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## Development of spectrophotometric method to assay irbesartan in pure and in pharmaceutical dosage form using diazotization reaction

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**Abstract**--This study includes the development of an accurate and cost-effective spectrophotometric method for the determination of irbesartan in pure form and in its formulation dosages. In this strategy, m-Aminophenol reagent was displaced with an excess amount of sodium nitrite in an acidic medium and the resulting diazonium salt followed by coupling with irbesartan in the base arrangement of NaOH to create a water-soluble yellow azo dye has maximum absorption at 444 nm versus reagent blank. Under optimum conditions, the linearity of the method obeyed Beer's law in the concentration range 1- 8 µg/ml with an excellent determination coefficient ( $R^2 = 0.9993$ ) and molar absorptivity  $9.856 \times 10^4$  l/mol.cm. and sandell's sensitivity index value equal to  $0.00434 \mu\text{g}/\text{cm}^2$ . The detection limit (LOD) and quantification limit (LOQ) have also been estimated and their values were found to be 0.452 and 0.876 µg/ ml, respectively. A relative error% was calculated and found in the range - 0.5% to 2.64%, while the precision (RSD) was estimated as  $\leq 2.17\%$ . The stoichiometry of the resulting azo dye was found to be 1:1 irbesartan : m-aminophenol. The suggested procedure was applied to the analysis of irbesartan in tablets.

**Keywords**--irbesartan, diazotization, m-aminophenol, spectrophotometry.

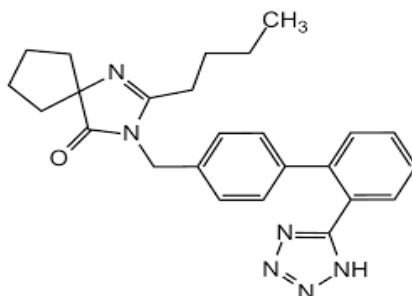
### Introduction

Irbesartan, (IRBN) is chemically called 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, (IRBN) is a potent,

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long-acting angiotensin II (AII) receptor antagonist, with high selectivity for the AT1 subtype (Fazlul Huq, 2007). (IRBN) 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. IRBN is slightly soluble in alcohol and methylene chloride and practically insoluble in water (Laxmi *et al.*, 2013).



$C_{25}H_{28}N_6O$

M.Wt. = 428.53 g/mol

IRBN is receptor agonists shielded and is probably lower average than previous classes of drugs used to treat hypertension, and it has shown good response in the treatment of diabetic nephropathy IRBN actually lowers pulse at daily dose and biotransformation regimen in human. (Theodore *et al.*, 1998). IRBN is mainly used to treat high blood pressure and congestive heart failure. and chronic kidney failure. (Weber, 1997),

A survey of the literature revealed that several methods have been used to estimate IRBN both purely and in pharmaceutical preparations, directly or indirectly for IRBN, and they include: spectrophotometric methods (Biyani and Yadav, 2019), (Khalid *et al.*, 2017), (Jadhav *et al.*, 2014) and (Syeda *et al.*, 2011), HPLC (Alanazi *et al.*, 2014), (Koyuturk *et al.*, 2014), (Goswami, 2014) and (Vujić *et al.*, 2012), LC-MS (Qui *et al.*, 2014) and (Chi and Chia 2011). The primary objective of the current review was to improve a rational, rapid and modest method for testing irbesartan in its pharmaceutical preparations for routine quality control examination, given spectroscopic analysis, furthermore the procedure has been effectively applied for assurance of IRBN in tablets.

## Experimental Apparatus

A double beam UV-visible spectrophotometer (JASCOV-630) with 1.0-cm quartz cells was used for all absorbance measurements and absorption spectra. While a professional Benchtop pH meter BP3001 was used for the pH measurements.

## Chemical reagents

All synthetic substances utilized were of the greatest virtue accessible and were acquired from Fluka, BDH and Merck organizations. The standard material of IRBN was supplied by the organization to sedate businesses and clinical apparatuses (Dar Al Dawa), JORDAN.

