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Analytical Method Development and Method Validation for the Estimation of Irbesartan in Tablet Dosage Form by HPLC Method

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ABSTRACT

A simple, sensitive and specific HPLC method with UV detection was developed for the estimation of irbesartan in tablet dosage form. Separation was achieved by Lithosphere 100 RP-18e, 5µ column having 250x4.0 mm internal diameter with mobile phase containing phosphate buffer and acetonitrile in 50:50 (v/v) with an ambient temperature. The flow rate was 0.8mL min^{-1} and eluent was monitored at 220 nm. The proposed method was found to be rectilinear over theconcentration range of 20µg/ml to 60µg/ml**.** This method was validated for system suitability, Specificity, Linearity, Precision, Accuracy, Range, Stability studies, Ruggedness, Robustness and Filter validation. The results of all the validation parameters were well within their acceptance values. Therefore the proposed method can be used for routine analysis of estimation of Irbesartan in its tablet formulation

Keywords: Irbesartan, Tablet formulation, Antihypertensive, HPLC, Validation

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INTRODUCTION

Irbesartan is used mainly for the treatment of hypertension[1-4]. Hypertension is an independent risk factor for cardiovascular disease and is associated with an increased incidence of stroke and coronary heart disease. Angiotensin II antagonists are the major development in hypertension management in over a decade. Their excellent lower side effect profile and specificity in the action provide good condition for patient compliance as well as effectiveness. Therefore, these drugs are used as first-line to treat essential hypertension[5]. Irbesartan is an orally active non-peptide specific angiotensin II receptor antagonist (AT1 subtype) used, as a hypotensive agent does not require biotransformation into an active form. Irbesartan is chemically described as a 2-butyl-3-[*p*-(*o*-1*H*-tetrazol-5ylphenyl)benzyl] -1,3-diazaspiro[4.4]non1 en-4-one (Figure. 1).

Literature survey revealed that few analytical methods have been reported for determination of irbesartan in pure drug, pharmaceutical dosage forms and in biological samples using spectrophotometry[6-8] and liquid chromatography[9-18] either in single or in combined forms**.** Present study involves development of HPLC method and validates a simple, fast and reliable method with UV detection for the determination of irbesartan in tablet dosage form. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the determination of irbesartan in tablet dosage forms.

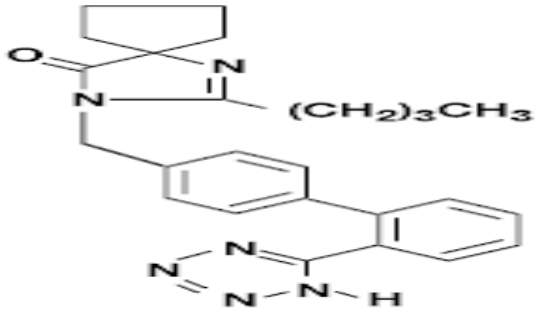

Figure 1Chemical structure of Irbesartan

MATERIALS AND METHODS

Materials

All the solvents used were of HPLC grade. Irbesartan working standard and irbesartan tablet 150mg were obtained from Dr.Reddy's Laboratories, Hyderabad, India.

Method development

Chromatographic conditions

A Lithosphere column 100RP-18e, having internal diameter of 150×4.0 mm and 5µ particle size was used at ambient temperature. The mobile phase consisting of phosphate buffer (pH 3.2) which

was prepared by mixing 5.5 ml of orthophosphoric acid in 950 ml of water. The contents were mixed well and pH was adjusted to 3.2 with treithyl amine. The resultant solution was filtered through 0.22µ Pall Pharma lab nylon 66 membrane filter or 0.22µ Durapore PVDF hydrophilic membrane filter and the above buffer solution and acetonitrile were mixed in the ratio of 50:50 v/v and degassed in a sonicator for 10 minutes. The flow rate was maintained at 0.8ml/min. The elution was observed at 220nm. Injection volume and run time were 10µl and 15 minutes respectively.

Standard preparation:

An accurately weighed amount of about 50 mg of irbesartan working standard or reference standard was transferred into a 250ml volumetric flask, dissolved and diluted to volume with methanol and mixed well. 5.0ml of the above solution was pipette into 25ml volumetric flask and diluted to volume with Methanol and mixed well. Then, filtered through 0.45µ Pall Pharma lab nylon 66 membrane filter or 0.45µ Durapore PVDF hydrophilic membrane filter.

