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A NEW RP-HPLC METHOD DEVELOPMENTAND VALIDATION OF RP - HPLC METHOD FOR THE ESTIMATION OF IRBESARTAN FROM PHARMACEIICAL DOSAGE FORM

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ABSTRACT

A simple, precise, rapid and reproducible RP -HPLC method was developed and validated for the determination of Irbesartan in pharmaceutical dosage forms. Chromatography was performed on a Phenomenax LunaC_{18} (ODS) column (250 mm X 4.6 mm i.d. Particle size 5µ) water: Acetonitrile in the ratio 50:50 (v/v) as a mobile phase at a flow rate of 1 ml/ min. The detection was carried out at 226 nm using analytical Tech. UV-Visible detector SpD-10AVP. The obtained calibration curve was linear in the concentration range of 10–60µg/ ml. The limit of detection and quantification was found to be 0.2645 µg/ml and 0.6656 µg/ml respectively. It was found that the amount of Irbesartan present in the formulation (IROVEL) was 99.95%. The method was validated statistically using SD, %RSD and SE and the values are found to be within the limits. The recovery studies were performed and the percentage recovery was found to be 99.65 %.

Key words: Irbesartan**,** RP-HPLC, UV detection, Isocratic Elution, Development and validation of method in bulk and pharmaceutical formulations.

INTRODUCTION

Irbesartan is used mainly for the treatment of hypertension.1-7Irbesartan (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 is $C_{25}H_{28}N_6O$[1-7].

Literature survey revealed that numerous methods have been reported for estimation of Irbesartan in pharmaceutical formulations. Present study involves the 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. The important features and novelty of the proposed method included sonication of sample at ambient temperature treatment with sonication of small amount of powder sample at ambient temperature.

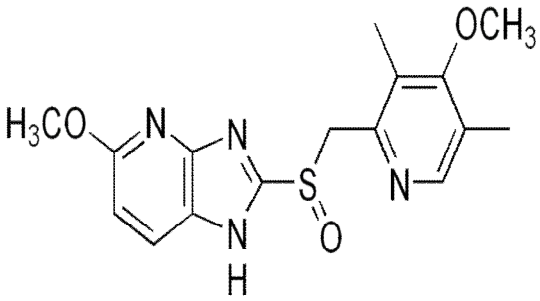

Fig.1 chemical Structure of Irbesartan

Experimental

Chemicals and reagents

HPLC grade Acetonitrile and water was purchased from SD fine Chemicals (Mumbai, India). Irbesartan standard sample was provided by Dr.Reddy's Laboratories (Hyderabad, India). Irovel commercial formulation (sunpharma-mumbai) was procured from local market. The tablet dosage forms obtained was containing 300 mg of IRB for oral administration.

Instrumentation and analytical conditions

The HPLC system (Analytical Technologies, Gujarath india) consisted of a pump.The Analytical column,a Phenomenax LunaC_{18}(250mm×4.6mmi.d., 5µ particle size) was operated at ambient temperature (20 ± 1°c). Isocratic elution with Acetonitrile: Phosphate buffer (45:55% v/v pH2.5) was used at a flow rate of 1ml/ min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use (Soltec, Soluzioni tecnologiche, Luglio, Italy). The UV spectrum of IRB for selecting the working wavelength detection was taken using UV -Visible spectrophotometer (Analytical Tech Gujarat).

Stock and working standard solutions

Stock standard solution of 1000µg/ ml of IRB was prepared freshly by accurately weighing 25mg of IRB into 25ml volumetric flask. Dissolved and made up to the volume with

Phosphate buffer (pH 2.5). The solution was diluted by pipetting 1ml into 25ml volumetric flask to obtain 300 µg/ ml solution.

The solution was further diluted with mobile phase in 10ml volumetric flask to obtain six working standards in the concentration of 10-60 µg/ ml of IRB. All the solutions were prepared in triplicates. Before being subjected to analysis, all the working standard solutions were filtered through 13mm membrane syringe filter (Pore size 0.2 µm).

