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# Development and validation of a simple bio-analytical HPLC-UV method for estimation of irbesartan in human plasma

Ashok P[1], Narenderan S T[1], Meyyanathan S N[*1], Babu B[1], Jawahar N[2]

[1]Department of Pharmaceutical Analysis, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Nilgiris, Tamil Nadu, India
[2]Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Nilgiris, Tamil Nadu, India

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ABSTRACT

The present study was aimed to develop and validate a simple, sensitive and economical bio-analytical high-performance liquid chromatographic-ultraviolet method for the determination of irbesartan in human plasma. The method involves the use of simple precipitation method for the determination of irbesartan, using methanol as precipitating agent and losartan as internal standard. The separation was achieved using Zorbax $C_{18}$ column (150 x 4.6 mm, 5$\mu$m), mobile phase consists of methanol and 0.2% formic acid in water at the ratio 85:15, v/v using detection wavelength of 237 nm. Further, the developed method was validated as per US-FDA guidelines for accuracy, precision, linearity, stability, detection and quantification limit. The method developed was found to be linear over the concentration ranging from 5 to 500 ng/ml with a correlation coefficient of 0.9987. The LOD and LLOQ of the method were found to be 1 ng/ml and 5 ng/ml, respectively.

*Corresponding Author

Name: Meyyanathan S N
Phone: +91-7010551923
Email: snmeyyanathan@jssuni.edu.in

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## INTRODUCTION

Irbesartan is a hypertension drug chemically known as 2-butyl-3-[p-(o-1H-tetrazol-5- ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one (Figure 1). Irbesartan is widely prescribed to lower blood pressure in patients due to its effect on angiotensin receptor. It competes with angiotensin II for binding at the AT1 receptor subtype (Coyle *et al.*, 2007; Bae *et al.*, 2009).

Literature review revealed few HPLC chromatographic methods for the determination of irbesartan in plasma samples using tedious solid-phase extraction (SPE) (González *et al.*, 2002; Chang *et al.*, 1997; Caudron *et al.*, 2004), solid-phase microextraction (SPME) (Nie *et al.*, 2005) and liquid-liquid extraction (LLE) (Shakya *et al.*, 2007). However, the reported methods require costly (Erk, 2003; González *et al.*, 2002) and tedious extraction methods (Nie *et al.*, 2005; Caudron *et al.*, 2004) and also require a large volume of plasma samples for the determination irbesartan (Tutunji *et al.*, 2010; Wani and Zargar, 2015; Mhaske *et al.*, 2012). Hence, our objective is to develop a simple, rapid method for the determination of irbesartan in human plasma by HPLC-UV method using economical sample preparation method.

## MATERIALS AND METHODS

### Chemicals and reagents

Irbesartan and losartan (IS) were procured from Sigma-Aldrich (St. Louis, Missouri, United States).

**Table 1: Precision and accuracy results (n = 6).**

| Spiking Concentration levels (ng/ml) | Mean concentration found ± SD | Intra-day | | Inter-day | |
|---|---|---|---|---|---|
| | | Accuracy (%) | Precision (% RSD*) | Accuracy (%) | Precision (% RSD) |
| 5 | 4.87 ± 0.35 | 97.40 | 7.18 | 96.41 | 9.74 |
| 15 | 14.78 ± 1.12 | 98.54 | 7.57 | 97.09 | 9.64 |
| 150 | 147.95 ± 13.74 | 98.63 | 9.28 | 97.88 | 8.74 |
| 400 | 397.24 ± 27.85 | 99.31 | 7.01 | 98.47 | 8.01 |

*% RSD: Percentage relative standard deviation

**Table 2: Stability results for the developed method**

| Stability test | Spiking Concentration levels (ng/ml) | Mean ± SD; % RSD* |
|---|---|---|
| Short-term (24 h at room temperature) | 5 | 4.86 ± 0.33; 6.79 |
| | 15 | 14.71 ± 1.13; 7.68 |
| | 150 | 147.94 ± 12.64; 8.54 |
| | 400 | 397.19 ± 26.35; 6.63 |
| Long-term (30 days at -20°C) | 5 | 4.66 ± 0.46; 9.87 |
| | 15 | 14.01 ± 1.22; 8.70 |
| | 150 | 145.74 ± 13.71; 9.40 |
| | 400 | 394.17 ± 27.61; 7.00 |
| Freeze-thaw (6 cycles) | 5 | 4.86 ± 0.33; 6.79 |
| | 15 | 14.77 ± 1.13; 7.65 |
| | 150 | 147.94 ± 13.71; 9.26 |
| | 400 | 397.23 ± 27.79; 6.99 |
| Stock solution stability (12 h at room temperature) | 5 | 4.95 ± 0.13; 2.62 |
| | 15 | 14.90 ± 0.87; 5.83 |
| | 150 | 149.00 ± 9.51; 6.38 |
| | 400 | 398.97 ± 17.09; 4.28 |