IRBN stock standard solution 500  $\mu\text{g}/\text{ml}$  0.050 g of pure IRTN was dissolved in about 5 mL of ethanol by heating and shaking, and then volume was made up to 100 ml with ethanol in a volumetric flask. This solution has been kept in a dark bottle. Similarly, a working buffer solution ( $100 \mu\text{g}/\text{ml} = 2.3335 \times 10^{-4} \text{ M}$ ) of IRBN was freshly prepared by diluting 10 ml of the stock solution with distilled water in a titrated 100 ml beaker.

m-Aminophenol (m-AP) solution  $100 \mu\text{g}/\text{ml}$  ( $9.163 \times 10^{-4} \text{ M}$ ): A 0.010 g of m-AP reagent was dissolved in small portions of distilled water then the volume was completed to 100 ml in a standardized flask with the same solvent. The solution was then transferred to a dark bottle.

Sodium nitrite solution 0.5 % : This solution was prepared by dissolving 0.5 g of  $\text{NaNO}_2$  in 100 ml distilled water using a volumetric flask. The solution was then transferred to a dark bottle.

Urea solution 1 % : prepared by dissolving 1 g of urea in 10 ml distilled water with shaking and lightly heating, then completed the volume to 100 ml with distilled water using a volumetric flask, then solution was then transferred to a dark bottle.

hydrochloric acid 1 mol/L : It was prepared by diluting 8.47 ml of concentrated hydrochloric acid to 100 ml with distilled water.

Sodium hydroxide solution (1M) was also prepared.

### **Recommended procedure and calibration curve**

Into a series of 25 ml calibrated flasks, 2 ml of  $100 \mu\text{g}/\text{ml}$  m-AP reagent, 0.5 ml of 0.5 %  $\text{NaNO}_2$  and 1 ml of 1M hydrochloric acid then kept constant at room temperature for 5 min followed by adding 0.5 ml of 1% urea and add 0.1 to 4 ml of  $100 \mu\text{g}/\text{ml}$  pure IRBN solution. The contents were mixed thoroughly and 1.5 ml of 1M sodium hydroxide solution. The contents are shaken well and fill it up to the mark with distilled water. The absorbance of the azo dye formed was recorded at 444 nm against blank solution that was prepared similarly but without drug. A linear relationship between absorption and IRBN concentration in the range 25–200  $\mu\text{g}$  of IRBN/25 ml was obtained (Fig.1). The apparent molar absorptivity and Sandell's sensitivity were equal to be  $9.856 \times 10^4 \text{ l}/\text{mol}\cdot\text{cm}$ . and  $0.00434 \mu\text{g}/\text{cm}^2$ , respectively.

