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BIOANALYTICAL DETERMINATION OF IRBESARTAN IN BULK POWDER AND PHARMACEUTICAL PREPARATION USING SPECTROPHOTOMETRIC METHOD (FIRST ORDER)

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ABSTRACT

Irbesartan is chemically 2-butyl-3-({4-[2-(2H-1,2,3,4-tetrazol-5-yl) phenyl] phenyl} methyl) -1,3-diazaspiro [4.4] non-1-en-4-one. Irbesartan is an Angiotensin II receptor antagonist effective in the treatment of Hypertension. It is also effective in the treatment of High blood pressure. It is also effective when used alone or in combination with other drugs. Objective of the present study is to develop a simple, sensitive, accurate, precise and rapid first order derivative spectrophotometric method for the estimation of irbesartan in pure form. For the estimation of irbesartan, solvent system employed was 50% v/v aqueous ethanol and wavelength of detection (λ_{det}) was 237 nm. The linearity was obtained in the range 8 – 18 µg/ml, with a regression coefficient, R^2 = 1. The LOD & LOQ were found to be 0.5 µg/ml and 1.63 µg/ml respectively. Obtained results showed that there is minimum intra day and inter day variation. The developed method was validated and recovery studies were also carried out. Sample recovery 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. First order derivative spectroscopy method is simple, rapid and reproducible and further it can be used for the analysis.

Keywords – *Irbesartan, first order, derivative spectroscopy, UV spectroscopy, Validation.*

1. INTRODUCTION

Irbesartan is chemically 2-butyl-3-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl) -1,3-diazaspiro [4.4] non-1-en-4-one. Irbesartan is an Angiotensin II receptor antagonist effective in the treatment of Hypertension[1]. It is also effective in the treatment of High blood pressure[1]. It is also effective when used alone or in combination with other drugs. Irbesartan is a nonpeptide tetrazole derivative and an angiotensin II antagonist that selectively blocks the binding of angiotensin II to the AT1 receptor. In the renin-angiotensin system, angiotensin I is converted by angiotensin-converting enzyme (ACE) to form angiotensin II. Angiotensin II stimulates the adrenal cortex to synthesize and secrete aldosterone, which decreases the excretion of sodium and increases the excretion of potassium. Angiotensin II also acts as a vasoconstrictor in vascular smooth muscle[1]. Irbesartan, by blocking the binding of angiotensin II

to the AT1 receptor, promotes vasodilation and decreases the effects of aldosterone. The negative feedback regulation of angiotensin II on renin secretion is also inhibited, but the resulting rise in plasma renin concentrations and consequent rise in angiotensin II plasma concentrations do not counteract the blood pressure–lowering effect that occurs. The action of ARBs is different from ACE inhibitors, which block the conversion of angiotensin I to angiotensin II, meaning that the production of angiotensin II is not completely inhibited, as the hormone can be formed via other enzymes. Also, unlike ACE inhibitors, irbesartan and other ARBs do not interfere with response to bradykinins and substance P, which allows for the absence of adverse effects that are present in ACE inhibitors (e.g. dry cough)[1].

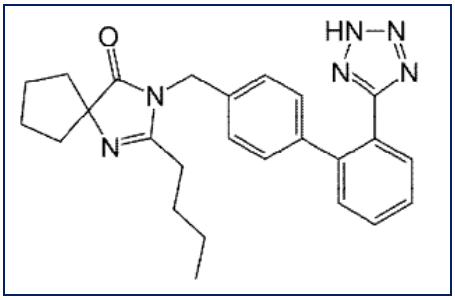

Fig.1 : Structure of Irbesartan

UV spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (190-380nm). The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy.

Literature review suggested several analytical methods that have been reported for the estimation of Irbesartan in bulk or pharmaceutical formulations include High Performance Liquid Chromatography, and UV-Visible Spectrophotometry. Literature review also revealed that there is no bioanalytical method development by first order derivative Spectroscopic method. The objective of the work was to develop simple, accurate, precise and economic first order derivative Spectroscopic method to estimate the candesartan in bulk. The method should be simple, accurate, precise, reproducible and statistically valid.

