Research Article

DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR ASSAY OF IRBESARTAN IN PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A novel stability-indicating gradient reverse phase-high performance liquid chromatographic (RP-HPLC) method was developed for the determination of Irbesartan in pure and pharmaceutical dosage form was developed and validated. The chromatographic conditions comprised of a reversed-phase C_{18} column (250 x 4.6 mm), 5 µ with a mobile phase consisting of a mixture of Acetonitrile: Buffer solution (0.03M KH_2PO_4) solution in the ratio (15:85v/v) and pH adjusted to 3. Flow rate was 1 mL / min. Detection was carried out at 275 nm. The retention time of Irbesartan was 5.54 min. Irbesartan was subjected to acid and alkali hydrolysis, oxidation, photochemical degradation and thermal degradation. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range 2-12µg/ml. The value of correlation coefficient, slope and intercept were, 0.9998, 99.839 and 0.3321, respectively. The method was validated for precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, photochemical 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.

Keywords: Irbesartan, chromatography, stability indicating, degradation.

INTRODUCTION

Irbesartan, chemically it is 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 is an angiotensin II receptor antagonist. It has also been tested for use in the treatment of high blood pressure (hypertension) [1, 2, 3] (**Figure: 1**). Literature survey reveals that LC, HPTLC, HPLC for determination of content uniformity and simultaneous estimation of Irbesartan is reported[4, 5, 6], but there is no stability indicating high-performance liquid chromatography (HPLC) method for the determination of Irbesartan from its tablets, as its Pharmaceutical dosage form.

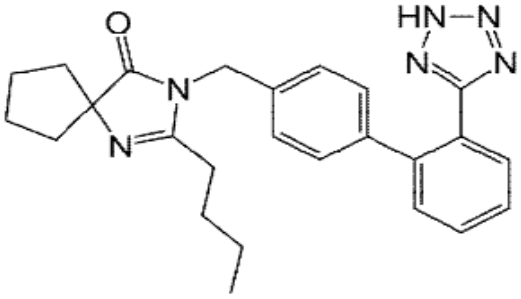


Figure 1: Chemical structure for irbesartan

The International Conference on Harmonization (ICH) guideline entitled 'Stability Testing of New Drug Substances and Products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidation is one of the required tests[7, 8] (ICH, 1993, 1996). The hydrolytic and the photolytic stability are also required. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products. A very viable alternative for stability-indicating analysis of Irbesartan is HPLC.

The aim of the present work was to develop an accurate, specific, reproducible, and stability indicating method for the determination of Irbesartan in the presence of its degradation products and related impurities as per ICH guideline.

MATERIALS AND METHODS

Materials

Irbesartan was supplied by Sun Pharma and tablets (Label Claim: 150 mg per tablet, Product Name: APROVEL and Manufacturer: Sanofi Aventis) was procured from the market. Acetonitrile, Potassium dihydrogen phosphate AR Grade, Orthophosphoric acid LR Grade were purchased from RFCL Ltd., New Delhi, India and Water HPLC Grade were used. High purity water was prepared by using Millipore Milli-Q plus water purification system.

Instrument used

The HPLC used was a shimazdu RP- HPLC LC-20AT series with SPD-20A UV photodiode array detector and Borwin software, Japan was used for all the experiments. The column used was XTerra® RP18, 250 x 4.6 mm, 5 µ (water Ireland) and Luna C8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Thermal Stability studies were

performed in a dry air oven (Thermo labs, India). Micrositer syringer- 50 µL (Hamilton Company, USA).