Assay of irbesartan tablets

20 irbesartan tablets 150mg were weighed and average weight was calculated. All the tablets were crushed and made into a fine powder and the weighed tablet powder equivalent to 100mg of irbesartan was transferred into a 250ml volumetric flask. About 25ml of water was added and the sample was soaked in water for 5 minutes. Then the flask was shake for 10 minutes. 100ml of methanol was added and stirred on magnetic stirrer for 30 minutes, again another 100ml of methanol was added and stirred on magnetic stirrer for another 30 minutes and then sonicated for 30 minutes. Then the solution was cooled to room temperature. Diluted up to mark with methanol and filtered through 0.45µ (Nylon) membrane filter (Pall Pharma). 5 ml of above solution was diluted to 50ml with methanol and mixed well. 10 µl portion of methanol as a blank, standard preparation (five times) and test preparation were injected into the chromatograph, the chromatogram was recorded and the peak response was measured.

METHOD VALIDATION

The developed method was validated as per the ICH (International Conference on Harmonization) guidelines with respect to Linearity, System suitability, Precision, Specificity, Accuracy, Range, Ruggedness and Robustness.

Linearity

The linearity of the method was evaluated by analyzing different concentrations of the drugs. According to ICH recommendations, at least five concentrations must be used. In this study five concentrations were chosen, in the range of 20µg/ml to 60 µg/ml of irbesartan.

Accuracy

The accuracy of the method was determined by calculating recovery of Irbesartan in the presence of excipients with various concentrations of Irbesartan spiked ranging from 50% of lower strength to 200% of higher strength, the concentration ranging from 0.0025mg/ml to 0.02mg/ml i.e., 25% to 200% of target concentration, either by spiking of drug substance or by solution spiking. It was prepared six times at 25% and 200% remaining all in triplicate at each level.

System suitability

The Standard solution was prepared by using Irbesartan working standard as per test method and injected 5 times into the HPLC system. The % RSD for peak areas of five replicate injections of standard preparation and the asymmetry of Irbesartan peak were found to be within limits.

Precision

The precision of method was checked and verified by repeatability and intermediate precision. Repeatability was evaluated by assaying the six different individual weights of the Irbesartan tablets equivalent to 100 mg and analyzed as per test method. The % assay and the Relative standard deviation of Irbesartan were found. For intermediate precision the method precision was repeated by different analyst using different lot of column on different day.The precision of test method on six individual samples prepared from finished product as per test method was determined. Each preparation was injected once into a chromatograph. The % Assay and % Relative standard deviation for six preparations were calculated.

Specificity:

The specificity of the method was evaluated by assessing the following parameters:

Placebo Interference:

The selectivity of the method was evaluated by assessing whether excipients present in the pharmaceutical formulations interfere with the analysis. A study to establish the interference of placebo was conducted. Assay was performed on placebo (Placebo contains excipients without Irbesartan API) equivalent to about the weight of placebo present in portion of test preparation as per test method.

Interference from Degradation Products:

A study was conducted to demonstrate the effective separation of degradants from Irbesartan peak. Separate portions of drug product and placebo were exposed to stress conditions mentioned in Table 4 to induce degradation. Stressed samples were injected into the HPLC system with photodiode array detector by following the test method conditions. All degradant peaks were

resolved from Irbesartan peak in all degradation samples. The chromatograms of the stressed samples were evaluated for peak purity of Irbesartan.

Range

The specified range was derived from precision and accuracy studies.

Precision

Six replicate samples of lower spike level (at 50% of target concentration) and higher spike level (at 150% of target concentration) were analyzed as per test method; the % assay and the Relative standard deviation of Irbesartan were found.

Accuracy

Sample solutions were prepared in triplicate for lower and higher spike level (50% and 150%) and assayed as per test method. The mean % recovery at each spike level was found to be within the limits.

Syringe Filter evaluation study

Filter validation is a study to establish the suitability of filters. It was conducted using filter 0.22µ Durapore hydrophilic membrane (PVDF) filters and 0.22µ Pall Nylon membrane filter. Test preparations prepared in duplicate were centrifuged and filtered through different filters, and calculated similarity factor of test solution against standard solution. The % Assay difference of filtered test solution was also found.

Ruggedness

Ruggedness was determined by assessing system to system variability, column to column variability, bench top stability of standard, test and mobile phase. System to system variability study, column to column variability study was conducted on two different HPLC systems and two different columns respectively by assaying six different individual weights of irbesartan Tablets 150mg as per the test method. The system suitability parameters, the % assay with a relative standard deviation were evaluated on both the systems and columns.

