

# New Spectrophotometric Methods for the Simultaneous Determination of Irbesartan and Hydrochlorothiazide in Combined Dosage Forms

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## ABSTRACT

The combination of Irbesartan and Hydrochlorothiazide is mainly used for the treatment of hypertension. New spectrophotometric methods have been developed and validated for the simultaneous determination of Irbesartan and Hydrochlorothiazide in pharmaceutical formulations (Tablets) by two methods i.e. simultaneous equation method and absorbance ratio method (Q-analysis) in phosphate buffer (pH 7.5). Irbesartan and Hydrochlorothiazide have shown linearity over the concentration range 5-35 µg/ml and 0.2-40 µg/ml in both the methods.

**Key words:** Hydrochlorothiazide, Irbesartan, Spectrophotometric, Tablets, Validation.

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## INTRODUCTION

Irbesartan (IRB) (Figure 1) is an angiotensin II receptor antagonist used mainly for the treatment of hypertension. It delays the progression of diabetic nephropathy and is also indicated for the reduction of renal disease progression in patients with type 2 diabetes.<sup>1</sup> Hydrochlorothiazide (HTZ) (Figure 2) is a diuretic drug used for the treatment of high blood pressure and swelling due to fluid build-up.<sup>2</sup> HTZ is administered by oral route and combined with other blood pressure medications as a single pill to increase the effectiveness.

Analytical techniques such as LC-MS,<sup>3</sup> UPLC,<sup>4</sup> HPLC,<sup>5-10</sup> Micro emulsion LC,<sup>11</sup> HPTLC,<sup>12</sup> spectrofluorimetric<sup>13</sup> and spectrophotometric<sup>14-18</sup> methods were established for the simultaneous determination of Irbesartan and Hydrochlorothiazide in biological fluids as well as pharmaceutical formulations. In the present study the authors have proposed two spectrophotometric methods for the simultaneous determination of Irbesartan and Hydrochlorothiazide in tablets.

## MATERIALS AND METHODS

### Chemicals and reagents

The combined dosage form of Irbesartan and Hydrochlorothiazide is available as film-coated tablets with brand names AVALIDE (Sanofi Aventis Pharma, India), XARB-H (Nicholas, India) for oral administration containing 150 mg of Irbesartan and 12.5 mg of Hydrochlorothiazide. Methanol (MERCK), Di-Hydrogen phosphate (Rankem) and Potassium Di-Hydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) (Rankem) were purchased and used as received. All the chemicals are of analytical grade.

### Instrumentation

Spectral and absorbance measurements were made on an UV-1800 SHIMADZU double beam UV-Visible Spectrophotometer with 1 cm matched quartz cells. SHIMADZU electronic balance was used for weighing the samples.

### Preparation of stock solution

Stock solutions (1000 µg/ml) of Irbesartan and Hydrochlorothiazide were prepared by dissolving about 25 mg of each of Irbesartan and

Hydrochlorothiazide in two separate 25 ml volumetric flasks in methanol. Working standard solutions were prepared from the stock solution with phosphate buffer as per the requirement.

### Preparation of phosphate buffer (pH 7.5) solution

6.8 gm of potassium di-hydrogen orthophosphate and 1.56 gm of sodium hydroxide were weighed accurately in a 1000 mL volumetric flask. First 900 ml of double distilled water was added to mix them thoroughly by Sonication for 15 minutes, then adjust the pH 7.5 with sodium hydroxide solution and dilute with water to produce 1000ml.