Methodology

i. Chromatographic conditions

Chromatographic separation was achieved at ambient temperature on a reversed phase column using a mobile-phase consisting of a mixture of Acetonitrile: Buffer solution (Dissolve 0.03 M potassium dihydrogen phosphate in water. Adjust pH of solution to 3.0 ± 0.05 with orthophosphoric acid) in the ratio (15:85 v/v). The mobile phase so prepared was filtered through 0.22 µm nylon membrane filter and degassed by sonication. Flow rate of 1 mL / min was maintained. Detection was carried out at 275 nm. The injection volume was 20 µL for assay and degradation level.

ii. Standard preparation

100 mg of Irbesartan working standard was accurately weighed and transferred to a 100 mL volumetric flask. Solution was sonicated and diluted up to the mark with mobile phase.

iii. Sample preparation

20 tablets were weighed and finely powdered. Blend equivalent to 100 mg of Irbesartan was transferred to a 100 mL volumetric flask. About 60 mL of mobile phase was added and the solution was sonicated for 15 min and make up to the mark with mobile phase. The resulting solution was filtered through 0.22µm nylon membrane filter. The solution was mixed well and centrifuged at 2500 rpm for 10 min.

Method validation

i. Linearity

The linearity of response for Irbesartan assay method was determined by preparing and injecting solutions with concentrations of about 2, 4, 6, 8, 10 and 12µg/ml of Irbesartan.

ii. 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 (6 µg / mL for standard application). Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marketed sample (6 µg / mL for sample application). It showed very low % relative standard deviation (% RSD) of peak area of Irbesartan.

iii. Accuracy

Accuracy (Recovery) study was performed by spiking 30, 50 and 70% of Irbesartan working standard to a preanalysed sample. The preanalysed sample was weighed in such a way that final concentration is half or 50% of the sample preparation before spiking. The percentage sum level of preanalysed sample and spiked amount of drug should be 80, 100 and 120% of simulated dosages nominal or target concentration of sample preparation. The accuracy of the analytical method was established in duplicate across its range according to the assay procedure.

$$\% \text{ Recovery} = \frac{\%Amount\,\text{Recovered}}{\%SumLevel} \times 100$$

iv. 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, instrument and analyst. The robustness and ruggedness of the method was established as the % deviation from mean assay value obtain from precision study is less than ±2.0%.

iv. Analysis of marketed formulation

20 tablets were weighed and finely powdered. Transfer blend equivalent to 100 mg of Irbesartan to a 100 mL volumetric flask. Add about 60 mL of mobile phase and sonicate for 15 min and make up volume with mobile phase. Mix well and centrifuge the solution at 2500 RPM for 10 min. Dilute the solution up to the desired concentration and inject it into the HPLC system.

Forced degradation studies

i. Preparation of acid and based- Induced degradation product

Tablet powder equivalent to 100 mg of Irbesartan was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 1 N HCl was added and 5 mL of 1 N NaOH were added separately. The sample was heated on a boiling water bath for 60 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. The acidic forced degradation and the alkaline forced degradation was performed in dark in order to exclude the possible degradative effect of light. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

ii. Preparation of hydrogen peroxide - induced degradation product

Tablet powder equivalent to 100 mg of Irbesartan was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 3.0% H_2O_2 was added. The sample was heated on a boiling water bath for 60 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well, and injected into the HPLC system.

iii. Photodegradation and Thermal degradation product

Tablet powder equivalent to 100 mg of Irbesartan (previously kept in UV light for 24 hr) was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking and diluted up to the mark with mobile phase. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

Tablet powder equivalent to 100 mg of Irbesartan was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. The sample was heated on a boiling water bath for 60 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC. The specificity degradation study data for the determination of Irbesartan and its degradants in pharmaceutical dosage form is given in Table: 5 & 6. The no stress treatment sample (as control) has been evaluated relative to the standard concentration where as rest of the stressed condition samples (Figures: 2 to 8) is evaluated relative to the control sample with respect to the % assay and % degradation. The percentage degradation results are calculated by area normalization method.

