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Research Article

VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF IRBESARTAN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of irbesartan in bulk and tablet dosage form. Isocratic elution at a flow rate of 0.8mL/min was employed on a Symmetry C8 (150x4.6mm I.D., 5μm particle size) at ambient temperature. The mobile phase consisted of buffer (0.01M sodium dihydrogen orthophosphate, pH was adjusted to 3.0 with orthophosphoric acid): acetonitrile (50:50v/v). The UV detection wavelength was 209nm and 20μL of sample was injected. The retention time for irbesartan was 4.121min. The % recovery was within the range between 98.3% and 101.4%. The percentage RSD for precision and accuracy of the 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 and its formulations.

Keywords: Irbesartan, RP-HPLC, UV detection.

INTRODUCTION

Hypertension is the most prevalent cardiovascular disease in the developed as well as developing countries, affecting as many as one quarter of the adult population. Furthermore, 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 treatment for essential hypertension. Irbesartan is an orally active non-peptide specific angiotensin II receptor antagonist (AT_1 subtype) used, as a hypotensive agent does not require biotransformation into an active form[1]. Irbesartan is chemically described as a 2-butyl-3-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one (Fig. 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[2-3] and liquid chromatography [4-7] either in single or in combined forms**.** The aim of the present work is to develop and validate a simple, fast and reliable isocratic RP-HPLC method with UV detection for the determination of irbesartan in bulk and in tablet dosage forms. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the determination of irbesartan in bulk and tablet dosage forms.

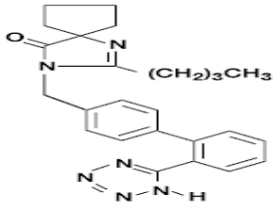

Fig. 1: Chemical structure of irbesartan

EXPERIMENTAL

Chemicals and reagents

HPLC grade acetonitrile, water and sodium dihydrogen orthophosphate was purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Irbesartan standard sample was provided by Dr. Reddy's Laboratories Ltd., Hyderabad, India.

Instrumentation and analytical conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase C8 column (150x4.6mm; 5µm), a 2695 binary pump, a 20µL injection loop and a 2487 dual absorbance detector and running on Waters Empower2 software. Isocratic elution with buffer: acetonitrile (50:50v/v, pH 3.0 adjusted with orthophosphoric acid) was used at a flow rate of 0.8mL/min. The mobile phase was prepared freshly and degassed by sonicating for 5min before use. The UV spectrum of irbesartan was taken using a Elico SL-159 UV-Visible spectrophotometer.

Stock and working standard solutions

Accurately weigh and transfer 10mg of irbesartan working standard into a 100mL volumetric flask, add about 70mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1mL of the above stock solution into a 10mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter. The calibration curve was plotted with the five concentrations of the 5-25µg/mL working standard solutions. Chromatogram was recorded thrice for each dilution. Calibration solutions were prepared daily and analyzed immediately after preparation.

Assay of irbesartan tablets

Weigh 20 irbesartan tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10mg of irbesartan into a 100mL volumetric flask. Add about 70mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 1mL of the above stock solution into a 10mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter. An aliquot of this solution was injected into HPLC system. Peak area of irbesartan was measured for the determination.

Validation procedure

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. Standard plots were constructed with five concentrations in the range of 5-25µg/mL prepared in triplicates to test linearity. The peak area of irbesartan was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared irbesartan test solution in the same equipment at a concentration value of 100% (10µg/mL) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of irbesartan was determined and precision was reported as %RSD.

Method accuracy was tested (%recovery and %RSD of individual measurements) by analyzing sample of irbesartan at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of irbesartan recovered in the samples. Sample solution short term stability was tested at ambient temperature (20±10^{0}C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re-injected after 24 and 48hrs at ambient temperature and compared with freshly prepared solutions.

RESULTS AND DISCUSSION

Selection of the detection wavelength

The UV spectra of irbesartan in 50:50v/v mixture of buffer and acetonitrile was scanned in the region between 200 and 400nm and shows λmax at 209nm.

