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Research paper

Development and validatation of HPTLC method for estimation of irbesartan in bulk and tablet dosage form

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Abstract

A simple, rapid and accurate High performance thin layer chromatography is described for the Development and validation of HPTLC method for Irbesartan in bulk and Pharmaceutical dosage form. The separation is carried out on Merck TLC aluminum sheets of silica gel 60 F254 using Ethyl acetate: Chloroform (6.5:3.5v/v) mobile phase. Quantification was done by Densitometric scanning at 254nm. The linearity was found to be the range of 100-500ng/spot for Irbesartan with the correlation coefficient of 0.9992. The regression equation was found to be Y=7.2733x+703.15. The Rf value of Irbesartan was found to be 0.55. The LOD and LOQ were found to 8.24 and 24.74 respectively. Average recovery was found to be 99.66% which show that the method was free from interference from excipients present in the formulation. Simultaneously the Percentage relative standard deviation was well within the range of 2%. The above method was validated according to the ICH guidelines. The established method enabled accurate, precise and applied to the analysis of Irbesartan in bulk and Pharmaceutical dosage form.

Keywords: *Irbesartan; HPTLC; Ethyl Acetate and Chloroform in the Ratio of 6.5:3.5.*

1. Introduction

Irbesartan is an angiotensin II receptor antagonist used mainly for treatment of Hypertension. It was developed by Sanofi research. It is a reasonable initial treatment for high blood pressure. It is an orally active nonpeptidetetrazole derivative and selectively inhibits angiotensin II receptor. Angiotensin II receptor type 1 antagonist has been widely used in treatment of disease like hypertension, heart failure, myocardial infarction and diabetic nephropathy [1].

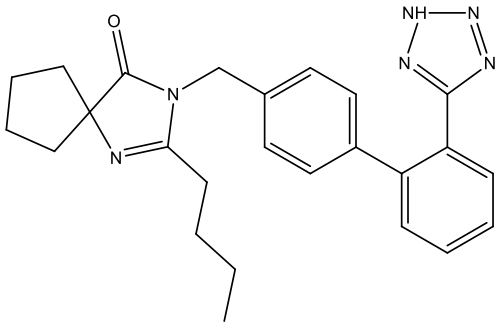

Fig. 1: Chemical Structure of Irbesartan.

Irbesartan is chemically 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, It has molecular formula $C_{25}H_{28}N_6O$ and molecular weight is 428.53g/mol. Irbesartan is practically insoluble in water, sparingly soluble in methanol and slightly soluble in methylene chloride. The melting point of Irbesartan is 180-181˚C [2].
Literature survey shows that there were few analytical methods have been reported for the determination of Irbesartan in pure and pharmaceutical dosage forms by using HPTLC [3-8], HPLC [9-13] and UV Spectrophotometric. [14-22]
The main aim of present work is to develop and validate novel, rapid, simple, precise and specific HPTLC method for estimation of Irbesartan in bulk and tablet dosage form.

2. Materials and methods

2.1. Chemicals and reagents

Irbesartan pure drug was obtained as gift sample from Apotex research private limited Bangalore and its pharmaceutical dosage form Irovel 150mg manufactured by Sun Pharma laboratories Ltd Assam. Batch no: EST0957 procure from local pharmacy, Methanol, Ethyl acetate and Chloroform obtained from Bharathi college of pharmacy, Mandya.

2.2. Solvent

Ethyl acetate and Chloroform (6.5:3.5)

2.3. Instrumentation

The samples were spotted in the form of bands of 6 mm width with a Camag micro liter syringe (100/µl) on pre-coated silica gel aluminum plates 60 F-254 (10 cm × 10 cm with 250 mm thickness, E. Merck, Wilmington, USA) using a Camag Linomat-5 applicator. The plates were pre-washed with methanol and activated at 100°C for 5 min prior to chromatography. The slit dimension was kept at 6.00 mm × 0.45 mm (micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of Ethyl acetate: Chloroform (6.5:3.5v/v) and 10ml of mobile phase was used. Linear ascending development was carried out in a 10 cm × 10 cm twin trough glass chamber (Camag, Switzerland) saturated with the mobile phase. The chamber saturation time for the mobile phase was 25 min at room temperature (25°C ± 2). The length of the chromatogram run was around 8 cm. Then the thin layer chromatography plates were dried in a current of air with the help of an air dryer. Densitometric scanning was done on a Camag TLC scanner 3 and which was operated by winCATS software.