RESULTS AND DISCUSSION

Method of 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® RP18, 250 x 4.6 mm, 5 µ (water, Ireland) and Luna C8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Luna C8 gave good peak shape but a lower retention. XTerra® RP18 column had given a good peak shape with response at affordable retention. The chromatographic conditions finally comprised of a mobile-phase in the ratio of Acetonitrile: Buffer solution (Dissolve 0.03 M potassium dihydrogen phosphate in water. Adjust pH of solution to 3.0 ± 0.05 with ortho-phosphoric acid) in the ratio (15:85 V/V) at a flow rate of 1 mL / min using XTerra® RP18 column; 250 x 4.6 mm; 5 µ (G. L. Sciences, Japan) at 275 nm.

Validation of the method

i. Linearity

These results indicate that the response is linear over the range of 2, 4, 6, 8, 10 and 12µg/ml of Irbesartan with coefficient of regression, R^2, value as 0.9998 the data were shown in Table: 1. The value of correlation coefficient, slope and intercept were 0.9998, 99.839 and 0.3321 respectively.

Table 1: Regression characteristics of the proposed HPLC method.

S. No	Drug	Irbesartan
1.	Range (µg/ml)	2-12 µg/ml
2.	Detection wave length (λ max)	275nm
3.	Mean 'R^2' value	0.9998
4.	Slope (m)	99.839
5.	Intercept (c)	0.3321
6.	Run time	10min
7.	Retention Time (min)	5.54
8.	Theoretical Plates (N)	3576
9.	Tailing Factor	1.034

ii. Precision

The %RSD for repeatability of sample preparation is 1.11%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than ± 2.0%. Table: 2 represent the precision of method.

Table 2: Method precision of Irbesartan.

Sample Preparation	% Assay Irbesartan	% Deviation From Mean Assay value Irbesartan
1	99.7	1.02
2	98.4	-0.28
3	100.3	1.62
4	97.6	-1.08
5	98.9	0.22
6	97.2	-1.48
Mean	98.68	
±SD	1.09	
%RSD	1.11	

iii. Ruggedness and robustness of the method

Method robustness and ruggedness was determined by analyzing the same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst. The deliberate aforementioned changes in parameters alter the result of Irbesartan 0.04% to method precision study, which is not a significant change. The robustness and ruggedness of the method is established as the % deviation from mean assay value obtain from precision study is less than ±2.0%. Table: 3 represent the ruggedness and robustness of the method.

Table 3: Ruggedness and Robustness of Irbesartan.

Parameter	Normal (Original)	Changed conditions
Column make	X Terra® RP18 column; 250 x 4.6 mm; 5 µ	Luna C_8 (Octylsilane), 250 x 4.6 mm; 5 µ
Flow rate	1 mL/min	1.2 mL/min
Mobile phase Composition	Acetonitrile: 0.03 M KH_2PO_4 (15:85 % v/v) pH adjusted to 3.0-4.5	Acetonitrile: 0.03 M KH_2PO_4 (20:80 % v/v) pH adjusted to 3.0-4.5
Pump	Jasco PU-2080 plus series	Shimazdu LC-20AT
Detector	Jasco UV-2075 plus series	Shimazdu UV-VIS detector
Analyst	Narasimha.V	Kalyan.B
% assay of Irbesartan	98.68%	98.62%
% deviation from mean assay value obtained in method precision studies for Irbesartan : 0.04%		

Table 4: Recovery (Accuracy) studies of the Irbesartan.

Sample preparation	% simulated dosage normal	% sum level	% amount recovered	% recovery	Mean% recovery
Preanalysed sample				99.78	
1	80	80.57	81.23	100.64	101.01
2	80	79.56	81.34	101.37	
1	100	100.34	101.52	100.71	100.76
2	100	101.54	101.65	100.81	
1	120	121.62	121.91	100.89	100.83
2	120	121.68	121.87	100.77	

Table 5: Stressed study data of Irbesartan.

