Research Article

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DEVELOPMENT AND VALIDATION OF REVERSE PHASE LIQUID CHROMATOGRAPHY METHOD FOR ESTIMATION OF IRBESARTAN IN BULK DRUG

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ABSTRACT:

A simple, precise and reversed phase liquid chromatographic (RP-LC) method was developed and validated for estimation of irbesartan in bulk drug. The separation was achieved on Acquity HSS T-3 1.8µ, (2.1 X 100) mm, Make: Waters, analytical column with mobile phase consisted of buffer (10mM Potassium dihydrogen phosphate with 0.05% triethylamine in water, adjust pH 3.0 with dilute phosphoric acid) : Acetonitrile (50:50v/v) at isocratic flow of 0.35ml/min with UV detection wavelength was at 205 nm and 3µl of sample volume was injected. The retention time of irbesartan was found to be 1.9 minute. The method was successfully validated in accordance to ICH guidelines for accuracy, precision, specificity, linearity. The linear regression analysis data for calibration plots showed good linear relationship in the concentration range 25-75µg/mL for irbesartan. The % Recovery/Accuracy was within the range between 98% and 102%. The percentage RSD for precision method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of irbesartan in bulk samples.

KEYWORDS:

Irbesartan, RP-LC, validation, ICH, Isocratic, Assay

INTRODUCTION

Irbesartan is an angiotensin II receptor (AT1 subtype) antagonist. Irbesartan is used for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents. Irbesartan is a non-peptide compound, chemically described as a 2-butyl-3-[p-(o-1H-tetrazol-5-ylphenyl) benzyl]-l, 3-diazaspiro [4.4] non-l -en-4-one. Its empirical formula is $C_{25}H_{28}N_6O$, and the structural formula is shown in **fiureg-I.** Irbesartan is a white to off-white crystalline powder with a molecular weight of 428.5. Irbesartan is slightly soluble in alcohol and methylene chloride and practically insoluble in water [1-4].

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The intended use of analytical methods is to assess product quality and validation is the process of generating experimental data that provides evidence that the performance of an analytical method is adequate for reliably assessing product quality [5-8]. Method validation acceptance criteria necessarily reflect what we believe is "adequate performance." The validation procedure has been performed by using ultra performance liquid chromatography. The method has been validated for linearity, precision (system repeatability, method repeatability, and method reproducibility), accuracy, range, specificity, and solution stability [9-11]. Literature survey indicates that there is no RP-LC short run time method available for assay determination of Irbesartan [12-15], thus we aimed to develop it. Liquid chromatography is a new technique used in analytical chemistry for separating and analyzing substances. Chromatography depends on the distribution of the mixture between two phases, one of them is fixed and is called Stationary phase while the other is not fixed and is called the Mobile phase. The mixture is dissolved in the moving phase and passed over a stationary phase. When a mixture of components is introduced in to a LC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated [15-21].

MATERIALS AND METHODS

Chemical and Reagents:- Pure samples of irbesartan were obtained as gift. LC grade Acetonitrile, potassium dihydrogen phosphate, phosphoric acid and triethylamine were purchased from Merck Company Mumbai. High purity deionised water was obtained from [Millipore, Milli-Q] purification system.

LC instrumentation and conditions:- The analysis of the drug was carried out on a Waters Acquity UPLC (Ultra performance liquid chromatography) Binary Gradient System, 10µL injection loop column with auto injector. Column compartment having temperature

control and for detection Ultraviolet Detector was employed throughout the analysis.

Chromatographic conditions:- Acquity HSS T-3 1.8µ, (2.1 X 100) mm, Make: Waters, analytical column was used for separation. Mobile phase consisted of buffer (10mM Potassium dihydrogen phosphate with 0.05% triethylamine in water adjust pH 3.0 with dilute phosphoric acid): Acetonitrile (50:50 v/v). Mix well and filter through 0.22µm filter. The mobile phase was prepared freshly and degassed by sonicating for 5min before use. Water: Acetonitrile (50:50 v/v) was used as diluent. The analysis was done on isocratic flow of 0.35ml/min with UV detection wavelength was performed at 205 nm at ambient temperature using 3.0 µL injection volumes with auto injector.