Before injecting solutions, the column was equilibrated for at least 60 min with the mobile phase flowing through the system. The calibration curve was plotted with the six concentrations of the 10-60 µg/ ml working standard solutions. Chromatogram was recorded thrice for each dilution. Calibration solutions were prepared daily and analyzed immediately after preparation.

Assay of sample preparation

The contents of twenty commercial tablets (labeled concentration 300 mg of Irovel) were weighed and their mean mass was determined. After grinding the tablets into a fine powder in a glass mortar, an accurately weighed quantity of the tablet powder equivalent to 25 mg of IRB was quantitatively transfer into a 25 ml volumetric flask with about 20 ml of phosphate buffer pH 2.5. The solution was sonicated for 10 min, brought to the volume with phosphate buffer, mixed well and filtered through 13mm membrane syringe filter (pore size 0.2 µm). 1 ml test solution was transferred into 25 ml volumetric flask and made up to the volume with mobile phase (100 µg/ ml). 1.5 ml aliquot was transferred into a 10 ml volumetric flask. The theoretical IRB concentration after dilution was 30µg/ ml (100% of IRB). An aliquot of this solution was filtered through a 13mm membrane syringe filter (Pore size 0.2 µm) prior to the injection into the HPLC system. Peak area of Irbesartan was measured for the determinations.

Validation procedure

The objective of 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, short term stability and system suitability.

Standard plots were constructed with six concentrations in the range of 10 - 60 µg/ ml of IRB prepared in triplicates to test linearity. The peak area of IRB 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 IRB test solution in the same equipment at a concentration of 100% 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 IRB was determined and precision was reported as % R.S.D. Method accuracy was tested (% recovery and % R.S.D. of individual measurements) by analyzing samples of IRB at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of IRB recovered in the samples.

Sample solution short term stability was tested at ambient temperature (20 ± 1°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 48 hrs at ambient temperature and compared with freshly prepared solutions.

RESULTS AND DISCUSSION

Screening and optimization

Selection of the detection wavelength

The UV spectra of IRB in 50:50 v/v mixtures of phosphate buffer and Acetonitrile in the region between 200 and 3000 nm are shown in Fig 2. It shows that at 226 nm, IRB have maximum absorbance. Hence λ max of IRB in mobile phase was selected as an optimum detection wavelength for the quantification of IRB.

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends upon the nature of the sample, molecular weight and solubility. The drug IRB is a non polar. Non polar compounds preferably analyzed by reverse phase columns. Among C_8 and C_{18}, C_{18} 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 Acetonitrle was selected as mobile phase and the effect of composition of mobile phase on the retention time of IRB was thoroughly investigated. The concentration of acetonitrile (30-50%v/v) and water (300-50%v/v) were optimized to give symmetric peaks with short run

time (Fig 3). A short run time and the stability of peak asymmetry were observed in the ratio of 55:45 % v/v of phosphate buffer and Acetonitrile. It was found to be optimum mobile phase concentration.

Validation of methods

Linearity

Six point's calibration graphs were constructed covering a concentration range 10-60µg/ ml (Three independent determinations were performed at each concentration. Linear relationships between the of peak area signal of IRB & the corresponding drug concentration was observed as shown in Fig 4. The standard deviations of the slope and intercept were low. The determination coefficient (r^2) exceeded 0.9999.The statistical analysis of calibration is shown in table 1.

Precision

The validated method was applied for the assay of commercial tablets containing 300 mg of IRB: Irovel. Sample was analyzed for six times after extracting the drug similar procedure and 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, was found to be 99.95 ± 1.02 for Irovel showing that the content of IRB in tablet formulations confirmed to the content requirements (95 - 105 %) of the label claim. Low values of standard deviation denoted very good repeatability of the measurement.