Robustness

Robustness of the method was studied by changing the composition of organic phase (Acetonitrile ±10%) in the mobile phase composition, by varying the pH of buffer in mobile phase, by changing the flow rate and varying the column temperature.

Stability study

A study to establish the stability of Irbesartan in test and standard preparation on bench top was conducted at initial time, after 24 hours and after 48 hours. The assay of irbesartan in test preparation (duplicate) and in standard preparation was estimated against a freshly prepared

standard each time and similarity factor for standard preparations were estimated.

The stability of mobile phase was studied for a period of about 5 days on bench top. Irbesartan tablets test samples were prepared in duplicate and the assay was performed as per test method at initial time, after 1 day, after 2 days and after 5 days. The standard solution was prepared as per test method and system suitability parameter and the % assay of irbesartan were determined from initial to 5 days.

RESULTS AND DISCUSSION

Linearity:

The method gave a linear response to irbesartan drug within the concentration range of 20-60µg/ml with r^2= 0.999 as shown in Figure. 2.

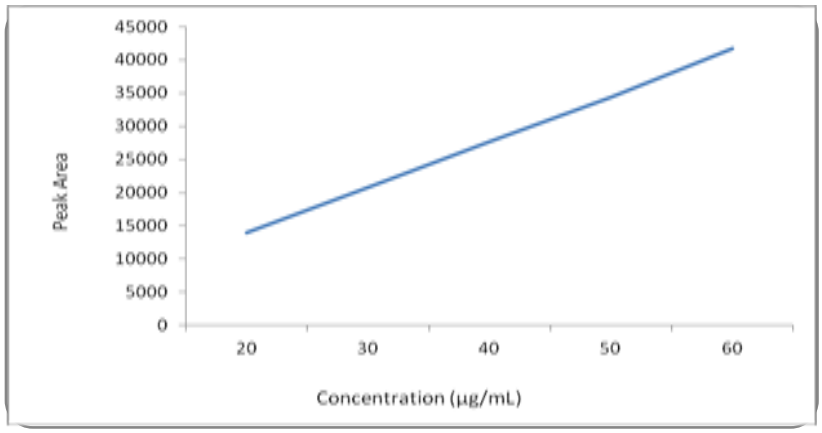

Figure 2Graphical representation of linearity of Irbesartan.

Accuracy

Table 1: Accuracy Study

Sample no.	Spike level	"mg" added	"mg" found	% Recovery	Mean % Recovery
1	50%	51.1	50.5	98.8	
2	50%	51.0	50.3	98.6	99.2
3	50%	50.2	50.3	100.2	
1	80%	80.1	80.3	100.3	
2	80%	79.9	81.7	102.3	101.0
3	80%	81.5	81.9	100.5	
1	100%	101.6	103.6	102.0	
2	100%	101.1	100.8	99.7	100.5
3	100%	100.4	100.1	99.7	
1	125%	120.9	120.6	99.7	
2	125%	118.7	116.6	98.2	99.0
3	125%	121.0	119.8	99.0	
1	150%	151.3	153.7	101.6	
2	150%	150.9	151.7	100.5	101.6
3	10%	150.8	155.0	102.8	

The data for accuracy were expressed in termsof percentage recoveries of irbesartan in the realsamples. The mean recovery data of irbesartanin real sample were within the range of 99.0 and101.6% satisfyingthe acceptance criteria for the study. It wasproved that there is no interference due toexcipients used in tablet formulation. Hence theaccuracy of the method was confirmed. Theresults were furnished in Table 1.

System suitability

Various system suitability parameters were also calculated. It was observed that all the values were within the limits is shown in Table.2.

Table 2: System suitability study

System suitability	Observed value	Acceptance criteria
Asymmetry for Irbesartan peak from standard solution	1.29	NMT 2.0
Relative standard deviation for peak areas of Irbesartan	0.55	NMT 2.0%

Precision

Low values of percentage relative standard deviation of percentage assay (0.68) denoted very good repeatability of the measurement Table 3a. Thus it was showing that the equipment used for the study was correct and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the different analyst working on different lot of column on different days and all the results were found to be within limits Table. 3b,3c. This indicates good method precision.