### Procedure

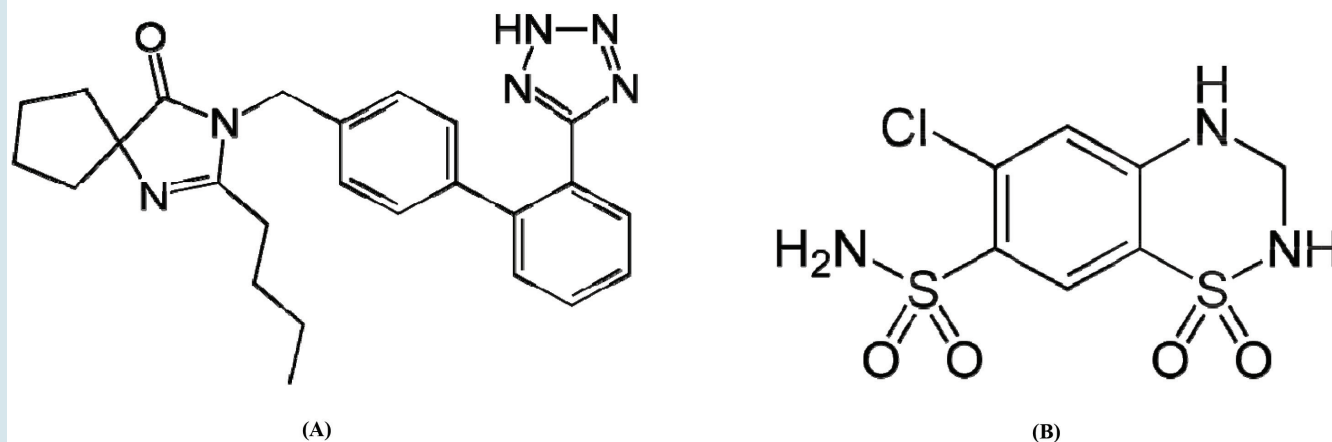
A series of solutions of Irbesartan (5-35 µg/ml) and Hydrochlorothiazide (0.2-40 µg/ml) were prepared from their stock solutions and scanned (200- 400 nm) against the reagent blank i.e. phosphate buffer pH 7.5. Two methods were used i.e. simultaneous equation method and absorbance ratio method (Q-analysis).

### Method A: Simultaneous Equation Method

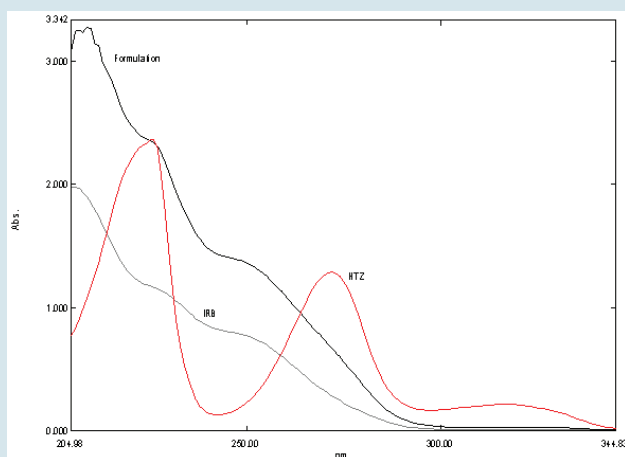
The absorption spectrum shows that Irbesartan has  $\lambda_{max}$  at 205 nm whereas Hydrochlorothiazide has at 272 nm respectively. For the simultaneous equation method, two wavelengths i.e.  $\lambda_{max}$  of the two drugs were selected and the absorbance as well as the absorptivity values were calculated from their individual spectra. Absorbance was noted against each concentration at 205 and 272 nm for both the drugs from their individual spectra and their absorptivity values were calculated.

### Method B: Absorbance ratio Method (Q Analysis)

Irbesartan has shown  $\lambda_{max}$  at 205 nm and Hydrochlorothiazide at 272 nm respectively in their absorption spectra. Three isosbestic (iso-absorptive) points were observed at 214.09, 231.28, and 258.75nm in the overlay spectra of Irbesartan and Hydrochlorothiazide. For the Q-analysis method, two wavelengths such as  $\lambda_{max}$  of one of the drugs and the isosbestic point were selected and the absorbance as well as the absorptivity values were calculated from their individual spectra. By using the simultaneous equation method and absorbance ratio method Irbesartan and Hydrochlorothiazide were determined in bulk and in its pharmaceutical



**Figure 1:** Chemical structures of Irbesartan (A) and Hydrochlorothiazide (B)



**Figure 2:** Overlay absorption Spectrum of IRB (20 µg/ml), HTZ (20 µg/ml) and mixture of IRB+HTZ (Formulation) in phosphate buffer pH-7.5

formulations (Tablets) using phosphate buffer pH 7.5 and the proposed method was statistically validated.