Thus showing that the equipment used for the study worked correctly for the developed analytical method and being highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day and on three consecutive days (n=3) indicated a R.S.D. of 0.0455 and 0.03995% respectively. Both values were far below to 2%, the limit percentage indicated a good method precision. The results of analysis are shown in table 2 and table 3.

Accuracy

The data for accuracy were expressed in terms of percentage recoveries of IRB in the real samples. These results are summarized in table 4. The mean recovery data of IRB in real sample were within the range of 98.60 and 101.05 %. Mean % R.S.D. was 0.7269 %, satisfying the acceptance criteria for the study. It is proves that there is no interference due to excipients used in tablet formulation .Hence the accuracy of the method was conformed.

Stability

The stability of IRB in standard and sample solutions are determined by storing the solutions at ambient temperature (20 ± 1°C). The solutions were checked in triplicate after 3 successive days of storage and the data were compared with freshly prepared samples. In each case, it has noticed the solutions were stable for 48 hrs, as during this time the results did not decrease below 98%. This denotes that IRB is stable in standard and sample solutions for at least 2 days at ambient temperature.

System suitability

The system suitability parameter like capacity factor, asymmetric factor, tailing factor, HETP and No. of theoretical plates also calculated. It was observed that all the values are within the limits (table 5).

The statistical evaluation of the proposed method revealed its good linearity, reproducibility and its validation parameters for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of IRB in tablet formulation.

Table-01. Statistical analysis of calibration curves in the HPLC determination of Irbesartan (n=6)

Parameters	**Values**
λ_{max}(nm)	226
Linearity range	10-60
Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U)	1.41E065
Correlation coefficient (r)	0.9996
Regression equation (y=mx+c)	Y=382547.9X-166399.43
Slope(m)	386487.9
Intercept(c)	164399.53
LOD (µg/ml)	0.2505722
LOQ (µg/ml)	0.6623
Standard error of mean	13654.53

Table 2: Repeatability of IRB

S. No	Labelled claim (mg/tab)*	Amount found(mg)*	% purity obtained *	Average (%)	S.D.	% RSD	S.E
1.	300	299.96	99.96	99.95	1.02	1.656	0.623
2.	300	300.01	100.01				
3.	300	299.36	100.23				
4.	300	299.63	99.65				
5.	300	299.99	99.23				
6.	300	299.56	101.96				

* Mean of SIX observations

Table 4: Accuracy study for Irbesartan (n =9)