Table 3a: Repeatability Study

Sample No.	% Assay
1	97.9
2	97.4
3	97.0
4	98.0
5	97.3
6	98.9
MEAN	97.8
% RSD	0.68

Table 3b: Test for intermediate precision (System suitability)

System suitability Parameters	Observed value		Acceptance Criteria
	Analyst 1	Analyst 2	
Asymmetry of Irbesartan peak in standard preparation	1.29	1.23	NMT 2.0
Relative standard deviation of Irbesartan peak area from five replicate injections of standard	0.55%	0.36%	NMT 2.0%

Table 3c: Test for intermediate precision (Assay)

Sample no.	% Assay of Irbesartan	
	Analyst-1	Analyst-2
1	97.9	99.3
2	97.4	98.6
3	97.0	101.0
4	98.0	101.9
5	97.3	100.2
6	98.9	101.8
Mean	97.8	100.5
%RSD	0.67	1.35

Specificity

Chromatograms of placebo showed no peak at the retention time of Irbesartan. This indicates that the excipients used in the formulation do not interfere in estimation of irbesartan in irbesartan tablets. For all degradation studies peak purity of irbesartan was found to be NLT 990. This indicates that there was no interference from degradants in quantitating the Irbesartan in Irbesartan tablet.

Table 4: Degradation studies of Irbesartan tablets

Stress Condition	DEGRADATION	
	% Degradation	Peak Purity of Irbesartan
Refluxed with 0.5M HCI solution for about 24 hours at 80°C.	1.0	1000
Refluxed with 0.5M NaOH solution for about 4 hours at 80°C.	16.2	1000
Refluxed with 0.5% Hydrogen peroxide (1-1202) for about 8hours at 80°C.	2.6	1000
Refluxed with purified water for about 24 hrs at 80°C.	4.9	1000
Exposed to Sunlight for about 1.2 Million Lux hours for 7 days	Nil	1000
Exposed to UV light both at 254 nm for about 10 days	Nil	1000
Dry heating done at 105°C for about 24 hours.	13.5	1000
Exposed to humidity at 25°C, 90% RH for about 7 days.	Nil	1000

Range

The specified range was normally derived from linearity studies and depends on the intended application of the procedure. It will be established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within the extremes of the specified range of the analytical procedure. Based on the Linearity, precision and accuracy results, the range of the method was determined as 50% to 150% of the target assay concentration. Range results were shown in Table 5a and 5b.

Table 5a: Precision study

Sample No.	Spike Level	Mean % Recovery
1	50%	100.1
2	150%	101.4

Table 5b: Accuracy study

Injection Number	% Assay	
	At Lower spike Level (50%)	At Higher Spike Level (150%)
1	98.8	101.6
2	98.6	100.5
3	100.2	102.8
4	101.9	101.6
5	100.5	100.0
6	100.5	101.8
Average	100.1	101.4
%RSD	1.22	0.93

Syringe Filter Evaluation Study

Study revealed that the % recovery obtained with the sample filtered through different filter paper was closer to each other. Table 6 shows the filter paper evaluation study.

Table 6: Syringe Filter Evaluation

Sample No.	Centrifuged	0.2µ Pall nylon		0.2µ millipore	
	%release	% release	Difference	% release	Difference
1	99.9	99.5	0.4	98.2	1.7
2	99.9	99.2	0.7	97.9	2.0

Robustness

Robustness of the method was investigated by changing flow rate, changing organic phase composition in mobile phase, column temperature and changing pH of buffer solution of mobile phase and results were summarized in Table 7. It was observed that the small changes in these operational parameters did not lead to changes of retention time of the peak interest. The degree of reproducibility of the results proved that the method was robust.

Table 7 Robustness study

System Suitability Parameters	Observed value with Flow rate (ml/min)			Observed value at column oven temperature (^{o}c)			Observed value at effect of Variation In Mobile Phase Composition (% of organic phase)			Observed value at pH			Acceptance criteria
	0.6	0.8	1.0	20	25	30	90	100	110	3.0	3.2	3.4	
Asymmetry for Irbesartan peak	1.29	1.31	1.28	1.32	1.31	1.22	1.32	1.31	1.22	1.27	1.32	1.30	NMT 2.0

from standard solution													
Relative standard deviation for peak areas of Irbesartan from five replicate injections of standard	0.83	0.72	0.56	0.41	0.72	0.47	0.41	0.72	0.47	0.08	0.50	0.11	**NMT 2.0%**

Ruggedness

Comparison of the results obtained on two different systems and two different columns shown that the assay method was rugged (Table 8a, 8b).

