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RP-HPLC-DAD METHOD FOR DETERMINATION OF IRBESARTAN IN BULK AND TABLETS EXPOSED TO FORCED CONDITIONS

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ABSTRACT

A simple, selective, precise and stability indicating RP-High Performance Liquid Chromatographic (HPLC) method of analysis of Irbesartan in pure and pharmaceutical dosage form was developed and validated. The chromatographic conditions comprised of a reversed phase C₁₈ column (4.6 x 150mm, 3.5 µm, Make: XTerra), with a mobile phase composed of Methanol: Phosphate buffer (50:50, Adjusted the pH to 3.0 with ortho Phosphoric acid).Flow rate was 1.0 mL / min. Detection was carried out at 209 nm. The retention time of Irbesartan was 4.11 min. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range 5-25 µg/ml. The values of correlation coefficient, slope and intercept were, 0.999, 8.3 and -0.86, respectively. The method was successfully validated in accordance to ICH guidelines acceptance criteria for specificity, linearity, precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, peroxide and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

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Key Words

Irbesartan, RP-HPLC,
Degradation studies.

INTRODUCTION

Irbesartan is chemically described as 2-butyl-3-[p-(o-1H tetrazol-5-ylphenyl) benzyl]-1, 3-diazaspiro [4.4] non-1-en-4-one. Its empirical formula is $C_{25}H_{28}N_6O$, its molecular weight is 428.5. A thorough literature survey revealed that the reported analytical procedures describing a stability indicating HPLC method for Irbesartan were more economical.

OBJECTIVE

The Objective of this study was to develop the method with less economical, precise, simple and sensitive and determination of Irbesartan in the presence of its degradation products. Here direct use of the mobile phase as diluent for formulations in quantitative analysis minimizes errors that occur during tedious extraction procedures. From the best of our knowledge via literature search, this is the first known RP-HPLC method that can separate all the related compounds of Irbesartan from each other and from Irbesartan with less economical and is therefore suitable to conduct stability studies of Irbesartan.

EXPERIMENTAL SECTION

MATERIALS

Irbesartan was supplied by SMS Pharmaceuticals Limited and Product Name: Irovel (150mg). Methanol (HPLC grade) purchased from Rankem Ltd., New Delhi, India. High purity water was prepared by using Millipore Milli-Q plus water purification system.

INSTRUMENT

The HPLC used was WATERS HPLC with photodiode array detector and Empower software. The column used was XTerra® RP 8, 4.6 x 150mm, 3.5 μ . Thermal Stability studies were performed in a dry air oven (Thermo labs, India).

METHODOLOGY

CHROMATOGRAPHIC CONDITIONS

Chromatographic separation was achieved at ambient temperature on a reversed phase column. The mobile phase consist of Methanol-Phosphate buffer solution (50:50v/v) at a flow rate of 1.0 mL/min. Monobasic potassium phosphate solution was prepared by dissolving 7 gms KH_2PO_4 in 1000ml double distilled

water. Final pH of the mobile phase was adjusted to 3.5 with orthophosphoric acid. The mobile phase so prepared was filtered through 0.22 μ m nylon membrane filter and degassed by sonication. Detection was carried out at 209 nm. The injection volume was 20 μ L for assay and degradation level.

STANDARD PREPARATION

Accurately weigh and transfer 20mg of Irbesartan into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). A series of standard solutions in the concentration range of 5, 10, 15, 20, 25 μ g/mL were prepared followed by a suitable dilution of stock solution with the mobile phase.

SAMPLE PREPARATION

Weigh 20 Irbesartan Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 20 mg of Irbesartan into a 10 mL volumetric flask. Add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 μ m filter.

METHOD VALIDATION

LINEARITY

The linearity response was determined by preparing and injecting solutions with concentrations of about 5, 10, 15, 20, 25 μ g/ml of Irbesartan.

PRECISION

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same standard concentration (30 μ g/mL for standard application). Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marketed sample (30 μ g/mL for sample application). It showed very low % relative standard deviation (% RSD) of peak area of Irbesartan.