S. No	Percentage	% recovery	Mean ± S.D	% RSD	S.E
1	50%	98.60	99.55± 0.7211	0.7269	0.4196
2	100%	101.05			
3	150%	99.00			

* Mean of three observations

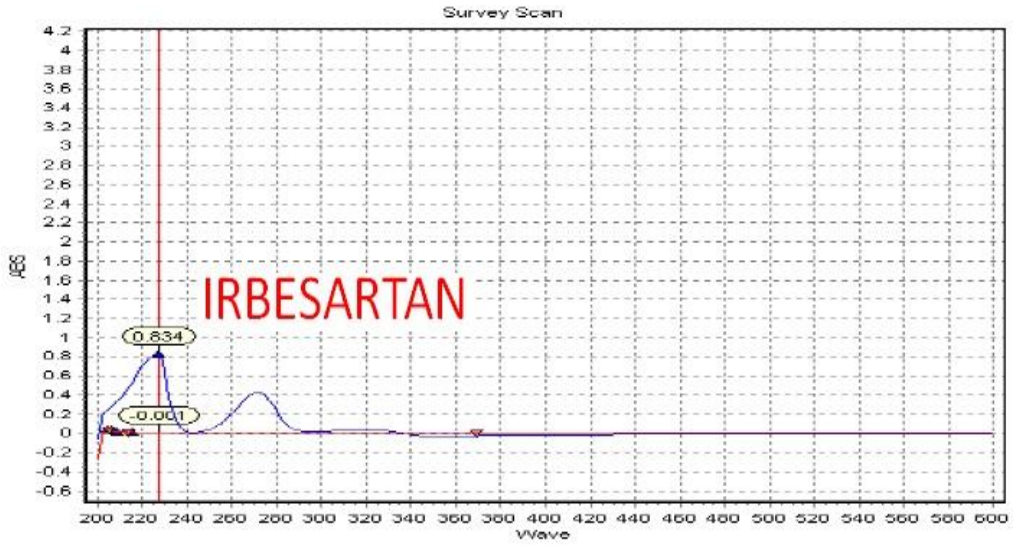

Fig.2. Absorption Spectrum of Irbesartan.

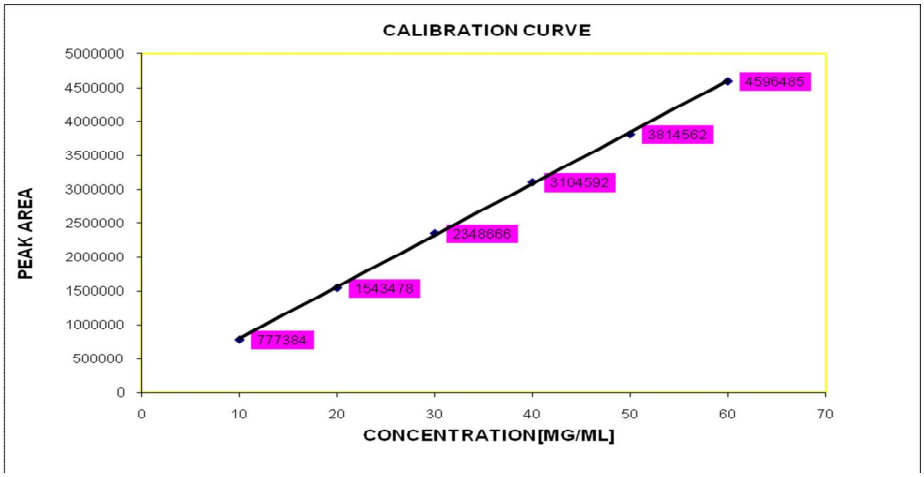

Fig.3 Calibration curve of Irbesartan

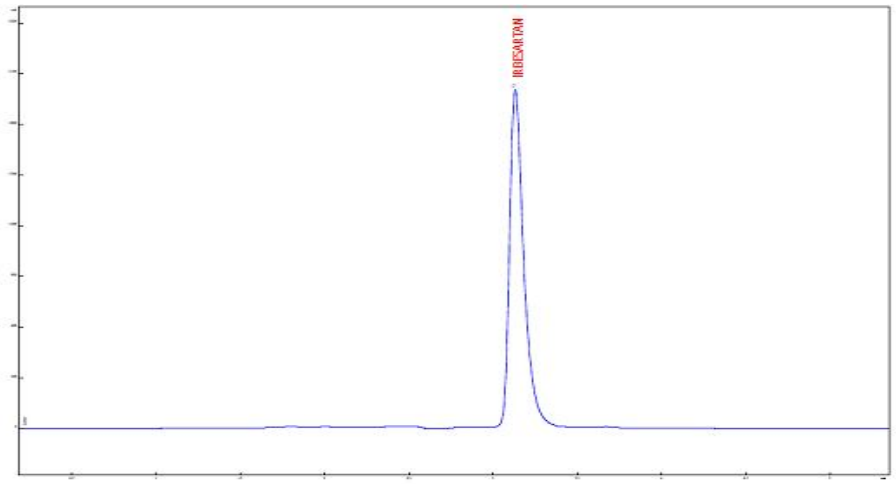

Fig.4 Irbesartan Optimization of chromatogram

CONCLUSION

A validated isocratic HPLC - UV 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 7 min 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. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as GC-MS that is complicated, costly and time consuming rather than a simple HPLC-UV method. Hence the proposed method could be useful for the national quality control laboratories in developing countries.

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