## Validation<sup>19</sup>

### Calibration curve (Linearity)

The series of solutions of Irbesartan (5-35 µg/ml) and Hydrochlorothiazide (0.2-40 µg/ml) prepared were scanned in the UV region (as the solutions were colorless) against the reagent blank i.e. phosphate buffer pH 7.5 and the absorbance was noted. Their selected wavelength for the two methods i.e. simultaneous equation method and absorbance ratio method (Q-analysis). A graph was drawn by taking the concentration on the x-axis and the corresponding absorbance values on the y-axis for the two drugs at the selected wavelength.

### Accuracy

Recovery studies were carried out by the standard addition method for the determination of accuracy of the proposed methods A and B. 80%, 100%, and 120% of pure bulk samples of Irbesartan and Hydrochlorothiazide were added to that of the pre-analyzed formulation and the % recovery as well as the % RSD were calculated.

### Precision

The intra-day and inter-day precision studies of the method was performed at three different concentration levels (10, 20 and 30 µg/mL) at three different intervals on the same day (Intra-day) and on three different days (Inter-day) respectively and the %RSD was calculated.

### Assay of Irbesartan and Hydrochlorothiazide combined dosage forms (Tablets)

The combined dosage forms of Irbesartan and Hydrochlorothiazide (Tablets) are available with brand names AVALIDE, XARB-H containing 150 mg of Irbesartan and 12.5 mg of Hydrochlorothiazide and were procured from the local pharmacy store. 20 tablets of each brand were weighed and powdered and powder equivalent w.r.t. 12.5 mg of Hydrochlorothiazide was taken and dissolved in a 100 ml volumetric flasks containing methanol and sonicated for 30 minutes. The volume was made up to the mark with methanol and filtered. These solutions were further diluted with phosphate buffer as per the requirement for the two methods and the percentage purity was determined.

## RESULTS AND DISCUSSION

The authors have developed two spectrophotometric methods, simultaneous equation method (Method A) and absorbance ratio method (Method B) for the simultaneous determination of Irbesartan and Hydrochlorothiazide in phosphate buffer (pH 7.5). The literature was thoroughly reviewed and the spectrophotometric methods developed till date were compared with the presently proposed method in Table 1.