S. No	Condition	% assay Irbesartan	Retention time of drug	% Degradation
1	No stress treatment	98.68	5.6	Nil
2	Acid	99.82	3.0, 3.88, 4.95	0.06
3	Alkali	99.43	3.21	0.04
4	H_20_2	98.45	2.94, 3.57, 4.65	0.07
5	UV	99.52	4.45	0.17
6	Thermal	99.04	4.2	0.03

Table 6: Summary of forced degradation results

S. No	Stress condition	Time	%Assay of active substance	Mass balance (%assay + % degradation products)	Remarks
1	Acid degradation (1 N HCl)	1 hr	99.82	99.9	No degradation products formed
2	Alkali degradation (1N NaOH)	1 hr	99.43	99.5	No degradation products formed
3	H_2O_2 degradation (3%)	1 hr	98.45	98.5	Mild degradation formed
4	UV degradation	24 hr	99.52	99.7	No degradation products formed
5	Thermal degradation (60 ^{0}C)	1 hr	99.04	99.1	Mild degradation formed

iv. Accuracy

The accuracy of the method was established by recovery studies. Results indicate that the mean% recovery of Irbesartan ranges from 100.76 % to 101.01. The recovery of Irbesartan by proposed method is satisfactory as % relative standard deviation is not more than ± 2.0% and mean recovery between 98.0 - 102.0%. Table: 4 represent the accuracy of method.

Stability- Indicating property

The chromatogram of no stress treatment of control and sample showed no additional peaks (Figure: 2 & 3), data were shown in table: 5. The retention time (RT) of standard and sample were 5.54 min and 5.6 min respectively. The chromatogram of acid degraded sample showed additional peaks at retention time (RT) of 3.00, 3.88 and 4.95 min, respectively (Figure: 4). The chromatogram of alkali degraded sample showed additional peaks at RT of 3.21 min, respectively (Figure: 5). The chromatogram of hydrogen peroxide degraded sample showed additional peaks at RT of 2.94, 3.57 and 4.65 min respectively (Figure: 6). The chromatogram of UV degraded sample showed additional peak at RT of 4.45 (Figure: 7). The chromatogram of thermal degraded sample showed additional peak at RT of 4.2 min (Figure: 8), results were shown in table: 6. Rest of the peaks, if any, were from its blank or placebo in each of these specified conditions. In each forced degradation samples where additional peaks were observed, the response of the drug was changing from the initial control sample. This indicates that the drug is susceptible to acid-base hydrolysis degradation, hydrogen peroxide degradation, UV degradation and thermal degradation. The lower RT of the degraded component indicated that they were more polar than the analyte itself.

Figure 2: The simple chromatogram of standard Irbesartan.

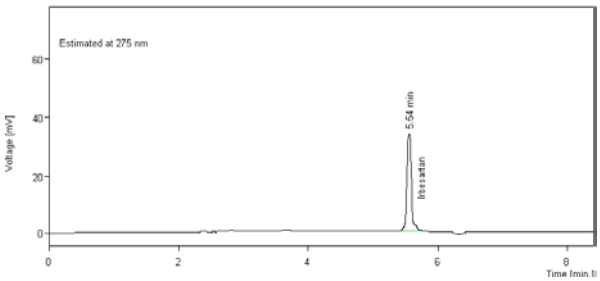


Figure 3: The simple chromatogram of test Irbesartan.

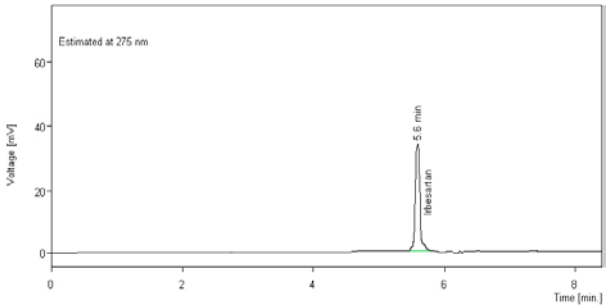


Figure 4: The simple chromatogram of acid degraded sample.