Table 8a:Ruggedness (system to system variation)

Sample no.	**Assay of Irbesartan as % of labeled amount**	
	System – 1	**System – 2**
1	97.9	99.3
2	97.4	98.6
3	98.0	101.9
4	97.3	100.2
5	98.9	101.8
Mean	97.8	100.5
% RSD	0.67	1.35

Table 8b:Ruggedness (column to column variation)

System suitability Parameters	**Observed value**		Acceptance Criteria
	Column – 1	**Column – 2**	
Asymmetry of Irbesartan peak in standard preparation.	1.29	1.23	NMT 2.0
Relative standard deviation of Irbesartan peak area from five replicate injections of standard	0.55%	0.36%	NMT 2.0%

Stability study

From the solution stability study it was observed that the test, standard and mobile phase were found to be stable. Table 9a, 9b, 9c shows the results of stability of test and standard preparation, stability of mobile phase and system suitability parameters for mobile phase respectively.

Table 9a: Bench top stability of test and standard solution

Time in days Similarity Factor of Standard Preparation		**%Assay of test preparation**		**Difference**	
		Test -1	**Test-2**	**Test-1**	**Test-2**
Initial	NA	99.7	99.2	NA	NA
24 Hours	1.0	99.1	98.1	0.9	1.1
48 Hours	1.0	99.6	99.1	0.1	0.1

Table 9b: Bench top stability of mobile phase

Approximate time	Average % Assay	Difference from initial
Initial	99.5	NA
24 hrs	98.4	1.1
48 hrs	100.8	1.3
5 days	98.0	1.5

Table 9c:System suitability parameters

System suitability parameters	Observed value				Acceptance Criteria
	Initial	After 24 Hrs	After 48 Hrs	After 5 days	
Asymmetry of Irbesartan peak in standard preparation.	99.7	99.2	NA	NA	NMT 2.0
Relative standard deviation of Irbesartan peak area from five replicate injections of standard preparation	99.1	98.1	0.9	1.1	NMT 2.0%