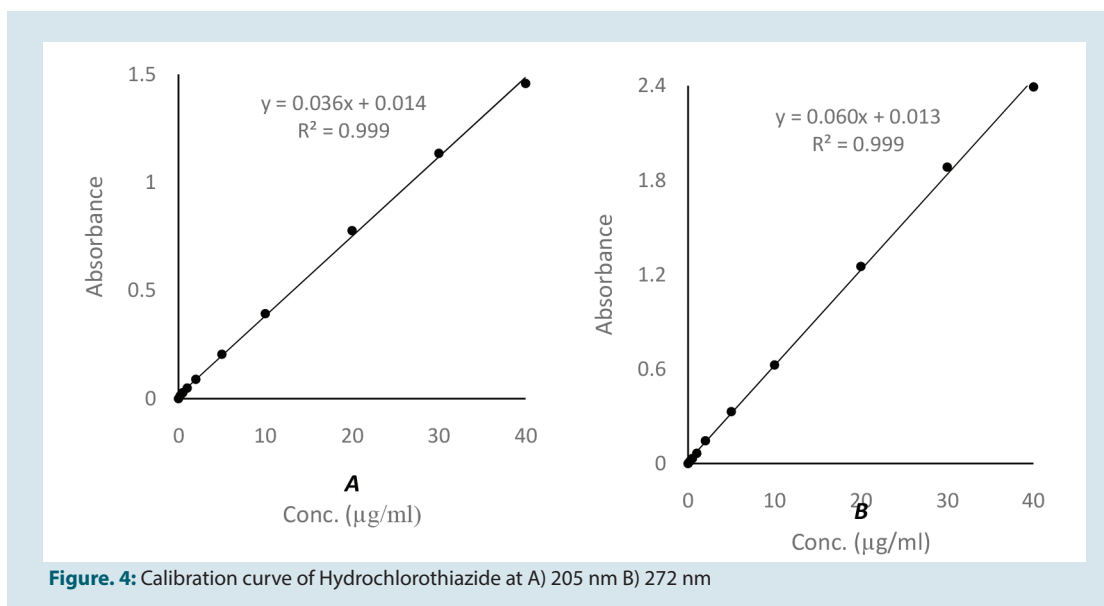
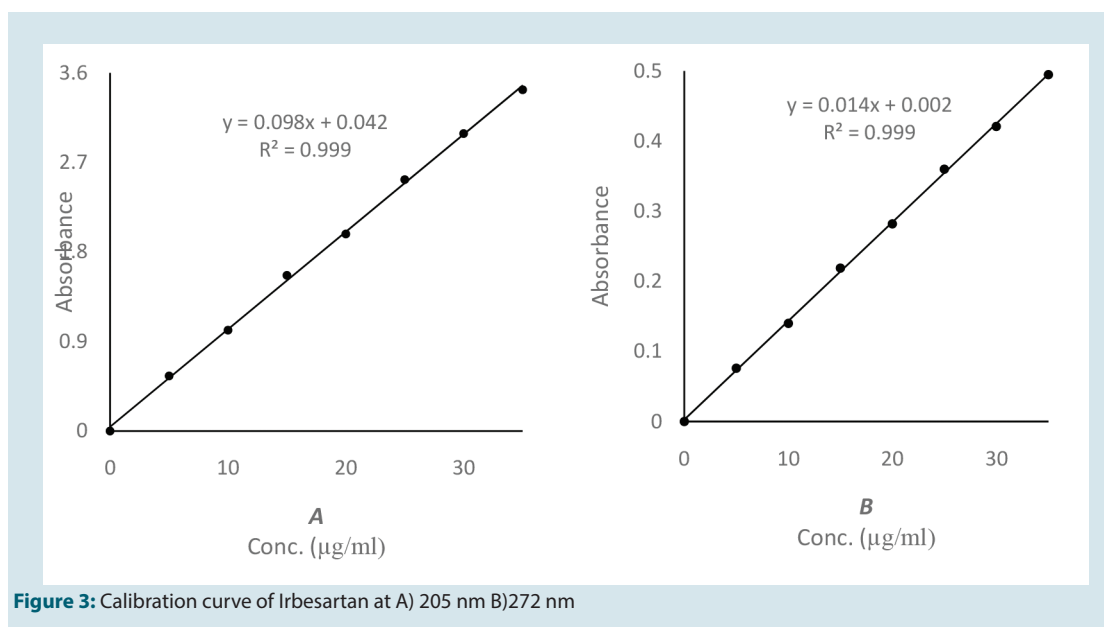
### Method A: Simultaneous Equation Method

For the simultaneous determination of two drugs by simultaneous equation method, specific absorptive values of the two drugs at the selected wavelengths were determined. The overlay absorption spectrum of Irbesartan and Hydrochlorothiazide was shown in Figure 3. The absorption spectrum of Irbesartan has shown  $\lambda_{\max}$  at 205 nm and that of Hydrochlorothiazide at 272 nm respectively.

The specific absorptivity value of a drug is the absorbance of the drug shown by a 1%, i.e. g/100ml solution and the absorptivity values obtained were incorporated in the simultaneous equations.

$$\text{At 205 nm, } A_1 = 1019.77 C_{\text{IRB}} + 464.925 C_{\text{HTZ}}$$

$$\text{At 272 nm, } A_2 = 143.06 C_{\text{IRB}} + 650.323 C_{\text{HTZ}}$$



where  $A_1$  and  $A_2$  are absorbance's of the mixture solution at 205 nm and 272 nm, respectively; 1019.77 and 143.06 are the absorptivity's of Irbesartan at 205nm and 272 nm, respectively and 464.925 and 650.323 are the absorptivity's of Hydrochlorothiazide at 205 nm and 272 nm, respectively;  $C_{IRB}$  and  $C_{HTZ}$  are the concentrations of Irbesartan and Hydrochlorothiazide, respectively in g/100ml.

#### Method B: Absorbance ratio Method (Q Analysis)

Irbesartan and Hydrochlorothiazide shows  $\lambda_{max}$  at 205 nm 272 nm respectively. Three isosbestic (iso-absorptive) points were observed at 214.09, 231.28, and 258.75 nm in the overlay absorption spectrum of Irbesartan and Hydrochlorothiazide (Figure 3).