Stock and working standard solutions:- Accurately weigh and transfer 25mg of irbesartan working standard into a 50mL volumetric flask, add about 30mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same. Further pipette out 5mL of the above stock solution into a 50mL volumetric flask and dilute up to the mark with diluent and filter through 0.22µm filter. Obtain solution concentration was 50µg/ml.

Assay of irbesartan sample:- Accurately weigh and transfer equivalent to 25mg of irbesartan sample into a 50mL volumetric flask, add about 30mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same. Further pipette out 5mL of the above stock solution into a 50mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.22µm filter. Obtain solution concentration was 50µg/ml. This solution was injected into LC system. For the determination, Peak area of irbesartan was measured. Calculate % Irbesartan by following formulae.

Calculation

$$\textbf{IRBESARTAN}\ (\%) = \frac{A_1 \times C_2}{A_2 \times C_1} \times P$$

Where,

A_1 = Area of Irbesartan in sample

A_2 = Area of Irbesartan in standard

C_1 = Concentration of Irbesartan in sample (mg/ml)

C_2 = Concentration of Irbesartan in Standard (mg/ml)

P = Potency of Standard

METHOD VALIDATION

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability.

Linearity:- The linearity of an analytical procedure is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. It should be established initially by visual examination of a plot of signals as a function of analyte concentration of content. If there appears to be a linear relationship, test results should be established by appropriate statistical methods (e.g., by calculation of a regression line by the method of least squares). In some cases, to obtain linearity between the response of an analyte and its concentration, the test data may have to be subjected to a mathematical transformation. Data from the regression line itself may be helpful for providing mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares should be submitted.

Five standard solutions of Irbesartan were prepared from three stocks in the range of 50% to 150 % of the nominal concentration and injected once. Linearity regression analysis demonstrated the acceptability of the method for quantitative determination of Irbesartan over the concentration range of about 25ppm to 75ppm of the nominal concentration. Linearity graph was shown in **figure-2** and slope, intercept correlation factor and Regression equation were shown in **table-I**

Precision:-The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or repeatability of the analytical method under normal operating conditions. The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations and three replicates of each concentration, or a minimum of six determinations at 100% of the test concentration).

System Repeatability:- Standard solution is prepared 50ppm were injected in six times and RSD of areas and retention times were calculated. The percentage RSD of areas was less than 1.0% and the % RSD of retention times was less than 5.0 %.

Result are presented in **Table-2**

Method repeatability:- Six preparation of Irbesartan sample was analyzed from sample preparation to final results by the same analyst and the percentage RSD of obtained results was less than 2% and obtained result were within given range 100 ± 2. Result are presented in **Table-3**

System Reproducibility:- Three Irbesartan sample are analysed by this method in duplicate preparation and obtain result are in **Table-4**

Accuracy: It is defined as the closeness of test results obtained by the method to the true value. It may often be expressed as percent recovery by the assay of known, added amounts of analyte. Accuracy is a measure of the exactness of the analytical method. The ICH documents recommend that accuracy be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration). The three different concentrations of Irbesartan standard solutions were determined from three replicate injections, using the linear regression lines (linearity section). The deviations of the obtained results

(expressed as percentage accuracy) were calculated from the true values were presented in **table-5.**

Each deviation should be calculated according to the following formula:

$$d = \frac{Calculated - Added}{Added} * 100$$

The average deviations from true value are less than 2.0 %.

Specificity:- The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay result is unaffected by the presence of these extraneous materials. The specificity of the method was verified by testing the blank, standard and sample (un-spiked and spiked), determined the resolution factors between analyte peak (Irbesartan) and the nearest peak. Sample of Irbesartan sample spiked with Irbesartan diamide impurity.

No significant interfering peak appeared in the blank, System suitability and standard chromatogram at the retention times of the analyte peaks.