ACCURACY

Accuracy (Recovery) study was performed by spiking 50, 100 and 150% of Irbesartan working standard to a preanalysed sample. The accuracy of the analytical method was established in triplicate across its range according to the assay procedure.

RUGGEDNESS AND ROBUSTNESS

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. Method Robustness was carried by a deliberate change in the Flow rate and change in Mobile Phase composition, was made to evaluate the impact on the method.

FORCED DEGRADATION STUDIES**ACID DEGRADATION**

Accurately weighed and transferred 10mg of Irbesartan Working standard into a 100mL volumetric flask. To it 10mL of 0.1N HCl was added and sonicated for 5minutes. Refluxed under heat at 60 degrees in a heating mantle for 2 hours. The sample solution was neutralized using 0.1N NaOH and diluted up to the mark with Mobile phase. Further pipette 1 mL of the above solution into a 10mL volumetric flask and diluted up to the mark with Mobile phase. Mixed well and filtered through 0.45µm filter and injected into HPLC system.

BASE DEGRADATION

Accurately weighed and transferred 10mg of Irbesartan Working standard into a 100mL volumetric flask. To it 10mL of 0.1N NaOH was added and sonicated for 5minutes. Refluxed under heat at 60 degrees in a heating mantle for 2 hours. The sample solution was neutralized using 0.1N HCl and diluted up to the mark with Mobile phase. Further pipette 1 mL of the above solution into a 10mL volumetric flask and diluted up to the mark with Mobile phase. Mixed well and filter through 0.45µm filter and injected into HPLC system.

THERMAL DEGRADATION

Accurately weighed and transferred 10mg of Irbesartan Working standard into a 100mL volumetric flask and oven under heat at 105 degrees for 12 hours. Further pipette 1 mL of the solution into a 10mL volumetric flask and diluted up to the mark with Mobile phase. Mixed well and filter through 0.45µm filter and injected into HPLC system.

PEROXIDE DEGRADATION

Accurately weighed and transferred 10mg of Irbesartan Working standard into a 100mL volumetric flask. To it 10mL of 3% Hydrogen Peroxide (H₂O₂) and sonicated for 5minutes and Refluxed under heat at 60 degrees in a

heating mantle for 2 hours. Further pipette 1mL of the solution into a 10mL volumetric flask and diluted up to the mark with Mobile phase. Mixed well and filter through 0.45µm filter and injected into HPLC system.

RESULTS**METHOD DEVELOPMENT**

The chromatographic conditions were optimized with a view to develop a stability- indicating assay method. Two different columns were tried as under chromatographic conditions namely, XTerra® RP C₁₈, 4.6 x 150mm, 3.5 µ. (water, Ireland) and Luna C₁₈ (Octylsilane), 150 x 4.6 mm, 3.5 µ (Phenomenax, USA). XTerra® RP C₁₈ column had given a good peak shape with response at affordable retention time than Luna C₁₈. The chromatographic conditions finally comprised of Methanol: Potassiumdihydrogen phosphate solution (50:50 v/v) at a flow rate of 1.0ml/min using XTerra® RP C₁₈ column at 209 nm.

VALIDATION OF THE METHOD**LINEARITY**

These results indicate that the response is linear over the range of 5, 10, 15, 20, 25 µg/mL of Irbesartan. The results were shown in **Table: i**.

Table: i Regression characteristics of the proposed RP-HPLC method

S.NO.	Regression characteristics	Irbesartan
1.	Range (µg/mL)	5-25
2.	Detection wave length(λ _{max})	209
3.	Mean 'R ² ' value	0.999
4.	Slope (m)	8.3
5.	Intercept (c)	-0.86
6.	Run time(min)	6
7.	Retention time(min)	4.11
8.	Theoretical plates(N)	4337
9.	Tailing factor	1.17

Table: ii Ruggedness and robustness of Irbesartan

Parameters	Normal (Original)	Changed conditions
Column make	XTerra RP-C ₁₈ ; 4.6 x 150mm; 3.5 µ	Luna RP-C ₁₈ 150 x 4.6 mm; 3.5 µ
Flow rate	1.0	0.8
Mobile phase Composition	Buffer and Methanol (50:50v/v)	Buffer and Methanol (30:50v/v)
Analyst	Sujana.K	Bala souri.O
% assay of	99.25%	99.22%

