*Pharmacophore* 2011, Vol. 2 (2), 108-112 ISSN 2229 – 5402

Pharmacophore
(An International Research Journal)
Available online at http://www.pharmacophorejournal.com/
Original Research Paper

# DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE ESTIMATION OF IRBESARTAN IN PHARMACEUTICAL DOSAGE FORM

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## ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Irbesartan in tablet dosage form. An Inertsil ODS C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing methanol, acetonitrile and 2% OPA (40:40:20,v/v/v) was used. The flow rate was 1.5 ml/min and effluents were monitored at 260 nm. The retention time for Irbesartan was 4.5 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found 10 ng and 30 ng respectively and recovery of Irbesartan from tablet formulation was found 100.61%. The proposed method was successfully applied for the quantitative determination of Irbesartan in tablet formulation.

**Keywords:** Irbesartan, HPLC, Linearity, Validation, Tablet form.

## INTRODUCTION

Irbesartan is used mainly for the treatment of hypertension.[1-7]Irbesartan (INN) pronounced is an angiotensin II receptor antagonist. Irbesartan IAPUC name is 2-butyl-3-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1,3-diazas-piro[4.4]non-1-en-4-one and molecular formula $C_{25}H_{28}N_6O$.

Literature survey revealed that numerous methods have been reported for estimation of Irbesartan in pharmaceutical formulations has been reported. Present study involves development of HPLC method [8-12] using simple mobile phase which is sensitive and rapid for quantification of Irbesartan in tablet dosage forms as well as subsequent validation of developed method according to ICH guide lines.

## MATERIALS AND METHODS

### Instrument

The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Intersil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle

size. Shimadzu electronic balance (AX-200) was used for weighing purpose.

## Reagents and Materials

Methanol of HPLC grade was purchased from E.Merck, Mumbai, India. HPLC grade water was obtained by double distillation and purification through mille-Q water purification system. Potassium dihydrogen phosphate of analytical grade was procured from qualigens, Mumbai, India.

## Preparation of Standard Stock Solution

A stock solution of Irbesartan was prepared by accurately weighing 10mg of drug, transferring to 10ml of volumetric flask, containing 10ml of mobile phase dissolving it to obtain final standard solution of 1mg/ml of Irbesartan. Resultant solution was filtered through Ultipor N66 Nylon 6, 6 membrane sample filter paper.

## Preparation of Sample Solution

The formulation tablets of Irbesartan were crushed to give finely powdered material. Powder equivalent to 10mg of Irbesartan was taken in 10 ml of volumetric flask containing 10ml of solvent and was shaken to dissolve the drug and then filtered through Ultipor N66 Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent and was further diluted to obtain concentration of 100µg/ml.

## Chromatographic Conditions

The mobile phase consisting of methanol, acetonitrile and 2% OPA were filtered through 0.45µ Ultipor N66 Nylon 6,6 membrane solvent filter, degassed and were pumped from the solvent reservoir in the ratio of 40:40:20,v/v/v and was pumped into the column. The flow rate of mobile phase was maintained at 1.5ml/min and detection wavelength was set at 260nm with a run time of 6min. The volume of injection loop was 20µl prior to injection of the drug solution the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

## Calibration Curve

Appropriate aliquots of standard Irbesartan stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 10, 20, 30,40,50,60 and 70µg/ml of Irbesartan. These solutions were injected into chromatographic system, chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Irbesartan was constructed by plotting peak area ratio versus applied concentration of Irbesartan and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Irbesartan in tablet sample was found out using regression equation.

## Method Validation

The method was validated for accuracy, precision, linearity, specificity, limit of detection, limit of quantitation and robustness by following procedures.

### *Accuracy*

The accuracy of the method was determined by calculating recovery of Irbesartan by the method of standard addition. Known amount of Irbesartan was added to a pre quantified sample solution and the amounts of Irbesartan was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve (Table 1). The recovery studies were carried out three times over the specified concentration range and amount of Irbesartan was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

### *Precision*

The intra-day precision study of Irbesartan was carried out by estimating the correspondence responses six times on the same day with 100µg/ml concentration and inter-day precision study of Irbesartan was carried out by estimating the correspondence responses six times next day with 100µg/ml concentration.