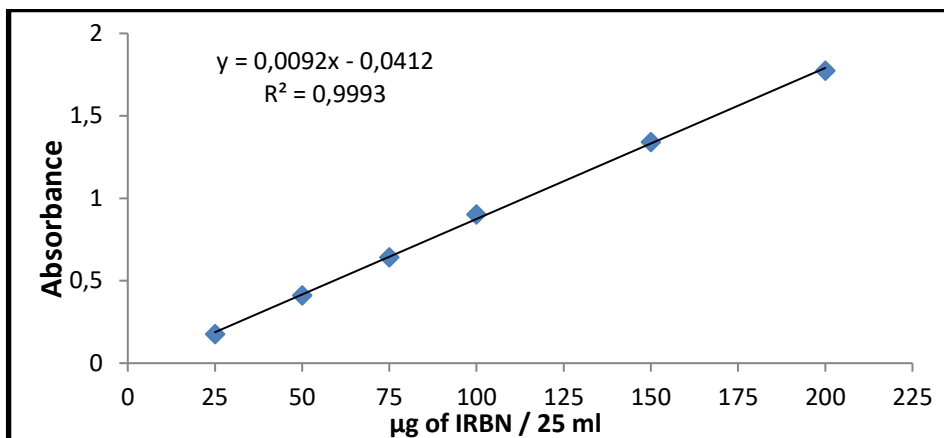


Fig. 1: The calibration curve for IRBN determination

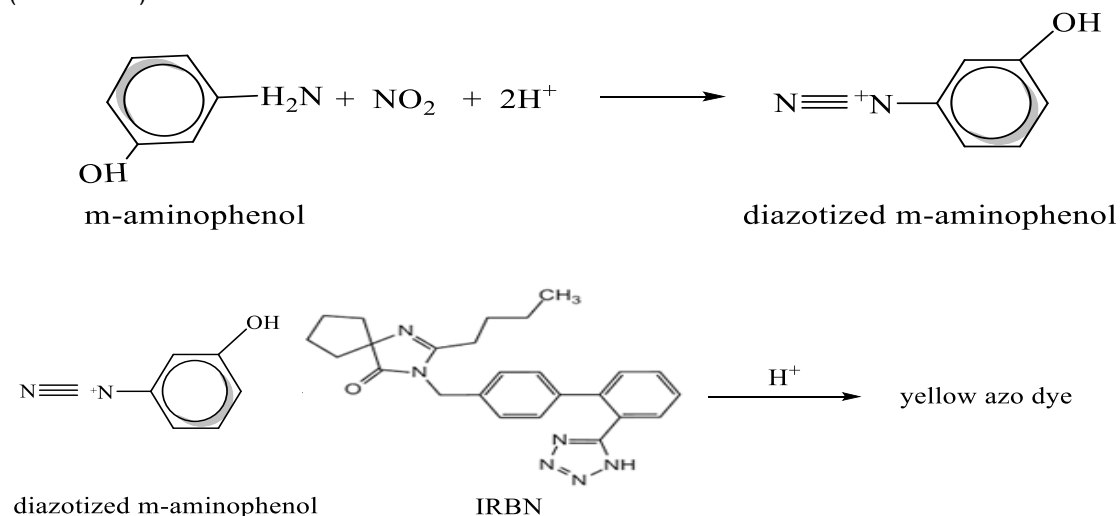
### Recommended procedure to assay IRBN in drug

IRBN tablets ( GIZAN ) solution 100 µg/ml : Five tablets were weighed and crushed into a fine powder, then a portion 0.017g from the powder equivalent to 0.01 g of pure IRBN was weighed and dissolved in 5 ml ethanol by heating and will be stirred, then filtered with filter paper, the filter transferred to a 100 ml volumetric flask then the volume is completed to the mark using water distilled; This solution was treated as a generic procedure.

### Results and Discussion

#### Preliminary conditions

Under the response condition, m-aminophenol was reacted with an excess of  $\text{NaNO}_2$  in acidic solution to give the relating diazonium salt, which goes through a diazotization procedure with IRBN as a coupling agent to produce the azo dye (scheme 1):



**Scheme 1**

The azo dye gave maximum absorption at 444 nm against blank solution .The dye intensity has been found to be relative to the IRBN amount that originally present in the solution.

### Optimum conditions for the reaction

#### Effect of different amounts of various acids on the absorbance

The reaction of diazotisation requires acid solution . the effect of different amounts of various acidic solutions (1M) such as HCl , HCOOH , HNO<sub>3</sub> and CH<sub>3</sub>COOH has been examined on the dye colored . The experimental results are shown in Fig.2 .

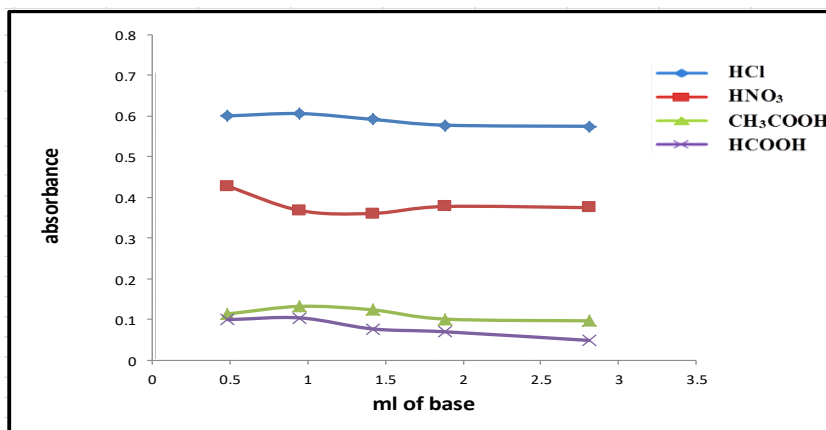


Fig. 2: Effect of different amounts of various acids on absorbance of azo dye

The data in Figure 2 shows that HCl was suitable as an acidic medium to achieve maximum stability and sensitivity and that 1 mL of 1M HCl was the best amount that could be used for this method.