*% RSD: Percentage relative standard deviation; SD: Standard deviation, n= 6

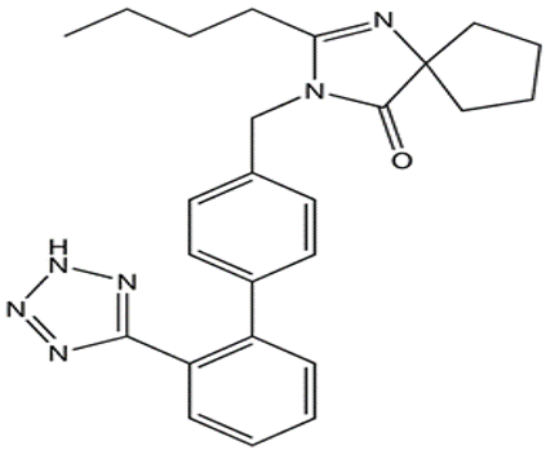


**Figure 1: Structure of irbesartan**

HPLC grade methanol was procured from Rankem, Lab Chemicals (Mumbai, India). Analytical grade salts and reagents were procured from SD Fine chemicals Ltd. (Mumbai, India) and ultra-pure water was obtained from Milli-Q RO system.

### Preparation of standard and quality control (QC) samples

The stock solutions were prepared by accurately weighing irbesartan and losartan (IS) with methanol to produce a stock solution (1 mg/ml). The stock solutions were further diluted and spiked to drug-free human plasma to produce the calibration standards of drug 5, 10, 25, 50, 75, 100, 250, 500 ng/ml and IS 100 ng/ml. The four QC samples were prepared similarly by spiking the drug (5, 15, 150, 400 ng/ml) and IS (100 ng/ml) into drug-free human plasma.