2.4. Standard solutions

Irbesartan (150 mg) was accurately weighed into a 100 ml volumetric flask and dissolved in a minimum volume of methanol, and diluted to the required volume with methanol to furnish a solution of concentration 1000ng/µl. This was used as stock solution. Stock solution containing 1 mg/ml Irbesartan was prepared in methanol. Calibration solutions were prepared by diluting the stock solution so that application of 2 µl volumes gave a series of spots covering the range 100 to 500 ng of Irbesartan.

2.5. Sample preparation

Twenty tablets were weighed and powdered. Tablet powder equivalent to 100 mg of Irbesartan was transferred to a 100 ml calibrated volumetric flask and extract it with 70 ml of methanol for 15 min by the use of ultra sonicate and then diluted up to the mark with the same solvent, and filtered. 10 ml of tablet stock solution was then diluted up to 10 ml with methanol to give final concentration of 1000 µg/ml. 2 µl of this solution was spotted on HPTLC plate to give a concentration of 100-500 ng/spot of Irbesartan.

2.6. Calibration curve

Separate stock standard solutions of Irbesartan were used for the preparation of calibration standard solutions. All calibration standards were prepared freshly every day and were found to be stable during the analysis time. The plate was developed, dried and scanned as described above. After densitometric scanning, the peak area was recorded for each concentration and a calibration plot was obtained by plotting average peak area against concentration of Irbesartan (ng/spot). The slope and correlation coefficient were also determined.

2.7. Method validation

The method was validated according to ICH guidelines. [23]

2.7.1. Linearity

The linear regression data for the calibration curves showed a good linear relationship over the concentration range 100-500ng/band. The results were depicted in table 1. Linear regression equation was found to be $Y= 7.2733x + 703.15$ and $R^2 = 0.9992$. The calibration curve was shown in the figure 2.

2.7.2. Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the different band. The intra- and inter-day variation for the determination of Irbesartan was carried out at six different concentration levels of 100, 200, 300, 400 and 500ng/spot.

2.7.3. Limit of detection and limit of quantification

In order to determine the limit of detection (LOD) and limit of quantification (LOQ), Irbesartan concentrations in the lower part of the linear range of the calibration curve were used. Irbesartan solutions of 100-500 ng/ band were prepared and applied on the plate. The LOD and LOQ were calculated using the equation $LOD = 3.3 \times N/B$ and $LOQ = 10 \times N/B$, where, N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

2.7.4. Specificity

The specificity of the method was determined by a comparing the standard drug and sample. The spot for Irbesartan in the sample was confirmed by comparing the R values and spectra of the spot with that of the standard. The peak purity of Irbesartan was determined by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

2.7.5. Ruggedness

Ruggedness of the method was performed by spotting 300ng/band of Irbesartan by two different analysts keeping the same experimental and environmental conditions.

2.7.6. Accuracy

The samples were spotted with extra 50, 100 and 150% of standard Irbesartan, and the mixture was analyzed by the proposed method. This was done to determine the recovery of the drug in the formulations.

2.7.5. Robustness

By making small & deliberate changes in the environment and the mobile phase composition, the effects on the results were examined. The Mobile phases having different compositions of Ethyl acetate: Chloroform (6.5:3.5v/v) was tried and chromatograms were run. The volumes of mobile phase, temperature and relative humidity were varied in the range of ± 5%. The plates were pre-washed by methanol and activated at 80 ± 10°C for 2, 5 and 7 min prior to chromatography. The spotting Time to chromatography and from chromatography to scanning was varied from 10, 15 and 20 min.