#### Effect of amount and time for sodium nitrite

The diazotization process of m-AP was investigated by adding different amounts 0.2-2.0 ml of NaNO<sub>2</sub> solution (0.5 %) at different times .The results are shown in Fig.3.

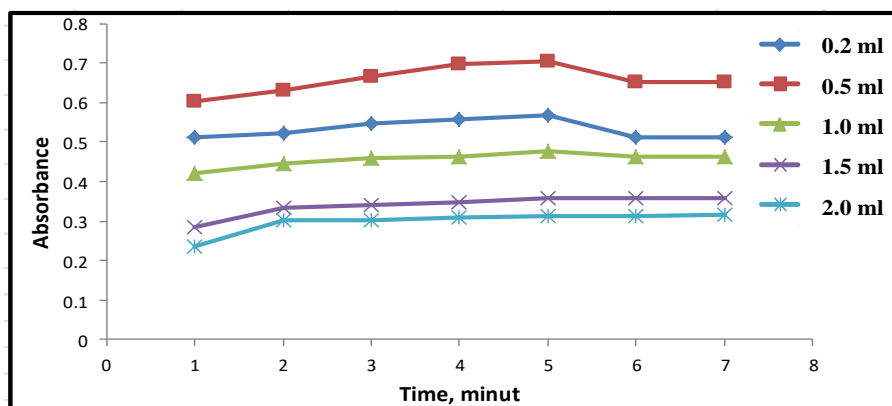


Fig. 3: Effect of amount of sodium nitrite and time

The results in above figure indicate that the diazotization process of m-AP was completed after 5 minutes when 0.5 ml of 0.1%  $\text{NaNO}_2$  solution was added because this amounts show maximum absorption therefore, they have been selected for subsequent experiment.

#### Effect of Urea amount

In this step we study the effect of different amounts 0.1-1.0 ml of 1% Urea on absorbance of the resulting azo dye. Fig.4 show that 0.5 ml of 1% Urea was enough to destroy the excess of sodium nitrite (Clayden, 2001) and it recommended for the all subsequent experiments.

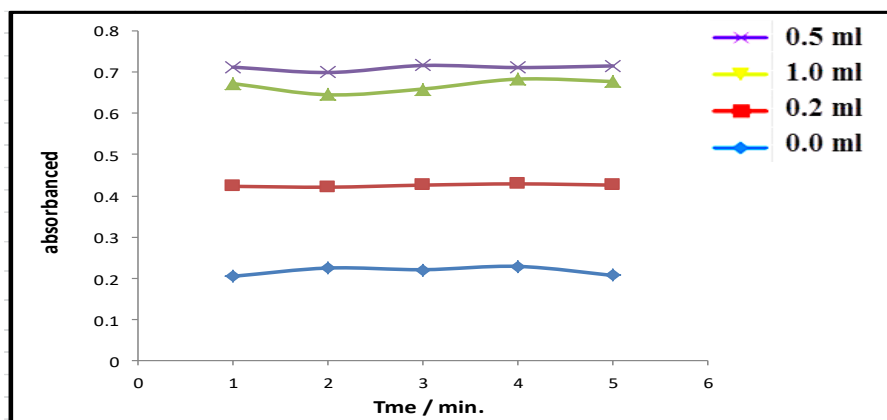


Fig. 4: Effect of Urea amount and the time on absorbance

#### Effect of m-AP amount

The influence of various amounts (0.5-3.0) ml of 100  $\mu\text{g}$  m-AP reagent on the azo dye absorbance has been investigated. The results in Fig.5 indicated that 2 ml of m-AP are the most suitable volume to give high absorbance and good determination coefficient value ( $R^2=0.9996$ ) therefore, it was selected for coupling reaction.