Irbesartan

PRECISION

Method Precision was evaluated by injecting the standard solution of 30 µg/mL six times and %RSD was 0.33%. System precision (repeatability) was evaluated by performing six consecutive injections of the 30 µg/mL standard solution, giving a low R.S.D. value of 0.16% and no change in retention time of the drug. The Irbesartan contents were found in the tablet formulations using the proposed method. The low R.S.D. values indicate that the proposed method is precise.

RUGGEDNESS AND ROBUSTNESS OF THE METHOD

Method robustness and ruggedness was determined by analyzing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate and analyst. The deliberate aforementioned changes in parameters alter the result of Irbesartan 0.01% to method precision study, which is not a significant change. The robustness

Table: iii Recovery of Irbesartan

*Average of Three determinations

%Concentration (at specification Level)*	Area	Amount Added (mg)	Amount Found (mg)	% Recovery*
50%	679971	5.1	10.5	101.4%
100%	1314775	10.0	10.0	100%
150%	1874690	14.5	14.26	98.3%
Mean				99.9
± Standard deviation				1.55
% Relative standard deviation				1.5

ANALYSIS OF MARKETED FORMULATIONS

The drug content was found to be 99.22% with a % RSD of 0.87%. It was noted that no degradation of Irbesartan had occurred in the marketed formulation that was analyzed by this method. The low RSD value indicated the suitability of this method for routine analysis of Irbesartan in pharmaceutical dosage form.

and ruggedness of the method shows assay value less than $\pm 2.0\%$. **Table: ii** represent the ruggedness and robustness of the method.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

The S/N Ratio values of LOD and LOQ concentrations were found to be 2.90 and 10.2 respectively.

ACCURACY

The accuracy of the method was established by recovery studies. Results indicate that the individual recovery of Irbesartan ranges from 101.4% to 98.3% with mean recovery of 99.9 % and % relative standard deviation of 1.5%. The recovery of Irbesartan by proposed method is satisfactory as % relative standard deviation is not more than $\pm 2.0\%$ and mean recovery between 101.4% to 98.3%. **Table: iii** represent the accuracy of method.

STABILITY- INDICATING STUDIES

The chromatogram of no stress treatment sample (as control) showed no additional peak (**Figure: i**). The retention time (RT) of standard and sample were 4.12 min respectively

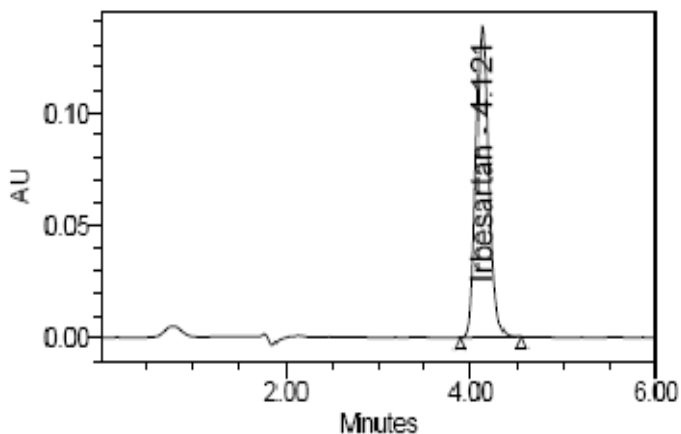


Fig i. The simple chromatogram of standard Irbesartan
 The chromatogram of acid degraded sample ,alkali degraded sample ,thermal degraded sample ,hydrogen peroxide degraded sample shows an additional peak at

different retention times (**Fig: ii,iii,iv&v**) and the values were shown in **Table: iv**.