Range: - The range obtained from Linearity, Precision and Accuracy is summarized - ibesartan-25ppm to 75ppm (50% to 150% of nominal sample concentration)

Solution Stability:- The time period and storage conditions of testing the stability of standard and sample solutions will be according to accumulated knowledge. Stability shall be verified in the glassware specified for the particular solution in the method, e.g. transparent or amber glass. Solution stability was verified by retesting the solutions after 4 hours stored in transparent vials. The stability of the solution was evaluated by calculating the differences between the obtained results of solution stored in vials at room temperature with the freshly prepared solution results. The comprehensive results of this study are presented in the **table-6**.

No new degradation peak was observed. The obtained results demonstrated a good stability of the sample solution stored at room temperature in vial for at least 4 hours.

RESULT AND DISCUSSION

From the linearity calibration curve, this method is linear for determination of assay content concentration ranges of 50 to150 % of Irbesartan nominal sample concentration. Method repeatability is performed and got satisfactory % RSD is less than 1%. Obtained accuracy is within range 98-102.

Conclusion:- The method validation demonstrated that The Method "Determination of Irbesartan Assay content by liquid Chromatography is selective, precise, linear, and accurate along with very short run time" for performing routine basis analysis in quality control.

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Representative chromatograms and table

Linearity Data	
Irbesartan Concentration(ppm)	**Irbesartan Area**
25.09ppm	1209777
40.15ppm	1871359
49.87ppm	2312555
59.84ppm	2738281
73.76ppm	3310271
Slope	**43294**
Intercept	**135678**
Correlation factor	**0.999**

Table-1 Linearity Data

System Repeatability		
Concentration (ppm)	**Retention time (min)**	**Area**
49.87	1.888	2312554
49.87	1.888	2319382
49.87	1.888	2316536
49.87	1.888	2324532
49.87	1.890	2327427
49.87	1.889	2322082
Average	**1.889**	**2320419**
STDEV	**0.001**	**5419**
%RSD	**0.04**	**0.23**

Table-2 System Repeatability data

Method Repeatability			
Concentration (ppm)	**Retention time (min)**	**Area**	**% Irbesartan**
50.11	1.889	2327749	101.0
50.21	1.891	2334935	101.2
49.97	1.890	2339536	101.9
50.17	1.888	2327262	100.9
50.37	1.890	2336411	101.0
49.95	1.889	2327862	101.4
Average	**1.889**		**101.2**
STDEV	**<0.01**		**0.37**
%RSD	**0.056**		**0.37**

Table-3 Method Repeatability data

Method Reproducibility				
S No.	**Concentration (ppm)**	**Area**	**% Irbesartan**	**% Irbesartan Average**
Sample-I Pre-I	51.20	2345474	99.8	99.75
Sample-I Pre-II	50.92	2332112	99.7	
Sample-II Pre-I	51.10	2343829	100.0	99.82
Sample-II Pre-II	50.82	2328230	99.7	
Sample-III Pre-I	50.50	2330695	100.6	100.44
Sample-III Pre-II	50.56	2330107	100.3	

Table-4 Method Reproducibility data

Accuracy/recovery data					
Injection No	**Level**	**Concentration(ppm)**	**Area**	**Calculated concentration (ppm)**	**Accuracy (%)**
1	80 %	39.96	1871359	40.11	99.90
2			1875086	40.20	100.11
3			1874795	40.19	100.09
Average			**1873747**		**100.0**
1	100 %	49.95	2312554	50.30	100.86
2			2319382	50.49	101.18
3			2316536	50.39	101.05
Average			**2316157**		**101.0**
1	120 %	59.50	2738281	60.13	101.07
2			2738930	60.15	101.09
3			2741870	60.22	101.21
Average			**2739693**		**101.1**

Table-5 Accuracy test data of Irbesartan

Solution Stability after 4 hours		
S. No.	**Solution**	**(%) Irbesartan**
1	Old	101.34
2	Fresh	101.18
% Difference		**0.28**