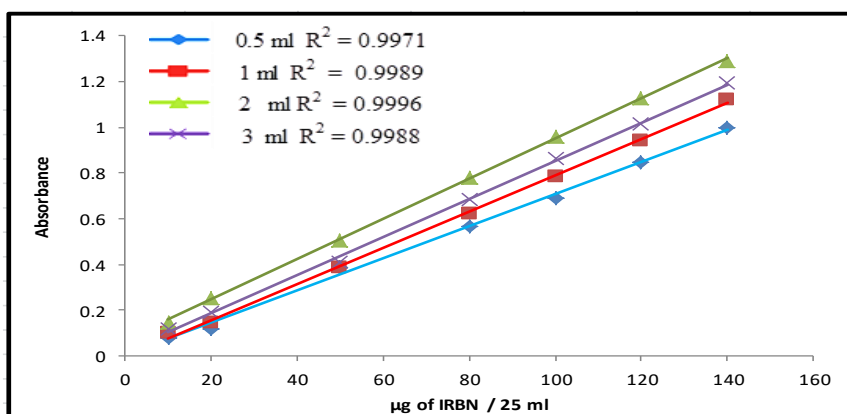


Fig. 5: Effect of reagent amounts on absorbance

### Effect of alkaline solution

The effect of different amounts of 0.25-3 ml of different alkaline solutions (1M) such as sodium hydroxide, sodium carbonate, sodium bicarbonate and potassium hydroxide was investigated for the purpose of producing azo dye with intense color and low vacuum value. The results in Fig.6 showed higher volume of sensitivity and stability were obtained, while sodium carbonate and sodium bicarbonate showed low sensitivity which may be due to the pH difference. 1.5 ml of 1M NaOH (pH = 11.9) was found to be optimal.

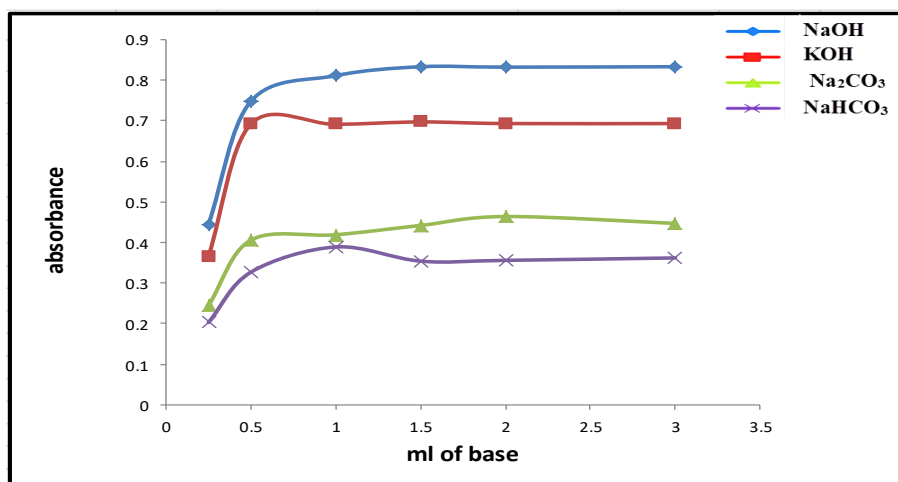


Fig. 6: Effect of different amounts of various bases (1M) on absorbance

### Development time and stability period

Under the optimal experimental conditions the influence of time on the stability of the colored azo dye at 444 nm has been carried out by preparing two different amounts 50 and 150 µg of IRBN. The absorbance was measured at dissimilar pauses of time up to 60 minutes. The results in Fig.7 illustrate that the absorbance of azo dye reached maximum value after the reaction mixture solution

was mixed and the absorbance remained stable for at least 60 minutes at room temperature .

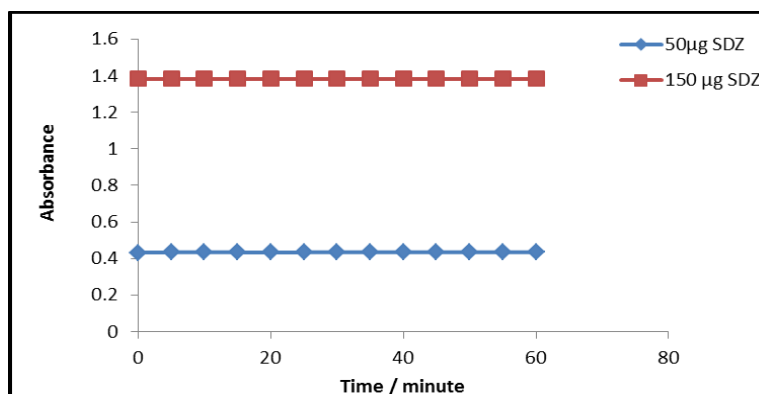


Fig. 7: Effect of time on absorbance.