Thus, the objectives of project were:

I. To develop a simple, precise, accurate method, less time consuming & economical derivative spectroscopic method.
II. Under derivative spectroscopy, the bioanalytical method development by first Order derivative Method.
III. Validation of developed method using common parameters.

2. MATERIALS AND METHODS

2.1 Drug

The standard sample of IBRESARTAN was obtained as gift sample from Dr. Reddy's Laboratory Pvt. Ltd., Hyderabad, A.P., India. The irbesartan tablets were procured from local market.

2.2 Animal model

Wistar Rats (200 – 250 g), for bioanalytical method development by first order derivative spectroscopy, were acquired from M/S Ghosh Enterprises, Kolkata.

Note: Permission has been obtained from IAEC for performing experiment on animal models, Form B, per rule 8(a).

Reg. no. 1188; Protocol No: SRIP/IAEC/2011-12/04 dated 13/02/2012.

2.3 Instrument used (specifications)

UV Spectrophotometer, Shimadzu, Model 1800.

2.4 Chemicals and reagents used

Methanol obtained from local market, manufactured by Merck Pharmaceuticals.

2.5 Preparation of stock solution

The stock solution of irbesartan is prepared by dissolving 100 mg of drug in 100 ml methanol in volumetric flask with continuous shaking; 0.08 ml of sample was withdrawn and diluted to 10 ml methanol to get 8 µg/ml of solution. The solution was than scanned in UV range between 200-400 nm UV-VIS Spectrophotometer, Shimadzu, Japan to determine the absorption maxima of the drug against blank as methanol. Convert the spectra to first order derivative by software UV Probe 2.34.

2.6 Extraction from plasma

A suitable amount of standard solution of Salmeterol was added to 0.5 ml plasma sample. Methanol was used as the precipitating agent and was added to drug spiked plasma. The solutions were vortex mixed for 1minute and centrifuged at 5000 rpm for 15 mins using an Eltek, Model TC 650 D centrifugal device. The clear supernatant liquid was separated, the solutions were then filtered with 0.45 µ filter and the extracted Salmeterol was reconstituted with the solvent methanol. The appropriate dilutions were prepared in the above stated manner. And transfer it to quartz cell for analysis.

2.7 Wavelength scanning and determination of absorption maximum

With the stock solution of Salmeterol, known concentration of 10µg/ml plasma standard is prepared by suitable dilution with methanol. Wavelength scanned for the maximum absorption of drug solution using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against blank methanol. Convert the normal mode obtained spectra to first order derivative. The wavelength that shows the peak with a highest absorbance is considered as absorbance maximum of the drug. The result is presented in fig. 2

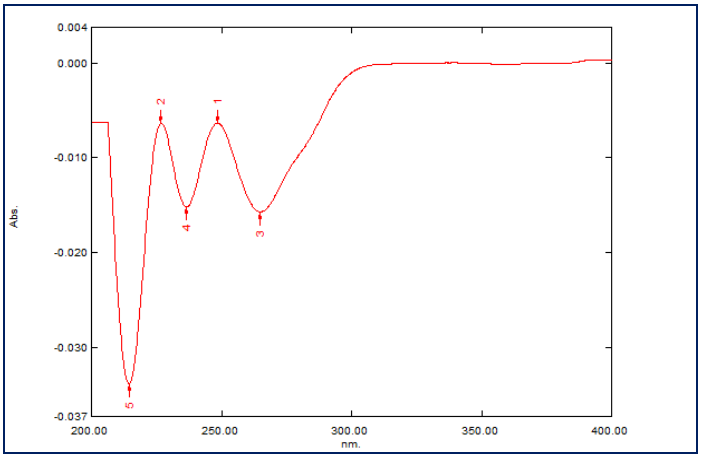

Fig. 2. Wavelength scanning and determination of absorption maximum

2.8 Method validation

The validation of method was carried out by establishing linearity, limit of detection (LOD) and limit of quantification (LOQ) and within- and between- day precision and accuracy.