Table-6 Solution stability

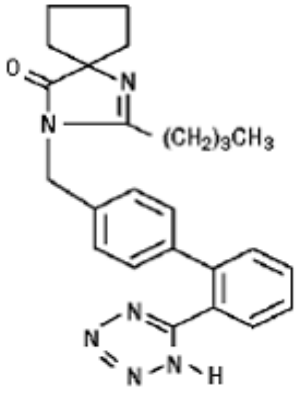


Figure-I Irbesartan Structute

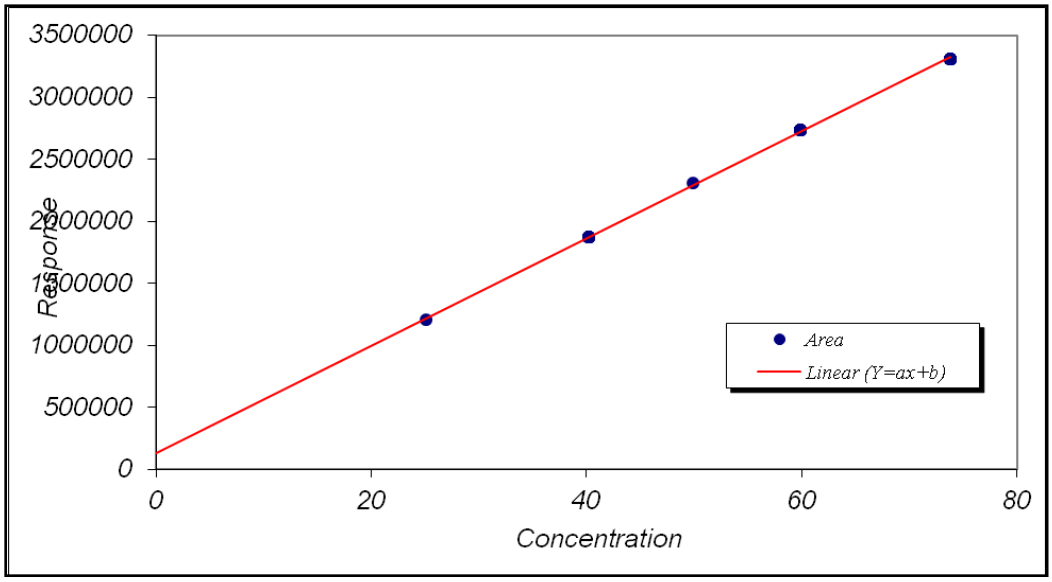


Figure-2 Linearity graph

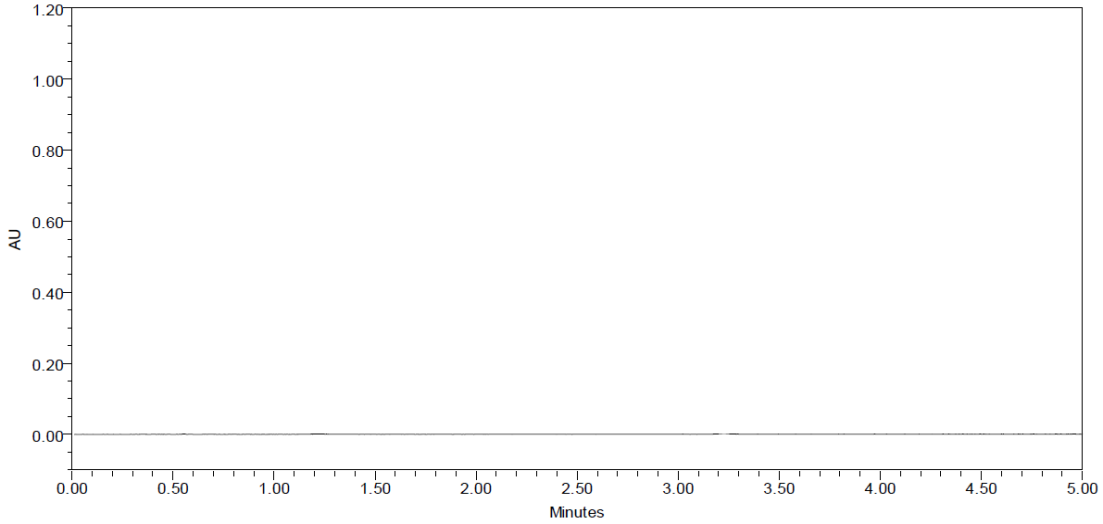
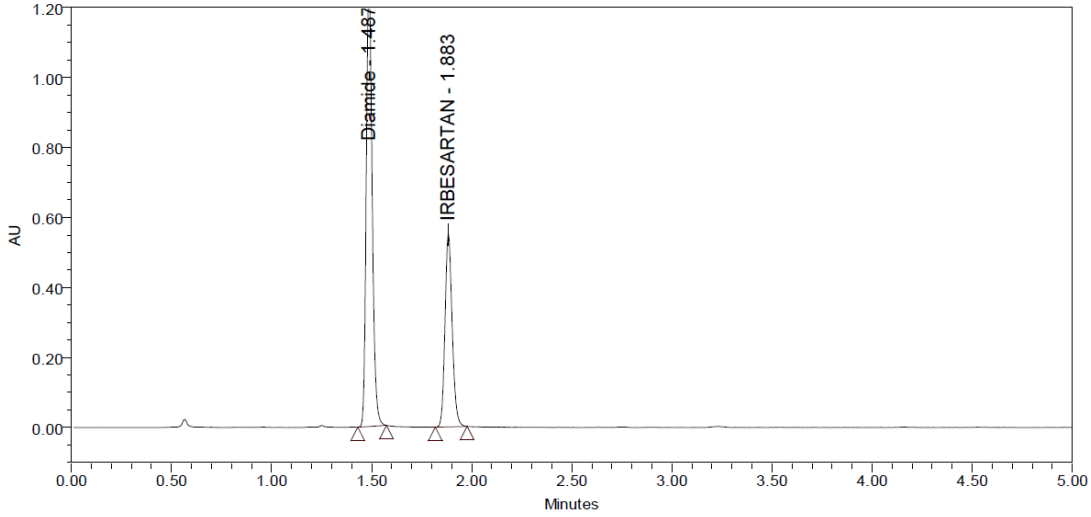