### *Linearity and range*

The linearity of the method was determined at six concentration levels ranging from 10-70µg/ml for Irbesartan.

### *Specificity*

Commonly used excipients (colloidal silicon dioxide, lactose, magnesium stearate, starch and talc) were spiked into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantity of drug was determined.

### *Limit of detection and limit of quantification*

Limit of detection = 10ng

Limit of quantitation = 30ng

### *Stability*

In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 8 hours at room temperature.

### *Robustness*

Robustness of the method was studied by changing the composition of organic phase by ±4% and the PH by ± 0.1, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

## RESULTS AND DISCUSSION

The UV spectra of Irbesartan showed that the drug absorbs appreciably at 260nm was selected as the detection wave length in liquid chromatography. Optimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase methanol, acetonitrile and 2% OPA (40:40:20, v/v/v). The retention time of Irbesartan was found to be 4.561 min, which indicates a good baseline (Figure3).

The number of theoretical plates was found to be 3871, which indicates efficient performance of the column. The calibration curve for Irbesartan was obtained by plotting the peak area ratio versus the concentration of Irbesartan over the range of 10-70µg/ml, and it was found to be linear with r 2=0.998. The regression equation of Irbesartan concentration over its peak area ratio was found to be y = 1.49150 + 5190.804 x, where x is the concentration of Irbesartan and Y is the respective peak area. The data of regression analysis of the calibration curve was shown in table 1. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The limit of detection and limit of quantification for Irbesartan was found to be 10ng and 30ng, indicates the sensitivity of the method. The system suitability and validation parameters were given in (Table 2).The high percentage of recovery of Irbesartan was found to be 100.61% indicates that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Irbesartan in tablet formulation (Figure 2). The result for Irbesartan was comparable with a corresponding labeled amount (Table 3). The absence of additional peaks indicates no interference of the recipients used in the tablets.

## 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 shows that the method is 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.

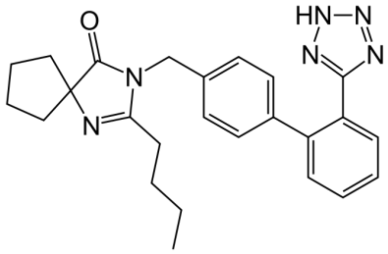
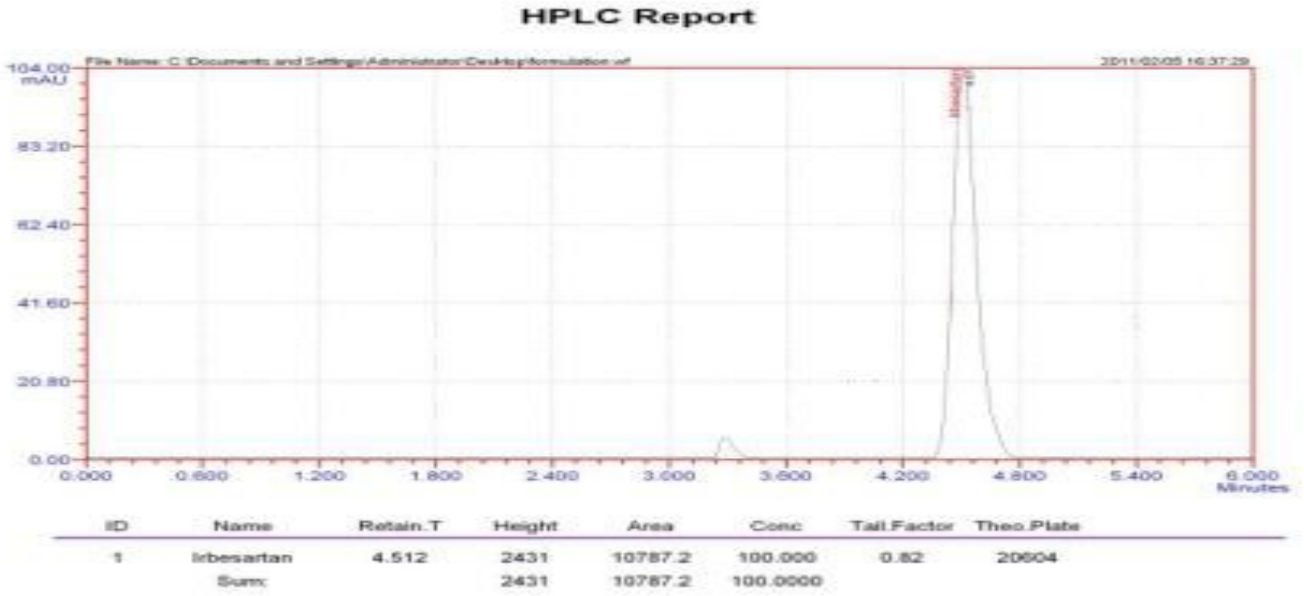