### Absorption spectrum

Under the optimum reaction conditions and in 25 ml volumetric flask the 2ml of 100 µg/ml m-AP reagent was reacted with 0.5 ml of 0.5 % NaNO<sub>2</sub> solution in presence of 1ml of 1M HCl solution and after standing for 5 minutes we add 0.5 ml of 1% urea get rid of unreacted NaNO<sub>2</sub> and give the corresponding diazonium salt. The diazonium salt was then coupled with 1ml of 100 µg/ml IRBN in 1.5 ml of a basic solution of 1M NaOH. A yellow water soluble azo dye was obtained which presented maximum absorption at 444 nm versus the colorless reagent blank (Fig.8). The intensity of the azo dye formed was found to be relative to the amount of IRBN originally present in the solution.

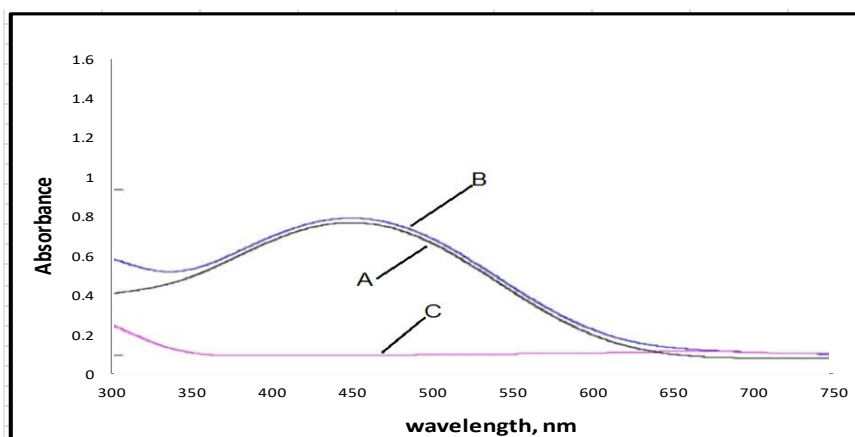


Fig. 8. Absorption spectra of 100 ppm IRBN treated according to the recommended procedure versus (A) reagent blank , (B) distilled water and (C) blank solution measured versus distilled water

## Quantification

The limits of Beer's law, molar absorptivity ( $\epsilon_{\max}$ ), accuracy (recovery%), precision (RSD), conditional stability constant (Hargis, 1988), The LOD and LOQ values were calculated and the results are shown in Table 1, and these results indicate that the proposed method is sensitive, accurate and accurate. The linearity of the method was studied by calculating the regression equation and the corresponding estimation factor ( $R^2$ ) for the IRBN determined by the proposed method represents good linearity.

Table 1. optical characteristics and statistical data for the proposed method

Parameters	Data
Limits of Beer's law , $\mu\text{g/ml}$	0.1-8
$\lambda_{\max}$ , nm	444
$\epsilon_{\max}$ , l/mol.cm	$9.856 \times 10^4$
Range of recovery* , %	99.5% to 102.64%
Relative error range* , %	- 0. 5% to 2.64%
RSD* , %	$\leq 2.17\%$
Sandall's sensitivity , $\mu\text{g/cm}^2$	0.00434
Determination coefficient ( $R^2$ )	0.9993
Average of stability constant (K) , l/mol	$0.52 \times 10^7$
LOD , $\mu\text{g/ml}$	0.452
LOQ , $\mu\text{g/ml}$	0.876
Slope (a)#	0.0092
Intercept (b)#	0.0412

# Regression equation  $X = aY + b$  , where Y is [IRBN] in  $\mu\text{g/ml}$ .

\*Average of five determinations

## Composition of the azo dye

The final composition of the product was examined under predetermined optimal conditions by applying the methods of continuous variance (Job's) and molar ratio (Delevie, 1997). In the continuous contrast method, 0.5-4.5 mL volumes of (100  $\mu\text{g/mL}$ )  $9.163 \times 10^{-4}$  M m-AP reagent (VS) were denatured using 0.5 mL of 0.5% sodium nitrite and 1 mL of 1M HCl and paired according to the recommended procedure. It with the corresponding additional volume of  $2.3335 \times 10^{-4}$  M portions of IRBN solution (VR) to give a total volume of 5 ml for (VS + VR) Then we added 1.5 ml of 1M NaOH and diluted with distilled water to 25 ml.

