parameter such as retention time, theoretical plate and asymmetry factor. then the %RSD of all parameter were calculated.

System suitability parameters	Irbesartan
Retention time	3.257 min
Theoretical plate no.	9234
Tailing factor	1.23
Resolution	0.0

Table 4. System suitability parameters for Irbesartan

Assay of the Developed RP-HPLC Method:

Sr. No.	Concentration (µg/ml)	Area of Standard	Area of Sample	% Assay(w/v)
1	10	2327476	2129410	91.490%

Table 5 Assay of the Developed RP-HPLC Method

Validation of the Developed Method: A. Linearity:

Linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range.

The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration. Percentage curve fittings are calculated.

Conc.of Irbesartan Standard (µg/ml)	Area	Conc.of Irbesartan Sample (µg/ml)	Area
60	1874532	60	1612912
70	2159314	70	2048572
80	2535040	80	2438745
90	2910603	90	2735040
100	3210538	100	3015112

Table No. 6. Data of Linearity for Irbesartan

From the linearity study of Irbesartan was found to be linear in the concentration ranges from 60-100 µg/ml. Correlation coefficient value(R2)were found to be Irbesartan respectively.

B. Accuracy (%recovery):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte.

The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay.

Drug	Conc. (µg/ml)	Amount added (mg)	Area	Amount recovered (mg)	% Recovery	SD and %RSD
Std drug of Irbesartan	60	10	2639654	9.95	99.5	0.91 & 0.92
	80	10	2867513	9.85	98.5	
	100	10	3497122	10.12	100.1	
Tablet sample of Irbesartan	60	10	2113076	9.88	98.8	1.12 & 1.14
	80	10	2009078	9.77	97.7	
	100	10	1822153	9.96	99.6	

Table No. 7 Accuracy result of Irbesartan

From the results shown in the accuracy table it was found that recovery value of pure and sample drugs were between 98.0% to 102%. Mean recovery was found 98.7% for Irbesartan standard and 99.36% for Sample.

C. Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Precision of the method was determined by Repeatability (intraday) and Intermediate Precision (inter-day) studies. Precision study was carried out by injecting a sample into HPLC without changing the assay procedure and the result are shown the %RSD is less than 2% for Meclizine hydrochloride and Caffeine. The low RSD value indicate that the method was precise.

i. Repeatability (intraday):

This study was performed with a minimum of three replicate measurement of sample solution at morning and evening in a same day.

Drug	Area	Amount added (mg)	Amount recovered (mg)	% Recovery	SD and %RSD
Std drug of Irbesartan	2329410	10	9.94	99.4	0.132 & 0.052
	2327221	10	9.92	99.2	
	2327238	10	9.91	99.1	

Tablet sample of Irbesartan	2100556	10	9.88	99.5	0.106 & 0.125
	2095046	10	9.98	99.8	
	1991893	10	9.96	99.6	

Table No. 8 Repeatability (intra-day) result of Irbesartan

The relative standard deviation values for repeatability precision was found less than 2%. %RSD of repeatability was 0.052% for standard and 0.125% for sample.

ii. Intermediate precision (inter-day):

Intermediate precision was performed by injecting the sample solution in to HPLC at two different day.

Drug	**Area**	**Amount added (mg)**	**Amount recovered (mg)**	**% Recovery**	**SD and %RSD**
Std drug of Irbesartan	2335654	10	9.98	99.8	0.387 & 0.154
	2328949	10	9.88	98.8	
	2329952	10	9.96	99.6	
Std drug of Irbesartan	2113070	10	10.1	100	0.256 & 0.220
	2091289	10	9.95	99.5	
	2197590	10	9.97	99.7	

Table No. 9 inter-day result of Irbesartan

The relative standard deviation values for intermediate precision was found less than 2%. %RSD of intermediate precision was 0.154% for standard and 0.220% for sample.

CONCLUSION :

The developed RP-HPLC method offers several advantages such as rapidity, usage of simple mobile phase and easy sample preparation steps. From the present study, it can be concluded that the proposed method is simple, specific, sensitive, precise, accurate and reproducible. Results of validation parameters demonstrated that the analytical procedure is suitable or appropriate for its intended purpose. Further, improved sensitivity makes it and reliable specific for its intended use. Hence, this method can be applied for the analysis of pharmaceutical dosage forms and pure drug.

ACKNOWLEDGEMENT :

The authors express their gratitude to the Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Vilad Ghat, Ahmednagar for providing all the facilities and Macleods Pharmaceutical Ltd Sarigram Gujarat for providing me the gift samples of Irbesartan.

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