3. Results and discussion

3.1. Development of optimum mobile phase

The TLC procedure was optimized with a view to developing a stability-indicating assay method. Initially, Ethyl acetate: Chloroform: methanol (5:3:2v/v) gave good resolution but high Rf value of 0.95 for Irbesartan, to reduce the Rf value then the mobile phase was optimized to Ethyl acetate: Chloroform (6.5:3.5v/v) Finally, the mobile phase consisting of gave a sharp and well-defined peak at Rf value of 0.55 [Figure 2]. The spots were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature.

3.2. Validation of the method

The summary of validation parameters are in the table 2.

3.2.1. Linearity and range

The linear regression data for the calibration curves showed a good linear relationship over the concentration range 100-500ng/band. The results were depicted in table 1. Linear regression equation was found to be Y= 7.2733x + 703.15 and R^2 = 0.9992. The calibration curve was shown in the figure 2.

Table 1: Linearity

SI. NO	CONCENTRATION	PEAK AREA	RF
1	100ng/spot	1401	0.57
2	200ng/spot	2170.5	0.56
3	300ng/spot	2908.7	0.56
4	400ng/spot	3645.2	0.55
5	500ng/spot	4300.3	0.54

*Mean of six estimations.

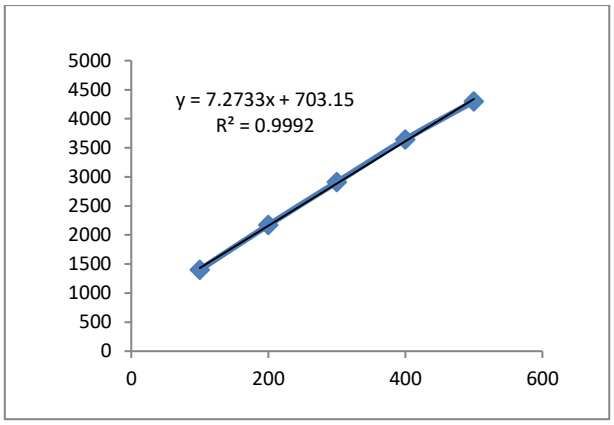

Fig. 2: Calibration Graph of Irbesartan.

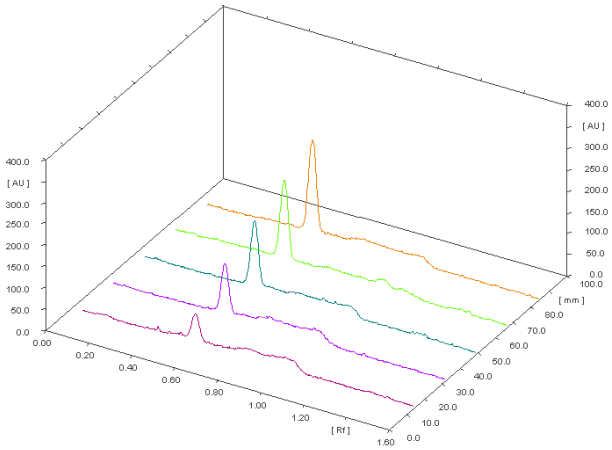

Fig. 3: 3D View of Irbesartan by HPTLC Method.

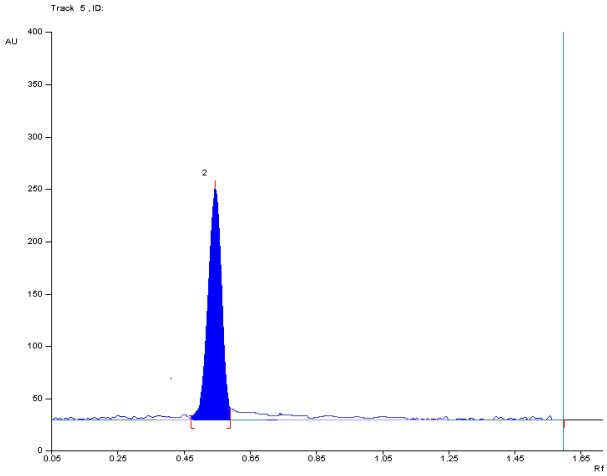

Fig. 4: HPTLC Chromatogram of Irbesartan with Corresponding Rf Value at 254 Nm.