The absorptivity values obtained at the selected wavelengths were incorporated in the following equation.

$$Cx = \frac{Qm - Qy}{Qx - Qy} \times \frac{A_1}{ax_1}$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} \times \frac{A_2}{ay_1}$$

'Cx' = the concentration of Irbesartan

'Cy' = the concentration of Hydrochlorothiazide.

' $A_1$ ' = the absorbance at iso-absorptive wavelength 258.75 nm.

' $A_2$ ' = the absorbance at wavelength 272 nm.

' $ax_1$ ' = the mean absorptivity of Irbesartan at 258.75 nm.

' $ay_1$ ' = the mean absorptivity of Hydrochlorothiazide at 258.75 nm.

Qm = the ratio of absorbance of sample solution at 258.75 & 272 nm.

Qx = the ratio of absorptivity of Irbesartan at 258.75 & 272 nm.

Qy = ratio of absorptivity of Hydrochlorothiazide at 258.75 & 272 nm.

## Validation

### Calibration curve (Linearity)

Irbesartan and Hydrochlorothiazide have shown linearity over the concentration range 5-35  $\mu\text{g/ml}$  and 0.2-40  $\mu\text{g/ml}$  in phosphate buffer ( $p^H$  7.5) in both simultaneous equation method and absorbance ratio method.

**Table 1: Comparison of previously published spectrophotometric methods with the proposed methods**

Method	$\lambda$ (nm)	Linearity ( $\mu\text{g/ml}$ )	Reference
Ratio subtraction	305	-	13
Derivative Ratio subtraction	262		
Simultaneous equation	224, 272	2-20	14
AUC	225-230	0.5-10	
	258-265		
Simultaneous equation	250	2-36	15
Q analysis	270.6	1-18	
Multi component mode	234	10-26	16
	272	5-13	
Simultaneous equation	215, 230	2-16	17
H-point standard addition	295.12	0.5-3	18
	216.32		
	228.41		
Simultaneous equation	205, 272	5-35	Present work
Q analysis	272	0.2-40	

**Table 2: Accuracy study of IRB and HTZ**

Drugs	Spiked Conc ( $\mu\text{g/ml}$ )	Formulation Conc. ( $\mu\text{g/ml}$ )	Method A		Method B	
			%RSD	%*Recovery	%RSD	%*Recovery
IRB	12 (80%)	15	0.76	99.25	0.45	98.50
	15 (100%)	15	0.89	99.80	0.57	98.85
	18 (120%)	15	0.56	99.87	0.87	98.61
HTZ	1.00 (80%)	1.25	0.66	98.88	0.45	99.30
	1.25 (100%)	1.25	0.87	99.40	0.27	99.50
	1.50 (120%)	1.25	0.33	98.31	0.76	98.31

\*Mean of three replicates.

**Table 3: Precision study of IRB and HTZ**

Drug	Conc. $\mu\text{g/ml}$	Intra-day precision				Inter-day precision			
		Method A		Method B		Method A		Method B	
		*Conc. obtained ( $\mu\text{g/ml}$ ) $\pm$ SD (RSD)	*% Recovery	*Conc. obtained ( $\mu\text{g/ml}$ ) $\pm$ SD (RSD)	*% Recovery	*Conc. obtained ( $\mu\text{g/ml}$ ) $\pm$ SD (RSD)	*% Recovery	*Conc. obtained ( $\mu\text{g/ml}$ ) $\pm$ SD (RSD)	*% Recovery
IRB	10	9.98 $\pm$ 0.03 (0.30)	99.8	9.96 $\pm$ 0.05 (0.56)	99.6	9.90 $\pm$ 0.09 (0.95)	99.0	9.8 $\pm$ 0.09 (0.99)	98.0
	20	19.93 $\pm$ 0.12 (0.60)	99.6	19.97 $\pm$ 0.15 (0.78)	99.8	19.82 $\pm$ 0.19 (0.98)	99.2	19.89 $\pm$ 0.23 (1.19)	99.4
	30	29.98 $\pm$ 0.26 (0.90)	99.9	29.96 $\pm$ 0.08 (0.29)	99.8	29.95 $\pm$ 0.36 (1.21)	99.5	29.95 $\pm$ 0.39 (1.31)	99.8
HTZ	10	9.97 $\pm$ 0.02 (0.28)	99.7	9.98 $\pm$ 0.02 (0.25)	99.8	9.93 $\pm$ 0.09 (0.98)	99.8	9.95 $\pm$ 0.09 (0.98)	99.5
	20	19.94 $\pm$ 0.11 (0.56)	99.7	19.99 $\pm$ 0.09 (0.49)	99.9	19.86 $\pm$ 0.22 (1.15)	99.3	19.89 $\pm$ 0.20 (1.04)	99.4
	30	29.99 $\pm$ 0.22 (0.76)	99.9	29.93 $\pm$ 0.16 (0.55)	99.7	29.92 $\pm$ 0.36 (1.23)	99.7	29.93 $\pm$ 0.36 (1.23)	99.7