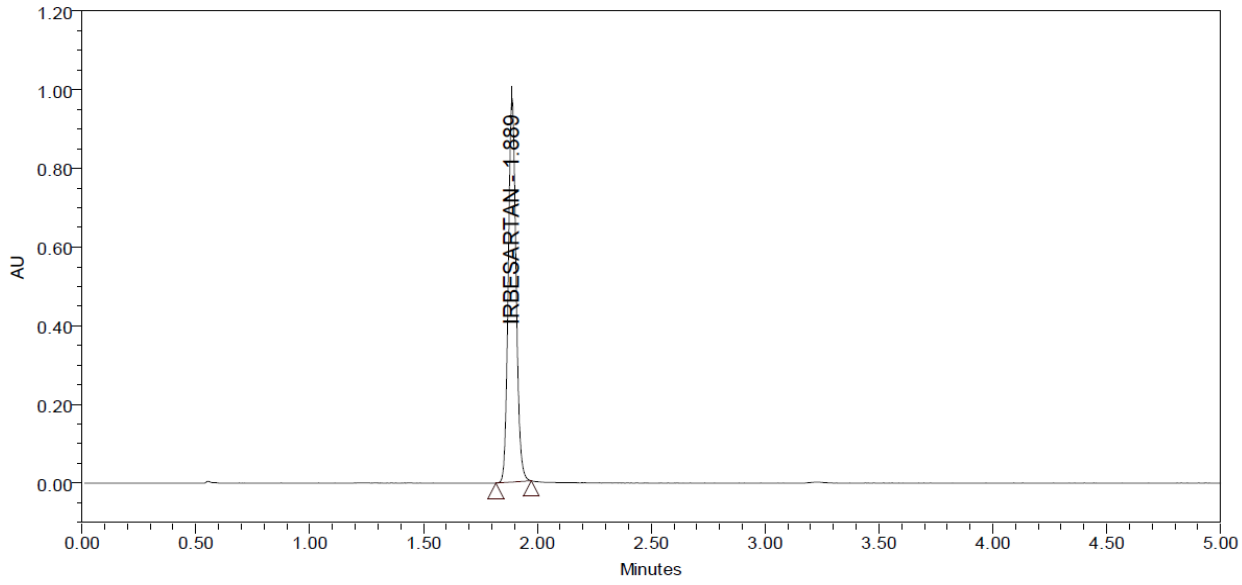
Fig-3 Typical Blank Chromatogram



Peak Results

	Name	RT	Area	% Area	RT Ratio	Resolution	USP Tailing
1	Diamide	1.487	2609095	65.55	0.79		1.20
2	IRBESARTAN	1.883	1371023	34.45	1.00	6.7	1.16
Sum			3980117.8				