**Figure1:** Molecular structure of Irbesartan




| ID | Name | Retain.T | Height | Area | Conc | Tail.Factor | Theo.Plate |
|---|---|---|---|---|---|---|---|
| 1 | Irbesartan | 4.512 | 2431 | 10787.2 | 100.000 | 0.82 | 20604 |
| | Sum: | | 2431 | 10787.2 | 100.0000 | | |

**Figure2:** HPLC Chromatogram of Irbesartan formulation


| ID | Name | Retain.T | Height | Area | Conc | Tail.Factor | Theo.Plate |
|---|---|---|---|---|---|---|---|
| 1 | api | 4.561 | 66664 | 690031.1 | 100.000 | 1.31 | 3871 |
| | Sum: | | 66664 | 690031.1 | 100.0000 | | |

**Figure3:** HPLC chromatogram of Irbesartan

**Table 1:** Regression analysis of the calibration curve

| Parameters | Values |
|---|---|
| Calibration range | 10-70µg/ml |
| Slope | 5190.804 |
| Intercept | 1.49150 |
| Correlation coefficient ($r^2$) | 0.9982 |

**Table 2:** System suitability and validation parameters

| Parameters | Results |
|---|---|
| Theoretical plates (N) | 3871 |
| Retention time (min) | 4.561 |
| LOD (ng) | 10ng |
| LOQ (ng) | 30ng |
| Accuracy (%) | 97.162 |
| R.S.D. (%) | 0.8179 |

**Table 3:** Assay result of tablet formulation

| Formulation | Labelled claim (mg) | % Recovery |
|---|---|---|
| Irovel | 300mg | 55.38% |

## REFERENCES


1. Lewis, EJ; Hunsicker, LG; Clarke, WR; Berl, T and Pohl, MA *et al.*(2001), "Collaborative Study Group: Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes", ***N Engl J Med***, 345(12), 851-60.
2. Rossi, S (2006), "***Australian Medicines Handbook***".
3. Massie, BM; Carson, PE; McMurray, JJ and Komajda, M (2008), "Irbesartan in patients with heart failure and preserved ejection fraction", ***N. Engl. J. Med.***, 359 (23).
4. ***http://www.medicinenet.com/irbesartan/article.htm***
5. Suzanne, Oparil and Michael, A (2005), "***Renal Out Come Trials***", A Weber-2005, 384-872.
6. Jonathan,Upfal (2006), "***The Australian Drug Guide***", 372-832.
7. Aronson, JK (2009), "Irbesartan; Angiotensin II receptor antagonists", General Information, 121-829.
8. Bae, SK; Kim, MJ; Shim, EJ and Shin, JG (2009), "HPLC determination of Irbesartan in human plasma: its application to pharmacokinetic studies", ***Biomedical Chromatography***, 23(6), 568-72
9. Praveen, KM *et al.* (2011), "Development and validation of a stability indicating RP-HPLC method for assay of Irbesartan in pure pharmaceutical dosage", ***IJPSC Review and Research***, Vol. 6(1), 100-4.
10. Ashok, KS *et al* (2003), "Simultaneous determination of Irbesartan and Hydrochlorothiazide in human plasma by liquid chromatography", ***Journal of Chromatography B***, Vol.784(1)195-201.
11. M,Ganesan (2010), "Method development and validation of Irbesartan using LCMS/MS: Application to pharmacokinetic studies", ***J. Chem. Pharm. Res.***,2 (4),740-746
12. (2008),"Simultaneous determination of Olmesartan Medoxomil and Irbesartan and Hydrochlorothiazide in pharmaceutical formulations and human serum using high performance liquid chromatography" ***Chinese Journal of Chromatography***, Vol. 26(5),544-549