Table: iv Stressed study data of Irbesartan

S. No	Condition	Time(hrs)	% assay of Irbesartan	Retention time of drug
1.	No stress treatment	-	99.6	4.12
2.	Acid	2	99.61	4.42
3.	Alkali	2	99.61	4.42
4.	H2O2	2	99.61	4.42
5.	Thermal	12	99.61	4.42

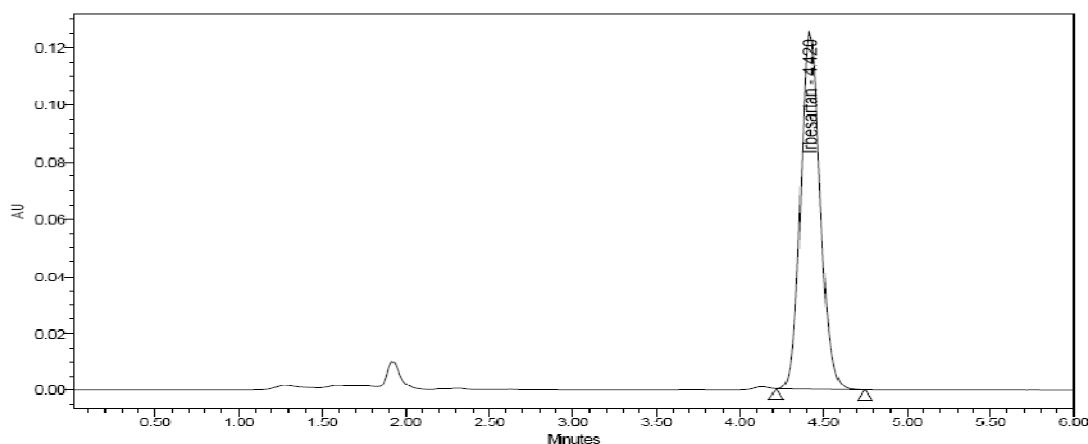


Fig ii. The simple chromatogram of Acid degraded sample.

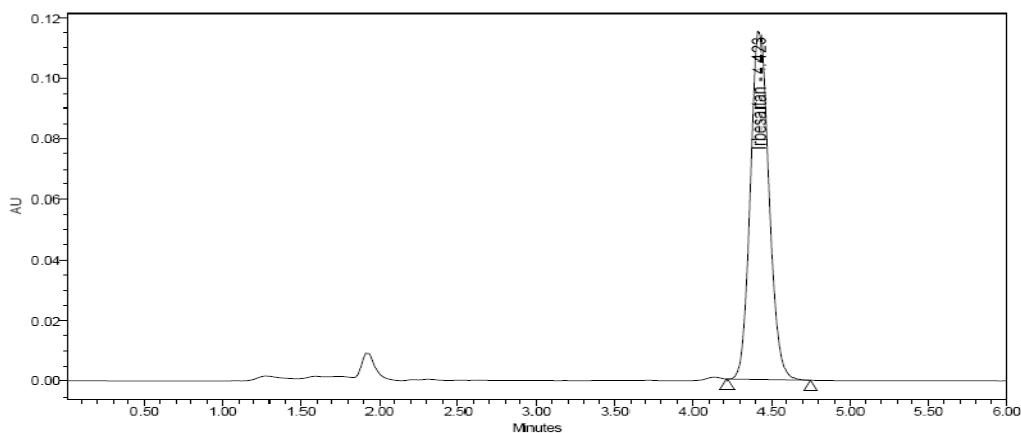


Fig iii. The simple chromatogram of Alkali degraded sample.