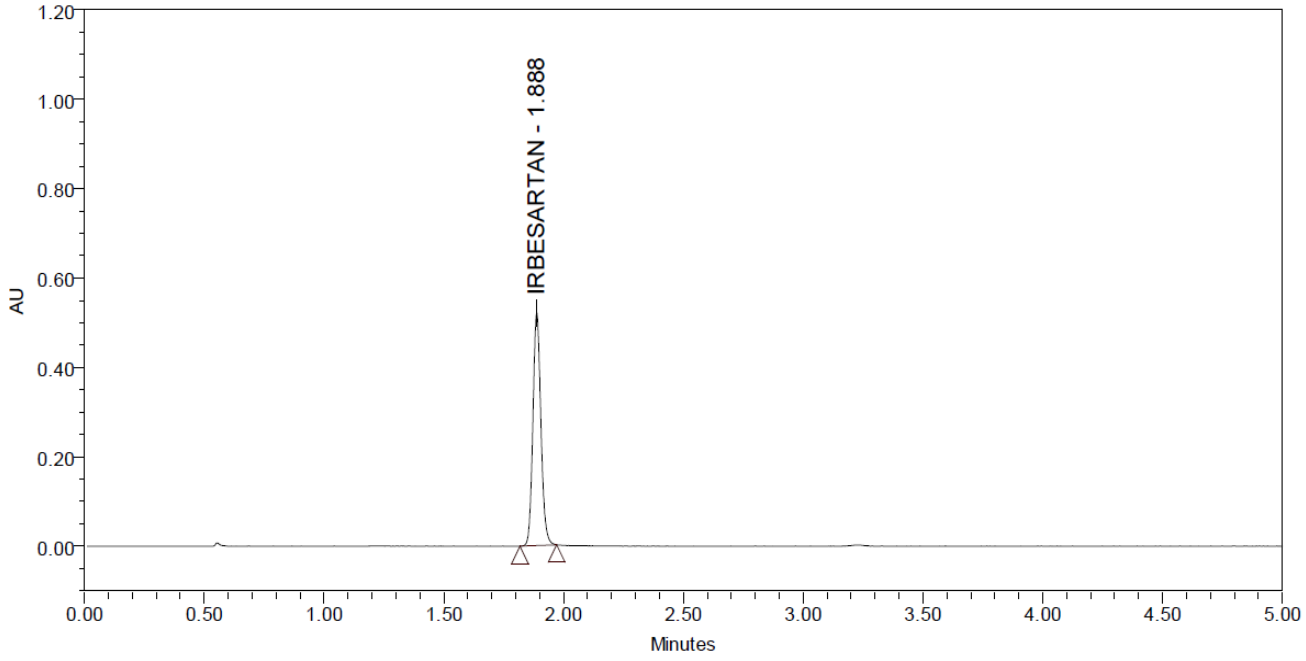
Fig-4 Typical System Suitability Chromatogram



