RESEARCH ARTICLE

METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF IRBESARTAN IN BULK DRUG AND PHARMACEUTCAL DOSAGE

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ABSTRACT:

Simple, sensitive, accurate, precise and rapid ultraviolet (UV) spectrophotometric method was developed for the estimation of Irebesartan in pure form, its formulations and stability samples. Sample recovery in both the formulations using the above method was in good agreement with their respective labeled claims, thus suggesting the validity of the method and non-interference of formulation excipients in the estimation. Detection wavelength was selected as 263 nm Linearity in response was observed in the range of 10-100 µg/ml having $R^2 = 0.999$. ($R^2 =$ not less then .996) The regeration equ. y=mx+c was calculated   as Y = The sandell's sensitivity for the developed method was found to be1.6269. The values were founds to be within the official limit. The percentage purity of three different samples, 12, 14, and 16µg/ml was found to be 92.44 ,87.49, 83.47respectively. The percentage recovery results revealed that the value is 100%, which indicates that the proposed method is accurate as the results are within the official limits.

Keywords: ultraviolet (UV) spectrophotometric method, Irebesrtan, recovery experiment, determination of linearity

INTRODUCTION:

AVAPRO® (irbesartan) is an angiotensin II receptor (AT1 subtype) antagonist. Irbesartan is a non-peptide compound, chemically described as a 2-butyl-3-[p-(o-1Htetrazol-5-ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one.

Its empirical formula is $C_{25}H_{28}N_6O$, and the structural formula:

Irbesartan is a white to off-white crystalline powder with a molecular weight of 428.5. It is a nonpolar compound with a partition coefficient (octanol/water) of 10.1 at pH of 7.4. Irbesartan is slightly soluble in alcohol and methylene chloride and practically insoluble in water. AVAPRO is available for oral administration in unscored tablets containing 75 mg, 150 mg, or 300 mg of irbesartan. Inactive ingredients include: lactose, microcrystalline cellulose, pregelatinized starch, croscarmellose sodium, poloxamer 188, silicon dioxide, and magnesium. It has also been tested for use in the treatment of high blood pressure (hypertension) Literature survey reveals that LC, HPTLC, HPLC for determination of content uniformity and simultaneous estimation of Irbesartan is reported, but there is no stability indicating high-performance liquid chromatography (HPLC) method for the determination of Irbesartan from its tablets, as its Pharmaceutical dosage form. The International Conference on Harmonization (ICH) guideline entitled 'Stability Testing of New Drug Substances and Products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidation is one of the required tests(ICH, 1993, 1996). The hydrolytic and the photolytic stability are also required. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products. A very viable alternative for stability-indicating analysis of Irbesartan is HPLC. The aim of the present work was to develop an accurate, specific, reproducible, and stability indicating method for the determination of Irbesartan in the presence of its degradation products and related impurities as per ICH guideline. Few analytical methods for the estimation of Irebesartan from plasma2-3, and metabolites including HPTLC4-5, HPLC6-7, and GC8 are reported. To the best of our knowledge, a very few Spectrophotometric methods have been reported. In view of the above fact, some rapid and sensitive analytical methods are in need for its quantitative estimation. The present work describes two simple and accurate spectrophotometric methods for the estimation of Irbesartan in bulk and dosage form stearate.

MATERIALS AND METHODS:

Drug: The standard sample of Irebesrtan was obtained as gift sample from Dr. Reddy's Laboratory Pvt. Ltd., Hyderabad, A.P., India. The Irebesrtan ablets were procured from local market, manufactured by RANBAXY Laboratories, India.

Instrument specifications: UV Spectrophotometer, Shimadzu, model 1800.

Chemicals and reagents used: Methanol obtained from local market, manufactured by Merck Pharmaceuticals.

Analytical assay - The samples were weighed on electronic analytical balance (Contech Model CB-50)

Preparation of Standard Solutions

The 10 mg of standard Irbesarten was weighed accurately and transferred into 100 ml volumetric flask. It was dissolved in methanol and diluted up to the mark by using

the same solvent to obtain a final concentration of 100μg/ml. The resulting solution was used as a working standard solution.