2.8.1 Linearity studies

Plasma standard solutions were prepared of concentrations 8µg/ml, 10µg/ml, 12µg/ml 14µg/ml, 16µg/ml, 18µg/ml 20µg/ml. Convert the normal mode obtained spectra to first order derivative. The results are tabulated and the linearity curve was constructed by plotting concentration vs. D^1 value. The result is presented in table 1 and fig. 3. Figure 4 presents the linearity obtained from the derivative spectra.

Table 1. Linearity of Irbesartan

S.No.	Concentration (µg/ml)	D^1 value at detection wavelength (237 nm)
1	8	0.012
2	10	0.015
3	12	0.018
4	14	0.021
5	16	0.024
6	18	0.027

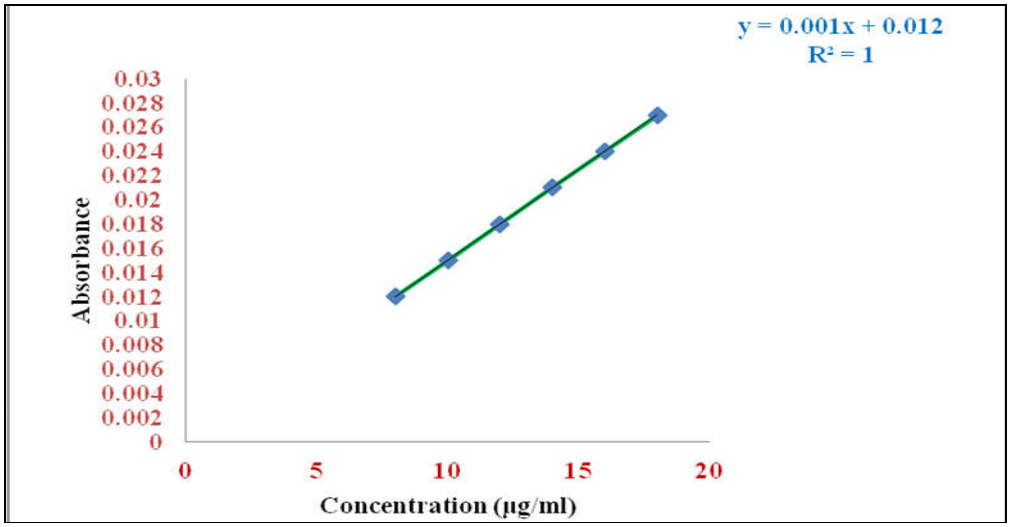

Fig. 3. Standard graph of Irbesartan

2.8.2 Precision

The precision of method was ascertained; the percent relative standard deviation were calculated and presented.

2.8.3 Inter day and intraday studies

The prepared stock solution was subsequently diluted to get 8µg/ml, 10µg/ml, 12µg/ml 14µg/ml, 16µg/ml and 18µg/ml. The resulting solutions absorbance was measured at detection wavelength of 237 nm using double beam UV spectrophotometer against blank of methanol. The findings was made at different time intervals in a day times in a day and performed continuously for six days. Convert the normal mode obtained spectra to first order derivative. The results obtained were tabulated and studied for inter day and intraday variation. The results are presented in table 2 (a) and 2 (b).

Table 2(a). Results of intraday precision

S. No.	Concentration (µg/ml)	D^1 value at detection wavelength (237 nm)				
		Time (Mins)	I	II	III	Mean
1	14	0 min.	0.020	0.020	0.020	0.020
2	14	15 min	0.020	0.020	0.021	0.02033
3	14	30 min	0.020	0.020	0.021	0.02033
4	14	60 min	0.020	0.021	0.020	0.02033
5	14	120 min	0.021	0.020	0.020	0.02033
6	14	180 min	0.021	0.021	0.021	0.021
7	14	240 min	0.020	0.020	0.020	0.020
					Mean =	**0.020**
					SD =	**0.000333**
					% RSD =	**1.66**