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. The drug irbesartan is non-polar. Non-polar compounds preferably

analyzed by reverse phase columns. Among C8 and C18, C8 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of phosphate buffer and acetonitrile was selected as mobile phase and the effect of composition of mobile phase on the retention time of irbesartan was thoroughly investigated. The concentration of buffer and acetonitrile were optimized to give symmetric peak with short run time (Fig. 2). A short run time and the stability of peak asymmetry were observed in the ratio of 50:50% v/v of phosphate buffer and acetonitrile. It was found to be optimum mobile phase concentration.

Validation of method

Linearity

Five points calibration graphs was constructed covering a concentration range 5-25µg/mL (Three independent determinations were performed at each concentration). Linear relationships between the peak area signals of irbesartan the corresponding drug concentration was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 1.

Precision

The validated method was applied for the assay of commercial tablets containing irbesartan. Sample was analyzed for six times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented in good agreement with the labeled content. Assay results, expressed as the percentage of label claim, and was found to be 101.14 showing that the content of irbesartan in tablet formulations confirmed to the content of requirements (95 – 105%) of the label claim. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day on three consecutive days (n=3) indicated a RSD of 0.11. This indicates good method precision.

Accuracy

The data for accuracy were expressed in terms of percentage recoveries of irbesartan in the real samples. The mean recovery data of irbesartan in real sample were within the range of 98.3 and 101.4%. The mean %RSD was 99.4% satisfying the acceptance criteria for the study. It was proved that there is no interference due to excipients used in tablet formulation. Hence the accuracy of the method was confirmed. The results are furnished in Table 2.

Stability

The stability of irbesartan in standard and sample solutions containing determined by storing the solutions at ambient temperature (20±10^{0}C). The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48hrs, as during this time the results did not decrease below 98%. This denotes that irbesartan is stable in standard and sample solutions for at least 2days at ambient temperature.

System suitability

Various system suitability parameters were also calculated. It was observed that all the values are within the limits (Table 3). The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of irbesartan in tablet formulation. The results are furnished in Table 4.

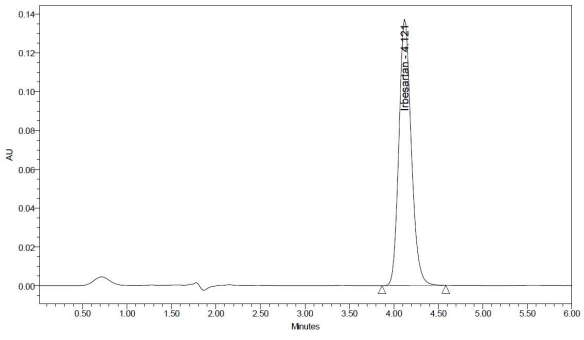

Fig. 2: Typical chromatogram of irbesartan

Table 1: Linearity of irbesartan

S. No.	Linearity Level	Concentration	Area
1	I	5µg/ml	686482
2	II	10µg/ml	1327024
3	III	15µg/ml	1880002
4	IV	20µg/ml	2487061
5	V	25µg/ml	3092080
Correlation coefficient			1.000

Table 2: Recovery studies of irbesartan

% Concen-tration	Area	Amount added (mg)	Amount found (mg)	% Recovery	% Mean Recovery
50	680637	5.1	5.17	101.4	99.4
100	1315694	10.15	9.99	98.4	
150	1877061	14.5	14.26	98.3	

Table 3: System stability parameters

Parameters	Values
λmax (nm)	209
Beer's law limit (µg/mL)	5-25
Correlation coefficient	1.000
Retention time (min)	4.121
Theoretical plates	4276
Tailing factor	1.17
Limit of detection (µg/mL)	0.01
Limit of quantitation (µg/mL)	0.04

Table 4: Assay result of tablet formulations

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Irovel	10	10.01	99.90
Xarb	10	9.98	100.20

CONCLUSION

A validated RP-HPLC method has been developed for the determination of irbesartan in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6min allows the analysis of a large number of samples in a short period of time. Therefore, it is suitable for the routine analysis of irbesartan in pharmaceutical dosage form.

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