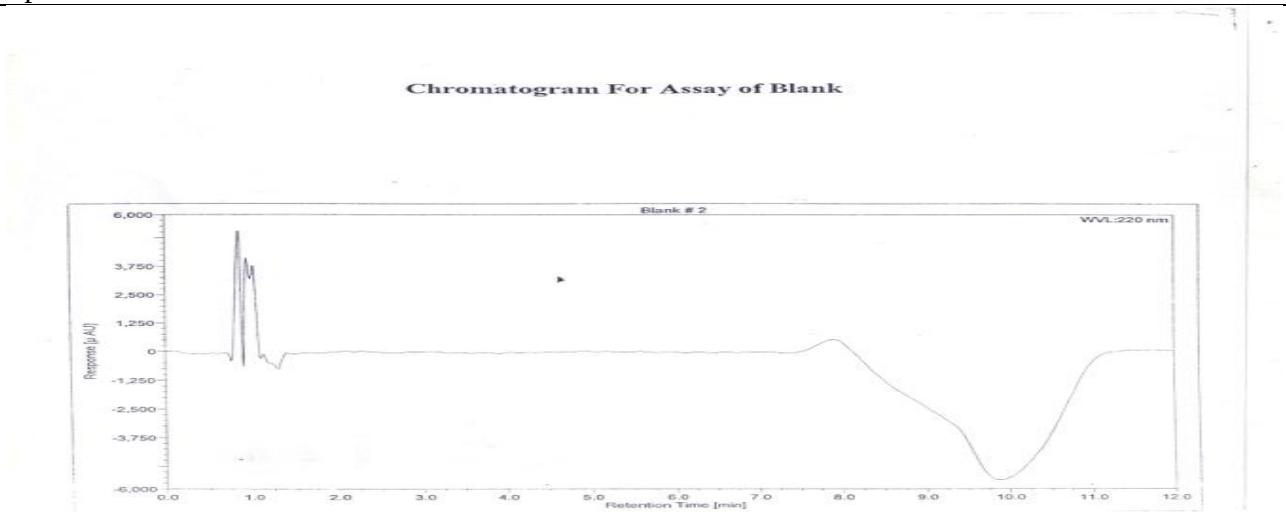

Figure 4 Chromatogram for assay of Blank

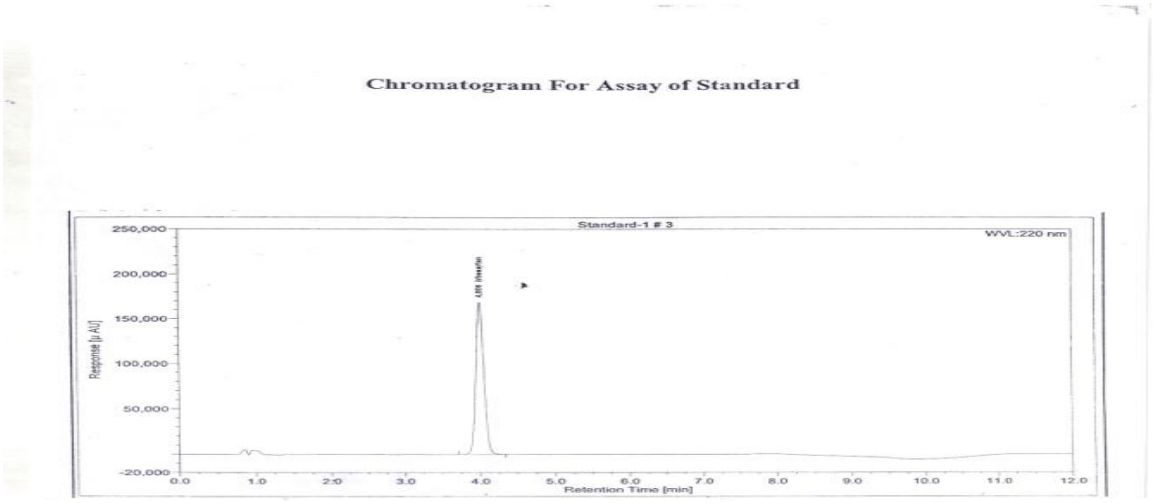

Figure 5 Chromatogram for assay of Standard

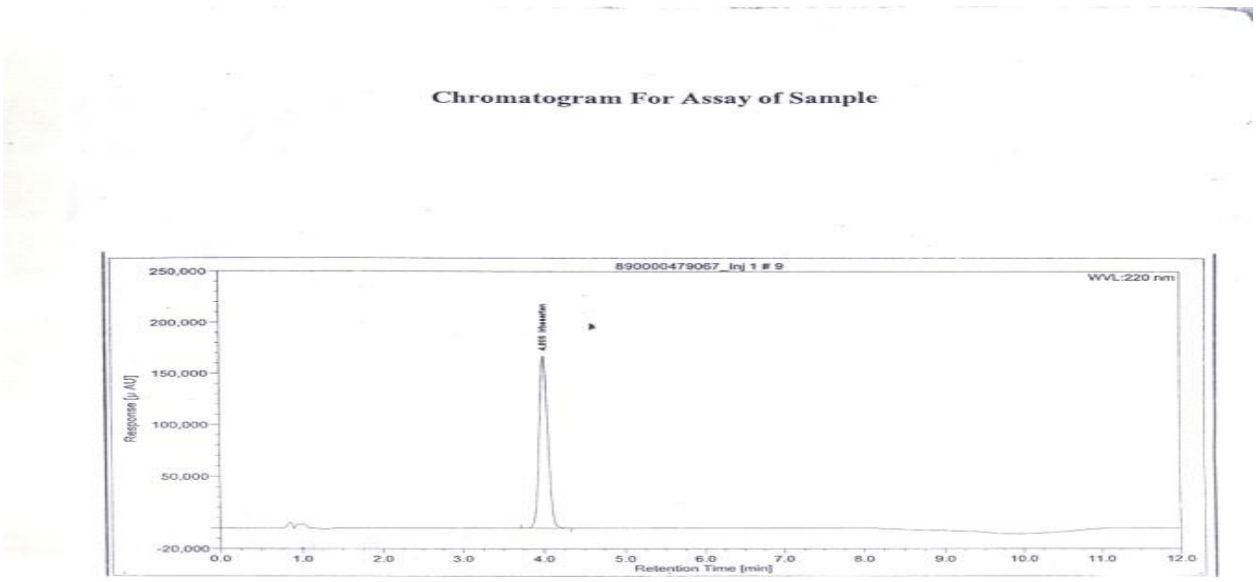

Figure 6 Chromatogram for assay of Sample

CONCLUSION

Proposed study describes new HPLC method for the estimation of Irbesartan in tablet formulation. The method was validated and found to be simple, sensitive,accurate and precise. Percentage of recovery has shown that the method was free from interference of the excipients used in the formulation. Therefore the proposed method can be used for routine analysis of estimation of Irbesartan in its tablet formulation.

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