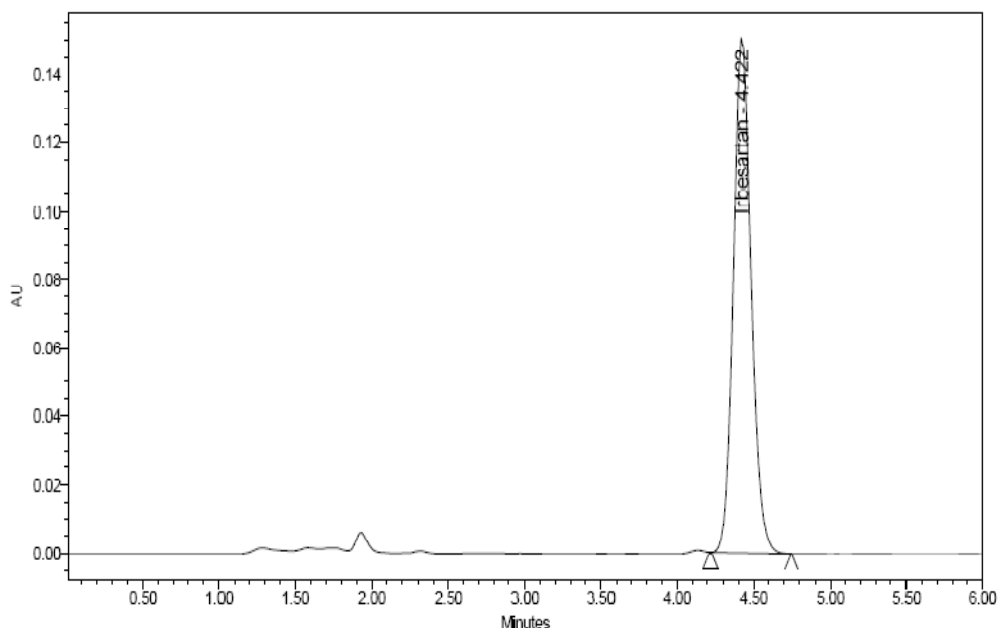


Fig iv. The simple chromatogram of Thermal degraded sample.

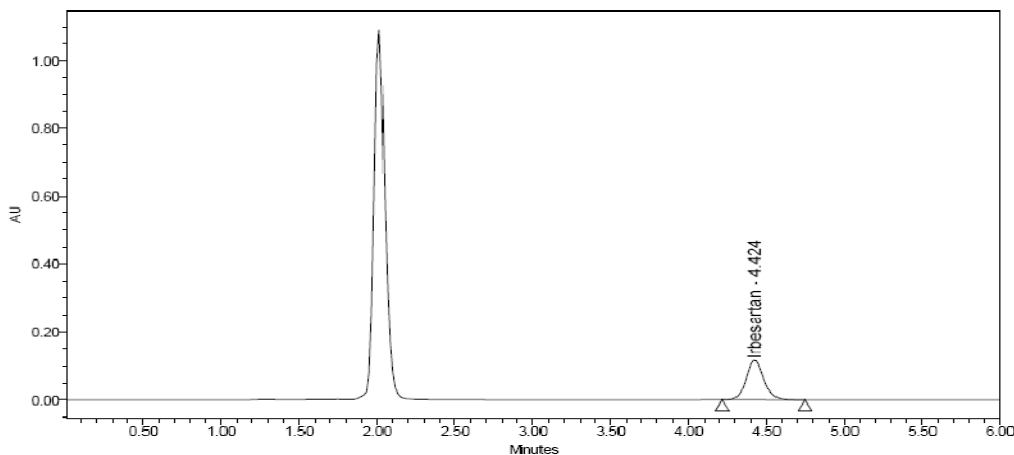


Fig v. The simple chromatogram of Hydrogen Peroxide degraded sample.

DETECTION OF RELATED IMPURITIES

The sample solution showed no additional peak other than principal peak. Hence, related impurities are not present in the market sample.

CONCLUSION

The developed HPLC technique is precise, specific, accurate and stability-indicating. Statistical analysis proves that the method is suitable for the analysis of Irbesartan as bulk drug and in pharmaceutical formulation without any interference from the excipients. This study is a typical example of a stability-

indicating assay, established following the recommendations of ICH guidelines. The method can be used to determine the purity of drug available from various sources by detecting any related impurities. The method has been found to be better than previously reported methods, because of use of a less economical and readily available mobile phase, lack of extraction procedures, no internal standard, and use of the same mobile phase for washing of the column. All these factors make this method suitable for quantification of Irbesartan in bulk drugs and in pharmaceutical dosage forms. It can therefore be concluded that use of the

method can save much time and money and it can be used in small laboratories with very high accuracy and a wide linear range.

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