Peak Results

	Name	RT	Area	% Area
1	IRBESARTAN	1.889	2327749	100.00
Sum			2327748.9	

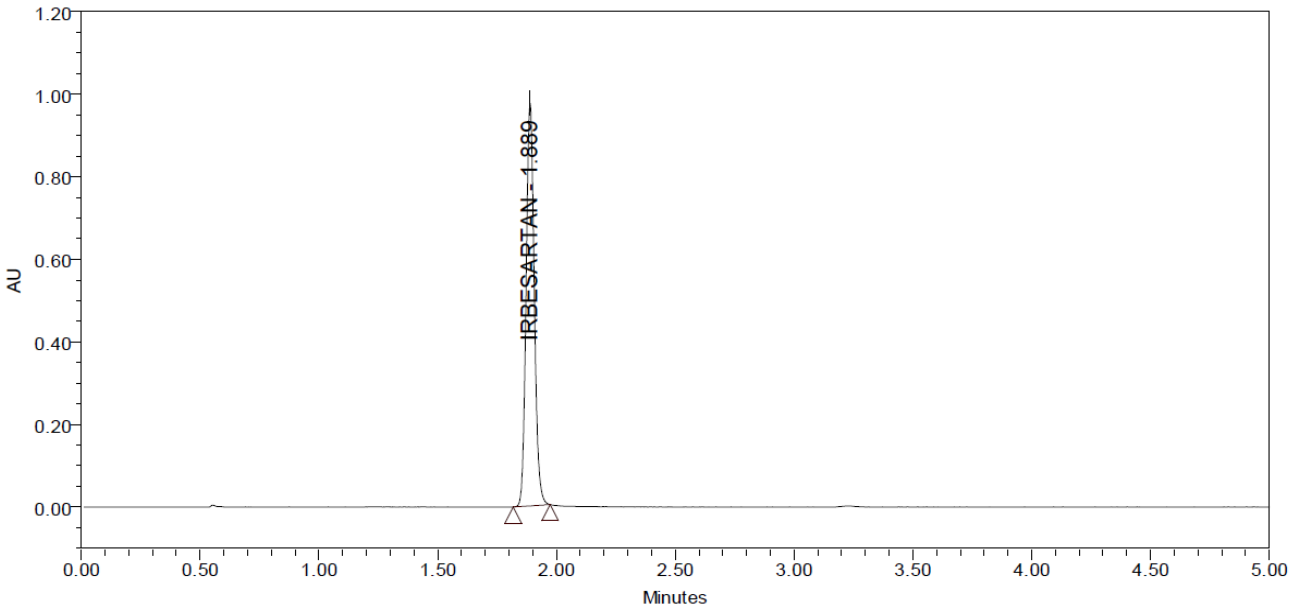
Fig-5 Typical Standard Chromatogram (50ppm)



Peak Results

	Name	RT	Area	% Area
1	IRBESARTAN	1.888	1209777	100.00
Sum			1209776.7	

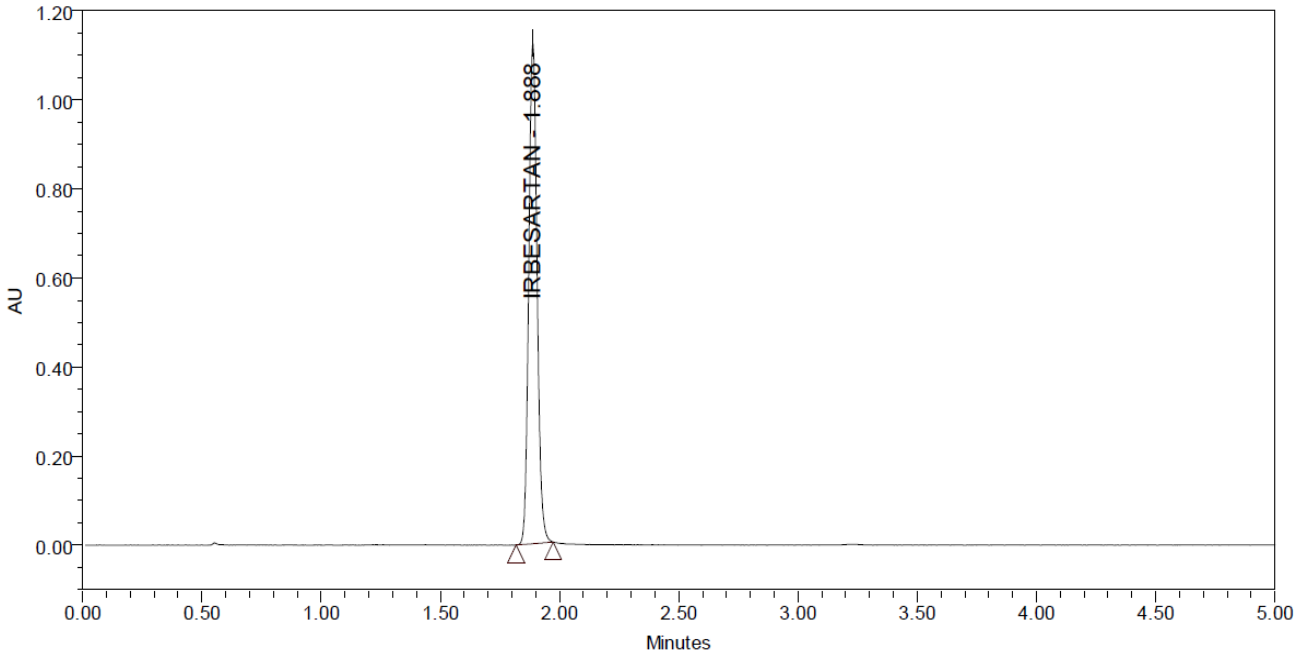
Fig-6 Typical Standard Chromatogram (25ppm)