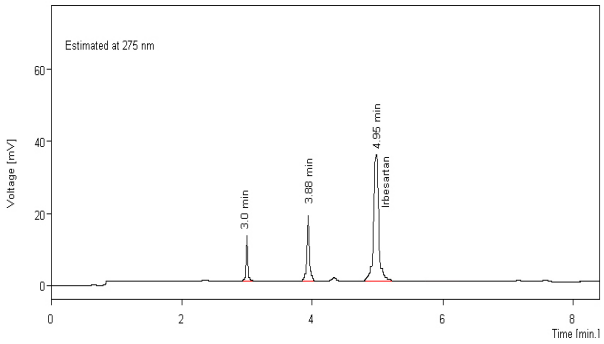


Figure 5: The simple chromatogram of alkali degraded sample.

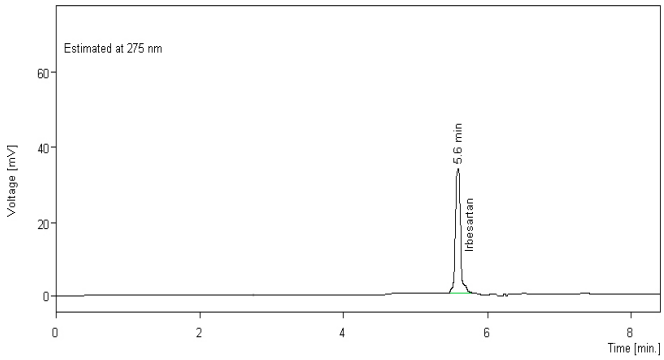


Figure 6: The simple chromatogram of Hydrogen Peroxide degraded sample.

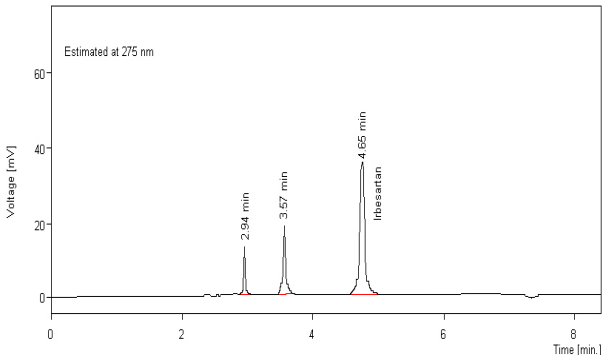


Figure 7: The simple chromatogram of UV degraded sample.

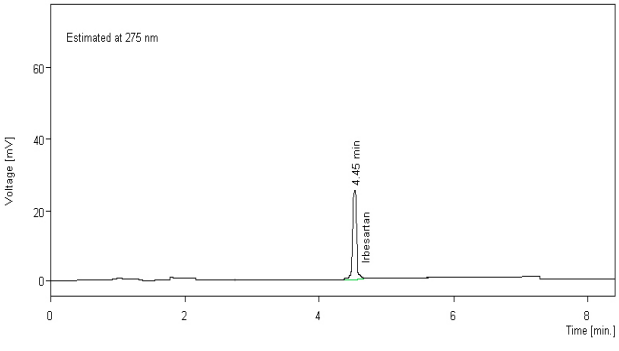
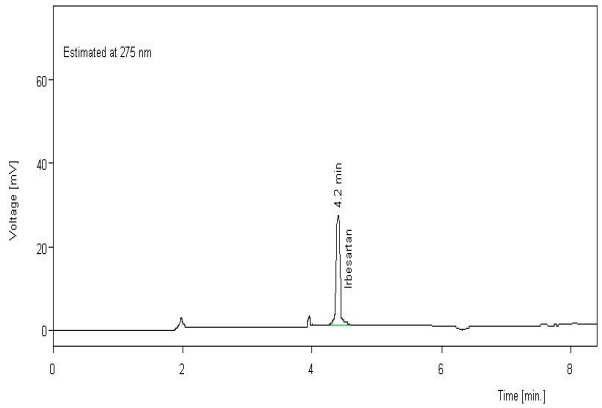


Figure 8: The simple chromatogram of Thermal degraded sample.



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