### Instrumentation

The Shimadzu high-performance liquid chromatog-

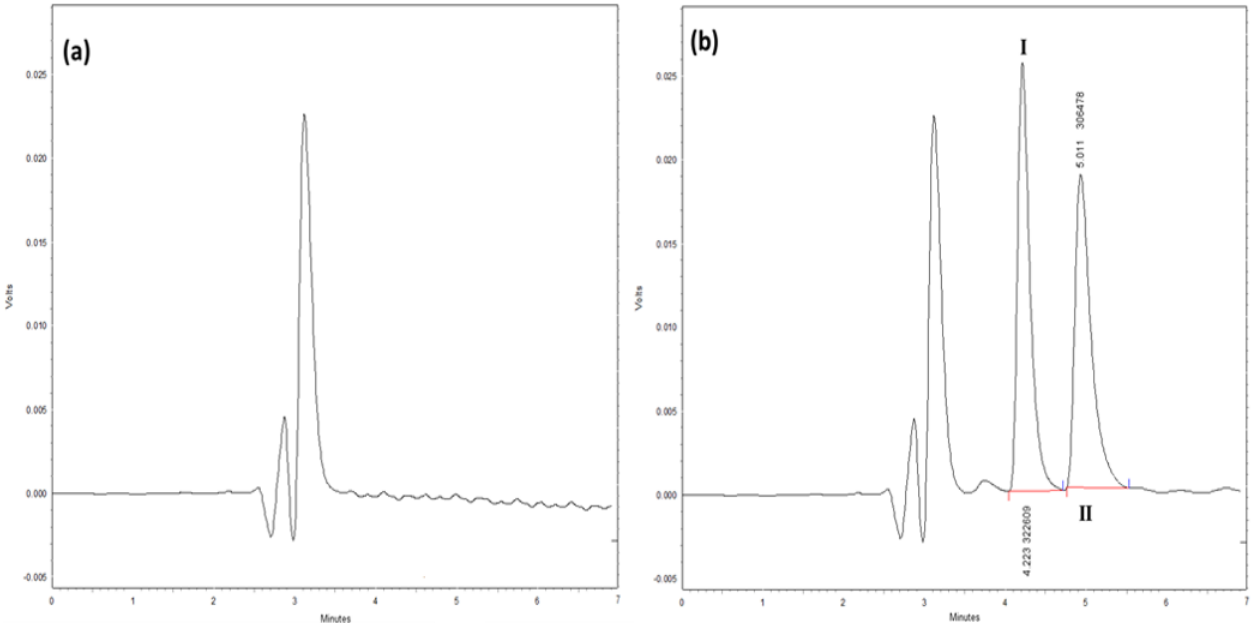


**Figure 2: Chromatogram of (a) Blank and (b) blank human plasma spiked with LLOQ level of irbesartan(I) with IS (II)**

raphy consists of LC/10 AT/VP solvent delivery

system, 7725i rheodyne injector with 20$\mu$l loop, SPD M/10A VP UV detector and Class VP data station software. The chromatographic separation was achieved using Zorbax $C_{18}$ column (150 x 4.6 mm, 5$\mu$m), mobile phase (isocratic) consists of methanol and 0.2 % formic acid in water at the ratio 85:15, v/v using detection wavelength of 237 nm. The separation was achieved in 7.0 min with a retention time of 4.22 min for irbesartan and 5.01 min for IS.

### Preparation of plasma sample

To an eppendorf tube, 100 $\mu$l of human plasma samples, 400 $\mu$l of methanol was added (containing 100 ng/ml of IS), vortexed (1 min) and centrifuged at 5000 rpm for 15 minutes. The supernatant was transferred to another eppendorf tube (centrifuged at 5000 rpm for 10 min) and 10 $\mu$l aliquot was analyzed by the developed HPLC method.

### Method validation

The developed method was validated for specificity, accuracy, precision, linearity, stability and detection limit as per the USFDA guidelines for the validation of bioanalytical method (USFDA, 2018).

The method specificity was established by comparing chromatograms of blank plasma and samples spiked with irbesartan and IS. Four QC samples of lower limit (LLOQ), low (LOQ), medium (MQC) and high-quality control (HQC) samples of 5, 15, 150, 400 ng/ml were analyzed at six replicates to determine the precision and accuracy (intra- and inter-day) of the method. The linearity of the method was established by spiking in the blank plasma over a concentration range of 5 to 500 ng/ml. The limit of detection and LLOQ of the method was established based on signal-to-noise ratio of 3:1 and 5:1, respectively with acceptable precision and accuracy. Further, the stability of the method was analyzed at the four QC levels at six replicates for short-term (24 h at room temperature), long-term (30 days at -20°C), freeze-thaw (6 cycles) and stock solution stability (12 h at room temperature).

## RESULTS AND DISCUSSION

### Method development

The chromatographic conditions were optimized to elute the analytes in human plasma. The chromatographic conditions such as column, flow rate, column temperature, buffer pH and strength were optimized; the developed method was sensitive, rapid and accurate to determine irbesartan in human plasma. The optimized conditions include, Zorbax $C_{18}$ column (150 x 4.6 mm, 5$\mu$m), mobile phase (isocratic) consists of methanol and 0.2 % formic acid in water at the ratio 85:15, v/v using detection wavelength of 237 nm. The detection was carried out with a total runtime time of 7.0 min (Figure 2).

### Method validation

The chromatograms obtained indicate that no endogenous interferences were found at the elusion time of irbesartan (4.22 min) and IS (5.01 min), which indicates that the developed method is specific for the determination of the analytes

 

(Figure 2). The method developed was found to be linear over the concentration ranging from 5 to 500 ng/ml with a correlation coefficient of 0.9987. The LOD and LLOQ were the method found to be 1 ng/ml and 5 ng/ml respectively, indicating the high sensitivity of the developed method. The intra- and inter-day accuracy and precision results are shown in Table 1 . The precision (intra- and inter-day) results were represented in terms of percentage relative standard deviation (% RSD) with less than <10.0 %, indicating the precision of the method. The accuracy (intra- and inter-day) results (represented as % recovery) were obtained over the range of 96.41 to 99.31 %. Further, the stability studies were performed and the results obtained indicate the stability of the solutions and the results are summarized in Table 2.

## CONCLUSIONS

A sensitive, rapid and accurate HPLC-UV method was developed for the determination of irbesartan in human plasma. The developed method was validated as per the USFDA guidelines for specificity, accuracy, precision, linearity, stability and detection limit. When compared with the reported methods, the developed method involves the use of simple protein precipitation method with low plasma volume (100 $\mu$l), short analysis time (7.0 min) with good accuracy and precision.

### Conflict of Interest

None.

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None.

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