Peak Results

	Name	RT	Area	% Area
1	IRBESARTAN	1.889	2327749	100.00
Sum			2327748.9	

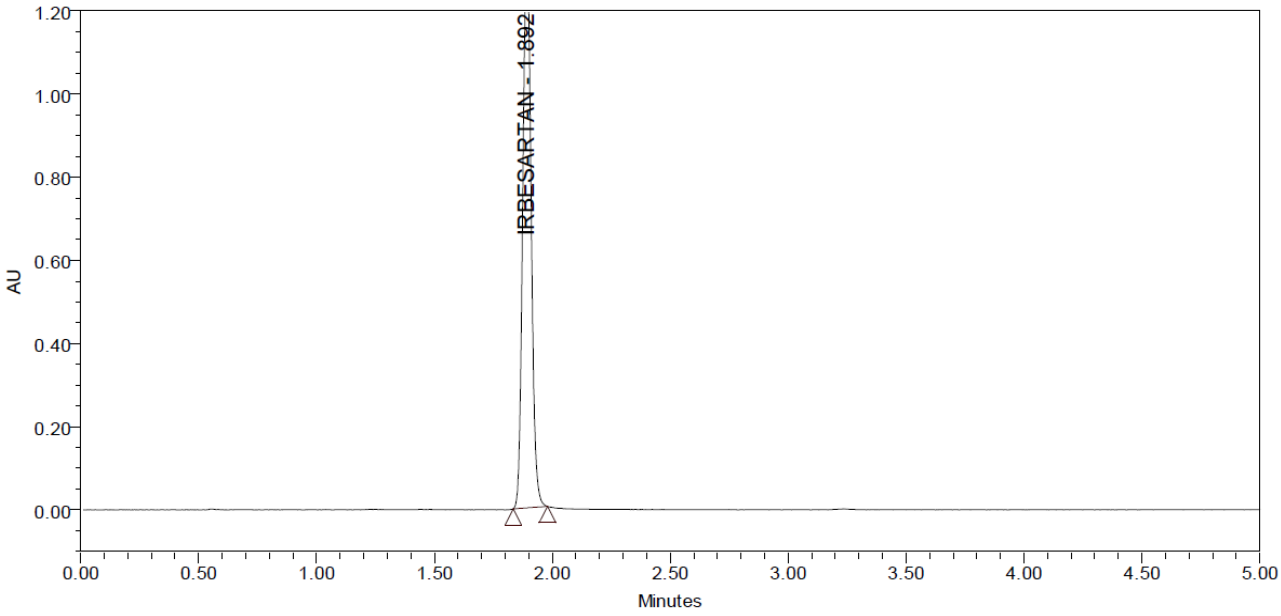
Fig-7 Typical Standard Chromatogram (40ppm)



Peak Results

	Name	RT	Area	% Area
1	IRBESARTAN	1.888	2738281	100.00
Sum			2738281.0	

Fig-8 Typical Standard Chromatogram (60ppm)



Peak Results

	Name	RT	Area	% Area
1	IRBESARTAN	1.892	3310271	100.00
Sum			3310271.1	

Fig-9 Typical Standard Chromatogram (75ppm)

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