Preparation of Sample Solutions

For the sample solution each tablet containing 150mg of Irebesartan 20 tablets were taken and weighed, their mean weight was determined and finely powdered. An equivalent weighed (10mg) of the tablet content was transferred into a100ml volumetric flask containing 50ml of ethanol, sonicated for 30 min and diluted to 100ml with ethanol. The resulting solution was sonicated for 30 min and filtered through Whatmann filter paper no.0.45. This solution was used as test solution the methods.

Method for the Determination of detection wavelength

Standard stock solution of Irebesartan: 150 mg of Irebesartan was accurately weighed and transferred into a clean and dry 100 ml volumetric flask, dissolved with sufficient volume of methanol (AR grade). The volume was then made up to 100 ml with methanol to obtain the concentration of 1000 μg/ml (1mg/ml). Working standard solution: 1 ml of the stock solution was further diluted up to 10 ml volumetric flask with methanol to get a concentration 100 μg/ml. Serial dilutions of concentration range 2-30 μg/ml were prepared from the working standard solution. These dilutions were scanned in the UV range of 200 to 400 nm using methanol as blank. The normal mode graphs were converted to first order derivative by software UV probe Shimadzu 2.34. The detection wavelength was thus selected. Is shown in fig no – 1

Figure 1: Wavelength scanning and determination of absorption maximum

Detection wavelength was selected as 263 nm

Determination of linearity

Standard stock solution of Irebesartan: 150 mg of Irebesartan was accurately weighed and transferred into a clean and dry 100 ml volumetric flask, dissolved with sufficient volume of methanol (AR grade). The volume

was then made up to 100 ml with methanol to obtain the concentration of 1000 μg/ml (1mg/ml). Working standard solution: 1 ml of the stock solution was further diluted up to 10 ml volumetric flask with methanol to get a concentration 100 μg/ml. Serial dilutions of concentration range 2-100 μg/ml were prepared from the working standard solution. These dilutions were scanned at the detection wavelength of 263 nm using methanol as blank. The normal mode graphs were converted to first order derivative by software UV probe Shimadzu 2.34. The regression equation, Y- intercept and correlation coefficient were calculated. The linearity was thus determined, and a concentration range was selected

Figure 2: Standard Graph Of Irbesartan

Linearity in the response was obtained in the range of 10-100μg/ml and regration equation was calculated as y=mx+c

$$y=0.0253x+0.0278$$

$$R2=0.999$$

Figure 3: Derivative graph of the concentration range for Linearity

Determination of Sandell's Sensitivity

Standard stock solution of Irebesartan: **150** mg of Irbesartan was accurately weighed and transferred into a clean and dry 100 ml volumetric flask, dissolved with

sufficient volume of methanol (AR grade). The volume was then made up to 100 ml with methanol to obtain the concentration of 1000 µg/ml (1mg/ml). Working standard solution: 1 ml of the stock solution was further diluted up to 10 ml volumetric flask with methanol to get a concentration 100 µg/ml. Serial dilutions of the concentration range (10-20 µg/ml) were prepared from the working standard solution (100µg/ml). Each of the dilutions was scanned a several times by changing the dilution in the curette during scan at the detection wavelength 263 nm. Convert the obtained normal mode graphs to first order derivative. Note down the observations. From this observation table take the mean of the columns note down the observations in a table and calculate the Sandell's sensitivity from the formula below:

Sandell's Sensitivity (л) = Conc. (µg/100 ml) x $0.001/D_1$ value

Table 1: Sandell's Sensitivity

S .NO.	CONCENTRATION (µg/ml)	ABSORBANCE	SENSITIVITY	MEAN SENSITIVITY
1	10	0.4831	1.6801	1.6269
2	20	0.6120	1.6265	
3	30	0.7324	1.6212	
4	40	0.8031	1.6327	
5	50	1.2134	1.6089	
6	60	1.3215	1.6361	

The sandell's sensitivity for the developed method was found to be 1.6269 The values were founds to be within the official limit.

Limit of Detection and Limit of Quantitation

The LOD and LOQ were separately determined based on calibration curve. The residual standard deviation of a regression line or the standard deviation of y- intercepts of regression lines were used to calculate the LOD and LOQ From the observation table of Sandell's sensitivity, the regression equations, slope an intercept were calculated. The observations were noted down in observation table, the detection limit and quantification limit was calculated by statistical formula.