\*Mean of three replicates.

**Table 4: Assay of IRB and HTZ by simultaneous equation method (Method A) and absorbance ratio method (Method B)**

Formulation Brand	Drug	Label claim(mg)	Method A		Method B	
			*Amount found	*Recovery (%)	*Amount found	*Recovery (%)
I	IRB	150	148.95	99.30	147.67	98.45
	HTZ	12.5	12.49	99.95	12.15	97.23
II	IRB	150	149.19	99.46	147.87	98.58
	HTZ	12.5	12.46	99.73	12.34	98.76

\*Mean of three replicates.

### Accuracy

The percentage recovery of Irbesartan and Hydrochlorothiazide were found to be 99.25-99.87 and 98.31-99.88 for method A; 98.50-98.85 and 98.31-99.50 respectively for method B. The % RSD for Irbesartan and Hydrochlorothiazide by the two methods is found to be less than 2.0 indicating that the methods are accurate (Table 2).

### Precision

The intra-day and inter-day precision studies were performed by the two methods A and B and the results were shown in Table 3. The percentage of recovery of Irbesartan and Hydrochlorothiazide were found to be 99.60-99.90 and 99.70-99.90 for method A; 98.00-99.80 and 98.40-99.70 respectively for method B. The % RSD for Irbesartan and Hydrochlorothiazide by the two methods is found to be less than 2.0 indicating that the methods are precise (Table 3).

### Assay of Irbesartan and Hydrochlorothiazide (Tablets)

The combined dosage forms of Irbesartan and Hydrochlorothiazide (Tablets) available with brand names AVALIDE (Brand I), XARB-H (Brand II) were evaluated by the two methods and the results were incorporated in Table 4. The percentage of purity of Irbesartan and Hydrochlorothiazide were found to be 99.30-99.95 and 99.46-99.73 for method A and 97.23-98.45 and 98.58-98.76 respectively for method B respectively.

## CONCLUSION

The proposed methods were validated and can be used for the determination of Hydrochlorothiazide and Irbesartan in tablets. The methods were found to be precise and accurate.

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## CONFLICTS OF INTEREST

The authors have no conflict of interest.

## ABBREVIATION USED

**HTZ:** Hydrochlorothiazide; **LC-MS:** Liquid Chromatography Mass Spectroscopy; **UPLC:** Ultra Pressure Liquid Chromatography; **HPLC:** High Performance; **HPTLC:** High Pressure Thin Layer Chromatography; **IRB:** Irbesartan; **UV:** Ultraviolet; **RSD:** Relative Standard Deviation; **ICH:** International Conference on Harmonization.

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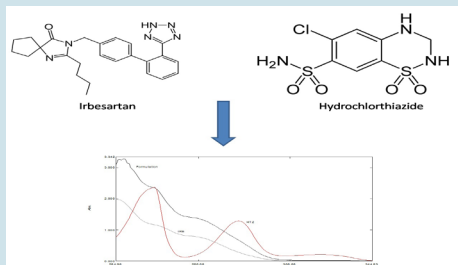
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### PICTORIAL ABSTRACT



### SUMMARY

- Two simple, precise and accurate spectrophotometric methods have been developed for the simultaneous determination of Hydrochlorothiazide and Irbesartan using phosphate buffer (pH 7.5) and validated.
- Simultaneous equation method and absorbance ratio method (Q-analysis) were adopted for the analysis of Irbesartan and Hydrochlorothiazide in combined dosage forms (Tablets).

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