In the molar ratio method, volumes are increased from 1 to 9 mL of (100  $\mu\text{g/mL}$ )  $9.163 \times 10^{-4}$  M of the reagent reacted by 0.5 mL of 0.5% sodium nitrite in the presence of 1 mL of 1M hydrochloride (VR) , to 1 ml of  $2.3335 \times 10^{-4}$  M IRBN (VS), 1.5 ml of 1M NaOH was added and the absorbance was recorded at 444 nm after diluting to the mark with distilled water. The results obtained in Figure 9 are revealed that the azo dye was formed in a 1:1 ratio of isolated IRBN to m-AP.



### Application of the method

The present method was applied successfully to the analysis of IRBN in pharmaceutical preparations containing IRBN (Tablets). The results are summarized in Table 2 reveal that the proposed procedure is in good agreement and with the declared content.

Table 2. Determination of IRBN in pharmaceutical preparations

Drug	IRBN Present ( $\mu\text{g}$ )	IRBN Found ( $\mu\text{g}$ )	Relative error (%) <sup>*</sup>	Recovery (%) <sup>*</sup>	RSD <sup>*</sup>
GIZLAN (Tablets) 300 mg IRBN / 1 tab (JORDAN)	40	39.80	-0.5	99.50	2.11
	70	70.84	1.2	101.2	1.74
	100	101.33	1.33	101.33	1.94
	150	153.96	2.64	102.64	2.17

\*Average of four determinations

### Evaluation of the suggested procedure

To validate the proposed procedure proving that the recommended method can be successfully applied for the determination of IRBN in pharmaceuticals without interventions the standard additive method was applied. The data are shown and included in Figure 10 :

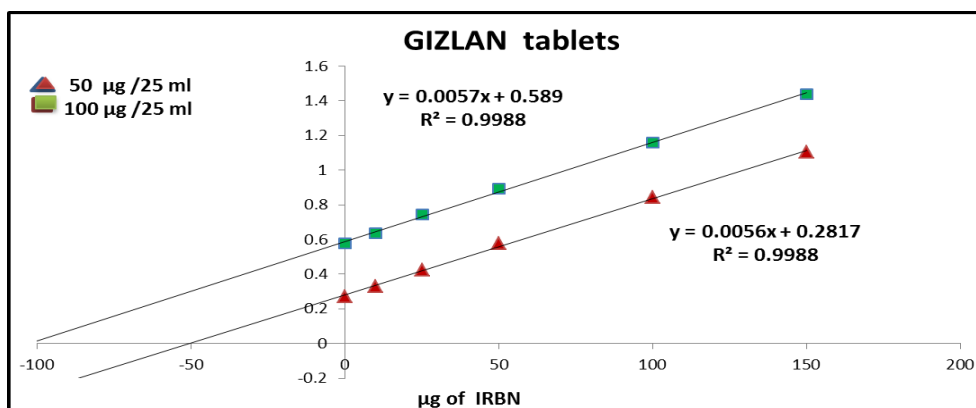


Fig. 10 : Calibration graphs of standard addition methods for analysis of IRBN in GIZLAN tablets

Table 3. The results of standard addition methods for analysis of IRBN

Drug	IRBN Present ( $\mu\text{g}$ )	IRBN Measured ( $\mu\text{g}$ )	Recovery (%)
GIZLAN (Tablets) 300 mg IRBN / 1tab. (JORDAN)	50	50.30	100.6
	100	103.33	103.33

## Conclusion

The proposed method for the determination of irbesartan in pharmaceutical samples is simple, sensitive, fast and inexpensive because it does not require temperature control or separation steps. The method is also accurate and precise enough to be successfully adopted as an alternative to the current spectrophotometric method for the determination of drugs in pharmaceuticals.

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