3.2.2. Precision

The precision of the above method was expressed in terms of %relative standard deviation (%RSD). The results depicted in Table 2.

Table 2: Precision

Concentration (µg/ml)	INTRA-DAY MEAN OF AREA	%RSD	INTER-DAY MEAN OF AREA±SD**	%RSD
100ng/spot	1397	0.38	1405	0.55
200ng/spot	2171	0.58	2169.9	0.36
300ng/spot	2902.4	0.18	2902.4	0.29
400ng/spot	3667.6	0.54	3622.9	0.50
500ng/spot	4326.7	0.76	4274	0.49

*Mean of three estimations.

3.2.3. Recovery study

The proposed method when used for extraction and estimation of Irbesartan from the pharmaceutical dosage form after over spotting with 50, 100 and 150% of additional drug, affording good recovery of Irbesartan. The % recovery is listed in Table 3.

Table 3: Recovery Studies

Spiked levels	Amount of sample (ng/spot)	Amount of standard (ng/spot)	Amount recovered	%Recovery ±SD**	%RSD
50	300	150	447.02	99.93±0.51	0.512
100	300	300	597.07	99.60±0.09	0.098
150	300	450	752.39	99.41±0.27	0.271

3.2.4. Ruggedness

When the method was performed by two different analysts under the same experimental and environmental conditions, it was found to be rugged [Table 4].

Table 4: Ruggedness

Analysts	Analyst-1	Analyst-2
Mean peak area	2905	2918
Standard deviation	9.58	10.62
%RSD**	0.33	0.364

*Mean of three estimations.

3.2.5. Robustness of the method

The standard deviation of peak areas was calculated for each parameter, and the %RSD was found to be less than 2% The low values of %RSD [Table 5] indicated that the method was robust.

Table 5: Results of Robustness for Irbesartan

Parameters	%RSD
Mobile phase volume(±2ml)	1.45
Development distance (±0.5cm)	1.62
Duration of saturation (±10min)	0.84
Time from spotting to chromatography(±10min)	0.47
Time from chromatography to Scanning(±10min)	0.14

*Mean of three estimations.

3.2.6. LOD and LOQ

Detection limit and quantification limit was calculated by the method described above. The LOQ and LOD were found to be 8.24 and 24.74 respectively. This indicated adequate sensitivity of the method.

Table 6: Summary of Validation Parameters

S.NO	PARAMETER	RESULTS
1	Linearity	100-500ng/spot
2	Regression equation	Y= 7.2733x + 703.15
3	Slope	7.2733
4	Intercept	703.15
5	Correlation coefficient(R^2)	R^2 = 0.9992
6	Limit of detection	8.24
7	Limit of quantification	24.74
8	Specificity	Specific
9	Recovery (n=6)	99.66

3.3. Analysis of the marketed formulation

A single spot at Rf 0.55 was observed in the chromatogram of the drug samples extracted from the tablets. There was no interference from the excipients. The %drug content and %RSD were calculated. The low %RSD value indicated the suitability of this method for the routine analysis of Irbesartan in pharmaceutical dosage forms.

4. Conclusion

This HPTLC method is simple, precise, specific and accurate. Statistical analysis proved that the method is reproducible and selective for the analysis of Irbesartan as the bulk drug and in tablet formulations

5. Acknowledgment

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6. Abbreviations

- HPTLC: High performance thin layer chromatography;
- ICH: International council for Harmonization;
- LOD: Limit of detection;
- LOQ: Limit of quantitation;
- RSD: Relative standard deviation.

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