Table 2: The observations table of LOD and LOQ

SET	SLOPE	INTERCEPT
1	0.022	0.69
2	0.022	0.63
3	0.022	0.68
4	0.022	0.65
5	0.022	0.68
6	0.022	0.68
	MEAN =0.022	SD =0.000521

I. Formula for LOD (µg/ml);

$$LOD = 3.3 \times SD / S$$

Where,

SD = The standard deviation of the response
S = The slope of the calibration curve (mean).

II. Formula for LOQ (µg/ml);

$$LOQ = 10 \times SD / S$$

Where,

SD = The standard deviation of the response
S = The slope of the calibration curve (mean).

Accuracy/Recovery Studies

The stock solution of the formulation was prepared by following method (1mg/ml), Weigh powder equivalent to 50 m of Irebesartan. Dissolve in approximately 30-40 ml of solvent (methanol). Sonicate for 20-30 mins. Make up the volume up to 50 ml with the solvent (methanol). Filter & use. A stock solution of pure drug sample was prepared (1mg/ml).

The three dilutions in the following way:

To the 0.1 ml of the standard stock solution, add 0.05 ml of the test stock (formulation).
To the 0.1 ml of the standard stock solution, add 0.1 ml of the test stock (formulation).
To the 0.1 ml of the standard stock solution, add 0.15 ml of the test stock (formulation).

I. The three dilutions were scanned at detection wavelength (263 nm), and the D^1 values were noted down.

II. Amount of test recovered was calculated by the formula:

$(D^1$ test/ D^1 std) x conc. of std

Table 3: Accuracy study

S.NO.	TEST (µg/ml)	STANDARD (µg/ml)	D^1 VALUE AT 263 nm	CONC. (µg/ml)	AMOUNT OF TEST RECOVERED (µg/ml)	% RECOVERY
1	5	10	0.015	15	5	100
2	10	10	0.020	20	10	100
3	15	10	0.025	25	15	100

The percentage recovery results revealed that the value is 100%, which indicates that the proposed method is accurate as the results are within the official limits. It also reveals that the commonly used excipients and additives in the formulation were not interfering with the proposed method.

Assay

A method to analyze or quantify a substance in a sample. An assay is an analysis done to determine:

1. The presence of a substance and the amount of that substance.
2. The biological or pharmacological potency of a drug.

Table 4: Depicting the assay study

S.NO.	CONC. (µg/ml)	D^1 VALUE AT 263 nm	AMOUNT OBTAINED (mg)	% PURITY (w/w)
1	12	0.010	6.32	92.44
2	14	0.014	5.26	87.49
3	16	0.019	4.38	83.47

The percentage purity of three different samples, 12, 14 and 16µg/ml was found to be 92.44%, 87.49% and 83.47% respectively.

RESULT AND DISCUSSION:

Develop some new and sensitive analytical methods for the determination and validation of Irebesartan in bulk and pharmaceutical dosage forms. Detection wavelength was selected as 263 nm Linearity in response was observed in the range of 10-100 µg/ml having R^2 = 0.999. ($R^{2=}$ not less then .996) The regeration equ. y=0.0253x+0.0278 was calculated as Y = The sandell's sensitivity for the developed method was found to be1.6269. The values were founds to be within the official limit. The percentage purity of three different samples, 12, 14, and 16µg/ml was found to be 92.44 ,87.49, 83.47respectively. The percentage recovery results revealed that the value is

100%, which indicates that the proposed method is accurate as the results are within the official limits. It also reveals that the commonly used excipients and additives in the formulation were not interfering with the proposed method

CONCLUSION:

In view of the results, it can be inferred that the UV Spectroscopy may be applied to the determination of Irbesartan as an alternative to the Other UV methods and analytical HPLC methods. Although the precisions of both methods are similar, estimation of Irebesartan is slightly more exact in the derivative approach. Thus, it can be concluded that the Method Developed by the Spectroscopy in the present investigation is simple, sensitive, accurate, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Irebesartan.

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