Table 2(b): Results of Inter day precision

S. No.	Conc. (µg/ml)	Days	I	II	III	D^1 at 237 nm (mean)
1	14	1st	0.021	0.021	0.020	0.021
2	14	2nd	0.020	0.020	0.021	0.02033
3	14	3rd	0.020	0.020	0.021	0.02033
4	14	4th	0.021	0.021	0.020	0.022066
5	14	5th	0.021	0.020	0.020	0.02033
6	14	6th	0.021	0.021	0.021	0.021
Mean =						**0.020**
SD =						**0.000329**
% RSD =						**1.64**

2.8.4 Accuracy studies

The accuracy/recovery studies were carried out with the commercial preparation of Salmeterol and percentage recoveries was calculated. Convert the normal mode obtained spectra to first order derivative. The reproducibility of estimation was determined by performing the drug content of different samples. The results of accuracy studies were expressed in %. The result is presented in table 3.

Table 3: Results of Accuracy studies

S. No.	Test (µg/ml)	Standard (µg/ml)	D^1 value at 237 nm	Conc. (µg/ml)	Amount of test recovered (µg/ml)	% Recovery
A	3	6	0.0136	9.06	3.06	102.2%
C	6	6	0.018	12.00	6.00	100.0%
3	9	6	0.022	14.66	8.66	96.22%

2.8.5 Assay studies

The assay studies were carried out with the help of commercial preparation of Salmeterol. The percentage purity was calculated. Convert the normal mode obtained spectra to first order derivative. The reproducibility of estimation was determined by performing the drug content of different samples. The results of assay studies were expressed in %. The result is presented in table 4.

Table 4. Results of Assay

S. No.	Conc. (µg/ml)	D^1 value at 268.8 nm	Conc. of drug in test solution (µg/ml)	% Purity (w/w)
1	14	0.021	14	100
2	14	0.021	14	100
3	14	0.021	14	100

2.8.6 Stability of Plasma samples

To determine the stability in plasma at ambient and refrigeration temperature, four sets of low (4 µg/ml), medium (7 µg/ml) and high (9 µg/ml) concentrations of the spiked calibration standards in plasma were divided in to 18 test tubes. One set of spiked samples was assayed immediately and was considered as standard (100 %). Two of the sets were stored at a temperature of -4^o C in a refrigerator for 24 hours and one week. Then one of the sets was stored at -20^o C in a deep freeze for one week. The remaining set was allowed to stand at ambient temperature for 24 hours. Stability measurements then carried out. The results were evaluated and comparing these measurements with those of standards and expressed as percentage deviation. The results are summarized in table 3. The plasma samples were found to be stable for over one week at -20^o C (deep freeze) and 24 hours at room temperature and at -4^o C

(refrigerator). The stability of analyte stock solution was tested for over 7 days in the same assay. The results indicated stability of stock solution for over 7 days at both room temperature and refrigerator temperature. The results are presented in table 5.

Table 5: Results of stability study

S. No.	Concentration (µg/ml)	Room temperature Stability (%) 24 h	Freeze thaw stability (%) 24 h	Freeze thaw stability (%) 1 week	Deep freeze stability (%) 1 week
1	8	100	100	100	100
2	12	100	100	90	88
3	16	100	100	90	90

3. CONCLUSION

For routine analytical purposes, it is always of interest to establish methods capable of analyzing a large number of samples in a short time period with due accuracy and precision. Spectrophotometric techniques can generate large amounts of data within a short period of analysis. In the present study a simple, accurate, precise and economic first order bioanalytical derivative spectroscopic method was developed to estimate the Salmeterol in bulk and pharmaceutical dosage forms. The developed bioanalytical method for Salmeterol by using first order derivative spectroscopy is found to simple, rapid and selective and the amount of drug recovered were same as the label claim and precise. It can be conveniently employed for the routine analysis and quantification of Salmeterol.

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